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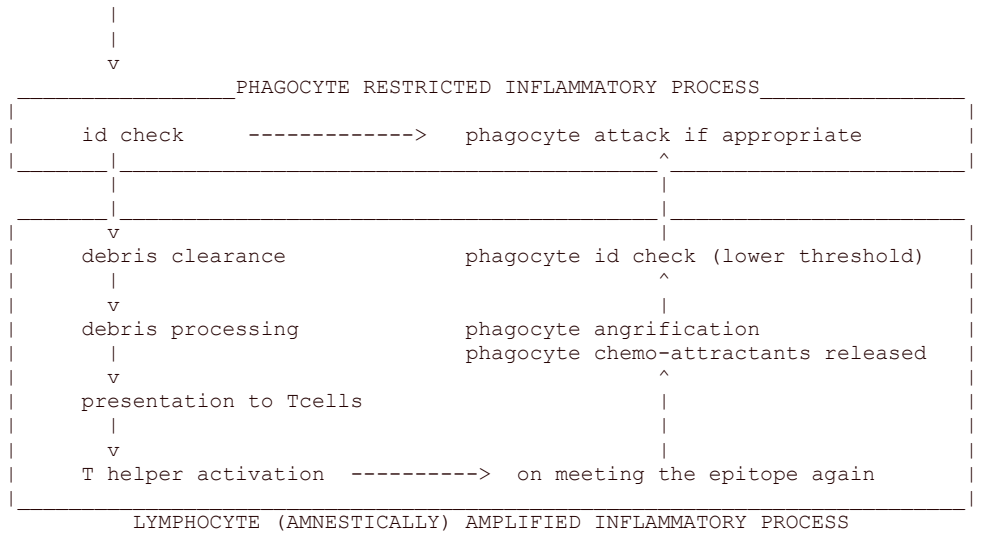

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MORPHOSTASIS and IMMUNITY Jamie Cunliffe Table

ABBREVIATIONS USED IN ARTICLE

UHS	Un-Healthy Self (cells)
NS	Non Self (organisms)
OTHS	Other Than Healthy Self (cells) (= UHS + NS)
ZDC	Zygote Derived Colony (or Clone)

JARGON USED IN ARTICLE

Epitope = the binding site on the Ag (recognised by the Ab)

Paratope = the binding site on the Ab (which recognises the Ag)

RELATIONSHIPS

HOMOLOGIES	between:	C3a C5a
	between:	beta-2-microglobulin constant region
	between:	homology unit constant region variable region
	between:	C1r and C1s
	between:	all parts C3,4,5
	between:	C2 and Bb
	between:	Ba and factor H
PHYLOGENY beta-2-microglobulin:		annelids cnidaria

	crustaceans
	frog
	birds
	mammals
complement	sharks
Mhc genes	frogs
	birds
	fish
	most mammals

All these molecules are spread across very broad phylae and orders. It looks as though their origins, especially that of NCAM, go back a long way and are preserved across these boundaries -- though NOT necessarily for the same functions.

EVOLUTION OF ZDCs from PROTOZOA to MAMMALS

(1) In the beginning, all cells express phagocytic behaviour

(2) Division of cells into phagocytes and soma and development of:
phagocyte LIGAND
somatic LIGAND(s)

(3) Evolution of a "vascular" system (locking out phagocytes till reqd)

(4) Progressive evolution and expansion of somatic LIGANDs

(5) The development of a "mix and match" process to generate a personal LIGAND unique to the individual (mitotic evolution!) to act as the phagocyte's id checker. This is an inversion of Tc cell activity (see text) and can be regarded as the generator of specificity (vs generator of diversity). The simultaneous evolution of Mhc LIGANDs with a method of creating a high level of population polymorphism in them.

(6) The inversion of the generator of specificity to generator of diversity. This would permit new cells (lymphocytic cells) to recognise virally infected cells (perhaps other intracellular pathogens too). These are the equivalent of Tc cells. They recognise class I antigens.

(7) The stage is now set to allow the evolution of Th cells on the basis of class II antigen recognition. The generator of specificity can now be adapted so that when the appropriate epitope is subsequently met the new T cell will attract and "angrify" large numbers of phagocytes.

(8) Simultaneous evolution of the tolerance principle is essential. Paratopes reactive against self will be mostly "mopped up" into Ts commitment. This happens because they are far more likely to be met in a non-inflammatory context. Newly generated self reactive paratopes are, however, able to be committed to Th activity if the inflammatory presentation occurs first. This is most likely to happen if the inflammatory process is prolonged and foreign antigen is sparse.

(9) The result is that disease will inevitably consist of a mixture of a reaction aimed exclusively at the pathogen (most likely not needing significant Th amplification) and a reaction aimed almost entirely at self: the latter occurring most significantly when the identification of clearly abnormal orgnisms/cells is not efficient.

CCCCCC

(10) Last of all, the Th function can now be adapted to produce the B-cell system and freely circulating antibodies. These help by opsonising organisms (preparing them as a "meal" for phagocytes). They are invaluble as a preemptive defence.

	MULTI-SYSTEM DISORDER						
COMPONENT DISORDER	SLE	PsA	RS	BS	UCA	CDA	Sa
ACNEIFORM LESIONS				+	+		
ANKYLOSING SPONDYLITIS	R	+	+	R	+	+	R
APHTHOUS ULCERS	+		+	+	+	+	
ARTHRITIS	+	+	+	+	+	+	+
ATOPY	+	+			+	+	+
ENCEPHALOMYELITIS (MS) (+MENINGITIS)	+		+	+	+		+
EPIDIDYMO-ORCHITIS				+			+
ERYTHEMA NODOSUM			+	+	+	+	+
NEUROSIS/PSYCHOSIS	+		+	+	+	+	+
OPTHALMITIS							
Conjunctivitis	+	+	+	+	+	+	+
Anterior Uveitis	+	+	+	+	+	+	+
Posterior Uveitis			+	+	+	+	+
Periphlebitis Retinae/Retinitis				+			+
Optic Neuritis			+	+	R	R	+
PERI/MYO-CARDITIS	+		+	+	+		+
PSORIASIS		+			+	+	
PUSTULES		+	+	+	+		
TENOSYNOVITIS			+	+			
TERMINAL ILEITIS/COLITIS				+	+	+	
THROMBOPHLEBITIS			+	+	+	+	
(NON-SPECIFIC) URETHRITIS			+	+			

+ = clinical association
R = recorded though significance of association unclear
SLE=SystemicLupus PsA=PsoriaticArthropathy RS=Reiter'sSyndrome
BS=Behcet'sSyndrome UCA=UlcColitis+Arthritis
CDA=Crohn'sDisease+Arthritis Sa=Sarcoisosis

ADJUVANT ARTHRITIS

Joint lesions	polyarthritis spondylitis tendinitis & tenosynovitis
Nodules	erythema nodosum like
Muco-cutaneous	pustules acanthosis parakeratosis & hyperkeratosis urethritis
Colon	non-specific diarrhoea inflammatory infiltration of the submucosa
Ocular	uveitis keratitis conjunctivitis
Heart	pericarditis myocarditis
Visceral	granulomata in liver and lungs
Neurological	focal encephalitis meningitis

TUBERCULOSIS	SERO-NEGATIVE ARTHRITIDES
ORAL ULCERS (up to 20% affected at autopsy)	RAU
EPIDIDYMO-ORCHITIS	BS Sa
ERYTHEMA-NODOSUM	BS RS UC CD Sa
INTESTINAL DISEASE with fistulation resembling Crohn's disease [78]	CD BS
ARTHROPATHY: a) mild non-bacterial b) bacterial involving SI joints, hips, knees, shoulders in descending order of prevalence c) Pott's disease of the spine d) TB tenosynovitis	All All have the same predilection for joints but no bacterial infection AS may masquerade as Pott's disease [79] RS BS
PLEURO-PERICARDO-PERITONITIS	SLE (all) & heart only in BS UC and Sa
ENCEPHALO-MYELITIS [80]	RS BS Sa SLE MS UC
APICAL PULMONARY CAVITATION	AS produces a clinically identical picture without TB bacillus infection [81]
LUPUS VULGARIS	Sa Discoid Lupus
OPHTHALMITIS a) phlyctenular conjunctivitis b) periphlebitis retinae	All associated with conjunctivitis BS Sa
ADDISON'S DISEASE	Idiopathic (auto-rejective) Addison's
predisposition	
STRESS PRECIPITATION and emotional factors [82]	Most
STEROID REPONSE - paradoxical initial improvement of X-rays and clinical condition with steroids	Steroids and immunosuppressives lead to amelioration ofthe acute features

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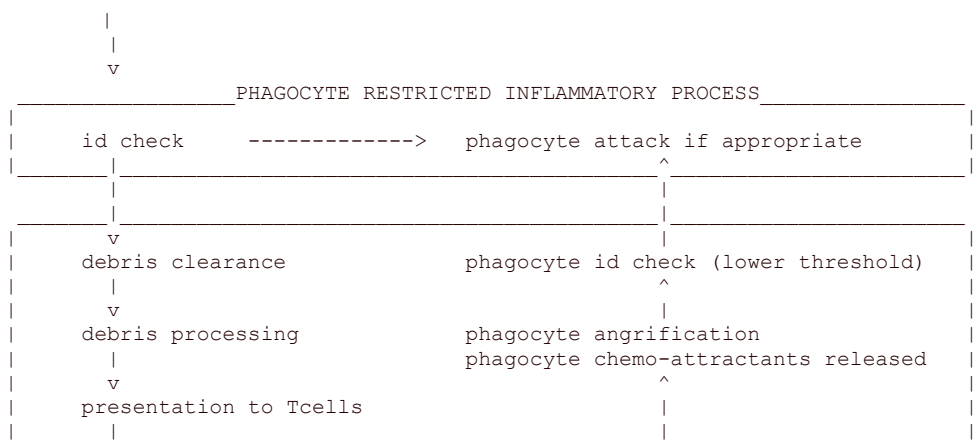

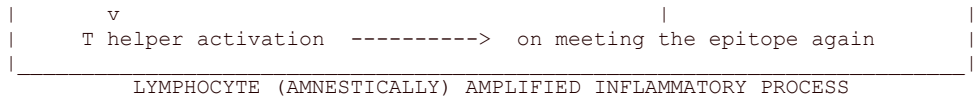

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Several points to make:

1) Cells developed GJs and used leaky membrane attack well before multicellulates started to get complicated. The GJs possibly evolved somehow from an adaptation of this attack. The two processes retain some functional links. It is possible that the membrane proteins specifying each of them are completely different but some aspects of their insertion and control are linked.

Evidence?

a) Logical

b) TNF and Lymphotoxin are related. TNF is preferentially less aggressive to cells in gap junctional contact.

c) Ig superfamily seems to be linked with the focal deposition of membrane holes (ie, either N-CAM or immunoglobulins).

d) N-CAM is necessary for gap junctional contact - it's essential for it to be expressed first.

e) Even yeasts use a similar membrane attack system.

f) IgSF CAMs probably create gap junctions selectively (selectivity rather than specificity - see Garrod). This selectivity probably account for developmental compartments (and they continue in the adult animal).

g) Two strategies are used. The first is to create an electrical synctium and this is of relatively low selectivity. It may be the main property assessed by phagocytes. The phagocyte has only to communicate (perhaps in its trailing membrane - via gjs) with the underlying tissues mass, and it becomes part of the local electrical synctium. The second strategy is to create morphogenetic fields. A brief look at Paramecium demonstrates how complex a single cell can be. It forms definite longitudinal, AP and Left/Right axes and clearly exercises some form of morphogenetic control within the cell. Developmental cell compartments are probably playing the same game but now, thanks to high density, wide lumen gap junctional plates, the whole compartment can act as one large block of cytoplasm (perhaps where homoeo genes fit in).

h) Cancer.

19920502_short

"There is only one constant element in immunity, whether innate or acquired, and that is phagocytosis. The extension and importance of this factor can no longer be denied."

Elie METCHNIKOFF 1905 [1]

"Immunology is an invention of the devil, who is making it up as he goes along

because he's not too clear about this stuff either.". . . . . ."Besides, immunology is what we North Americans call a Rube Goldberg system, referring to old cartoons about how to turn on the light, for example: you trip over a footstool, thus startling the cat, who bumps into the kitchen door, which swings shut, knocking over a chair that hits the light switch . . . you get the idea. There has to be an easier way."

Janice Hopkins TANNE 1990 [2]

INTRODUCTION:

The proposal I am about to make is stark: immunologists are missing the point. Their current perception of the immune process is flawed. Just as astronomers were once confident that the heavens revolved around the earth, so modern immunologists are generally confident that anamnestic immunity and its executors, the lymphocytes, are placed firmly centre stage, at the hub of the mammalian immune universe. In particular, it is current dogma that anamnestic aggression to non-self(epitopes) and tolerance of self(epitopes) is the source of self(cell)/non-self(cell) discrimination.

Let me see if I can shake your faith. The argument is fairly simple . I will describe the way I believe the system works and show how lymphocyte activity is probably the consequence rather than the source of self(cell)/non-self(cell) discrimination.

(1) MORPHOSTASIS:

Morphostasis is tissue homeostasis: it is manifestly efficient in all animals. This is the core function, the true centre of the metazoan universe. It is built upon cell to cell recognition and communication. Anamnestic immunity is but a branch of the morphostatic process and it has evolved to enhance morphostatic efficiency in vertebrates.

An animal is built from a large colony of cells all derived from one zygotic cell (a zygote derived colony - ZDC). This colony constructs itself a relatively inert skeleton of connective tissues which allows it a greatly enhanced versatility. The critical process in morphostasis is to discriminate Healthy Self (HS) cells from Other Than Healthy Self (OTHS) cells. OTHS includes both Unhealthy Self (UHS) cells and clearly foreign organisms. Morphostasis was needed from the moment that multicellular animal forms first evolved. It should be clear that the main need at that time was to develop a unique way of allowing healthy self cells to acknowledge each other and then of devising a means of abandoning this healthy self status when things went wrong.

Morphostasis (tissue homeostasis) can be maintained by:

(a) discriminating OTHS cells from HS cells.
(b) removing OTHS cells (UHS and foreign cells/organisms).
(c) replacing lost UHS cells with fresh HS cells (resurgent morphogenesis).

(2) HEALTHY SELF/OTHER THAN HEALTHY SELF DISCRIMINATION:

This hypothesis requires that individual cells MUST either have a fail-safe internal device for recognising that they have become unhealthy OR an ability to monitor a neighbouring cell's change in health (probably) by monitoring cell to cell communication. The announcement of an "OTHS foul" comes directly from an affected group of somatic cells. Inflammatory cells (mostly phagocytes) are only invited into the area at this group's request - a "call" is sent out to fetch the "police". Foreign organisms need not induce an inflammatory response UNLESS they unsuccessfully attempt communication with a HS cell, OR force their way between cells (and so disrupt communication), OR directly attack a cell and make it sick.

Several mechanisms may combine to contribute to HS identity; remember that one or more of the critical aspects which lead to HS recognition must be abandoned when the cell becomes sick:

(a) Lectins and the recognition of saccharides (eg, sialic acid).
(b) The inhibition of complement attack by proteins released from or displayed on the cell membrane (eg, factor H, DAF, MCP).
(c) Beta-2-microglobulin and Class 1 Mhc ligand expression.
(d) Cell to cell cytoplasmic joining - particularly electrical.

(3) INFLAMMATION:

The infiltration of somatic tissues by inflammatory cells is a ancient and virtually universal metazoan defence mechanism. These cells are clearly able to recognise most organisms (particularly those which are not dedicated pathogens) and, in the vast mass of animal life, they appear to do so without the aid of memory cells. They also remove aging and disordered self cells. In fact, they are ideally adapted to deal with OTHS. I propose that the prime function of the lymphocytic system (which evolved later) was to accelerate and accentuate the inflammatory process and, in turn, make the removal of OTHS by phagocytes more efficient. The discrimination of HS from OTHS by phagocytes remains a central and critical immune process. But HS/OTHS discrimination probably starts in general cell to cell communication.

Static (somatic) cells are attached to each other by several types of cell junction. Their cytoplasms are joined by gap junctions (GJs - except in those cells who's function depends on electrical excitability). When membrane junctions are split apart the disruptions in the cell membranes inevitably lead to the release of various eicosanoids (prostaglandins etc). This announcement of an OTHS event by somatic cells results in an inflammatory reaction (in tissues with few GJs, inflammation is less pronounced). Chemical messengers released at the OTHS site encourage the ingress of phagocytes (in mammals, through the endothelial cell linings of local post-capillary venules). Phagocytes now invade the OTHS site. They begin assessing cells on the basis of their HS status. Thus far, the basic process is the same for almost every, if not all, animal species. At this point, vertebrates enroll a new mechanism. Debris from local tissues is processed by phagocytes (or phagocyte related cells) and it is then presented, in local lymph nodes, to the anamnestic immune system as short representative peptides. The aim is to select representative epitopes and to retain a memory of them and their inflammatory environment so that, on their next encounter, this inflammatory environment can be rapidly and potently reproduced. This anamnestic response is under the full command of the morphostatic process and, in particular, largely under the control of phagocytes.

(4) THE GENERATION OF SPECIFICITY:

This hypothesis requires that (at the very least) a scavenger cell existed in the ancestry of modern vertebrates which was able to recognise a self cell on the basis that it expressed self Mhc "Class-I-like" ligands and, in so doing, it observed a "horror autotoxicus" to that self cell. This cell may still exist (a possible candidate is the natural killer lymphocyte - Tnk). This scavenger would have had a natural tendency to attack cell like structures UNLESS they could prove that they were healthy self cells. (Note that the result of complement component activity is very much in this style, with healthy self being "immune": and also that phagocytes synthesise enough of all but the terminal components to attack cells.) This putative cell would be naturally aggressive to all cellular structures and only switched into

non-aggressiveness by the presence of appropriate "Class-I-like" ligands. This action is an inversion of the activity of the Tc cell. Both phagocytes and lymphocytes are derived from marrow stem cells. They are closely related, adding weight to the proposition that a phagocyte like or derived cell might, at one stage, have evolved to have the ability to select/rearrange its genes so that it could specifically recognise healthy self ligands (Mhc "Class-I-like" ligands: note that N-CAM RNA is selected and rearranged).

Cell type	Receptors disabled	Receptors enabled	Normal state	Triggered state
Scavenger	non pure self	pure self	aggressive	passive
	GENERATOR OF SPECIFITY			
Tc cell	pure self	non pure self	passive	aggressive
	GENERATOR OF DIVERSITY			

This would neatly explain how the anamnestic immune system appears to have erupted onto the evolutionary scene so suddenly and so completely in the vertebrates. Even a repertoire of receptors as few as two would be useful in the generation of specificity whereas a large repertoire seems almost a "sine qua non" for effective T-cell functioning. So, RECEPTOR genes would have had ample time to expand their repertoire before being precipitously "flipped" around for use by an anamnestic immune system.

So why are there virtually no reports to suggest that a scavenger can still specifically recognise self cells on the basis of Class I Mhc ligands? Well, it may be that the lymphocyte based system has been so successful that it has largely obviated the need for a scavenger to rearrange its genes and the system relies on the more primitive phagocytic assessment of HS cells (see (6) below); there might even be a positive advantage in achieving the apparent recognition of HS(cells) by inverting the action into an attack on non-self(epitopes) by Tc lymphocytes (achieved by the clonal elimination of any lymphocyte capable of reacting with "pure self" Class 1 ligands); OR natural killer T-cells (Tnk) are the delegated scavengers which check that somatic cells possess Class I HS ligands (hence enabled/disabled rather that selected/deleted). A final possibility is that we are failing to observe specific recognition even though it exists.

Natural killer cells could certainly fulfil this function. They were first identified because F1 Tnk cells attacked parental cells (quite unlike the classical transplantation laws). These cells also preferentially attack cells expressing low levels of Class I antigen and beta-2-microglobulin. However, it seems that, at most, only a proportion of them rearange their receptor genes. This might imply that they either use different receptors to Tc cells, or, perhaps, most Tnk cells exercise a low specificity recognition (eg, to beta-2-microglobulin alone). Whatever, the observed properties of Tnk cells are at least partially consistent with the expected functions of an inverted Tc cell.

(5) MIMICRY:
Because morphostatic systems have always relied on self recognition, dedicated pathogens have had to use mimicry (or more subtle interferences with identity molecule expression and recognition) to gain access to and persist in the soma. Every animal needs to stay one step ahead of its competition. Constant pressure is exerted to expand the variety of identity molecules available within a species (pleomorphism). Somatic cells appear to recognise each other by developmental ligands (cell adhesion molecules, CAMs). When embryonic cells

from two mammalian species are disaggregated, mixed together and allowed to settle, they segregate into tissue type and not into species. Somatic ligands have probably needed to stay constant over countless meiotic generations. This makes them a sitting duck for determined pathogens. So, somatic cells need a backstop identity to be used as a second check when things go wrong (phagocyte based and Mhc Class 1 based). And until they do go wrong, inflammatory cells can be confined to the vascular system, locked out behind tight endothelial cell junctions until invited in. (Note that "loss of function" is a cardinal feature of the inflammatory process.) Some cell ligands (eg, N-CAM) are acknowledged members of the immunoglobulin supergene family and may even have been the originators of this family.

(6) ANAMNESTIC AMPLIFICATION:
So, what are lymphocytes doing? When T-cells are released from the thymus they are already committed in specificity (ie, they are committed to recognising a specific epitope). But, they are not committed in activity (aggression or suppression). It is only when they meet their respective epitope that they commit themselves. Self epitopes are, in general, encountered frequently and nearly always first in a "healthy self" (non-inflammatory) environment. So tolerance is generally favoured for those lymphocytes which recognise self molecules. Few self specific T-cells will remain uncommitted for more than a brief period while there is a relatively large pool of the relevant self epitope waiting to be encountered. On the other hand, because only small quantities of foreign or strange epitope are met, infrequently, in the body, most T-cells capable of recognising them will remain uncommitted until they meet the epitope in an inflammatory encounter. Inevitably, they are most often met in an inflammatory context and aggression is favoured. Furthermore, it seems that it may be easier to provoke older precursor lymphocytes into aggression. This further concentrates the aggressive response onto those epitopes that are most strange to the body. No veto is imposed on T-cells to prevent them becoming aggressive to self epitopes (except for "pure self" Mhc ligands - these are clonally disabled). Indeed, epitopes that are usually hidden behind tight endothelial cell junctions (like the eye and brain) are infrequently encountered and a larger pool of uncommitted T-cells is likely to be available. They are, consequently, more inclined to provoke an aggressive response when they are exposed during periods of intense inflammation. The thymus constantly produces new uncommitted T-cells. So, whenever clearly foreign epitopes are sparse and inflammation is intense, attention will gradually turn to self epitopes (eg tuberculosis). In summary, aggression is most likely to develop to clearly foreign (strange) epitopes and tolerance most likely to develop to self (frequently encountered) epitopes.

The overall effect is that lymphocytes remember the inflammatory or non-inflammatory context in which they first meet their respective epitope (and become committed); and they aim to recreate and caricaturise this memorised inflammatory milieu at the next encounter. Whenever Td cells provoke an inflammatory response they call large numbers of phagocytes (& Tnk cells?) to the epitope site. These are then switched into a heightened state of "anger". However, phagocytes (& Tnk cells?) STILL have to discriminate HS from OTHS. But now, the threshold at which aggression is considered is greatly reduced. Cells expressing a relatively low level of "HS identity" are now likely to be attacked. This amplification of the inflammatory response by lymphocytes has the potential to escalate catastrophically. It can slip into a strong positive feedback loop, particularly when the epitope is an abundant self Ag. When the local auto-rejective response becomes excessive, it must be down-regulated otherwise things will get disastrously out of hand. This could be done in a number of ways and these may account for many instances of anergy:

(a) inhibition of phagocyte ingression (chemotaxis)
(b) inhibition of phagocyte aggression

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| (c) inhibition of further aggressive lymphocyte activation |
| (d) a tightening of endothelial cell junctions             |
| (e) encapsulation in a fibrin sheath (fibrocytes later)    |
| (f) promotion of lymphocytic tolerance to typical Ag       |
| (g) production of auto-antibodies to the newly cloned,     |
|     locally reactive lymphocytes (lymphocytotoxic Abs)      |
|____________________________________________________________|
```

(7) MORPHOSTATIC EVOLUTION:

It is now easier to see how the morphostatic system may have evolved. It has been suggested that CAMs belonging to the immunoglobulin supergene family may have appeared early in the history of cell cooperation. If this proves to be the case then there is a clear path in the development of the morphostatic system from early multicellulates to man. Remember that ontogeny frequently retraces phylogeny. Though this trend cannot be regarded as an absolute blueprint for the evolutionary process, it is a useful pointer. Cell to cell recognition in embryos is likely to point towards HS/OTHS discrimination in the adult mammal. Imagine taking a journey through evolution:

EVOLUTION OF ZDCs from SIMPLE MULTICELLULATES to MAMMALS

(a) In the beginning, all cells in the colony express equally marked phagocytic behaviour.

(b) "SELF is established by making holes in the membranes of apposing cells and lining them up to create gap junctions. This allows cells to become electrically coupled and so to act as an electrical and, probably, a cytoplasmic continuum. This ability to couple membranes dates back to the very earliest multicellulates. It relies on the controlled, ordered, simultaneous adjacent membrane insertion of membrane holes. Cells learn, early on, to allow the uncoordinated, bigger, higgledy piggledy insertion of leaky holes into organisms which fail to demonstrate the membrane LIGANDs used as a focus for the tidy construction of gap junctions: electrical discontinuity and a lower membrane potential invite phagocytosis. Unhealthy self cells can elect to be rejected by uncoupling themselves and dropping their membrane potential: they also learn to abandon their membrane (self) LIGANDs."

(c) Cells now divide into phagocytes and soma. They selectively improve the specificity and efficiency of cell junction construction by facilitating and amplifying their construction at the site of cell LIGAND/RECEPTOR interaction. The resultant gap junctions are (perhaps) larger and more specific. They develop:

somatic LIGAND(s) - for recognition by resident scaffolders.
phagocyte LIGAND(s) - for recognition by itinerant scavengers.

(d) Dedicated phagocytes now evolve. They refine this cooperative gap-junctional communication with self and the runaway, leaky hole attack of non-self. The molecules used to do the second evolve into what we now recognise as the complement components. It is possible that these two construction cascades are related but become independant early in evolution. At this stage the complement components are secreted locally by phagocytes and their action is directed entirely at membranes. It is only much later that these components are co-opted into a humeral system and very much later that they are co-opted to interact with antibodies (probably an adaptation of specific Mhc recognition).

(e) A "vascular" system now evolves, locking out phagocytes till required. The alternative complement cascade can now be "humeralised" so that circulating C3 can mark clearly foreign organisms so that they can be more readily identified when they meet a phagocyte.

(f) There is now a progressive evolution and expansion of somatic LIGANDs leading to increased tissue compartmentalisation.

(g) Ig supergene like LIGANDs develop to act as a focus on which to grow highly specific gap junctional plates and create developmental compartments. The genes specifying these molecules are now copied then altered by a "mix and match" process to generate one set of LIGANDs which have a great variability within a herd. These pleomorphic LIGANDs now act as the final arbiters of healthy self in each individual. Over many meiotic generations, they have evolved into Mhc Class I LIGANDs. Newly developed scavenger cells are now able, when required, to electrically couple with any somatic cell that displays self specific LIGANDs and observe a horror autotoxicus to it. These scavengers need a mechanism to produce and/or select self specific RECEPTORs unique to each ZDC. This must be done post-meiotically over a number of mitotic generations - the "generation of specificity". (This possibly coincides with the appearance of "eggs".) These scavengers resemble natural killer cells.

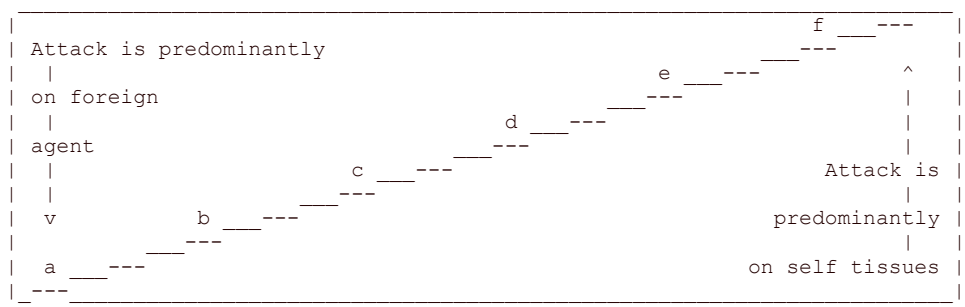
(h) By inverting the "generator of specificity" into the "generator of diversity" lymphocytic cells evolve which are able to recognise and attack cells who's Class I ligands have been altered. It is well recognised now that viruses and other intracellular pathogens interfere (by attachement) with ligand/receptor machinery. If these altered Class I ligands are processed, leaving representative peptides attached, viral particles in association with self Mhc can be remembered then, on their next encounter, attacked by an inverted scavenger (?Tnk). These are the equivalent of Tc cells and recognise Mhc "Class-I-like" ligands. Sometime between now and the evolution of free antibodies, the so called "alternative" complement pathway is extended into the "classical" pathway. C1 might be specialised for short range triggering of high density, single surface LIGAND/RECEPTOR complexes so that hole construction is now restricted to the target membrane rather than to a coordinated construction in apposing membranes.

(j) The stage is now set to allow the evolution of Td cells. Class II Mhc ligands evolve: the "intention" is to present these on the inner surface of phagocyte lysosomes where they are allowed to interact with cellular peptide debris picked up by phagocytes at inflammatory sites. These are then externalised as a Class II/debris combination ready for the attention of uncommitted T-cells. The "generator of diversity" can now be enrolled into memorising the inflammatory context of these epitopes. On re-encountering the epitope these T-cells can now rapidly attract large numbers of phagocytes to the site and "angrify" them: inflammation now has a memory. (Note that only a very limited set of cells - APCs, phagocytes and a few others - can present the combinant epitopes so this amplification of the inflammatory cascade can only start after OTHS has been processed.)

(k) The capacity to develop T-cell tolerance has to evolve simultaneously with Tc and Td cells. T-cells capable of recognising self epitopes are mostly decommissioned. This may be a co-operative process (Td/Ts cooperation akin to Th/B-cell co-operation). Whatever, agression is averted by having them "mopped up" by Ts commitment. This happens because these epitopes are more likely to be met in a non-inflammatory context. However, self specific T-cells continue to be released from the thymus and can become available for aggression. Aggression to self epitopes will be most likely to be induced and permitted when the inflammatory process is prolonged and foreign epitopes are sparse. Tolerance might be amplified by Ts cell clonal expansion and, perhaps, the release of anti-inflammatory agents at the site of epitope re-encounter. (Like Th and B-cell interaction, helper and suppressor epitopes tend not to overlap, suggesting a co-operative

mechanism: it may also reflect the preferential attention of Tc and Td cells to allotypes.)

(l) The result of all this is that any disease which evokes an inflammatory response has an element of auto-rejection. It inevitably consists of a varying mixture of attack directed exclusively at the pathogen (usually leading to mild inflammation) and attack directed almost entirely at self (often highly inflammatory): the latter occurs when organisms or cells provoke prolonged inflammation but do not provide or present clearly foreign looking (unusual) epitopes. Every disease that leads to cell damage will be also be accompanied by auto-rejection.



EXAMPLES
(a) Saprophyte
(b) Simple epithelial commensal
(c) Staphylococci and streptococci
(d) Tuberculosis and syphilis
(e)-(f) Multiple sclerosis and sero-negative arthritis

(m) Last of all, Th cells can now be enrolled into the system to create the B-cell system and freely circulating antibodies. The B-cells are also derived from a scavenger cell but designed, now, to secrete large quantities of circulating antibody. Antibodies help by opsonising organisms (preparing them as a "meal" for phagocytes). The classical complement cascade is now optimised to work within the vascular system and to interact with antibody tagged antigen. This system has proved invaluable as a preemptive defence.

SUMMARY:
The perception of immunity has been reshaped to encompass the broader principle of MORPHOSTASIS. The loss of healthy self is sensed and expressed by the malfunctioning cell itself or emanates from the site at which it makes contact with its immediate neighbours. This "foul" is broadcast by the release of inflammatory mediators. These invite phagocytes into the area to assess local cells. Phagocytes (and Tnk cells) then attack those cells with which they fail to become electrically contiguous. The time they have to make this connection varies with the "anger" of the phagocytes. Now phagocytes present cell debris to lymphocytes in local lymph nodes. The most foreign "looking" epitopes are selected to act as the pegs on which to hang a greatly accelerated inflammatory ingress on any subsequent encounter of these epitopes.

The concept of "horror autotoxicus" is now redefined and it is seen to be dependant on successful cell to cell communication. Both somatic and scavenger cells use this mechanism. The concept of immunological surveillance is also redefined. But now this surveillance is for any malfunctioning cell and not just for neoplasia. The evolution of a thymus dependant (anamnestic) lymphocytic system may have occurred at the expense of an increased prevalence of cancer, for intense focal suppression of surveillance now occurs whenever a

strong positive feedback leads to an exaggerated attack on self epitopes.

This explanation is undoubtedly simplistic and will prove to be inaccurate in a number of its more specific assumptions. Also, the immune system has gathered a great number of refinements throughout its evolution including various specialised phagocytes and permanently resident, non-itinerant antigen presenting cells: little has been said about these. However, I suggest that the "flavour" of the concept is essentially correct and the hypothesis will serve as a useful framework for refinement.

It should now be clear that the breaking of cellular junctions is probably an important event which leads to the declaration of an OTHS "foul". There are a number of close similarities between the insertion of gap junctions into self cell membranes and the insertion of complement membrane attack complexes into invaders. If it could be shown that there is a continuing or a distant relationship between their respective insertion mechanisms, then it would be reasonable to assume that HS is sensed by the speed with which both somatic cells and scavenger cells establish an electrical continuum with those cells that they encounter.

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"There is only one constant element in immunity, whether innate or acquired, and that is phagocytosis. The extension and importance of this factor can no longer be denied."

Elie METCHNIKOFF 1905 [1]

"Immunology is an invention of the devil, who is making it up as he goes along because he's not too clear about this stuff either.". . . . . "Besides, immunology is what we North Americans call a Rube Goldberg system, referring to old cartoons about how to turn on the light, for example: you trip over a footstool, thus startling the cat, who bumps into the kitchen door, which swings shut, knocking over a chair that hits the light switch . . . you get the idea. There has to be an easier way."

Janice Hopkins TANNE 1990 [2]

INTRODUCTION

The proposal I am about to make is stark: I believe immunologists are missing the point: their current perception of the immune process is flawed. Just as astronomers were once confident that the heavens revolved around the earth, so modern immunologists are generally confident that anamnestic immunity and its executors, the lymphocytes, are placed firmly centre stage, at the hub of

the (mammalian) immune universe. In particular, it is current dogma that lymphocytes are the commanders of self/non-self discrimination.

non-self epitopes could be better regarded as the result, rather than the source, of healthy-self(cell)/all-other(cell/organism) discrimination.

Few of the elements that I assume in this article are radically new. However, the emphasis of their perception IS and this fresh perception leads to a "paradigm shift".

THE EMERGENCE OF SELF(CELL)/NON-SELF(CELL) DISCRIMINATION

To set the scene, I would like to emphasise these points:

(1) When the first multicellulates evolved, they needed to recognise and discriminate self-cells from non-self-cells.

(2) We have become preoccupied with self(epitope)/non-self(epitope) discrimination, mainly as a result of the sequence of discoveries in immunology: this has blinkered our perceptions.

(3) In a large proportion of metazoans, lymphocytes are self-evidently NOT the source of self(cell)/non-self(cell) discrimination: they don't have any.

(4) It SHOULD be possible to discern gradual steps in immune evolution starting in primitive metazoans and leading to the sophisticated system found in mammals. So far, this progression has eluded immunologists.

(5) In development, ontogeny frequently appears to retrace phylogeny: whilst this is not an absolute blueprint for evolution, it can provide important pointers.

MORPHOSTASIS

Morphostasis is tissue homeostasis and it is well maintained in all animals [3]. It is a core process: the functional hub of the metazoan universe. It works efficiently because cells monitor their own health and keep a constant close communication with appropriate neighbours. Anamnestic immunity is a branch of the morphostatic process: it has evolved to enhance the effectiveness of morphostasis in vertebrates.

Remember, an animal is built of a large colony of cells all derived from one zygote cell (a zygote derived colony - ZDC). This colony constructs itself a skeleton of connective tissues which, while relatively inert, gives it great versatility (eg, the bony skeleton).

The critical function in morphostasis is discriminating Healthy-Self (HS) cells from all other cells and organisms (other than healthy self - OTHS cells). OTHS includes both Unhealthy Self (UHS) cells (eg, ectopic, sick, damaged, aging) and clearly foreign cells and/or organisms. Morphostasis was needed from the moment that multicellular animals first evolved. It should be clear that the main need at that time was to develop a unique way of tagging healthy self cells, so enabling them to acknowledge one another, and then to devise a means of abandoning this healthy self status when things went wrong.

Morphostasis (tissue homeostasis) can be maintained by:

(a) discriminating OTHS cells from HS cells.

(b) removing OTHS cells (UHS and foreign cells/organisms).

(c) replacing lost UHS cells with fresh HS cells (resurgent morphogenesis).

HEALTHY SELF/OTHER THAN HEALTHY SELF DISCRIMINATION:

This hypothesis requires that individual cells MUST either have a fail-safe internal device for recognising that they have become unhealthy and/or an ability to monitor a neighbouring cell's change in health (probably) by monitoring (appropriate) cell to cell communication. The announcement of an "OTHS foul" is issued directly from the affected (somatic) cells. Inflammatory cells (mostly phagocytes) are ONLY invited into the soma at this group's request - a "call" is sent out to fetch the "police". Foreign organisms need

not induce an inflammatory response UNLESS they unsuccessfully attempt communication with a HS cell, OR force their way between cells (and so disrupt communication), OR directly attack a cell and make it sick.

Several properties may combine to constitute HS identity; remember that one or more of the critical aspects which lead to HS recognition must be abandoned when the cell becomes sick. Here are some possible candidates:-

(a) Lectins and the recognition of saccharides (eg, sialic acid).
(b) The inhibition of complement attack by proteins released from or displayed on the cell membrane (eg, factor H, DAF, MCP).
(c) Beta-2-microglobulin and Class 1 Mhc ligand expression.
(d) Cell to cell cytoplasmic joining - particularly electrical.

CELL IDENTITY IN THE EMBRYO AND OTHER SYSTEMS

The cells in an embryo recognise each other through Cell Adhesion Molecules (CAMs) [4]. At the cell surface, like/like and ligand/receptor interactions of these CAMs lead to cell adhesion. This adhesion then rapidly progresses on to communication through gap junctions [5]. These CAMs are of three types: first, the cadherins, second the integrins and third, a group of CAMs which are members of the immunoglobulin superfamily (IgSF) of which NCAM is an example. The somatic cells of an embryo are able to recognise appropriate neighbours and navigate themselves into appropriate positions until they meet appropriate cell types. There are many examples of the specific recognition of cells in biology (see below).

Edelman has stated, "The origin of the entire Ig superfamily from an early N-CAM-like gene precursor has deep implications for the understanding of the role of adhesion in processes that are not concerned with morphogenesis but

rather with immune defense, inflammation and repair" [6].

Note that the transfer RNA molecules specifying NCAM are spliced by cells in a variety of different ways to produce a range of NCAM phenotypes.

Here are some specific examples of identity recognition [7]:

Protozoans recognise and discriminate food and sexual partners
Phagocytes are able to recognise their own pseudopodia and avoid self attack.
Simple multicellulates are known to reject allografts
Plants - pollination is highly selective against self
Reaggregation of disrupted foetal cells (see later)
Bacterial agglutination and conjugation can be highly specific to self and (in pathogens) to target tissues.
Plants - tree roots in a forest often fuse together. This is very frequent in roots from the same individual, frequent in the same species and far less frequent in unrelated species.
Molecular recognition is a fundamental biological principle (eg, nuclear enzymes).
Cell homing. For example, lymphocytes and injected marrow cells.

Self recognition could, therefore, be observed in several ways, each becoming progressively more specific to the individual animal:-

(a) species recognition (eg, gamete recognition)
(b) tissue type recognition (eg, embryo cell recognition)
(c) self recognition (ie, cells of the individual zygote derived clone. Useful for phagocytic defence)

MORPHOGENESIS

Morphogenesis is the process by which tissues and organs are sculptured from a zygote derived colony. It is most obvious in developing embryos: regeneration

is a resurgence of morphogenesis.

Since morphogenesis is an integral part of a morphostatic system, it is reasonable to expect that the component elements of morphostasis will use molecular machinery which is genetically related. They have (presumably) been closely associated through every epoch of metazoan evolution. The complete mechanism which leads to embryonic development remains unclear. However, CAMs and gap junctions appear to play central roles [8].

EMBRYOS, CAMs AND GAP JUNCTIONS

1) Gap junctional communication can be relatively non-specific (crossing species barriers) but it can also be highly selective (as below) [9].
2) Gap junctional communication is critical in development. Embryo development fails when GJ communication is disrupted [10].
3) When CAMs (cell adhesion molecules) interact with each other or their receptors, the ensuing cell adhesion appears to lead directly to gap-junctional communication. CAMs precede GJ insertion and both are necessary for normal development [11].
4) Embryos are made up of a number of compartments. Communication through gap junctions is constricted at their boundaries. These compartments correspond to important developmental fields [12]. They also correspond to fields of specific CAM expression [13].
5) The gap junctions in these compartments are of two sorts [14]. First, there are high permeability junctions joining each cell within a compartment. These allow the free passage of larger molecules: lucifer yellow is used to demonstrate this. I suspect that this "open" communication enables a group of cells to be organised, as if they were a single block of cytoplasm. This may be under the control of the appropriate segmental homoeotic gene (look at the complex structure of paramecium to see how this might work). Second, there are more restrictive junctions which join the cells at the boundaries

of these "open" compartments. These only allow small molecules to diffuse (eg, ions). These junctions allow ions to pass in either both or just one direction (ie, they are rectifying and correspond to junctions formed from hybrid connexons [15]). This directionality may be of significance in the way that embryonic cells sort, with endoderm to centre and ectoderm to the outside. These restrictive junctions are either insufficiently large or insufficiently extensive to allow lucifer yellow to diffuse freely.

6) Despite its name, N-CAM is not confined to neural tissues. Whilst it is expressed strongly and for long periods in neural development, it is also expressed, more transiently, in other sites [16]. It is a recognised IgSF member (Immunoglobulin Super Family). A number of authors have considered these IgSF CAMs to be the probable ancestors of immunoglobulins, T-cell receptors and histocompatibility antigens (Edelman for one [17])).

When embryo cells are disaggregated and allowed to resettle, they reaggregate into tissue layers, ectoderm to the outside, mesoderm to the middle, then endoderm to the centre [18]. When embryonic cells from two mammalian species are mixed, they reaggregate into tissue type rather than species type and this appears to be because the genes which specify the various CAMs are highly conserved across the species barriers [19].

MEMBRANE HOLES

It is now possible to make a stab at the general principle which governs HS/OTHS discrimination. I suspect it goes something like this:-

"SELF is established by making holes in the membranes of apposing cells and lining them up to create gap junctions. This allows cells to become electrically coupled and so to act as an electrical and, probably, a cytoplasmic continuum. This ability to couple membranes dates back to the very

earliest multicellulates. It relies on the controlled, ordered, simultaneous adjacent membrane insertion of membrane holes. Cells learned, from the start, to allow the uncoordinated, bigger, higgledy piggledy insertion of leaky holes into organisms which fail to demonstrate the membrane LIGANDs used as a focus for the tidy construction of gap junctions: electrical discontinuity and a lower membrane potential leads to an attack by scavengers. Unhealthy self cells can elect to be rejected by uncoupling from adjacent cells then dropping their membrane potential (by mobilising calcium ions from covalently bound calcium stores): they can also abandon the membrane LIGANDs which specify self. The mechanisms for constructing leaky holes (complement MACs) may be distantly related to the mechanisms for constructing gap junctions."

HORROR AUTOTOXICUS & MORPHOSTASIS

One result of relying on self(cell) recognition is that "horror autotoxicus" (HA - the horror of attacking self) will probably have evolved long before lymphocytes and their memory for previously encountered antigens (anamnesis). However, this HA must be based upon the possession of specific and recognisable cell surface markers ("flags"): these probably aid the co-operative "docking" of one cell with another. Furthermore, because infection, cell damage, mutation, aging, genetic errors and other cell disturbances can also be assumed to be ancient problems, cells of the ZDC probably learned, early on, to observe "horror autotoxicus" to HS cells whilst rejecting or ignoring OTHS (unhealthy self [UHS] and clearly foreign cells/organisms).

This interpretation of "horror autotoxicus" is quite different from the classic one, in which lymphocytes are deemed to be "denied" the right to attack self antigens. In this new interpretation, lymphocyte aggression towards self antigens is neither denied nor necessarily avoided. However, as will become apparent, once such auto-aggression has arisen, it will decay

unless other circumstances actively sustain it.

PHAGOCYTES and DOUBLE-THINK

There is a strange double-think that pervades immunology when it comes to the importance and centrality of phagocytes and the recognition of non-self and/or unhealthy self. Every medical student knows that phagocytes recognise dead, damaged, sick and effete cells. Every medical student knows they can recognise foreign organisms and eliminate them (particularly non-dedicated-pathogens). Every text book devotes its statutory (short) introductory opening to the importance of phagocytes and innate immunity: then, almost without fail and with indecent haste, authors are seduced into an intense dissection of the principles of anamnesis and lymphocyte function. What makes this more bizarre is that the anamnestic immune system isn't essential to prepare cells for phagocyte attention. The phagocytic system works well, even if slowly, in invertebrates: self/non-self discrimination works well in invertebrates.

There cannot be much doubt that the reason for this tendency to overlook the fundamental centrality of phagocytes is (a) a lack of understanding of the mechanisms of self/non-self discrimination by these cells and (b) the intense acceleration of the inflammatory process by lymphocytes. This greatly enhances the efficiency with which OTHS is removed and it has led us to regard lymphocytes as masters rather than servants of the system. There is, at the very least, a possibility that CAM interaction and junctional communication (between phagocytes and underlying somatic cells) may be the important factor in HS self cell recognition.

INFLAMMATION:

Metazoans have evolved an ancient and virtually universal defence mechanism which is to infiltrate somatic tissues with scavenger cells whenever required (mostly phagocytes). These scavengers are clearly capable of recognising most

organisms (particularly those which are not dedicated pathogens). And, in the vast mass of animal life, they appear to do so without the aid of cells which have the ability to "remember" epitopes. They also remove aging and disordered self cells. In fact, their behaviour is ideally suited to eliminating OTHS. I propose two things:

(a) In all complex metazoans, the discrimination of HS from OTHS by phagocytes REMAINS the central and crucial immune process.
(b) All other immune activities are geared to accelerating, accentuating and maximising this process. In consequence, the efficiency with which OTHS is removed by phagocytes can be greatly enhanced.

Even so (as you will see later) HS/OTHS discrimination does not begin in phagocytes but in somatic cells. It is the consequence of general cell recognition and communication. Inflammation is only established when somatic cells "decide" that they cannot cope alone and "invite" the scavengers in.

Static (somatic) cells are attached to each other by cell junctions. Their cytoplasms are joined by gap junctions (GJs - except in those cells who's function depends on electrical excitability). When membrane junctions are split apart the disruptions in the cell membranes probably lead to the release of various eicosanoids (prostaglandins etc). This announcement of an OTHS event, by somatic cells, results in an inflammatory reaction. (Note that in electrically excitable tissues which have few GJs, inflammatory responses are far less pronounced). Chemical messengers released at the OTHS site encourage the ingress of phagocytes (through the endothelial cell linings of local post-capillary venules). Phagocytes now invade the OTHS site. They begin assessing cells on the basis of their HS status. Thus far, the basic process is the same for almost every, if not all, animal species. At this point, vertebrates enrol a new mechanism. Debris from local tissues is processed by

phagocytes (or phagocyte related cells) and it is then presented, in local lymph nodes, to the anamnestic immune system as short representative peptides in combination with class II antigens. The aim is to select representative Class II/peptide epitopes and to retain a memory of them and their inflammatory environment so that, on their next encounter (which MUST, incidentally, follow phagocyte/APC processing), this inflammatory environment can be rapidly and potently reproduced and, more often than not, exaggerated. This anamnestic response is under the full command of the morphostatic process and, in particular, largely under the control of phagocytes.

MIMICRY:

Because morphostasis has always relied on self recognition, dedicated pathogens need to use mimicry (or more subtle interferences with identity molecule expression and recognition) to gain access to and persist in the soma [20]. Every animal needs to stay one step ahead of its competition. Constant pressure is exerted to expand the variety of identity molecules available within a species (pleomorphism). Somatic cells appear to recognise each other by developmental ligands (cell adhesion molecules, CAMs). When embryonic cells from two mammalian species are disaggregated, mixed together and allowed to settle, they segregate into tissue type and not into species. Somatic ligands have probably needed to stay constant over countless meiotic generations. This makes them a sitting duck for determined pathogens. So, somatic cells need a backstop identity to be used as a second check when things go wrong (phagocyte based and, perhaps, Mhc Class 1 based). And until they do go wrong, inflammatory cells can be confined to the vascular system, locked out behind tight endothelial cell junctions until invited in. (Note that "loss of function" is a cardinal feature of the inflammatory process.)

UNHEALTHY SELF ACTIONS: APOPTOSIS AND SELF SACRIFICE

When cells fail to establish communication, membrane reactions probably begin

which lead to the release of a variety of prostaglandins and other cytokines [21]. Similarly, when cells become unhealthy they break junctional communication and become prey to attack by both adjacent cells and the inflammatory cells which are (in consequence) called into the area [22]. When I first started thinking in these terms, I had found scant literature describing elective suicide and I even looked at plants for evidence of this (the hypersensitivity reaction [23]). However, interest and literature on this subject have become abundant recently and there are several recent articles, one in Adv Immunology [24], one in the Annual Review of Biology [25] and a very readable article in the New Scientist [26]. In synthesis, individual cells DO decide that they are sick and/or redundant. They DO have the capacity to invite attack by adjacent cells and also to invite phagocytes along to have themselves removed. There is no need to presume that antibodies and lymphocytes are the sole, let alone the prime, assessors of healthy self status.

Changes in the concentration of calcium ions within the cell are all important in this election for "disposal by consensus". Ca++ ions act as second messengers for a variety of cell processes including apoptosis, nuclear division, growth factor stimulation: and they are closely tied into the inositol-PO4/DAG/protein-kinase-C network of intracellular second messengers [27]. In this respect, cellular identity and cell health is all tied into proto-oncogene activity and this in turn into gap junction formation and communication competence [28]. Here is the promise of a much clearer understanding of cancer.

When cells are attacked by C9 or perforin, they are made leaky, their cytoplasmic membrane potential falls and Ca++ ions are allowed into the cell. Both these molecules contain sequence motifs similar to the LDL receptor and epidermal growth factor receptor. The significance of this escapes me at the

moment but one important feature is that both the receptors they seem to be related to are endocytosed in clathrin coated pits (like the Mhc molecules themselves).

THE GENERATION OF SPECIFICITY:

A major problem in understanding the evolution of anamnestic immunity is how such a complex system erupted onto the evolutionary scene, so suddenly and so completely, in the vertebrates. One explanation is that it evolved, not as a generator of receptor diversity but as a generator of receptor specificity.
The table below shows how a scavenger cell could be programmed only to cooperate with self cells which display ligands unique to that single ZDC. The specification of such a scavenger is an exact inversion of the specification of the cytotoxic T cell. Even a repertoire of receptors as few as two would be useful in specificity whereas, in diversity, it is difficult to see how any useful function could have evolved until there was a large repertoire of possible receptors. With a system which develops on the basis of specificity, there would be ample time to develop an extensive repertoire of possible receptors before being precipitously "flipped around" to service a generator of diversity. (Note that "pure self" is used to indicated unaltered, self Class I Mhc antigens.)

Cell type	Receptors disabled	Receptors enabled	Normal state	Triggered state
Scavenger	non pure self	pure self	aggressive	passive
	GENERATOR OF SPECIFITY			
Tc cell	pure self	non pure self	passive	aggressive
	GENERATOR OF DIVERSITY			

There are two possibilities. First, that the ancestors of the T cell receptor may have been used to recognise tissue CAM ligands: this could be the origin

of the V gene segments [29]. Secondly, a descendant of the simple scavenger (phagocyte) may have evolved to recognise a set of pleomorphic CAM like markers which were specifically evolved in a population to be used as a backstop identity check. Developmental CAMs seem to remain constant over countless generations and this is reflected in the way embryonic cells from different species reaggregate as germ layers and tissues rather than species. The "backstop" CAM like ligand (the precursor of the Class I Mhc antigens) could deliberately borrow bits and bobs from these developmental CAMs to form a unique looking ligand by using a genetic mix and match process.

There seems to be little question that phagocytes are unable to rearrange their genome to form specific receptors. And there is no significant evidence that they can selectively cooperate with cells carrying self Mhc antigens. Natural killer cells, however, might be such a candidate, particularly if they are composed of two populations: one with a lower specificity - perhaps based on beta-2-microglobulin expression - and another with highly specific receptors for self. Natural killer cells could fulfil this role. They were first identified because F1 Tnk cells attacked parental cells (unlike the classical transplantation laws) [30]. This would be consistent with specific (cooperative) recognition. These cells also preferentially attack cells expressing low levels of Class I antigen and beta-2-microglobulin [31]. It seems that, at most, only a proportion of Tnk cells rearrange their receptor genes [32].

Phagocytes, lymphocytes, fibroblasts and platelets are all derived from the same stem cell. They have almost certainly all evolved from a primitive scavenger. Each cell seems to have caricaturised a specific property of this general scavenger and refined it in order to make the mature mammal more versatile. This adds weight to the proposition that a phagocyte like or derived cell might, at one stage, have evolved to have the ability to

select/rearrange its genes so that it could specifically recognise healthy self ligands (Mhc "Class-I-like" ligands. The self receptors would have to be selected, in embryo, to be specific to each individual.

One possibility is that, now the lymphocyte system has evolved, this has been so successful that it has largely obviated the need for a scavenger to rearrange its genes to uniquely recognise self. There might even be a positive advantage in achieving the apparent recognition of HS(cells) by inverting the cooperative recognition of self cells into an attack on non-self(epitopes) by Tc lymphocytes. This can be achieved by the clonal elimination of any lymphocyte capable of reacting with "pure self" Class 1 ligands.

Note that complement activity is very much in the style of a horror autotoxicus, with healthy self being protected from attack by inhibitors: and also that phagocytes synthesise enough of all but the terminal components to attack undesirable cells unaided.

SOMA/SCAVENGER SEGREGATION

I have already alluded to soma/scavenger segregation. The important point to grasp is that somatic cells can and do deal adequately with a fair proportion of OTHS33. Provided the accumulation of OTHS is mild and the local cells can both recognise any loss of HS identity and discriminate foreign organisms from HS, then there is little need for a backstop identity check. HS here is established by displaying appropriate tissue CAMs which lead on to the establishment of a "synctial" communication through GJs. However, when UHS or foreign organisms fail to appear sufficiently OTHS to the local cells, then tissue damage will probably ensue as the foreign cells or UHS cells start to gain the upper hand. It is at this stage that scavengers are "invited" in and this is done by a failsafe device (the eicosanoid system - prostaglandins etc). These scavengers then establish HS status by employing a "backstop"

check on identity. If there is a scavenger which formally recognises HS Class 1 status then this would make the system highly specific (eg, the Tnk cell - see later).

Since inflammatory cells invade and disrupt the normal structure of tissues, this invasion leads to a loss of function. They are undesirable intruders in healthy tissues except in small numbers. Hence they need to be kept largely locked out, behind a tightly bound network of endothelial cells lining the blood vessel walls. This need for segregation is almost certainly the origin of the vascular system. The subsequent recruitment of the vascular system into distributing other "freight" has resulted in the phagocytes and their evolvents becoming adapted to such tasks as the encapsulation of the inflammatory process (by clotting factors and platelets), the distribution of fats in the blood (phagocytes) and the distribution of oxygen (red cells).

Now it is possible to add some concluding comments to the six points introduced earlier in the section "EMBRYOS, CAMs AND GAP JUNCTIONS":

7) In this hypothesis I have suggested that scavenger cells (phagocytes mostly) use a CAM receptor molecule to latch onto a respective CAM on self cells. This would lead on to an electrical connection with the underlying self cells. When a cytoplasmic finger from a scavenger cell encounters another cell it tries to establish direct electrical communication by forming gap junctions across the membranes separating the scavenger from the underlying cells. If it fails to establish communication, the scavenger may be triggered into aggression by the capacitative current which will flow as the membranes move close together. This could, in turn, trigger an action potential to arm this cytoplasmic finger of the scavenger cell. Additional recognition strategies may be employed. The changing of surface sugars in sick cells is one (loss of the negatively charged sialic acid

residues may increase the capacitive current above the triggering threshold). The phagocyte may well have a limited "hit list" of receptors which recognise markers which are indubitable evidence of their non-eucaryotic origin and which would, therefore, never be found as part of self. Dedicated pathogens will, of course, studiously avoid displaying these.

8) Now, the original self CAM may gradually be found to be inadequate as a backstop identity check because various pathogens discover ways of mimicking or interfering with its machinery. At this stage, a new cell is required (perhaps similar to the natural killer cell) which can recognise a more pleomorphic set of CAMs that are deliberately individualised in each animal of a population and more or less unique to each one. An appropriate set of specific receptors would have to be selected, in embryo, to recognise these unique ligands. These, I contend, may be the close ancestors the T cell receptor which led, by inversion of function, to the cytotoxic T cell. In this vein, note that TNF and lymphotoxin are selectively toxic to cells which are NOT communicating through gap junctions [34] .

ANAMNESTIC AMPLIFICATION

So, what is the function of lymphocyte system: what are lymphocytes doing? Direct killing is NOT the prime function in either delayed type hypersensitivity T-cells or helper cells T-cells. They are not remembering epitopes for the prime purpose of "killing" them. The precursor lymphocyte logs the context in which it first set eyes on its epitope. If it was inflammatory then at the next encounter it will recreate a rapid and potent inflammatory response rather than wait for the "cell damage-cytokine-inflammation" cascade to build up. "Tipped off" inflammatory cells can then settle down much more quickly and aggressively to their phylogenetically ancient task of sorting HS from OTHS. The main difference now

is that these phagocytes are doing it much more quickly and with better targeting. But they are also doing it more hamhandedly - they'll "bash" anything that looks remotely suspicious (hence the need to focalise this response). Tc cells are relatively more independent and kill directly but even these are only allowed to become aggressive if they have first been primed by IL-1 from APCs during an inflammatory encounter. And these, too, encourage a rapid inflammatory response once they start attacking cells.

Somatic cells probably show some specificity about which epitopes to present for Tc cell priming. The peptides they present in combination with Class I antigens have probably been shepherded through the cell by its garbage minders, the ubiquitins. Leaving this aside, it is still easy to imagine how self/non-self selectivity can occur. When T-cells are released from the thymus they are already committed in specificity (ie, they are committed to recognising a specific epitope). But, they are not committed in activity (aggression or suppression). It is only when they meet their respective epitope that this commitment is made. Self epitopes are, in general, encountered frequently and the first encounter (in embryo) is nearly always in a "healthy self" (non-inflammatory) environment. So tolerance is generally favoured for those lymphocytes which recognise self molecules. Few self specific T-cells will remain uncommitted for more than a brief period while there is a relatively large pool of the relevant self epitope waiting to be encountered.

On the other hand, because only small quantities of a foreign or strange epitope are infrequently met in the body, most T-cells capable of recognising them will remain uncommitted until they meet the epitope in an inflammatory encounter. Because they are part of OTHS, they will be met in an inflammatory context and aggression will be favoured. To enhance this, it seems that it is easier to provoke old rather than young precursor lymphocytes into aggression.

This further concentrates the aggressive response onto those epitopes that are most strange to the body. No veto need be imposed on T-cells to prevent them becoming aggressive to self epitopes (except for "pure self" Mhc ligands - these are clonally disabled). Indeed, epitopes from tissues that are usually hidden behind tight endothelial cell junctions (like the eye and brain) are infrequently encountered and there is likely to be a larger pool of uncommitted T-cells available. They are, consequently, more inclined to provoke an aggressive response when they are exposed during periods of intense inflammation.

The thymus constantly produces new uncommitted T-cells. So, whenever clearly foreign epitopes are sparse and inflammation is intense, attention can gradually turn to self epitopes (eg, as in tuberculosis). In summary, aggression is most likely to develop to clearly foreign (strange) epitopes and tolerance most likely to develop to self (frequently encountered) epitopes.

The overall effect is that lymphocytes remember the "inflammatory" or "healthy soma" context in which they first meet their respective epitope (and become committed); and they aim to recreate and caricaturise this memorised inflammatory or non-inflammatory milieu at the next encounter. Whenever Td cells provoke an inflammatory response they call large numbers of phagocytes (& Tnk cells?) to the epitope site. These are then switched into a heightened state of "anger". However, phagocytes (& Tnk cells?) STILL have to discriminate HS from OTHS. But now, the threshold at which aggression is considered is greatly reduced. Cells expressing a relatively low level of "HS identity" are now likely to be attacked. This amplification of the inflammatory response by lymphocytes has the potential to escalate catastrophically. It can slip into a loop of strong positive feedback, particularly when the epitope is an abundant self Ag. When the local auto-rejective response becomes excessive, it must be down-regulated otherwise

things will get disastrously out of hand. This could be done in a number of ways and these may account for many instances of clinical anergy [35]:

(a) inhibition of phagocyte ingression (chemotaxis)
(b) inhibition of phagocyte aggression
(c) inhibition of further aggressive lymphocyte activation
(d) a tightening of endothelial cell junctions
(e) encapsulation in a fibrin sheath (fibrocytes later)
(f) promotion of lymphocytic tolerance to typical Ag
(g) production of auto-antibodies to the newly cloned, locally reactive lymphocytes (lymphocytotoxic Abs)

OTHS PRESENTATION		HS PRESENTATION
Associated with an injurious or useless cell or situation	<<----------->>	Associated with a harmless or useful cell or situation
(Ag processed by APCs then presented to paratope: OR Tc cells given an aggressive kick by Il-1)		(Ag directly presented to paratope without processing: OR Tc cells not given aggressive kick by Il-1)
(INFLAMMATORY) Th Td Tc		(HEALTHY SOMA) Ts

AUTO-REJECTION

There is one important inference to be made from examining the structure of the sero-negative arthritides and particularly Behcet's syndrome [36]. This is that auto-rejective disease covers a wide spectrum of prevalence and severity. The mildest components are VERY common, suggesting that auto-rejection is a normal process (see the article "Clinical Morphostasis"). This leads to the conclusion that there is no automatic horror autotoxicus to self epitopes where T cells are concerned. When auto-rejection is so general, it has to have physiological as well as pathological significance: it must be a functioning element of the morphostatic mechanism.

ANTIBODIES - ICING ON THE CAKE

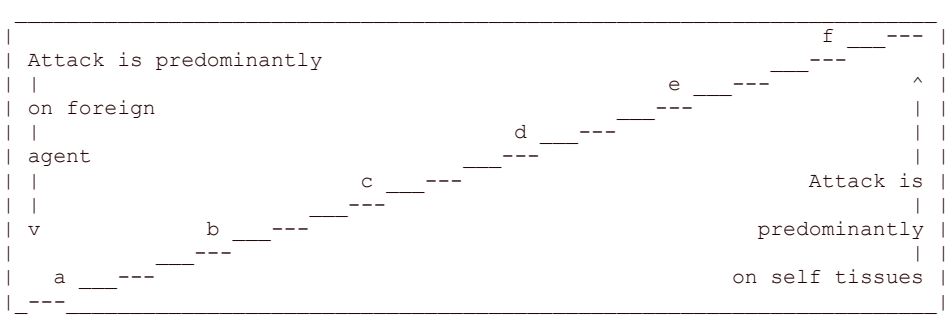
Antibodies are icing on the cake. Extremely useful, evidently important but

dominantly aimed at pre-empting the proliferation of blood borne pathogens and pathogens which colonise epi/endothelial surfaces. It's clear that the role of antibodies in tissue rejection (and hence auto-rejection) is minor if not minimal. The vast mass of animal life copes well without them. "Cell-mediated immunity clearly precedes humeral antibody production in phylogeny" (Manning and Turner [37]). We can safely put antibodies to one side until towards the end - which is more or less where they evolved. It appears to me that, to bother to look amongst antibodies for an explanation of how self/non-self discrimination evolved, would be manifestly Heath Robinson (or Rube Goldberg!).

In this vein, it is worth noting that the spleen may be specifically adapted to make the best of the difficult job of maintaining some semblance of morphostasis amongst the cells suspended in the highly mobile plasma.

THE CLINICAL IMPLICATIONS

The result of all this is that any disease which evokes an inflammatory response has an element of auto-rejection. It inevitably consists of a varying mixture of attack directed exclusively at the pathogen (usually leading to mild inflammation) and attack directed almost entirely at self (often highly inflammatory): the latter occurs when organisms or cells provoke prolonged inflammation but do not provide or present clearly foreign looking (unusual) epitopes. Every disease that leads to cell damage will induce auto-rejection.



EXAMPLES

(a) Saprophyte

(b) Simple epithelial commensal

(c) Staphylococci and streptococci

(d) Tuberculosis and syphilis

(e)-(f) Multiple sclerosis and sero-negative arthritis

This is explained in more detail in a separate article, Clinical Morphostasis".

MORPHOSTATIC EVOLUTION:

It is now easier to see how the morphostatic system may have evolved. Here is the probable path of the evolution of ZDCs from simple multicellulates to mammals.

(a) In the beginning, all cells in the colony express equally marked phagocytic behaviour.

(b) SELF is established by making holes in the membranes of apposing cells and lining them up to create gap junctions. Cells learn, early on, to allow the uncoordinated, bigger, higgledy piggledy insertion of leaky holes into organisms which fail to demonstrate the membrane LIGANDs used as a focus for the tidy construction of gap junctions.

(c) Cells now divide into phagocytes and soma. They selectively improve the specificity and efficiency of cell junction construction by facilitating and amplifying their construction at the site of cell LIGAND/RECEPTOR interaction. The resulting gap junctional plates are more "transparent" and more specific about where they form. They develop:

somatic LIGAND(s) - for recognition by resident scaffolders.

phagocyte LIGAND(s) - for recognition by itinerant scavengers.

(d) Dedicated scavengers (phagocytes) now evolve. They refine this cooperative gap-junctional communication with self and the runaway, leaky hole attack of non-self. The molecules used to do the second will eventually evolve into what we now recognise as the complement components. It is possible that the two construction cascades are related but become independent early in evolution. At this stage the complement components are only secreted locally by phagocytes and their action is directed entirely at membranes. It is a long time before these components are co-opted into a humeral system and very much later that they are co-opted to interact with antibodies (probably an adaptation of specific Mhc recognition).

(e) A "vascular" system now evolves, locking out phagocytes till required. The alternative complement cascade can now be "humeralised" so that circulating C3 can mark clearly foreign organisms so that they can be more readily identified when they meet a phagocyte.

(f) There is now a progressive evolution and expansion of somatic LIGANDs leading to increased tissue compartmentalisation.

(g) Ig supergene like LIGANDs develop to act as a focus on which to grow highly specific gap junctional plates and create developmental compartments. The genes specifying these molecules can now be copied then altered by a "mix and match" process to generate a set of LIGANDs which have a great variability within a herd. These pleomorphic LIGANDs will now act as the final arbiters of healthy self in each individual. Over many meiotic generations, they have eventually evolved into Mhc Class I LIGANDs. Newly developed scavenger cells are now able, when required, to electrically couple with any somatic cell that displays self specific LIGANDs and observe a horror autotoxicus to it. These scavengers need a mechanism to produce and/or select self specific RECEPTORs unique to each

ZDC. This must be done post-meiotically over a number of mitotic generations - the "generation of specificity". (This possibly coincides with the evolution of shell protected eggs.) These scavengers resemble natural killer cells.

(h) By inverting the "generator of specificity" into the "generator of diversity" lymphocytic cells can now evolve which are able to recognise and attack cells who's Class I ligands have been deliberately altered by the presenting cell so that they appear to be an allotype. These are the equivalent of Tc cells and recognise Mhc "Class-I-like" allotypes. Sometime between now and the evolution of free antibodies, the so called "alternative" complement pathway is extended into the "classical" pathway. C1 might be specialised for short range triggering of high density, single surface LIGAND/RECEPTOR complexes so that hole construction is now restricted to the target membrane rather than to a coordinated construction in apposing membranes.

(j) The stage is now set to allow the evolution of Td cells. Class II Mhc ligands evolve: the "intention" is to process short representative peptides from cellular debris picked up by phagocytes at inflammatory sites. These are then externalised as a Class II/debris combination ready for the attention of uncommitted T-cells. The "generator of diversity" can now be enrolled into memorising the inflammatory context of these processed epitopes. On re-encountering the processed epitope these T-cells can rapidly attract large numbers of phagocytes to the site and "angrify" them: inflammation now has a memory. (Note that only a very limited set of cells - APCs, phagocytes and a few others - can present these combinant epitopes so this amplification of the inflammatory cascade can only start after OTHS has been processed.)

(k) The capacity to develop T-cell tolerance has to evolve simultaneously with Tc and Td cells. T-cells capable of recognising healthy self epitopes are mostly decommissioned. This may be a co-operative process (Td/Ts

cooperation akin to Th/B-cell co-operation). Whatever, aggression is averted by having them "mopped up" by Ts commitment. This happens because these epitopes are more likely to be met in a non-inflammatory context. However, self specific T-cells continue to be released from the thymus and can become available for aggression. Aggression to self epitopes will be most likely to be induced and permitted when the inflammatory process is prolonged and foreign epitopes are sparse. Tolerance might be amplified by Ts cell clonal expansion and, perhaps, the release of anti-inflammatory agents at the site of epitope re-encounter. (Like Th and B-cell interaction, helper and suppressor epitopes tend not to overlap, suggesting a co-operative mechanism: it may also reflect the preferential attention of Tc and Td cells to allotypes.)

(m) Last of all, Th cells can now be incorporated into the system to prime the B-cell system and lead to freely circulating antibodies. The B-cells are also derived from a scavenger cell. This is designed to secrete large quantities of free, circulating antibody. Antibodies help by opsonising organisms (preparing them as a "meal" for phagocytes). The classical complement cascade is now optimised to work within the vascular system and to interact with antibody tagged antigen. This system has proved invaluable as a preemptive defence.

THE ADVANTAGES OF THIS PERCEPTION

By now I hope that you will be aware that all this suggests a clear path in self/non-self discrimination. Its beginnings can be seen in simple animals like sponges, which demonstrate differential cell reaggregation (for they, too, have gap junctions). And it proceeds through to the complex mammalian immune system. In this respect, it is interesting to read that differential sorting is, in embryos, a direct consequence of CAM expression (12). The reasons why embryonic cells sort according to tissues rather than according to species is that their CAMs have remained highly conserved across widely

separated species (13).

1) Seamless integration from embryonic development to anamnestic immunity.

2) The innate and the acquired immune system are no longer seen as fundamentally disparate entities. They are fused into a seamless whole.

3) A clearer understanding of preferential alloreactivity by T cells.

4) A clear evolutionary progression from organisms with no cellular differentiation, through simple organisms with phagocytes, then the evolution of a retinue of specialised cells all derived from the primitive scavenger. A "logical progression" would start with Tnk like cells, go to Tc like cells, then Td like cells, then Th like cells and finally B cells.

5) A far clearer perception of the cancerous process (not detailed here but there is good evidence that gap-junctional communication is involved [38].

6) The potential to explain the process of aging [39].

7) It all makes good biological sense. Indeed, it integrates so many biological, developmental and immunological mechanisms into a continuous whole that it holds out the promise of a "grand unification theory".

SUMMARY:

I have proposed reshaping the perception of immunity to encompass the broader principle of MORPHOSTASIS. The loss of healthy self is sensed and expressed by the malfunctioning cell itself or, at furthest, emanates from the membrane doublet where contact is established between this cell and its immediate neighbours. This "foul" is broadcast by the release of inflammatory mediators. These invite phagocytes into the area to assess the local population. Phagocytes (and perhaps Tnk cells) then attack those cells with which they fail to become electrically contiguous. The time they have to make this connection varies with the "anger" of the phagocytes. Phagocytes now present cell debris to lymphocytes in local lymph nodes. The epitopes which are most

strange to the lymphocytes are selected to act as the pegs on which to hang a greatly accelerated inflammatory infiltration on any subsequent encounter of these epitopes.

I have also proposed redefining the concept of "horror autotoxicus": it is established by successful cell to cell communication. Both somatic and scavenger cells use this mechanism. The concept of immunological surveillance is simultaneously redefined. But now surveillance is for any malfunctioning cell and not just for neoplasia. The evolution of a thymus dependant lymphocytic system with memory may have occurred at the expense of an increased prevalence of cancer, for intense focal suppression of surveillance now occurs whenever a strong positive feedback leads to an exaggerated attack on self epitopes.

This explanation is undoubtedly simplistic and I am sure it will prove to be inaccurate in many of its more specific assumptions. For example, the immune system has gathered a great number of refinements throughout its evolution including various specialised phagocytes and permanently resident, non-itinerant antigen presenting cells: little has been said about these. However, I suggest that the "flavour" of the concept is essentially correct and the hypothesis will prove to be a useful framework for refinement.

It should now be clear that the breaking of cellular junctions is probably an important event which leads to the declaration of an OTHS "foul". There are a number of close similarities between the insertion of gap junctions into self cell membranes and the insertion of complement membrane attack complexes into invaders. If it could be shown that there is a continuing or a distant relationship between their respective insertion mechanisms, then it would be reasonable to assume that HS is sensed by the speed with which both somatic cells and scavenger cells establish an electrical continuum with those cells

that they encounter.

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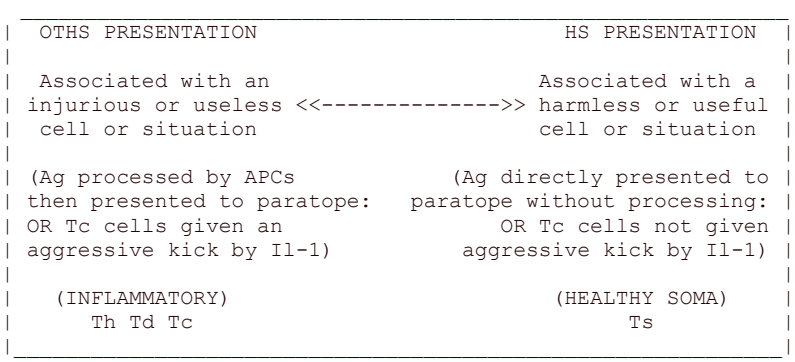

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"There is only one constant element in immunity, whether innate or acquired, and that is phagocytosis. The extension and importance of this factor can no longer be denied."

Elie METCHNIKOFF 1905 [1]

"Immunology is an invention of the devil, who is making it up as he goes along because he's not too clear about this stuff either.". . . . . "Besides, immunology is what we North Americans call a Rube Goldberg system, referring to old cartoons about how to turn on the light, for example: you trip over a footstool, thus startling the cat, who bumps into the kitchen door, which swings shut, knocking over a chair that hits the light switch . . . you get the idea. There has to be an easier way."

Janice Hopkins TANNE 1990 [2]

INTRODUCTION

The proposal I am about to make is stark: I believe immunologists are missing the point: their current perception of the immune process is flawed. Just as astronomers were once confident that the heavens revolved around the earth, so modern immunologists are generally confident that anamnestic immunity and its executors, the lymphocytes, are placed firmly centre stage, at the hub of the (mammalian) immune universe. In particular, it is current dogma that lymphocytes are the commanders of self/non-self discrimination.

Let me see if I can shake your faith. I will describe how T-cell aggression to non-self epitopes could be better regarded as the result, rather than the source, of healthy-self(cell)/all-other(cell/organism) discrimination.

Few of the elements that I assume in this article are radically new. However, the emphasis of their perception IS and this fresh perception leads to a "paradigm shift".

THE EMERGENCE OF SELF(CELL)/NON-SELF(CELL) DISCRIMINATION

To set the scene, I would like to emphasise these points:

(1) When the first multicellulates evolved, they needed to recognise and discriminate self-cells from non-self-cells.

(2) We have become preoccupied with self(epitope)/non-self(epitope) discrimination, mainly as a result of the sequence of discoveries in immunology: this has blinkered our perceptions.

(3) In a large proportion of metazoans, lymphocytes are self-evidently NOT the source of self(cell)/non-self(cell) discrimination: they don't have any.

(4) It SHOULD be possible to discern gradual steps in immune evolution starting in primitive metazoans and leading to the sophisticated system found in mammals. So far, this progression has eluded immunologists.

(5) In development, ontogeny frequently appears to retrace phylogeny: whilst this is not an absolute blueprint for evolution, it can provide important pointers.

MORPHOSTASIS

Morphostasis is tissue homeostasis and it is well maintained in all animals [3]. It is a core process: the functional hub of the metazoan universe. It works efficiently because cells monitor their own health and keep a constant close communication with appropriate neighbours. Anamnestic immunity is a branch of the morphostatic process: it has evolved to enhance the effectiveness of morphostasis in vertebrates.

Remember, an animal is built of a large colony of cells all derived from one

zygote cell (a zygote derived colony - ZDC). This colony constructs itself a skeleton of connective tissues which, while relatively inert, gives it great versatility (eg, the bony skeleton).

The critical function in morphostasis is discriminating Healthy-Self (HS) cells from all other cells and organisms (other than healthy self - OTHS cells). OTHS includes both Unhealthy Self (UHS) cells (eg, ectopic, sick, damaged, aging) and clearly foreign cells and/or organisms. Morphostasis was needed from the moment that multicellular animals first evolved. It should be clear that the main need at that time was to develop a unique way of tagging healthy self cells, so enabling them to acknowledge one another, and then to devise a means of abandoning this healthy self status when things went wrong.

Morphostasis (tissue homeostasis) can be maintained by:

(a) discriminating OTHS cells from HS cells.
(b) removing OTHS cells (UHS and foreign cells/organisms).
(c) replacing lost UHS cells with fresh HS cells (resurgent morphogenesis).

HEALTHY SELF/OTHER THAN HEALTHY SELF DISCRIMINATION:
This hypothesis requires that individual cells MUST either have a fail-safe internal device for recognising that they have become unhealthy and/or an ability to monitor a neighbouring cell's change in health (probably) by monitoring (appropriate) cell to cell communication. The announcement of an "OTHS foul" is issued directly from the affected (somatic) cells. Inflammatory cells (mostly phagocytes) are ONLY invited into the soma at this group's request - a "call" is sent out to fetch the "police". Foreign organisms need not induce an inflammatory response UNLESS they unsuccessfully attempt communication with a HS cell, OR force their way between cells (and so disrupt communication), OR directly attack a cell and make it sick.

Several properties may combine to constitute HS identity; remember that one or more of the critical aspects which lead to HS recognition must be abandoned when the cell becomes sick. Here are some possible candidates:-

(a) Lectins and the recognition of saccharides (eg, sialic acid).
(b) The inhibition of complement attack by proteins released from or displayed on the cell membrane (eg, factor H, DAF, MCP).
(c) Beta-2-microglobulin and Class 1 Mhc ligand expression.
(d) Cell to cell cytoplasmic joining - particularly electrical.

CELL IDENTITY IN THE EMBRYO AND OTHER SYSTEMS
The cells in an embryo recognise each other through Cell Adhesion Molecules (CAMs) [4]. At the cell surface, like/like and ligand/receptor interactions of these CAMs lead to cell adhesion. This adhesion then rapidly progresses on to communication through gap junctions [5]. These CAMs are of three types: first, the cadherins, second the integrins and third, a group of CAMs which are members of the immunoglobulin superfamily (IgSF) of which NCAM is an example. The somatic cells of an embryo are able to recognise appropriate neighbours and navigate themselves into appropriate positions until they meet appropriate cell types. There are many examples of the specific recognition of cells in biology (see below).

Edelman has stated, "The origin of the entire Ig superfamily from an early N-CAM-like gene precursor has deep implications for the understanding of the role of adhesion in processes that are not concerned with morphogenesis but rather with immune defense, inflammation and repair" [6].

Note that the transfer RNA molecules specifying NCAM are spliced by cells in a variety of different ways to produce a range of NCAM phenotypes.

Here are some specific examples of identity recognition [7]:

Protozoans recognise and discriminate food and sexual partners
Phagocytes are able to recognise their own pseudopodia and avoid self attack.
Simple multicellulates are known to reject allografts
Plants - pollination is highly selective against self
Reaggregation of disrupted foetal cells (see later)
Bacterial agglutination and conjugation can be highly specific to self and (in pathogens) to target tissues.
Plants - tree roots in a forest often fuse together. This is very frequent in roots from the same individual, frequent in the same species and far less frequent in unrelated species.
Molecular recognition is a fundamental biological principle (eg, nuclear enzymes).
Cell homing. For example, lymphocytes and injected marrow cells.

Self recognition could, therefore, be observed in several ways, each becoming progressively more specific to the individual animal:-

(a) species recognition (eg, gamete recognition)
(b) tissue type recognition (eg, embryo cell recognition)
(c) self recognition (ie, cells of the individual zygote derived clone. Useful for phagocytic defence)

MORPHOGENESIS

Morphogenesis is the process by which tissues and organs are sculptured from a zygote derived colony. It is most obvious in developing embryos: regeneration is a resurgence of morphogenesis.

Since morphogenesis is an integral part of a morphostatic system, it is reasonable to expect that the component elements of morphostasis will use molecular machinery which is genetically related. They have (presumably) been closely associated through every epoch of metazoan evolution. The complete mechanism which leads to embryonic development remains unclear. However, CAMs and gap junctions appear to play central roles [8].

EMBRYOS, CAMs AND GAP JUNCTIONS

1) Gap junctional communication can be relatively non-specific (crossing species barriers) but it can also be highly selective (as below) [9].
2) Gap junctional communication is critical in development. Embryo development fails when GJ communication is disrupted [10].
3) When CAMs (cell adhesion molecules) interact with each other or their receptors, the ensuing cell adhesion appears to lead directly to gap-junctional communication. CAMs precede GJ insertion and both are necessary for normal development [11].
4) Embryos are made up of a number of compartments. Communication through gap junctions is constricted at their boundaries. These compartments correspond

to important developmental fields [12]. They also correspond to fields of specific CAM expression [13].

5) The gap junctions in these compartments are of two sorts [14]. First, there are high permeability junctions joining each cell within a compartment. These allow the free passage of larger molecules: lucifer yellow is used to demonstrate this. I suspect that this "open" communication enables a group of cells to be organised, as if they were a single block of cytoplasm. This may be under the control of the appropriate segmental homoeotic gene (look at the complex structure of paramecium to see how this might work). Second, there are more restrictive junctions which join the cells at the boundaries of these "open" compartments. These only allow small molecules to diffuse (eg, ions). These junctions allow ions to pass in either both or just one direction (ie, they are rectifying and correspond to junctions formed from hybrid connexons [15]). This directionality may be of significance in the way that embryonic cells sort, with endoderm to centre and ectoderm to the outside. These restrictive junctions are either insufficiently large or insufficiently extensive to allow lucifer yellow to diffuse freely.
6) Despite its name, N-CAM is not confined to neural tissues. Whilst it is expressed strongly and for long periods in neural development, it is also expressed, more transiently, in other sites [16]. It is a recognised IgSF member (Immunoglobulin Super Family). A number of authors have considered these IgSF CAMs to be the probable ancestors of immunoglobulins, T-cell receptors and histocompatibility antigens (Edelman for one [17])).

EMBRYO CELL DISAGGREGATION

When embryo cells are disaggregated and allowed to resettle, they reaggregate into tissue layers, ectoderm to the outside, mesoderm to the middle, then endoderm to the centre [18]. When embryonic cells from two mammalian species are mixed, they reaggregate into tissue type rather than species type and this appears to be because the genes which specify the various CAMs are highly conserved across the species barriers [19].

MEMBRANE HOLES

It is now possible to make a stab at the general principle which governs HS/OTHS discrimination. I suspect it goes something like this:-

"SELF is established by making holes in the membranes of apposing cells and lining them up to create gap junctions. This allows cells to become electrically coupled and so to act as an electrical and, probably, a cytoplasmic continuum. This ability to couple membranes dates back to the very earliest multicellulates. It relies on the controlled, ordered, simultaneous adjacent membrane insertion of membrane holes. Cells learned, from the start, to allow the uncoordinated, bigger, higgledy piggledy insertion of leaky holes into organisms which fail to demonstrate the membrane LIGANDs used as a focus for the tidy construction of gap junctions: electrical discontinuity and a lower membrane potential leads to an attack by scavengers. Unhealthy self cells can elect to be rejected by uncoupling from adjacent cells then dropping their membrane potential (by mobilising calcium ions from covalently bound calcium stores): they can also abandon the membrane LIGANDs which specify self. The mechanisms for constructing leaky holes (complement MACs) may be distantly related to the mechanisms for constructing gap junctions."

HORROR AUTOTOXICUS & MORPHOSTASIS

One result of relying on self(cell) recognition is that "horror autotoxicus" (HA - the horror of attacking self) will probably have evolved long before lymphocytes and their memory for previously encountered antigens (anamnesis). However, this HA must be based upon the possession of specific and recognisable cell surface markers ("flags"): these probably aid the co-operative "docking" of one cell with another. Furthermore, because infection, cell damage, mutation, aging, genetic errors and other cell

disturbances can also be assumed to be ancient problems, cells of the ZDC probably learned, early on, to observe "horror autotoxicus" to HS cells whilst rejecting or ignoring OTHS (unhealthy self [UHS] and clearly foreign cells/organisms).

This interpretation of "horror autotoxicus" is quite different from the classic one, in which lymphocytes are deemed to be "denied" the right to attack self antigens. In this new interpretation, lymphocyte aggression towards self antigens is neither denied nor necessarily avoided. However, as will become apparent, once such auto-aggression has arisen, it will decay unless other circumstances actively sustain it.

PHAGOCYTES and DOUBLE-THINK

There is a strange double-think that pervades immunology when it comes to the importance and centrality of phagocytes and the recognition of non-self and/or unhealthy self. Every medical student knows that phagocytes recognise dead, damaged, sick and effete cells. Every medical student knows they can recognise foreign organisms and eliminate them (particularly non-dedicated-pathogens). Every text book devotes its statutory (short) introductory opening to the importance of phagocytes and innate immunity: then, almost without fail and with indecent haste, authors are seduced into an intense dissection of the principles of anamnesis and lymphocyte function. What makes this more bizarre is that the anamnestic immune system isn't essential to prepare cells for phagocyte attention. The phagocytic system works well, even if slowly, in invertebrates: self/non-self discrimination works well in invertebrates.

There cannot be much doubt that the reason for this tendency to overlook the fundamental centrality of phagocytes is (a) a lack of understanding of the mechanisms of self/non-self discrimination by these cells and (b) the intense acceleration of the inflammatory process by lymphocytes. This greatly enhances the efficiency with which OTHS is removed and it has led us to regard lymphocytes as masters rather than servants of the system. There is, at the very least, a possibility that CAM interaction and junctional communication (between phagocytes and underlying somatic cells) may be the important factor in HS self cell recognition.

INFLAMMATION:

Metazoans have evolved an ancient and virtually universal defence mechanism which is to infiltrate somatic tissues with scavenger cells whenever required (mostly phagocytes). These scavengers are clearly capable of recognising most organisms (particularly those which are not dedicated pathogens). And, in the vast mass of animal life, they appear to do so without the aid of cells which have the ability to "remember" epitopes. They also remove aging and disordered self cells. In fact, their behaviour is ideally suited to eliminating OTHS. I propose two things:

(a) In all complex metazoans, the discrimination of HS from OTHS by phagocytes REMAINS the central and crucial immune process.
(b) All other immune activities are geared to accelerating, accentuating and maximising this process. In consequence, the efficiency with which OTHS is removed by phagocytes can be greatly enhanced.

Even so (as you will see later) HS/OTHS discrimination does not begin in phagocytes but in somatic cells. It is the consequence of general cell recognition and communication. Inflammation is only established when somatic cells "decide" that they cannot cope alone and "invite" the scavengers in.

Static (somatic) cells are attached to each other by cell junctions. Their cytoplasms are joined by gap junctions (GJs - except in those cells who's function depends on electrical excitability). When membrane junctions are split

apart the disruptions in the cell membranes probably lead to the release of various eicosanoids (prostaglandins etc). This announcement of an OTHS event, by somatic cells, results in an inflammatory reaction. (Note that in electrically excitable tissues which have few GJs, inflammatory responses are far less pronounced). Chemical messengers released at the OTHS site encourage the ingress of phagocytes (through the endothelial cell linings of local post-capillary venules). Phagocytes now invade the OTHS site. They begin assessing cells on the basis of their HS status. Thus far, the basic process is the same for almost every, if not all, animal species. At this point, vertebrates enrol a new mechanism. Debris from local tissues is processed by phagocytes (or phagocyte related cells) and it is then presented, in local lymph nodes, to the anamnestic immune system as short representative peptides in combination with class II antigens. The aim is to select representative Class II/peptide epitopes and to retain a memory of them and their inflammatory environment so that, on their next encounter (which MUST, incidentally, follow phagocyte/APC processing), this inflammatory environment can be rapidly and potently reproduced and, more often than not, exaggerated. This anamnestic response is under the full command of the morphostatic process and, in particular, largely under the control of phagocytes.

MIMICRY:

Because morphostasis has always relied on self recognition, dedicated pathogens need to use mimicry (or more subtle interferences with identity molecule expression and recognition) to gain access to and persist in the soma [20]. Every animal needs to stay one step ahead of its competition. Constant pressure is exerted to expand the variety of identity molecules available within a species (pleomorphism). Somatic cells appear to recognise each other by developmental ligands (cell adhesion molecules, CAMs). When embryonic cells from two mammalian species are disaggregated, mixed together and allowed to settle, they segregate into tissue type and not into species. Somatic ligands have probably needed to stay constant over countless meiotic generations. This makes them a sitting duck for determined pathogens. So, somatic cells need a backstop identity to be used as a second check when things go wrong (phagocyte based and, perhaps, Mhc Class 1 based). And until they do go wrong, inflammatory cells can be confined to the vascular system, locked out behind tight endothelial cell junctions until invited in. (Note that "loss of function" is a cardinal feature of the inflammatory process.)

UNHEALTHY SELF ACTIONS: APOPTOSIS AND SELF SACRIFICE

When cells fail to establish communication, membrane reactions probably begin which lead to the release of a variety of prostaglandins and other cytokines [21]. Similarly, when cells become unhealthy they break junctional communication and become prey to attack by both adjacent cells and the inflammatory cells which are (in consequence) called into the area [22]. When I first started thinking in these terms, I had found scant literature describing elective suicide and I even looked at plants for evidence of this (the hypersensitivity reaction [23]). However, interest and literature on this subject have become abundant recently and there are several recent articles, one in Adv Immunology [24], one in the Annual Review of Biology [25] and a very readable article in the New Scientist [26]. In synthesis, individual cells DO decide that they are sick and/or redundant. They DO have the capacity to invite attack by adjacent cells and also to invite phagocytes along to have themselves removed. There is no need to presume that antibodies and lymphocytes are the sole, let alone the prime, assessors of healthy self status.

Changes in the concentration of calcium ions within the cell are all important in this election for "disposal by consensus". Ca++ ions act as second messengers for a variety of cell processes including apoptosis, nuclear division, growth factor stimulation: and they are closely tied into the

inositol-PO4/DAG/protein-kinase-C network of intracellular second messengers [27]. In this respect, cellular identity and cell health is all tied into proto-oncogene activity and this in turn into gap junction formation and communication competence [28]. Here is the promise of a much clearer understanding of cancer.

When cells are attacked by C9 or perforin, they are made leaky, their cytoplasmic membrane potential falls and Ca++ ions are allowed into the cell. Both these molecules contain sequence motifs similar to the LDL receptor and epidermal growth factor receptor. The significance of this escapes me at the moment but one important feature is that both the receptors they seem to be related to are endocytosed in clathrin coated pits (like the Mhc molecules themselves).

THE GENERATION OF SPECIFICITY:

A major problem in understanding the evolution of anamnestic immunity is how such a complex system erupted onto the evolutionary scene, so suddenly and so completely, in the vertebrates. One explanation is that it evolved, not as a generator of receptor diversity but as a generator of receptor specificity. The table below shows how a scavenger cell could be programmed only to cooperate with self cells which display ligands unique to that single ZDC. The specification of such a scavenger is an exact inversion of the specification of the cytotoxic T cell. Even a repertoire of receptors as few as two would be useful in specificity whereas, in diversity, it is difficult to see how any useful function could have evolved until there was a large repertoire of possible receptors. With a system which develops on the basis of specificity, there would be ample time to develop an extensive repertoire of possible receptors before being precipitously "flipped around" to service a generator of diversity. (Note that "pure self" is used to indicated unaltered, self Class I Mhc antigens.)

Cell type	Receptors disabled	Receptors enabled	Normal state	Triggered state
Scavenger	non pure self	pure self	aggressive	passive
	GENERATOR OF SPECIFITY			
Tc cell	pure self	non pure self	passive	aggressive
	GENERATOR OF DIVERSITY			

There are two possibilities. First, that the ancestors of the T cell receptor may have been used to recognise tissue CAM ligands: this could be the origin of the V gene segments [29]. Secondly, a descendant of the simple scavenger (phagocyte) may have evolved to recognise a set of pleomorphic CAM like markers which were specifically evolved in a population to be used as a backstop identity check. Developmental CAMs seem to remain constant over countless generations and this is reflected in the way embryonic cells from different species reaggregate as germ layers and tissues rather than species. The "backstop" CAM like ligand (the precursor of the Class I Mhc antigens) could deliberately borrow bits and bobs from these developmental CAMs to form a unique looking ligand by using a genetic mix and match process.

There seems to be little question that phagocytes are unable to rearrange their genome to form specific receptors. And there is no significant evidence that they can selectively cooperate with cells carrying self Mhc antigens. Natural killer cells, however, might be such a candidate, particularly if they

are composed of two populations: one with a lower specificity - perhaps based on beta-2-microglobulin expression - and another with highly specific receptors for self. Natural killer cells could fulfil this role. They were first identified because F1 Tnk cells attacked parental cells (unlike the classical transplantation laws) [30]. This would be consistent with specific (cooperative) recognition. These cells also preferentially attack cells expressing low levels of Class I antigen and beta-2-microglobulin [31]. It seems that, at most, only a proportion of Tnk cells rearrange their receptor genes [32].

Phagocytes, lymphocytes, fibroblasts and platelets are all derived from the same stem cell. They have almost certainly all evolved from a primitive scavenger. Each cell seems to have caricaturised a specific property of this general scavenger and refined it in order to make the mature mammal more versatile. This adds weight to the proposition that a phagocyte like or derived cell might, at one stage, have evolved to have the ability to select/rearrange its genes so that it could specifically recognise healthy self ligands (Mhc "Class-I-like" ligands. The self receptors would have to be selected, in embryo, to be specific to each individual.

One possibility is that, now the lymphocyte system has evolved, this has been so successful that it has largely obviated the need for a scavenger to rearrange its genes to uniquely recognise self. There might even be a positive advantage in achieving the apparent recognition of HS(cells) by inverting the cooperative recognition of self cells into an attack on non-self(epitopes) by Tc lymphocytes. This can be achieved by the clonal elimination of any lymphocyte capable of reacting with "pure self" Class 1 ligands.

Note that complement activity is very much in the style of a horror autotoxicus, with healthy self being protected from attack by inhibitors: and also that phagocytes synthesise enough of all but the terminal components to attack undesirable cells unaided.

SOMA/SCAVENGER SEGREGATION

I have already alluded to soma/scavenger segregation. The important point to grasp is that somatic cells can and do deal adequately with a fair proportion of OTHS33. Provided the accumulation of OTHS is mild and the local cells can both recognise any loss of HS identity and discriminate foreign organisms from HS, then there is little need for a backstop identity check. HS here is established by displaying appropriate tissue CAMs which lead on to the establishment of a "synctial" communication through GJs. However, when UHS or foreign organisms fail to appear sufficiently OTHS to the local cells, then tissue damage will probably ensue as the foreign cells or UHS cells start to gain the upper hand. It is at this stage that scavengers are "invited" in and this is done by a failsafe device (the eicosanoid system - prostaglandins etc). These scavengers then establish HS status by employing a "backstop" check on identity. If there is a scavenger which formally recognises HS Class 1 status then this would make the system highly specific (eg, the Tnk cell - see later).

Since inflammatory cells invade and disrupt the normal structure of tissues, this invasion leads to a loss of function. They are undesirable intruders in healthy tissues except in small numbers. Hence they need to be kept largely locked out, behind a tightly bound network of endothelial cells lining the blood vessel walls. This need for segregation is almost certainly the origin of the vascular system. The subsequent recruitment of the vascular system into distributing other "freight" has resulted in the phagocytes and their evolvents becoming adapted to such tasks as the encapsulation of the inflammatory process (by clotting factors and platelets), the distribution of fats in the blood (phagocytes) and the distribution of oxygen (red cells).

Now it is possible to add some concluding comments to the six points introduced earlier in the section "EMBRYOS, CAMs AND GAP JUNCTIONS":

7) In this hypothesis I have suggested that scavenger cells (phagocytes mostly) use a CAM receptor molecule to latch onto a respective CAM on self cells. This would lead on to an electrical connection with the underlying self cells. When a cytoplasmic finger from a scavenger cell encounters another cell it tries to establish direct electrical communication by forming gap junctions across the membranes separating the scavenger from the underlying cells. If it fails to establish communication, the scavenger may be triggered into aggression by the capacitative current which will flow as the membranes move close together. This could, in turn, trigger an action potential to arm this cytoplasmic finger of the scavenger cell. Additional recognition strategies may be employed. The changing of surface sugars in sick cells is one (loss of the negatively charged sialic acid residues may increase the capacitive current above the triggering threshold). The phagocyte may well have a limited "hit list" of receptors which recognise markers which are indubitable evidence of their non-eucaryotic origin and which would, therefore, never be found as part of self. Dedicated pathogens will, of course, studiously avoid displaying these.
8) Now, the original self CAM may gradually be found to be inadequate as a backstop identity check because various pathogens discover ways of mimicking or interfering with its machinery. At this stage, a new cell is required (perhaps similar to the natural killer cell) which can recognise a more pleomorphic set of CAMs that are deliberately individualised in each animal of a population and more or less unique to each one. An appropriate set of specific receptors would have to be selected, in embryo, to recognise these unique ligands. These, I contend, may be the close ancestors the T cell receptor which led, by inversion of function, to the cytotoxic T cell. In this vein, note that TNF and lymphotoxin are selectively toxic to cells which are NOT communicating through gap junctions [34] .

ANAMNESTIC AMPLIFICATION

So, what is the function of lymphocyte system: what are lymphocytes doing? Direct killing is NOT the prime function in either delayed type hypersensitivity T-cells or helper cells T-cells. They are not remembering epitopes for the prime purpose of "killing" them. The precursor lymphocyte logs the context in which it first set eyes on its epitope. If it was inflammatory then at the next encounter it will recreate a rapid and potent inflammatory response rather than wait for the "cell damage-cytokine-inflammation" cascade to build up. "Tipped off" inflammatory cells can then settle down much more quickly and aggressively to their phylogenetically ancient task of sorting HS from OTHS. The main difference now is that these phagocytes are doing it much more quickly and with better targeting. But they are also doing it more hamhandedly - they'll "bash" anything that looks remotely suspicious (hence the need to focalise this response). Tc cells are relatively more independent and kill directly but even these are only allowed to become aggressive if they have first been primed by IL-1 from APCs during an inflammatory encounter. And these, too, encourage a rapid inflammatory response once they start attacking cells.

Somatic cells probably show some specificity about which epitopes to present for Tc cell priming. The peptides they present in combination with Class I antigens have probably been shepherded through the cell by its garbage minders, the ubiquitins. Leaving this aside, it is still easy to imagine how self/non-self selectivity can occur. When T-cells are released from the thymus they are already committed in specificity (ie, they are committed to

recognising a specific epitope). But, they are not committed in activity (aggression or suppression). It is only when they meet their respective epitope that this commitment is made. Self epitopes are, in general, encountered frequently and the first encounter (in embryo) is nearly always in a "healthy self" (non-inflammatory) environment. So tolerance is generally favoured for those lymphocytes which recognise self molecules. Few self specific T-cells will remain uncommitted for more than a brief period while there is a relatively large pool of the relevant self epitope waiting to be encountered.

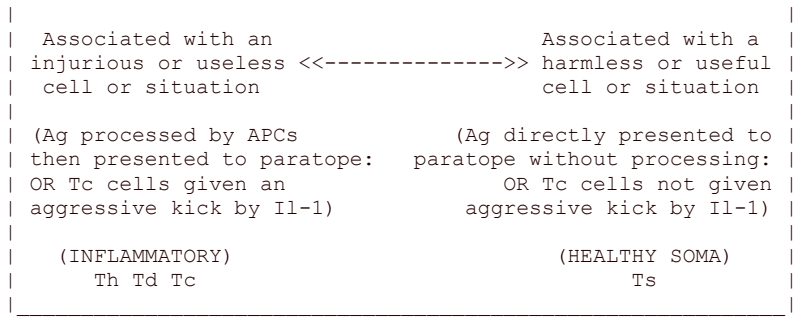
On the other hand, because only small quantities of a foreign or strange epitope are infrequently met in the body, most T-cells capable of recognising them will remain uncommitted until they meet the epitope in an inflammatory encounter. Because they are part of OTHS, they will be met in an inflammatory context and aggression will be favoured. To enhance this, it seems that it is easier to provoke old rather than young precursor lymphocytes into aggression. This further concentrates the aggressive response onto those epitopes that are most strange to the body. No veto need be imposed on T-cells to prevent them becoming aggressive to self epitopes (except for "pure self" Mhc ligands - these are clonally disabled). Indeed, epitopes from tissues that are usually hidden behind tight endothelial cell junctions (like the eye and brain) are infrequently encountered and there is likely to be a larger pool of uncommitted T-cells available. They are, consequently, more inclined to provoke an aggressive response when they are exposed during periods of intense inflammation.

The thymus constantly produces new uncommitted T-cells. So, whenever clearly foreign epitopes are sparse and inflammation is intense, attention can gradually turn to self epitopes (eg, as in tuberculosis). In summary, aggression is most likely to develop to clearly foreign (strange) epitopes and tolerance most likely to develop to self (frequently encountered) epitopes.

The overall effect is that lymphocytes remember the "inflammatory" or "healthy soma" context in which they first meet their respective epitope (and become committed); and they aim to recreate and caricaturise this memorised inflammatory or non-inflammatory milieu at the next encounter. Whenever Td cells provoke an inflammatory response they call large numbers of phagocytes (& Tnk cells?) to the epitope site. These are then switched into a heightened state of "anger". However, phagocytes (& Tnk cells?) STILL have to discriminate HS from OTHS. But now, the threshold at which aggression is considered is greatly reduced. Cells expressing a relatively low level of "HS identity" are now likely to be attacked. This amplification of the inflammatory response by lymphocytes has the potential to escalate catastrophically. It can slip into a loop of strong positive feedback, particularly when the epitope is an abundant self Ag. When the local auto-rejective response becomes excessive, it must be down-regulated otherwise things will get disastrously out of hand. This could be done in a number of ways and these may account for many instances of clinical anergy [35]:

(a) inhibition of phagocyte ingression (chemotaxis)
(b) inhibition of phagocyte aggression
(c) inhibition of further aggressive lymphocyte activation
(d) a tightening of endothelial cell junctions
(e) encapsulation in a fibrin sheath (fibrocytes later)
(f) promotion of lymphocytic tolerance to typical Ag
(g) production of auto-antibodies to the newly cloned, locally reactive lymphocytes (lymphocytotoxic Abs)

OTHS PRESENTATION	HS PRESENTATION



AUTO-REJECTION

There is one important inference to be made from examining the structure of the sero-negative arthritides and particularly Behcet's syndrome [36]. This is that auto-rejective disease covers a wide spectrum of prevalence and severity. The mildest components are VERY common, suggesting that auto-rejection is a normal process (see the article "Clinical Morphostasis"). This leads to the conclusion that there is no automatic horror autotoxicus to self epitopes where T cells are concerned. When auto-rejection is so general, it has to have physiological as well as pathological significance: it must be a functioning element of the morphostatic mechanism.

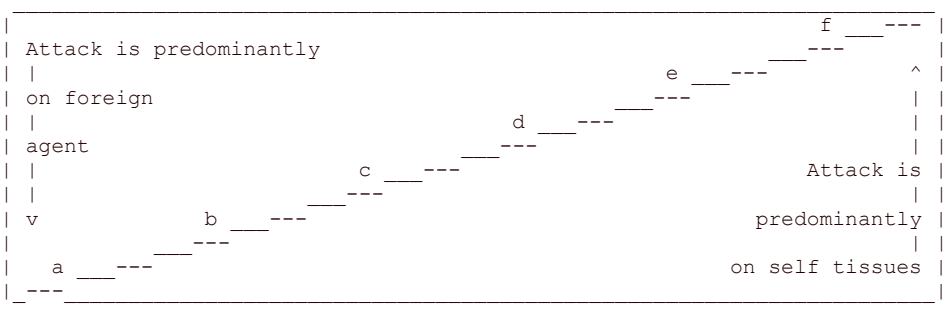
ANTIBODIES - ICING ON THE CAKE

Antibodies are icing on the cake. Extremely useful, evidently important but dominantly aimed at pre-empting the proliferation of blood borne pathogens and pathogens which colonise epi/endothelial surfaces. It's clear that the role of antibodies in tissue rejection (and hence auto-rejection) is minor if not minimal. The vast mass of animal life copes well without them. "Cell-mediated immunity clearly precedes humeral antibody production in phylogeny" (Manning and Turner [37]). We can safely put antibodies to one side until towards the end - which is more or less where they evolved. It appears to me that, to bother to look amongst antibodies for an explanation of how self/non-self discrimination evolved, would be manifestly Heath Robinson (or Rube Goldberg!).

In this vein, it is worth noting that the spleen may be specifically adapted to make the best of the difficult job of maintaining some semblance of morphostasis amongst the cells suspended in the highly mobile plasma.

THE CLINICAL IMPLICATIONS

The result of all this is that any disease which evokes an inflammatory response has an element of auto-rejection. It inevitably consists of a varying mixture of attack directed exclusively at the pathogen (usually leading to mild inflammation) and attack directed almost entirely at self (often highly inflammatory): the latter occurs when organisms or cells provoke prolonged inflammation but do not provide or present clearly foreign looking (unusual) epitopes. Every disease that leads to cell damage will induce auto-rejection.



EXAMPLES
(a) Saprophyte
(b) Simple epithelial commensal
(c) Staphylococci and streptococci
(d) Tuberculosis and syphilis
(e)-(f) Multiple sclerosis and sero-negative arthritis

This is explained in more detail in a separate article, Clinical Morphostasis".

MORPHOSTATIC EVOLUTION:
It is now easier to see how the morphostatic system may have evolved. Here is the probable path of the evolution of ZDCs from simple multicellulates to mammals.

(a) In the beginning, all cells in the colony express equally marked phagocytic behaviour.
(b) SELF is established by making holes in the membranes of apposing cells and lining them up to create gap junctions. Cells learn, early on, to allow the uncoordinated, bigger, higgledy piggledy insertion of leaky holes into organisms which fail to demonstrate the membrane LIGANDs used as a focus for the tidy construction of gap junctions.
(c) Cells now divide into phagocytes and soma. They selectively improve the specificity and efficiency of cell junction construction by facilitating and amplifying their construction at the site of cell LIGAND/RECEPTOR interaction. The resulting gap junctional plates are more "transparent" and more specific about where they form. They develop:

somatic LIGAND(s) - for recognition by resident scaffolders.
phagocyte LIGAND(s) - for recognition by itinerant scavengers.

(d) Dedicated scavengers (phagocytes) now evolve. They refine this cooperative gap-junctional communication with self and the runaway, leaky hole attack of non-self. The molecules used to do the second will eventually evolve into what we now recognise as the complement components. It is possible that the two construction cascades are related but become independent early in evolution. At this stage the complement components are only secreted locally by phagocytes and their action is directed entirely at membranes. It is a long time before these components are co-opted into a humeral system and very much later that they are co-opted to interact with antibodies (probably an adaptation of specific Mhc recognition).
(e) A "vascular" system now evolves, locking out phagocytes till required. The alternative complement cascade can now be "humeralised" so that circulating C3 can mark clearly foreign organisms so that they can be more readily identified when they meet a phagocyte.
(f) There is now a progressive evolution and expansion of somatic LIGANDs leading to increased tissue compartmentalisation.
(g) Ig supergene like LIGANDs develop to act as a focus on which to grow highly specific gap junctional plates and create developmental compartments. The genes specifying these molecules can now be copied then altered by a "mix and match" process to generate a set of LIGANDs which have a great variability within a herd. These pleomorphic LIGANDs will now act as the final arbiters of healthy self in each individual. Over many meiotic generations, they have eventually evolved into Mhc Class I LIGANDs. Newly developed scavenger cells are now able, when required, to electrically couple with any somatic cell that displays self specific LIGANDs and observe a horror autotoxicus to it. These scavengers need a mechanism to produce and/or select self specific RECEPTORs unique to each ZDC. This must be done post-meiotically over a number of mitotic generations - the "generation of specificity". (This possibly coincides

with the evolution of shell protected eggs.) These scavengers resemble natural killer cells.

(h) By inverting the "generator of specificity" into the "generator of diversity" lymphocytic cells can now evolve which are able to recognise and attack cells who's Class I ligands have been deliberately altered by the presenting cell so that they appear to be an allotype. These are the equivalent of Tc cells and recognise Mhc "Class-I-like" allotypes. Sometime between now and the evolution of free antibodies, the so called "alternative" complement pathway is extended into the "classical" pathway. C1 might be specialised for short range triggering of high density, single surface LIGAND/RECEPTOR complexes so that hole construction is now restricted to the target membrane rather than to a coordinated construction in apposing membranes.

(j) The stage is now set to allow the evolution of Td cells. Class II Mhc ligands evolve: the "intention" is to process short representative peptides from cellular debris picked up by phagocytes at inflammatory sites. These are then externalised as a Class II/debris combination ready for the attention of uncommitted T-cells. The "generator of diversity" can now be enrolled into memorising the inflammatory context of these processed epitopes. On re-encountering the processed epitope these T-cells can rapidly attract large numbers of phagocytes to the site and "angrify" them: inflammation now has a memory. (Note that only a very limited set of cells - APCs, phagocytes and a few others - can present these combinant epitopes so this amplification of the inflammatory cascade can only start after OTHS has been processed.)

(k) The capacity to develop T-cell tolerance has to evolve simultaneously with Tc and Td cells. T-cells capable of recognising healthy self epitopes are mostly decommissioned. This may be a co-operative process (Td/Ts cooperation akin to Th/B-cell co-operation). Whatever, aggression is averted by having them "mopped up" by Ts commitment. This happens because these epitopes are more likely to be met in a non-inflammatory context. However, self specific T-cells continue to be released from the thymus and can become available for aggression. Aggression to self epitopes will be most likely to be induced and permitted when the inflammatory process is prolonged and foreign epitopes are sparse. Tolerance might be amplified by Ts cell clonal expansion and, perhaps, the release of anti-inflammatory agents at the site of epitope re-encounter. (Like Th and B-cell interaction, helper and suppressor epitopes tend not to overlap, suggesting a co-operative mechanism: it may also reflect the preferential attention of Tc and Td cells to allotypes.)

(m) Last of all, Th cells can now be incorporated into the system to prime the B-cell system and lead to freely circulating antibodies. The B-cells are also derived from a scavenger cell. This is designed to secrete large quantities of free, circulating antibody. Antibodies help by opsonising organisms (preparing them as a "meal" for phagocytes). The classical complement cascade is now optimised to work within the vascular system and to interact with antibody tagged antigen. This system has proved invaluable as a preemptive defence.

THE ADVANTAGES OF THIS PERCEPTION

By now I hope that you will be aware that all this suggests a clear path in self/non-self discrimination. Its beginnings can be seen in simple animals like sponges, which demonstrate differential cell reaggregation (for they, too, have gap junctions). And it proceeds through to the complex mammalian immune system. In this respect, it is interesting to read that differential sorting is, in embryos, a direct consequence of CAM expression (12). The reasons why embryonic cells sort according to tissues rather than according to species is that their CAMs have remained highly conserved across widely separated species (13).

Let me tabulate the advantages of this way of perceiving the process:

1) Seamless integration from embryonic development to anamnestic immunity.
2) The innate and the acquired immune system are no longer seen as fundamentally disparate entities. They are fused into a seamless whole.
3) A clearer understanding of preferential alloreactivity by T cells.
4) A clear evolutionary progression from organisms with no cellular differentiation, through simple organisms with phagocytes, then the evolution of a retinue of specialised cells all derived from the primitive scavenger. A "logical progression" would start with Tnk like cells, go to Tc like cells, then Td like cells, then Th like cells and finally B cells.
5) A far clearer perception of the cancerous process (not detailed here but there is good evidence that gap-junctional communication is involved [38].
6) The potential to explain the process of aging [39].
7) It all makes good biological sense. Indeed, it integrates so many biological, developmental and immunological mechanisms into a continuous whole that it holds out the promise of a "grand unification theory".

SUMMARY:

I have proposed reshaping the perception of immunity to encompass the broader principle of MORPHOSTASIS. The loss of healthy self is sensed and expressed by the malfunctioning cell itself or, at furthest, emanates from the membrane doublet where contact is established between this cell and its immediate neighbours. This "foul" is broadcast by the release of inflammatory mediators. These invite phagocytes into the area to assess the local population. Phagocytes (and perhaps Tnk cells) then attack those cells with which they fail to become electrically contiguous. The time they have to make this connection varies with the "anger" of the phagocytes. Phagocytes now present cell debris to lymphocytes in local lymph nodes. The epitopes which are most strange to the lymphocytes are selected to act as the pegs on which to hang a greatly accelerated inflammatory infiltration on any subsequent encounter of these epitopes.

I have also proposed redefining the concept of "horror autotoxicus": it is established by successful cell to cell communication. Both somatic and scavenger cells use this mechanism. The concept of immunological surveillance is simultaneously redefined. But now surveillance is for any malfunctioning cell and not just for neoplasia. The evolution of a thymus dependant lymphocytic system with memory may have occurred at the expense of an increased prevalence of cancer, for intense focal suppression of surveillance now occurs whenever a strong positive feedback leads to an exaggerated attack on self epitopes.

This explanation is undoubtedly simplistic and I am sure it will prove to be inaccurate in many of its more specific assumptions. For example, the immune system has gathered a great number of refinements throughout its evolution including various specialised phagocytes and permanently resident, non-itinerant antigen presenting cells: little has been said about these. However, I suggest that the "flavour" of the concept is essentially correct and the hypothesis will prove to be a useful framework for refinement.

It should now be clear that the breaking of cellular junctions is probably an important event which leads to the declaration of an OTHS "foul". There are a number of close similarities between the insertion of gap junctions into self cell membranes and the insertion of complement membrane attack complexes into invaders. If it could be shown that there is a continuing or a distant relationship between their respective insertion mechanisms, then it would be reasonable to assume that HS is sensed by the speed with which both somatic cells and scavenger cells establish an electrical continuum with those cells that they encounter.

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GROWTH AND REGENERATION

It is inevitable that the rate at which generation (growth) and regeneration (mostly repair) can proceed is limited. Since these are essentially similar morphogenetic processes, auto-rejection as a morphostatic technique cannot be allowed to reach the level of intensity in a growing animal as that which can be permitted in a mature animal. If it does growth will be stunted. That is:-

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Generation + Regeneration  =  a set maximum

                Therefore:-

generation high ------> regeneration relatively restricted
generation low  ------> regeneration relatively unimpaired
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Put another way, the luxury of extensive auto-rejection, as part of a morphostatic technique, can only be fully afforded in adult animals. Thus, in order to avoid stunting of growth, those mechanisms which initiate and accelerate rejection (of all kinds) need to be less fierce in growing animals than they are in adults: lymphocytes must behave less aggressively and this is probably brought about by moderating the intensity with which APCs stimulate aggressive lymphocytes (APCs = antigen presenting cells) [30,31,31a]. Both CMI (cell mediated immunity) and IgG activity must be dampened (at least, for those IgGs capable of reaching the extracellular spaces even when there is no inflammation). The result of all this is to promote a relative immunological tolerance in very young animals. This impaired capacity to reject (and consequently autoreject) is apparent in the neonate in which the tolerance of grafts is much enhanced: the neonate can also tolerate a level of cerebral ischaemia which, in adults, would cause extensive tissue death (in large part an auto-rejective event). This relative incapacity to auto-reject is also a protection against the dangerous sequelae that follow virus infections (they may even have been a significant driving force to require it). These tend to produce their most severe effects when they first strike in adult life, eg, infectious mononucleosis [32], infectious hepatitis (both often mere URTIs in young children), mumps, chicken pox and measles; and an example from the mouse, lymphochoriomeningitis [33]. The sequelae, arthritis, jaundice, meningitis, orchitis & etc, can be prevented or at least ameliorated by immunosuppressives or steroids. From this point of view, "immunological immaturity" is a misleading term because the infant's immune system is likely to be perfectly adapted for an optimal compromise [new].

There are certain tissues where extensive auto-destruction could prove disastrous: such an event might seriously impair the ZDC's functionality and survivability. These include the eye and the nervous system. These sites enjoy a so called "immunological privilege". This priviledge seems to be achieved, at least in part, by locking out inflammatory cells behind tight endothelial cell junctions: the sparse population of local APCs is probably a direct consequence of this.

AUTO-REJECTIVE DISORDERS

Tissue rejection is largely accomplished by cells and cell mediated mechanisms. Whilst antibodies can affect the course of organ rejection, they cannot, on their own, precipitate it. In contrast, rejection can be provoked with injections of appropriately activated lymphocytes. Once it is apparent that disordered self cells are actively rejected, we are in a position to state the following:

Every disease which leads to an inflammatory reponse will have an auto-rejective element even if this is limited to a mildly increased tissue turnover.

So, there ought to be a group of disorders which are largely auto-rejective and who's pathogenesis is little, if at all, affected by humoral auto-immunity. Since immune function changes through life, the intensity of auto-rejection is likely to be dependent upon age. It will be at its climax in the healthy young adult. The initiation of auto-rejection is suppressed in the very young [30,31,new] and its execution becomes progressively impaired in the elderly [40]. Thus, a disease which is caused by extensive auto-rejection will be most likely to occur and also to be at its most severe in this central age range (figure 2). One likely cause of such disease is deliberate interference with and mimicry of aspects of the host's identity machinery. Micro-organisms, with their capacity for rapid genetic adaptation, are the most likely offenders. Where micro-organisms develop antigenic determinants close to some element of the host's identity machinery they will appear less foreign and gain easier access to the host's tissues and cytoplasm. Cells which are damaged in consequence of this should still signal malfunction (shout "foul"). However, because there may be a relative scarcity of clearly foreign antigen, the resultant inflammatory reaction will concentrate its enhanced attention on self Ags. Whenever these self Ags are reencountered, an amplified inflammation will ensue and the consequent auto-rejective attack will not necessarily remain confined to the initiating site.

Adjuvant arthritis is of interest because it produces a constellation of disease who's features are similar to those seen in the sero-negative arthritides and sarcoidosis. This experimental disease may be caused because clearly foreign antigen is sparse and the immune response is consequently concentrated upon local tissue antigens (eg, heat shock proteins or other mycobacterial antigens which cross react with the host) (table x). Whipple's disease may be an extreme example of this sort of disease (note the idiosyncratic infection [41,42] and familial aggregation of cases [42,43]).

The bacteria which colonise epithelial surfaces present a special hazard to the colony. It is well recognised that they have the ability to bind selectively to cells at particular epithelial sites [10]. Since they have evolved this specificity it is also highly likely that they have evolved some mimicry of and interference with the host's identity machinery (especially tissue/site LIGANDs). The clinical pattern and incidence of auto-rejective disease should be definable from basic principle: compatibility of organ transplants ranges from a common slight compatibility to a rare complete compatibility [13]. When this observation is extrapolated to microbial mimicry, one would expect to find minor mimicry often and extreme mimicry rarely. The seronegative arthritides and their component complications show just this sort of structuring (table 1). Their clinical pattern can be summed up by an axiom:-

The severity of any single patient's disease(*) is inversely proportional to its incidence in the population and directly proportional to the number of components found in association with one another. (*) - Whether it is an isolated component or a syndrome complex of more than one component.

For example, recurrent aphthous ulceration (RAU) occurs in about 5% of the population, oro-genital ulceration in about 0.5% or less and Behcet's syndrome (BS) in about 0.0001% (in Britain). As the apparent disease in any particular

patient is observed to be more severe, so we notice an expanding clinical overlap: more individual components coincide in one patient (table x). The pathogenesis of these disorders should be dominated by cell mediated immune aggression just as it is in non-acute graft rejection [44]: any contribution from circulating antibodies should simply be a bystander phenomenon. The pathological tempo of the individual components is often seen to increase with the severity of the syndrome disorder. Thus, in psoriasis, the prevalence of arthritis and iritis increases greatly in patients who have the exfoliative and the pustular forms of the disease [45]. On the basis of a personal study (in which the prime objective was to review the world literature on neurological Behcet's syndrome - unpublished) I believe that the meningo-encephalitis of multiple sclerosis should be regarded as the respective isolated component which becomes more severely expressed in the meningo-encephalitis that is encountered in BS (nb., MS is a meningo-encephalitis [46]).

The age incidences of all these disorders are typical [47]. The population incidences of the commoner conditions begin and peak earlier than in the rarer disorders. In the majority of components it is clear that they are constantly modulated by certain events: menstrual exacerbation, second and third trimester quiescence, puerperal exacerbation, stress precipitation and, finally, amelioration of symptoms with steroid and immunosuppressive therapy. (This pattern matches Tnk cell activity and numbers.)

At least two further disorders have features to suggest that they might legitimately be included amongst the (predominantly) auto-rejective disorders. These are sarcoidosis and systemic lupus erythematosis. Both of these demonstrate some clinical overlap with the sero-negative arthritides: and SLE has a similar component structuring. (Nb., high turnover granulomas are a recognised consequence of many cell mediated immune reactions [48]).

CANCER
Broadly speaking it can be surmised that cancer follows:-

(a) a triggering event (induction)
(b) a change in cell behaviour (promotion).
(c) a breakdown in surveillance (progression).

The event which finally trips an affected cell into loss of growth control need not concern us in this article other than to point out that it usually arises in a single cell from which the tumour then develops. A unifying feature is that a normal growth control gene starts being transcribed inappropriately (induction). But let's leave this to one side. I will, instead, focus attention on the reasons for the body's failure to identify the miscreant cell and its progeny (promotion/progression). Before proceeding, note how stark the contrast is between the Hayflick limit of about 50 doublings (in cultures of healthy cells) (footnote 4) and the apparent immortalisation of cell lines derived from many cancers.

Opportunistic infections and cancer should, presumably, be most prevalent when morphostatic surveillance is least effective. The cells making up an animal (there are around 10 to the power 13 of them in man!) are highly regimented and, presumably, intense cell co-operation has to be exercised to maintain such order within the ZDC's tissues. This implies that, by and large, disruptive cells (dead, damaged, dying, mutated and those with disordered growth control) are largely rejected. And, indeed, it has long been clear that phagocytes do recognise these cells and remove them. Our main attention here should be directed solely at those events which lead to the impairment and subsequent failure of surveillance. Focal anergy is likely to be one of these events and may well be the major contributor to the escape of malignant cells from

surveillance.

In mammals, this impairment of surveillance should (generally) be at the extremes of life or following prolonged focal auto-rejection and its consequent anergy. In the elderly, the increasing impairment of immunity coupled with the heightened susceptibility of epithelium to various noxiae (and thus auto-rejection) will predispose to a high incidence of carcinomas. Focal anergy on its own (consequent upon intense auto-rejection) may be a major cause of the predilection for certain cancers to strike young adult to middle aged patients (e.g., lymphomas and focal cancers like colonic cancer in ulcerative colitis or testicular tumours following mumps). In the very young there is a relative incapacity to reject tissues. It is worth noting, then, that the predisposition for epithelial cancers found in the elderly is not present in the young. Cancers are relatively common in the very young and there is evidence to suggest that many regress before they reach clinical significance [49]. (Note that, in general, carcinoma-in-situ is far commoner than overt cancer: the abnormal cells tend either to be kept in check or eliminated by lympho-monocytic cells.)

Cancer is characterised by a failure of growth control and the cells affected revert to a form of behaviour more typical of embryonic cells (retrodifferentiation [50]). Using a "reductio ab adsurdam" argument these changes are much more likely to happen when regeneration and/or proliferation are exuberant (eg, T-cells in lymphomas) rather than relatively quiescent (eg, cartiledge, neurones, macrophages). Note that lymphomas are relatively common in the years in which auto-rejection is most intense (16-45yrs) and also note that, in granulomatous disorders, lymphomas predominate over other cancers perhaps because local tissue regeneration is impaired [51,52].

The rate at which malfunctional cells arise (for any reason) probably increases with age. The net effect of this will be to cause a diffuse increase in the multiple foci of auto-rejection and, consequently, a gradual summation of focal anergy. This will eventually lead to a systemic spillover of this focal effect, a saturation effect. Epithelium is the tissue most exposed to infection, noxiae, regeneration and, in consequence, an increased probability of genetic divergence. Foci of anergy will be very frequent in this tissue form and carcinomas should consequently be more prevalent than sarcomas. Once initiated, cancer will itself lead to auto-rejection and, in turn, increased focal anergy. Thus, it is likely that there exists a critical mass and growth rate above which surveillance is irreparably blocked and the cancerous process becomes self perpetuating [53]. (Macrophages observed in vitro are clearly able to recognise malignant cells as abnormal [54,55].)

Now it is instructive to compare the age incidence profiles of various cancers with those of the auto-rejective disorders. However, before doing so it is important to establish which cancers are likely to flourish in the wake of intense auto-rejection (probable examples are lymphomas and testicular tumours [56,57,58]). These must be recognised as distinct from the commonest form of cancer (carcinoma) which seems to occur most frequently in the wake of age related impairment in immune surveillance. In general, these have a gradually rising incidence with age. Some cancers, particularly mesodermal malignancies, follow an incidence pattern showing a nadir in the middle years. It is interesting to note that the age incidence pattern of acute leukaemia is a complete inversion of the age incidence pattern of the auto-rejective disorders (figure 2). (See [59]).

It should now be clear that the lymphocytic system can have a dichotomous effect on cancer surveillance. It may enhance the focal accumulation of phagocytic cells and thus aid the (auto-)rejection of aberrant cells. However, the more aggressively it does this, the more likely it is to precipitate a

suppression of focal rejection in order to avert piecemeal self destruction. Indeed, in those animals that have evolved them, the possession of lymphocytes may have incurred an increased risk of cancer: cancer is relatively uncommon in primitive animals [60,61] and is relatively scarce in congenitally athymic mice [62,63] which have abundant aggressive phagocytes [64] and natural killer cells [65]. It is interesting to note that in the animal kingdom there is an inverse relationship between the capacity to extensively regenerate body form and the prevalence of cancer [66,67]: and that carcinogens may induce supernumerary structures in lower phylae (eg, limbs) [68,69].

Napolitano et al [70] report that tumour cells generally display less class I Mhc Ag at their surface. They draw attention to the fact that the more malignant the tumour is the less class I Ag it expresses. They interpret this as a cause of the malignant behaviour. However, I would interpret this as a cell adjustment going, pari passu, with the loss of HS identity. Macrophages in vitro have little trouble in identifying malignant cells [55]. So, it seems that some quirk is allowing the lymphocytic amplification system to become preoccupied with an inappropriately strong response to the "wrong" tissue Ags: this, in turn, has led to focal auto-aggression and focal anergy. The phagocytes' capacity to eliminate UHS (tumour) cells is thus impaired, permitting a (so far) dormant carcinoma-in-situ to grow to a critical mass where focal anergy will never subside: at this point, the focal impairment of phagocyte activity becomes irreversible and uncontrolled growth of the tumour proceeds unabated. This is consistent with the finding that tumour cells towards the centre of the tumour have a lower expression of class I Ags than tumour cells towards the outside. At the edges of the tumour, macrophage activity is likely to be much more active and successful in eliminating abnormal cells [55].

INFECTION

Infection can be defined as the survival and proliferation of an organism, not descended from the originating zygote, within the tissues of the ZDC. The colony need only remove these cells if they interfere with its structure or function (though the generality of the "dog eat dog" principle suggests that those that don't interfere will be highly specialised commensals or symbionts). Below I suggest four discrete ways in which surveillance can be overcome:-

(a) The first form of infection occurs when an organism acquires the ability to interfere, agonistically or antagonistically, with the host's machinery for establishing cell identity. Strategies based on species and tissue site identity can be cultured throughout the whole mass (surface mostly!) of a species and over its entire duration as a discrete species. The way in which foetal cells reaggregate into tissues rather than species [8,9] and the success, in nude mice, of skin transplants from distant species [71] suggests that tissue site identities may be broadly similar across widely separated species. A variety of infectious organisms could be interfering with this tissue site identity (eg, streptococci [72] and staphylococci). Others also show a clear species specificity (e.g., mycobacterium TB, bovine TB, avian TB etc, and various plant infections [73]). Interference with individual (Mhc) identities can only be evolved in a short timespan (about 60-70yrs in man) and in a small mass (about 60-70kg of which only a small proportion is actually epithelium). Should close mimicry of personal identity develop, this will facilitate that organism's access to the ZDC's tissues and, once there, there would be a relative lack of clearly foreign antigen to "attack". The resulting inflammatory response will tend to concentrate attention on tissue antigens common to both the organism and the host or just to the host. These self Ags will be selected as anchors for the subsequent lymphocyte accentuated inflammation, so leading to an accelerated rejection of self tissues. This kind of destructive attention to self is probably occurring in adjuvant arthritis [22,23]. This disorder has clinical features closely reminiscent of

the sero-negative arthritides and sarcoidosis (table 2). It is likely, therefore, that a highly idiosyncratic form of infection is a factor in the pathophysiology of the "auto-rejective disorders". Such disease could be precipitated by interference with the host's Mhc machinery by the microbe and this will probably have evolved in the lifetime of the animal. In biological systems, things are rarely black or white so the relative blend of the common/consensus and the idiosyncratic/individual response to infection will probably vary in a spectral manner (diag $). (Note that bacteria that manage to invade and survive within the cytoplasm could well pose a greater threat for this form of auto-rejective disease).

[Rejection will always be aimed at whatever is most apparently OTHS. The amount of auto-rejection will increase with the angrification of phagocytes, especially when clearly foreign OTHS is sparse. With the angrification of phagocytes, the threshold of HS expression required to avoid attack will be higher. In consequence, fewer self cells will continue to qualify as immune from self attack.]

(b) A second group of organisms manage to foil surveillance by virtue of their small size and obligate intracellular existence. The organisms of this group are the viruses. As soon as an infected cell is sufficiently compromised it should signal a malfunction so triggering inflammation and attracting phagocyte attention. This will lead to the activation of appropriate precursor lymphocyte clones. After an interval of 10-14 days a strong amnestic response to various viral*peptide+Mhc antigens will have developed. In the meantime, selected self Ags may be used to anchor an immune accelerated phagocyte accumulation at the affected site whilst waiting for the emergence of a more specific anti-viral activity. (In general, these are "hit and run" infections: they are soon suppressed or cleared from the system and those that persist do so by remaining dormant within cells.)

(c) The third group are the opportunistic infections. Whilst these may interfere with tissue and species identity mechanisms [74] their success is dependent on the depressions of focal surveillance which follow virus infections, burns, surgical incisions and trauma (etc.). Each of these noxiae lead to the auto-rejection of damaged and malfunctioning tissue with subsequent focal anergy [27]. Probable examples of such opportunistic infections include bacterial tonsillitis, otitis, sinusitis, bronchitis and various wound infections.

(d) The last group are organisms which set out to subvert the immune response by deliberately creating a field of intense focal anergy. They do so by maximally stimulating focal inflammation with the object of inducing intense focal auto-rejection. Mycobacterium TB is the example which will be considered here though syphilis is probably another. The properties of such an organism should include:

The result of these 3 properties is that intense focal inflammation and then auto-rejection is induced. In consequence, there is intense focal anergy and this leads to a field of surveillance impairment in which the bacterium flourishes, feeding upon the cell debris which is left in the wake of this auto-destruction [75,76]. Clinical mimicry of the auto-rejective disorders should be discernible: this, in fact, can be seen and is most noticeable in the middle years, an observation which is in keeping with the auto-rejective disorders (table 3).

When tuberculosis occurs outside these middle years it is, accordingly, different in its clinical expression. The lesions now tend to be miliary and disseminated and occur without the same intense tissue destruction. Instead, the pattern now resembles miliary cancer. At the extremes of life TB appears

to be acting more like an opportunistic infection. The overall age incidence of TB can, therefore, be regarded as a combination of the auto-rejective and the cancer type age incidence (figure 2).

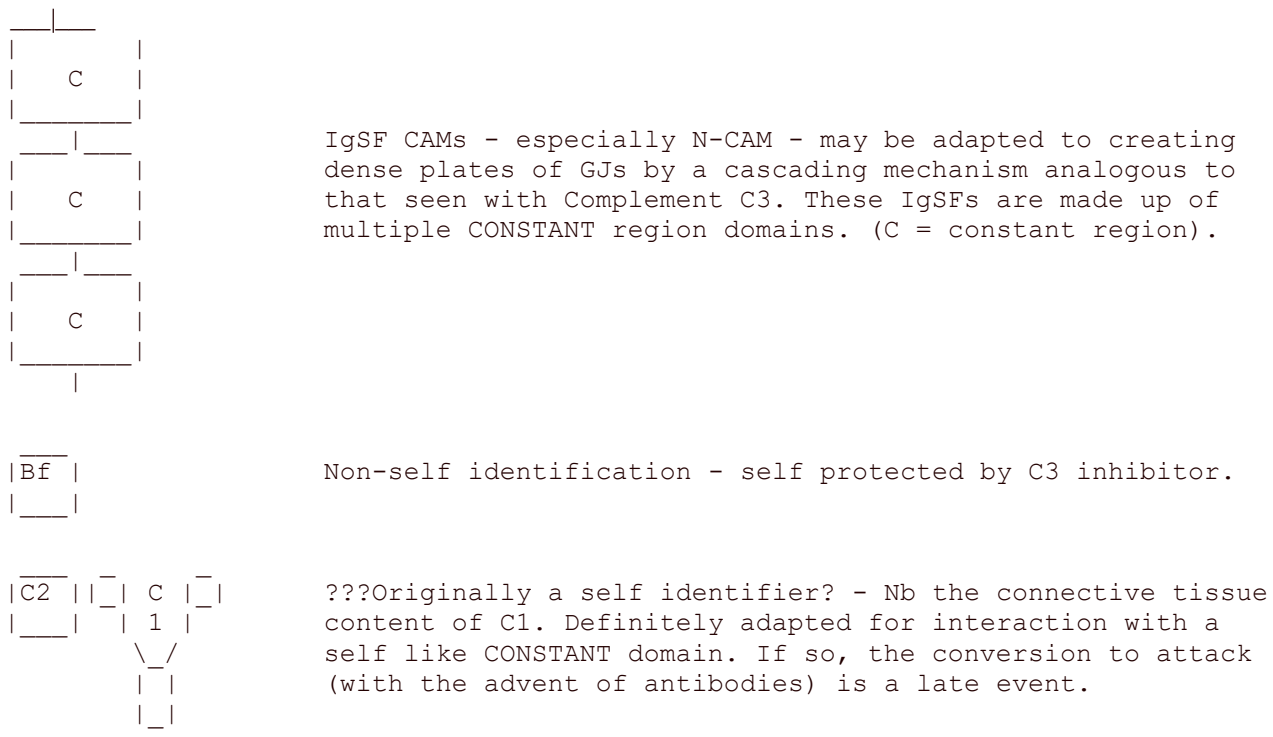
AUTO-IMMUNE DISORDERS

In several previous articles where immune surveillance has been discussed it has been suggested that cancer and auto-immunity might be expected to represent opposite poles of surveillance efficiency. However, the auto-immune title does not automatically imply auto-rejectiion. Rather than being dominantly auto-rejective, these disorders tend to result in one of two disturbances. The first is an interference with functional membrane molecules by the attachment to them of auto-antibodies (e.g., Graves disease, myaesthenia gravis). The second is a tissue destruction which is centred predominantly around (non-cellular) connective tissues (the "collagenoses") and is apparently exacerbated, if not caused, by excessive auto-antibody production and the widespread deposition of Ab/Ag immune complexes. Here, cell destruction is possibly secondary to the activation of macrophages in the locality of this connective tissue. Towards the end of life auto-immune disorders are relatively more common than the sero-negative arthritides. Their prevalence at these older ages may possibly be exacerbated by a decline in the efficiency with which phagocytes clear tissue debris: this, in turn, could lead to enhanced auto-antibody (immunoglobulin) production (the latter certainly appears to be a feature of many diseases causing widespread anergy, eg sarcoidosis [77]).

19930204_morphmol

THOUGHTS ON THE EVOLUTION OF HOLE CONSTRUCTION - shamefully conjectural!

CADHERIN CAMs These have no homology with the IgSF family. They perhaps lead to low density GJ formation without extensive plates. (Not having the capacity to "breed" many hole construction centres from one CAM.)

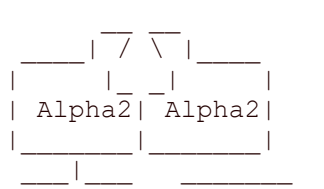


BBB

C3 amplification cascade. This lays down a carpet of hole construction centres - this must be analogous to what happens when GJs are laid down.

C4 is a specialised C3 like molecule used to link the C1/2 sequence into the conventional membrane attack sequence.

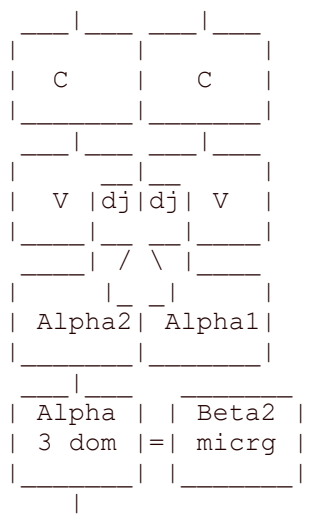
Is a CONSTANT region domain and it can trigger C1 just like antibody constant region domains. It is found in phylogenetically diverse species (eg, earthworm). Perhaps this was specialised to interact with Heat Shock Proteins in a complex intended for recognition.



BB

Perhaps beta-2-microglobulin was adapted to shadow HSPs and form a recognisable complex for phagocytes to recognise. Some specificity of the interaction may have excluded foreign HSPs from being interpreted as self. All because HSPs are involved in damage limitation - where they are, so is danger. The HSP peptide clasp appears to be associated with its function as a protein (re)naturer. At times of stress HSPs appear in profusion. The next step is to hoist the HSP gene onto a CONSTANT region gene so that a Class I like ligand appeared. The V region genes were evolved from CONSTANT region domains to recognise the new HSP like molecule. Initially, when Tnk like cells appeared, they were only looking at the none clasp part of the molecule. With the advent of the Tc cells, the

incorporation of the pincer mechanism into the recognition process was inevitable.



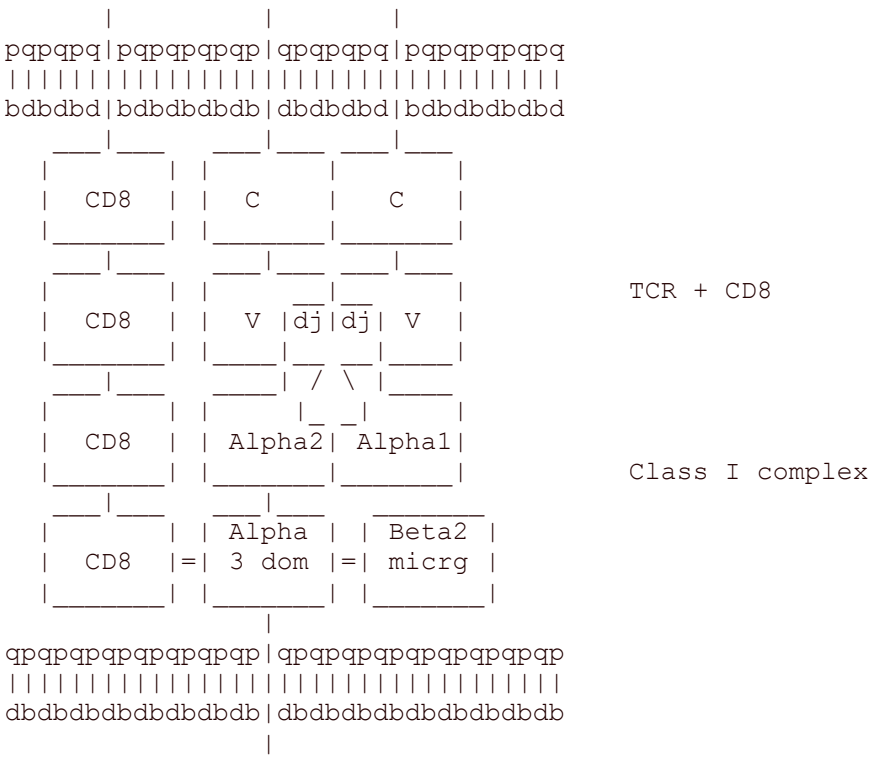
Eventual result of the TcR and Class I Ag interaction. The DJ region evolves to create the extra diversity to recognise the peptide-clasp section of the molecule.

Tnk EVOLUTION - Perhaps Tnk not interested in anything other than the non-clasp region of the HSP molecule (just beta-2-microg/HSP combination to start

and Class I to finish). Tnk seems to be most interested in cells expressing HSPs.

Tc INVERSION - ?? looking (in thymus) for cells with binding but not triggering of TCR (T-cell receptor). Non-binding not cultivated and triggering are clonally deleted. This would tend to pick out many "interlopers" who are trying to use mimickry as a means of defence breaching.

ANTIBODY EVOLUTION - Attaching a newly developed "self" CONSTANT domain marker to selected epitopes (antigens) means that the self recognising C1/C2 complement system can be brought in to be adapted to marking these antigens ready to trigger an an effector cascade.



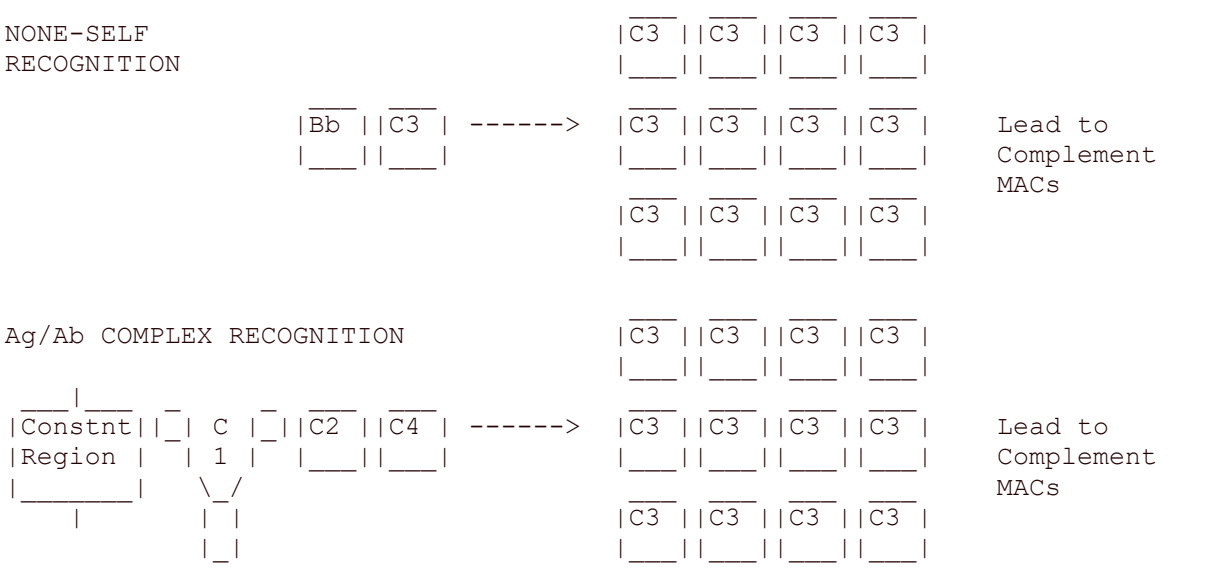
TCR + CD8

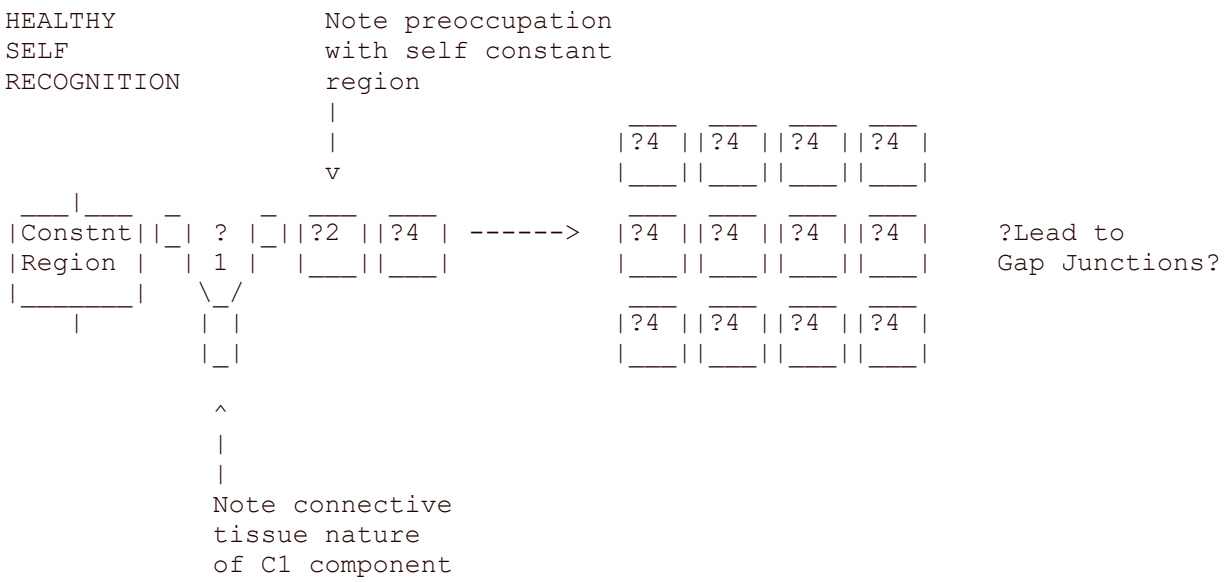
Class I complex

GJ/MAC DIVERGENCE This presumes that there is a relatiionship between GJs and MACs. When did it occur and how completely are their respective insertion mechanisms now duplicated and diverged? Reports of the complete absence of C1, C2 and C4 genes suggest that any molecules like these responsible for GJ insertion must be long since duplicated and that the GJ genes function independantly of MAC insertion genes.

The next set of diagrams indicate what may be happening. C2 (self recognition sequence) triggers the C4 based construction of holes. Could it be that there is a C4 like molecule that spawns hole construction centres, like C3, but constructed more slowly and tidily. The faster they are "zapped" into the membrane the bigger they can be, perhaps? (That might work with the the two kinds of GJs already noted in the text.) The trick in linking the C4 like slower construction for GJs into the faster C3 constuction for MACs would have been to fuse bits of the C4 like gene to

bits of the C3 like gene to form what is now the C4 gene.
This could be something to look for!





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"There is only one constant element in immunity, whether innate or acquired, and that is phagocytosis. The extension and importance of this factor can no longer be denied."

Elie METCHNIKOFF 1905

"Immunology is an invention of the devil, who is making it up as he goes along because he's not too clear about this stuff either.". . . . . "Besides, immunology is what we North Americans call a Rube Goldberg system, referring to old cartoons about how to turn on the light, for example: you trip over a footstool, thus startling the cat, who bumps into the kitchen door, which swings shut, knocking over a chair that hits the light switch . . . you get

the idea. There has to be an easier way."
Janice Hopkins TANNE 1990

ABSTRACT

I propose that the current perception of self/non-self discrimination is flawed. Most immunologists consider that lymphocytes are critically responsible for carrying out this discrimination. I propose that self/non-self discrimination is established in a different way and that the role of lymphocytes is one of servitude to the true self(cell)/non-self(cell) discriminator. The latter manipulates lymphocyte activity as a means to focus, caricaturise and amplify its own involvement at the next occurrence of a similar circumstance. All somatic cells are able to sense their neighbour's (healthy) self status. Individual self cells monitor their own health and generate a unique set of "healthy self (HS)" membrane "flags" and cytokines which act as signals to neighbouring HS cells to indicate that cooperation is safe and appropriate. In somatic tissues, minor breaches of HS identity can be dealt with by surrounding HS cells. When tissue damage is excessive, a second, "back stop", identity mechanism is brought to bear by inviting inflammatory cells into the area (mainly phagocytes). These phagocytes then assess local cells for HS status and will attack any cells (or organisms) that fail to exhibit it. Both somatic cells and the phagocytes which carry out this "back stop" check probably use an identity assessment similar to that used by somatic cells as they establish each others' identity when constructing an embryo. Individual helper lymphocytes simply remember the inflammatory or healthy soma context in which their respective epitope was first encountered and then they attempt to caricaturise this inflammatory or healthy soma environment on any fresh encounter. Using various clues, I go on to suggest that healthy self identity is emphasised strongly by groups of cells which are interconnected by gap junctions: these form extensive blocks of tissue which then behave as synctia of electrically and metabolically coupled "super-cells".

INTRODUCTION

The proposal I am about to make is stark: immunologists are missing the point: their current perception of the immune process is flawed. Just as astronomers were once confident that the heavens revolved around the earth, so modern immunologists are generally confident that anamnestic immunity and its executors, the lymphocytes, are placed firmly centre stage, acting as grand conductors in the (mammalian) immune universe. In particular, it has been an accepted dogma that lymphocytes are the major orchestrators of self/non-self discrimination.

Let me see if I can shake your faith. The T-cell's commitment to aggression is better regarded as a subservient response to, rather than the active source of, healthy-self(cell)/all-other(cell/organism) discrimination. Few of the component elements of this hypothesis are new. However, the emphasis on how they are perceived is and this new perception leads to a "paradigm shift".

THE EMERGENCE OF SELF(CELL)/NON-SELF(CELL) DISCRIMINATION

To set the scene, I would like to emphasise these points:

(1) When the first multicellulates evolved, they needed to recognise and discriminate self-cells from non-self-cells.
(2) We have become preoccupied with self(epitope)/non-self(epitope) discrimination, mainly as a result of the sequence of discoveries in immunology: this has blinkered our perceptions.
(3) In a large proportion of metazoans, lymphocytes are self-evidently not the source of self(cell)/non-self(cell) discrimination: they don't have any.
(4) It should be possible to discern gradual steps in the evolution of

immunity starting in primitive metazoans and leading on to the sophisticated system found in mammals. So far, no clear stepwise progression has been elucidated.

(5) In development, ontogeny frequently appears to retrace phylogeny: whilst this is not an absolute blueprint for evolution, it does provide important clues.

MORPHOSTASIS

Morphostasis is tissue homeostasis (Burwell, 1963) and it is well maintained in all animals. It is a core process, the functional hub of the metazoan universe. It works efficiently because cells monitor their own health and keep constant close communication with appropriate neighbours. Anamnestic immunity is a branch of the morphostatic process: it has evolved to enhance the effectiveness of morphostasis in vertebrates.

Remember, an animal is built of a large colony of cells all derived from one zygote cell (a zygote derived colony - ZDC). This colony constructs itself a skeleton of connective tissues which, while relatively inert, gives it great versatility (eg, the bony skeleton).

The critical function in morphostasis is discriminating Healthy-Self (HS) cells from all other cells and organisms (other than healthy self - OTHS cells). OTHS includes both UnHealthy Self (UHS) cells (eg, ectopic, sick, damaged, aging) and clearly foreign cells and/or organisms. Morphostasis was needed from the moment that multicellular animals first evolved. It should be clear that the main need at that time was to develop a unique way of tagging healthy self cells, so enabling them to identify and acknowledge one another, and then to devise mechanisms to abandon this healthy self status when things went wrong.

TABLE 1

Morphostasis (tissue homeostasis) can be maintained by:
(a) discriminating OTHS cells from HS cells.
(b) removing OTHS cells (UHS and foreign cells/organisms).
(c) replacing lost UHS cells with fresh HS cells (resurgent morphogenesis).

HEALTHY SELF/OTHER THAN HEALTHY SELF DISCRIMINATION

This hypothesis requires that individual cells must either have a fail-safe internal device for recognising that they have become unhealthy and/or an ability to monitor a neighbouring cell's change in health (probably) by monitoring (appropriate) cell to cell communication. The announcement of an "OTHS foul" can then be issued directly from the affected (somatic) cells. Inflammatory cells (mostly phagocytes) are only invited into the soma at this group's request - a "call" is sent out to fetch the "police". Foreign organisms need not induce an inflammatory response unless they unsuccessfully attempt communication with a HS cell, or force their way between cells (and so disrupt communication), or directly attack a cell and make it sick. Peaceful co-existence is an acceptable state.

Several properties may combine to specify HS (or UHS) identity; remember that one or more of the critical aspects which lead to HS (or UHS) recognition must be abandoned (or adopted) when the cell becomes sick. Here are some possible candidates:-

TABLE 2

(a) Lectins and the recognition of saccharides (eg, sialic acid).
(b) The inhibition of complement attack by proteins released from

	or displayed on the cell membrane (eg, factor H, DAF, MCP).
(c)	Beta-2-microglobulin and Class 1 Mhc ligand expression.
(d)	Cell to cell cytoplasmic joining - particularly electrical.
(e)	Various cytokines, particularly eicosanoids/prostaglandins.
(f)	Heat shock proteins and p53 are likely to be intimately involved in HS/UHS recognition and discrimination.

CELL IDENTITY IN THE EMBRYO AND OTHER SYSTEMS

The cells in an embryo recognise each other through Cell Adhesion Molecules (CAMs) (Edelman, 1986, 1987 & 1988, Edelman & Crossin, 1991, McClay & Ettenson, 1987). At the cell surface, both like/like and ligand/receptor interactions of these CAMs lead to cell adhesion. This adhesion then rapidly progresses on to communication through gap junctions (Keane et al., 1988). These CAMs are of three main types: first, the cadherins, second the integrins and third, a group of CAMs which are members of the immunoglobulin superfamily (IgSF) of which N-CAM is an example. Note that the transfer RNA molecules specifying N-CAM are spliced by cells in a variety of different ways to produce a range of N-CAM phenotypes. Edelman & Crossin (1991) state, "The origin of the entire Ig superfamily from an early N-CAM-like gene precursor has deep implications for the understanding of the role of adhesion in processes that are not concerned with morphogenesis but rather with immune defense, inflammation and repair".

The cells of an embryo are able to recognise appropriate neighbours: they navigate themselves into their designated locations where they meet their intended neighbours. There are many other observations of the specific recognition of cells and self in biology. Here are some specific examples:

TABLE 3

Protozoans recognise and discriminate food and sexual partners
Phagocytes are able to recognise their own pseudopodia and avoid self attack.
Simple multicellulates are known to reject allografts (1)
Plants - pollination is highly selective against self (2)
Reaggregation of disrupted foetal cells (see later) (3)
Bacterial agglutination and conjugation can be highly specific to self and (in pathogens) to target tissues. (4)
Plants - tree roots in a forest often fuse together. This is very frequent when they are from the same individual, not uncommon when they are from the same species and far less frequent when they are from unrelated species. (2)
Molecular recognition is a fundamental biological principle (eg, nuclear enzymes).
Cell homing: eg, lymphocytes and injected marrow cells. (5)

(1) Coombe et al., 1984
(2) Heslop-Harrison, 1988 and Lewis, 1979
(3) Garrod & Nicol, 1981 and Takeichi, 1990
(4) Reissig, 1977
(5) Hemler, 1990

Self recognition could, therefore, be observed in several ways, each becoming progressively more specific to the individual animal:-

TABLE 4

(a)	Tissue type recognition (eg, embryo cell recognition)
(b)	Species recognition (eg, gamete recognition)
(c)	Self ZDC recognition (ie, cells of the individual zygote derived clone. Useful as a "back stop" check of self)

MORPHOGENESIS

Morphogenesis is the process by which tissues and organs are sculptured from a zygote derived colony. It is most obvious in developing embryos: regeneration and repair are achieved by a resurgence of morphogenesis. Since morphogenesis is an integral part of a morphostatic system, it is reasonable to expect that it will share component elements of the same molecular machinery as those used by immune cells and phagocytes. These components have (presumably) been closely associated through every epoch of metazoan evolution. It remains unclear what the complete mechanisms are which lead to embryonic development. However, CAMs (as above) and gap junctions (Green, 1988) appear to play critical roles.

EMBRYOS, CAMs AND GAP JUNCTIONS

1) Gap junctional communication can be relatively non-specific (crossing species barriers) but it can also be highly selective (as below) (Kalima and Lo, 1989).
2) Gap junctional communication is critical in development. Embryo development fails when GJ communication is disrupted (Guthrie & Gilula, 1989).
3) When CAMs (cell adhesion molecules) interact with each other or their receptors, the ensuing cell adhesion appears to lead directly to gap-junctional communication. CAM interaction precedes GJ insertion and both are necessary for normal development (Jongen et al., 1991).
4) Embryos are made up of a number of compartments. Communication through gap junctions is constricted at their boundaries. These compartments correspond to important developmental fields (Kalima & Lo, 1989). They also correspond to fields of specific CAM expression (Keane et al., 1988) and homeotic gene expression (Coelho & Kosher 1991, Risek et al, 1992, Martinez et al, 1992).
5) The gap junctions in these compartments are of two sorts (Kalima & Lo, 1989). First, there are high permeability junctions joining each cell within a compartment. These allow the free passage of larger molecules: lucifer yellow is used to demonstrate this. I suspect that this "open" communication enables a block of cells to be organised, as if it was a single block of cytoplasm (a super-cell) . This may be under the control of the appropriate compartmental homoeotic genes (look at the complex structure of paramecium to see how structuring this block might work - the open cytoplasm of multinucleated drosophila eggs is similar). Second, there are more restrictive junctions which join the cells at the boundaries of these "open" compartments. These only allow small molecules to diffuse (eg, ions) so they are either insufficiently large or insufficiently extensive to allow lucifer yellow to diffuse freely. These junctions allow ions to pass in either both or just one direction. The second sort are rectifying and they correspond to junctions formed from hybrid connexons (Werner et al., 1989, Barrio et al., 1991). This directionality may be of significance in the way that embryonic cells sort, with endoderm to centre and ectoderm to the outside.
6) Despite its name, N-CAM is not confined to neural tissues. Whilst it is expressed strongly and for long periods in neural development, it is also expressed, more transiently, in other sites. It is a recognised IgSF member (Immunoglobulin Super Family). A number of authors have considered these IgSF CAMs to be the probable ancestors of immunoglobulins, T-cell receptors and histocompatibility antigens.

EMBRYO CELL DISAGGREGATION

When embryo cells are disaggregated and allowed to resettle, they reaggregate into tissue layers, ectoderm to the outside, mesoderm to the middle, then endoderm to the centre (Garrod & Nicol, 1981 and refs). When embryonic cells from two mammalian species are mixed, they reaggregate into tissue type rather than species type and this appears to be because the genes which specify the various CAMs are highly conserved across the species barriers (Takeichi, 1990).

MEMBRANE HOLES

It is now possible to make a stab at the general principle which governs HS/OTHS discrimination. I suspect it goes something like this:-

"SELF is established by making holes in the membranes of apposing cells and lining them up to create gap junctions. This allows cells to become electrically coupled and so to act as an electrical and, probably, a metabolic synctium. This ability to couple membranes dates back to the very earliest multicellulates. It relies on the controlled, ordered, simultaneous, adjacent membrane insertion of membrane holes. Cells learned, from the start, to encourage the uncoordinated, bigger, higgledy piggledy insertion of leaky holes into organisms which fail to demonstrate the membrane LIGANDs used as a focus for the tidy construction of gap junctions: the resulting electrical discontinuity and a lower membrane potential leads to an attack by scavengers. Unhealthy self cells can elect to be rejected by uncoupling from adjacent cells then dropping their membrane potential: they can also abandon the membrane LIGANDs which specify self. The mechanisms for constructing leaky holes (complement MACs) may, therefore, be distantly related to the mechanisms for constructing gap junctions."

HORROR AUTOTOXICUS & MORPHOSTASIS

One result of relying on self(cell) recognition is that "horror autotoxicus" (HA - the horror of attacking self) will probably have evolved long before lymphocytes and their memory for previously encountered antigens (anamnesis). However, this HA must be based upon the possession of specific and recognisable cell surface markers ("flags"): these probably aid the cooperative "docking" of one cell with another. Furthermore, because infection, cell damage, mutation, aging, genetic errors and other cell disturbances can also be assumed to be ancient problems, cells of the ZDC probably learned, early on, to observe "horror autotoxicus" to HS cells whilst rejecting, or sometimes just ignoring, OTHS (unhealthy self [UHS] and clearly foreign cells/organisms).

This interpretation of "horror autotoxicus" differs greatly from the classic one in which lymphocytes are deemed to be denied the right to attack self epitopes. In this new interpretation, lymphocyte aggression towards self epitopes is neither denied nor necessarily avoided. However, as will become apparent, once such auto-aggression has arisen, it will decay unless other circumstances actively sustain it.

PHAGOCYTES and DOUBLE-THINK

There is a strange double-think that pervades immunology when it comes to the importance and centrality of phagocytes and the recognition of non-self and/or unhealthy self. Every medical student learns that phagocytes recognise dead, damaged, sick and effete cells. They also learn that phagocytes can recognise foreign organisms and eliminate them (particularly non-dedicated-pathogens). Every text book devotes its statutory (short) introductory opening to the critical importance of phagocytes and innate immunity: then, almost without fail and with what I regard as indecent haste, authors are seduced into an intense dissection of the principles of anamnesis and lymphocyte function.

What makes this more bizarre is that the anamnestic immune system isn't essential to prepare cells for phagocyte attention. The phagocytic system works well, even if slowly, in invertebrates: and so does self/non-self discrimination.

There cannot be much doubt that the reason for this tendency to overlook the fundamental centrality of phagocytes is, first, a relative lack of understanding of the mechanisms of self/non-self discrimination by these cells and, second, the intense acceleration of the inflammatory process induced by lymphocytes. This greatly enhances the efficiency with which OTHS is removed and it has led us, for a long time, to regard lymphocytes as masters rather than servants of the system. There is, at the very least, a possibility that CAM interaction and junctional communication, between phagocytes and underlying somatic cells, may be the most important factor in (inflammatory) HS cell recognition. Furthermore, we have been preoccupied in looking for evidence of non-self recognition rather than healthy self recognition.

INFLAMMATION

Metazoans have evolved this ancient and virtually universal defence mechanism in which somatic tissues become infiltrated with scavenger cells (mostly phagocytes) whenever required. These scavengers are clearly capable of recognising most organisms, particularly those which are not dedicated pathogens. And, in the vast mass of animal life, they appear to do so without the aid of cells which have the ability to "remember" epitopes. They also remove aging and disordered self cells. In fact, their behaviour is ideally suited to eliminating OTHS. I propose two things:

(a) In all complex metazoans, the discrimination of OTHS from HS by phagocytes remains a central and crucial morphostatic process.

(b) All other immune processes are geared to accelerate, accentuate and maximise the discrimination of OTHS from HS by phagocytes. In consequence, the efficiency with which OTHS is removed is greatly enhanced.

Even so (as you will see later) HS/OTHS discrimination does not begin in phagocytes but in somatic cells. It is the consequence of general cell recognition and communication. Inflammation is only established when somatic cells "decide" that they cannot cope alone and "invite" these scavengers in. Static somatic cells are attached to each other at cell junctions. Their cytoplasms are joined by gap junctions (except in those cells who's mature function depends on electrical excitability). When membrane junctions are split apart the disruptions in the cell membranes probably lead to the release of various eicosanoids (prostaglandins etc). This announcement of an OTHS event, by somatic cells, results in an inflammatory reaction. Chemical messengers released at the OTHS site encourage the ingress of phagocytes through the endothelial cell linings of local post-capillary venules. Phagocytes now invade the OTHS site. They begin assessing cells on the basis of their HS status. Note that in electrically excitable cells, like neurones, their terminal differentiation requires that they uncouple from each other: it is left to unusually tightly bound endothelial cells to restrict the ingress of scavenger cells and thus reduce the susceptibility of these tissues to inflammation.

Thus far, the basic process is the same for almost every, if not all, animal species. At this point, vertebrates enrol a new mechanism. Debris from local tissues is processed by phagocytes (or phagocyte related cells) and it is then presented, in local lymph nodes, to the anamnestic immune system as short representative peptides in combination with class II antigens. The aim is to select representative Class II/peptide epitopes and then arrange to retain a

memory of them and their inflammatory environment so that, on their next encounter (which must, incidentally, follow phagocyte/APC processing), this inflammatory environment can be rapidly and potently reproduced and, more often than not, exaggerated. This anamnestic response is under the full command of the morphostatic process and, in particular, largely under the control of phagocytes.

MIMICRY

Because morphostasis has always relied on self recognition, dedicated pathogens need to use mimicry (or more subtle interferences with identity molecule expression and recognition) to gain access to and persist in the soma (eg, Lyampert & Danilova, 1975, Chakraborty, 1988, Vanderplank, 1982, Yoshino & Boswell 1986). Every animal needs to stay one step ahead of its competition. Constant pressure is exerted to expand the variety of identity molecules available within a species (pleomorphism). Somatic cells appear to recognise each other by developmental ligands (cell adhesion molecules, CAMs). When embryonic cells from two mammalian species are disaggregated, mixed together and allowed to settle, they segregate into tissue type and not into species. Somatic ligands have probably needed to stay constant over countless meiotic generations. This makes them a sitting duck for determined pathogens. So, somatic cells need a "back stop" identity to be used as a second check when things go wrong (phagocyte based and, perhaps, also Mhc Class 1 based (Versteeg, 1992)). And until they do go wrong, inflammatory cells can be confined to the vascular system, locked out behind tight endothelial cell junctions until invited in. Note that "loss of function" is a cardinal feature of the inflammatory process.

UNHEALTHY SELF ACTIONS: APOPTOSIS AND SELF SACRIFICE

When cells fail to establish communication, membrane reactions probably begin which lead to the release of a variety of eicosanoids and other cytokines (Bach, 1988). Similarly, when cells become unhealthy they break junctional communication and become prey to attack by both adjacent cells and the inflammatory cells which are (in consequence) called into the area (Loewenstein & Penn, 1967). When I first started thinking about self(cell) surveillance, I found scant literature describing elective suicide and I even looked at plants for evidence of this (the hypersensitivity reaction (Prusky, 1988, Fritig et al., 1987). However, interest and literature on this subject have become abundant recently (Bowen & Lockshin, 1981, Cohen, 1991, Ellis et al., 1991, Young, 1992). In synthesis, individual cells do decide that they are sick and/or redundant. They do have the capacity to invite attack by adjacent cells and also to invite phagocytes along to have themselves removed. There is no need to presume that antibodies and lymphocytes are responsible for the primary assessment of (healthy) self status.

Changes in the concentration of calcium ions within the cell are all important in this election for "disposal by consensus". Ca++ ions act as second messengers for a variety of cell processes including apoptosis, nuclear division, growth factor stimulation: they are closely tied into the inositol-PO4/DAG/protein-kinase-C network of intracellular second messengers (Hollywood, 1991, Evans & Graham 1990): and high Ca++ ion concentrations close down the gap junction channels between cells. In this respect, cellular identity and cell health is all tied into proto-oncogene activity and this in turn into gap junction formation and communication competence (Yamasaki et al, 1988, Yamasaki 1990). Here is the promise of a much clearer understanding of cancer.

When cells are attacked by C9 or perforin, they are made leaky, their cytoplasmic membrane potential falls and Ca++ ions are allowed into the cell. Both these molecules contain sequence motifs similar to the LDL receptor and epidermal growth factor receptor and there may be wider significance in this

(see Maldonado et al 1988). One important feature is that both these receptors are endocytosed in clathrin coated pits (like the Mhc molecules themselves).

THE GENERATION OF SPECIFICITY

A major problem in understanding the evolution of anamnestic immunity is how such a complex system erupted onto the evolutionary scene, so suddenly and so completely, in the vertebrates. One explanation is that it evolved, not as a generator of receptor diversity but as a generator of receptor specificity. The table below shows how a scavenger cell could be programmed only to cooperate with self cells which display ligands unique to that single ZDC. The specification of such a scavenger is an exact inversion of the specification of the cytotoxic T cell. Even a repertoire of receptors as few as two would be useful in specificity whereas, in diversity, it is difficult to see how any useful function could have evolved until there was a large repertoire of possible receptors. With a system which develops on the basis of specificity, there would be ample time to develop an extensive repertoire of possible receptors before being precipitously "flipped around" to service a generator of diversity. Note that "pure self" is used to indicate unaltered, self Class I Mhc antigens.

TABLE 5

Cell type	Receptors disabled	Receptors enabled	Normal state	Triggered state
Scavenger	non pure self	pure self	aggressive	passive
	GENERATOR OF SPECIFITY			
Tc cell	pure self	non pure self	passive	aggressive
	GENERATOR OF DIVERSITY			

There are two possibilities. First, that the ancestors of the T cell receptor may have been used to recognise tissue CAM ligands: this could be the origin of the V gene segments (Allison & Havrin, 1991). Secondly, a descendant of the simple scavenger (phagocyte) may have evolved to recognise a set of pleomorphic CAM like markers which were specifically evolved in a population for them to be used as a back stop identity check unique to each ZDC. Developmental CAMs seem to remain constant over countless generations and this is reflected in the way embryonic cells from different species reaggregate as germ layers and tissues rather than species. The "back stop" CAM like ligand (the precursor of the Class I Mhc antigens) could deliberately borrow bits and bobs from these developmental CAMs to form a unique looking ligand by using a genetic mix and match process.

There seems to be little likelihood that phagocytes are able to rearrange their genome to form specific receptors. And there is no substantive evidence that they can selectively cooperate with cells carrying self Mhc antigens. Natural killer cells, however, might be such a candidate, particularly if they are composed of two populations: one with a lower specificity - perhaps based on beta-2-microglobulin expression - and another with highly specific receptors for self. They were first identified because F1 Tnk cells attacked parental cells (unlike the classical transplantation laws). This would be consistent with specific (cooperative) recognition. These cells also preferentially attack cells expressing low levels of Class I antigen and beta-2-microglobulin. It seems that, at most, only a proportion of Tnk cells rearrange their receptor genes. (See Trinchieri, 1989 and Versteeg, 1992).

Phagocytes, lymphocytes, fibroblasts and platelets are all derived from the same stem cell. They have almost certainly all evolved from a primitive, ancestral scavenger. Each cell type seems to have caricaturised some specific property of this general scavenger and refined it in order to make the mature mammal's repertoire of responses more versatile. This adds weight to the proposition that a phagocyte like or derived cell might, at one stage, have evolved to have the ability to select/rearrange its genes so that it could specifically recognise healthy self ligands (Mhc "Class-I-like" ligands). The self receptors would have to be selected, in embryo, to be specific to each individual.

One possibility is that, now the lymphocyte system has evolved, this has been so successful that it has largely obviated the need for a scavenger to rearrange its genes to uniquely recognise self. There might even be a positive advantage in achieving the apparent recognition of HS(cells) by inverting the cooperative recognition of self cells into an attack on non-self(epitopes) by Tc lymphocytes. This can be achieved by the clonal elimination of any lymphocyte capable of reacting with "pure self" Class 1 ligands.

Note that complement activity is very much in the style of a horror autotoxicus, with healthy self being protected from attack by inhibitors: and also that phagocytes synthesise enough of all but the terminal components to attack undesirable cells without the aid of circulating complement.

SOMA/SCAVENGER SEGREGATION

I have already alluded to soma/scavenger segregation. The important point to grasp is that somatic cells can and do deal adequately with a fair proportion of OTHS (Young, 1992). Provided the accumulation of OTHS is mild and the local cells can both recognise any loss of HS identity and discriminate foreign organisms from HS, then there is little need for a back stop identity check. HS here is established by displaying appropriate tissue CAMs which lead on to the establishment of a "synctial" communication through GJs. However, when UHS or foreign organisms fail to appear sufficiently OTHS to the local cells, then tissue damage will probably ensue as the foreign cells or UHS cells start to gain the upper hand. It is at this stage that scavengers are "invited" in and this is done by a fail-safe device (the eicosanoid system - prostaglandins etc). These scavengers then establish HS status by employing a "back stop" check on identity. If there is a scavenger which formally recognises HS Class 1 status then this would give the system a highly specific way of recognising self once invoked (eg, the Tnk cell (Versteeg, 1992)).

Inflammatory cells invade and disrupt the normal structure of tissues and this invasion leads to loss of function. They are undesirable intruders in healthy tissues except in small numbers. Hence they need to be kept largely locked out, behind a tightly bound cylindrical pavement of endothelial cells lining the blood vessel walls. This need for segregation is almost certainly the origin of the vascular system. The subsequent recruitment of the vascular system into distributing other "freight" has meant that phagocytes and their evolvents have become adapted to such tasks as encapsulating the inflammatory process (by clotting factors and platelets), distributing fats in the blood (phagocytes), anamnestic immunity (lymphocytes) and transporting oxygen (red cells).

Now it is possible to add some concluding comments to the six points introduced earlier in the section "EMBRYOS, CAMs AND GAP JUNCTIONS":

7) In this hypothesis I have suggested that scavenger cells (phagocytes mostly) use a CAM receptor molecule to latch onto a respective CAM on self cells. The base of a phagocyte (uropod) remains attached to the underlying tissues. This base probably maintains electrical contact with the

underlying cells through GJs. The cytoplasmic fingers of a phagocyte (the lamellipod) constantly probe forward. If these fingers encounter a cell which is not in electrical continuity, the scavenger could be triggered into aggression by the capacitative current which flows as the membranes come close together. This could, in turn, trigger an action potential to arm the cytoplasmic finger of the scavenger cell. Additional recognition strategies may be employed. The changing of surface sugars in sick cells is one (loss of the negatively charged sialic acid residues may increase the capacitive current above the triggering threshold). The phagocyte may well have a limited "hit list" of receptors which recognise markers which are indubitable evidence of their non-eucaryotic origin and which would, therefore, never be found as part of self. Dedicated pathogens will, of course, studiously avoid displaying these.

8) Now, the original self CAM may gradually be found to be inadequate as a back stop identity check because various pathogens discover ways of mimicking or interfering with its machinery. At this stage, a new cell is required (perhaps similar to the natural killer cell) which can recognise a more pleomorphic set of CAMs that are deliberately individualised in each animal of a population and more or less unique to each ZDC. An appropriate set of specific receptors would have to be selected, in embryo, to recognise these unique ligands. These, I contend, may be the close ancestors the T cell receptor which led, by inversion of function, to the cytotoxic T cell. In this vein, note that tumour necrosis factor and lymphotoxin are selectively toxic to cells which are not communicating through gap junctions (Fletcher et al., 1987, Matthews & Neale 1989).

ANAMNESTIC AMPLIFICATION

So, what is the function of lymphocytes: what are they doing? An individual lymphocyte is simply following orders from an antigen presenting cell or phagocyte (in conjunction with an unhealthy somatic cell in the case of Tc cells). This instructs it to attach either an aggressive or a suppressive action to its paratope and to act accordingly on its next encounter with its respective epitope. Direct killing is not the prime function in either delayed type hypersensitivity T-cells (TH1) or helper T-cells (TH2). They are not remembering epitopes for the prime purpose of "killing" them. The precursor lymphocyte logs the context in which it first "sets eyes" on its epitope. If it was inflammatory then at the next encounter it will attempt to recreate a rapid and potent inflammatory response rather than wait for the "cell damage -> cytokine -> inflammation" cascade to build up. "Tipped off" inflammatory cells can then settle down much more quickly and aggressively to their phylogenetically ancient task of sorting HS from OTHS. The main difference now is that these phagocytes are doing it much more quickly and with better targeting. But, they are also doing it more hamhandedly - they'll "bash" anything that looks remotely suspicious (hence the need to focalise this response). Tc cells are relatively more independent and kill directly but even these are only allowed to become aggressive if they have first been primed by IL-1 released from APCs during an inflammatory encounter. And these, too, encourage a rapid inflammatory response once they start attacking target cells.

Somatic cells probably show some specificity for the epitopes that they present for Tc cell priming. The peptides that they present in combination with Class I antigens have probably been shepherded through the cell by its garbage minders, the ubiquitins. Even leaving this aside, it is still easy to imagine how self/non-self selectivity can occur. When T-cells are released from the thymus they are already committed in specificity (ie, they are committed to recognising a specific epitope) but, they are not committed in activity (aggression or suppression). It is only when they meet their respective epitope that this commitment is made. Self epitopes are, in general, encountered frequently and the first encounter (in embryo) is nearly

always in a "healthy self" (non-inflammatory) environment. So tolerance is generally favoured for those lymphocytes which recognise self molecules. Few self specific T-cells will remain uncommitted for more than a brief period while there is a relatively large pool of the relevant self epitope waiting to be encountered.

On the other hand, because only small quantities of a foreign or strange epitope are infrequently met in the body, most T-cells capable of recognising them will remain uncommitted until they meet the epitope, as part of OTHS, in an inflammatory encounter: aggression will be favoured. Furthermore, it seems that it is easier to provoke old rather than young precursor lymphocytes into aggression. This further concentrates the aggressive response onto those epitopes that are most strange to the body. No veto need be imposed on T-cells to prevent them becoming aggressive to self epitopes (except for "pure self" Mhc ligands - these must be clonally disabled). Indeed, epitopes from tissues that are usually hidden behind tight endothelial cell junctions (like the eye and brain), and are infrequently encountered, are more likely to provoke aggression as there will be a larger pool of uncommitted T-cells available. They are, consequently, more inclined to provoke an aggressive response when they are exposed during periods of intense inflammation. (Lymphocytes which have a paratope for recognising certain self Mhc/peptides are clonally deleted in the thymus: this deletion follows the disintegration of self cells in the thymic medulla.)

The bone marrow constantly produces new uncommitted T-cells. So, whenever clearly foreign epitopes are sparse and inflammation is intense and prolonged, attention can gradually turn to self epitopes (eg, as in tuberculosis). In summary, inflammatory acceleration is most likely to develop to clearly foreign (strange) epitopes and a "healthy soma tolerance" most likely to develop to self (frequently encountered) epitopes.

The overall effect is that lymphocytes remember the "inflammatory" or "healthy soma" context in which they first meet their respective epitope (and become committed); and they aim to recreate and caricaturise this memorised inflammatory or non-inflammatory milieu at the next encounter. Whenever TH1 cells provoke an inflammatory response they call large numbers of phagocytes (& Tnk cells?) to the epitope site. These are then switched into a heightened state of "anger". However, phagocytes (& Tnk cells?) still have to discriminate HS from OTHS but now, the threshold at which aggression is considered is greatly reduced. Cells expressing a relatively low level of "HS identity" are now likely to be attacked. This amplification of the inflammatory response by lymphocytes has the potential to escalate catastrophically. It can slip into a loop of strong positive feedback, particularly when the epitope is an abundant self Ag. When the local auto-rejective response becomes excessive, it must be down-regulated otherwise things will get disastrously out of hand. This could be done in a number of ways and these may account for many instances of clinical anergy (Dwyer, 1984, Meakins, 1988, Meakins & Christou, 1979, Normann et al., 1981, Ninneman, 1981):

TABLE 6

(a) inhibition of phagocyte ingression (chemotaxis)
(b) inhibition of phagocyte aggression
(c) inhibition of further aggressive lymphocyte activation
(d) a tightening of endothelial cell junctions
(e) encapsulation in a fibrin sheath (fibrocytes later)
(f) promotion of lymphocytic tolerance to typical Ag
(g) production of auto-antibodies to the newly cloned, locally reactive lymphocytes (lymphocytotoxic Abs)

TABLE 7

THE FOUR PRINCIPAL MODES OF EPITOPE PRESENTATION

	OTHER THAN HEALTHY SELF CONTEXT	HEALTHY SELF CONTEXT
SOMATIC CELL	Tc activation (Class I Mhc)	Ts activation (Direct??)
PHAGOCYTIC CELL	TH1 & TH2 activation GG GG (Class II Mhc)	Ts activation G (Like T/B cell co-operation? Th/Ts)

AUTO-REJECTION

Tissue rejection is largely accomplished by cell mediated mechanisms. Antibodies are generally bystanders. Similarly, the auto-rejection of abnormal cells will be accomplished predominantly by cell mediated immune mechanisms (eg, in various forms of necrosis like burns and infarction). There is one important inference to be made from examining the structure of the sero-negative arthritides and particularly Behcet's syndrome (based on a personal study). This is that auto-rejective disease covers a wide spectrum of prevalence and severity. The mildest components are VERY common, suggesting that auto-rejection is a normal process. This leads on to the conclusion that there is no automatic horror autotoxicus to self epitopes where T cells are concerned. When auto-rejection is so general, it has to have physiological as well as pathological significance: it must be a functioning element of the morphostatic mechanism.

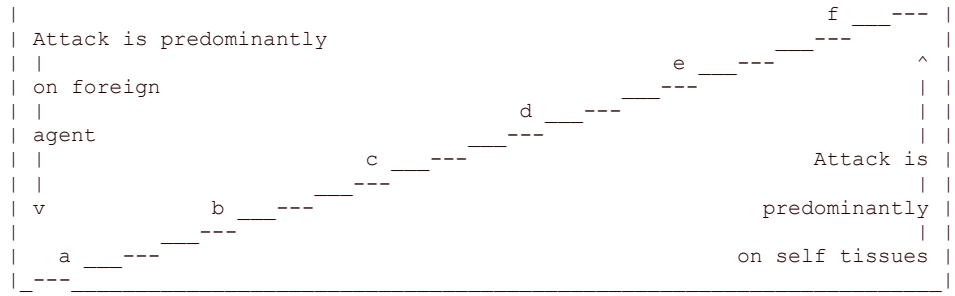
ANTIBODIES - ICING ON THE CAKE

Antibodies are icing on the cake. Extremely useful, evidently important but dominantly aimed at pre-empting the proliferation of blood borne pathogens and pathogens which colonise epi/endothelial surfaces. It's clear that the role of antibodies in tissue rejection (and hence auto-rejection) is minor if not minimal. The vast mass of animal life copes well without them. "Cell-mediated immunity clearly precedes humeral antibody production in phylogeny" (Manning and Turner, 1976 also emphasised by Cooper, 1982). We can safely put antibodies to one side until towards the end - which is more or less where they evolved. It appears to me that, to bother looking amongst antibodies for an explanation of how self/non-self discrimination evolved, would be manifestly Heath Robinson (or Rube Goldberg!). In this vein, it is worth noting that the spleen may be specifically adapted to make the best of the difficult job of maintaining morphostasis in the suspension of cells circulating in the highly mobile plasma.

THE CLINICAL IMPLICATIONS

The result of all this is that any disease which evokes an inflammatory response has an element of auto-rejection. It inevitably consists of a mixture which varies from an attack directed almost exclusively at the pathogen (usually leading to mild inflammation) to an attack directed almost entirely at self (often highly inflammatory): the latter occurs when organisms or cells provoke prolonged inflammation but do not provide or present clearly foreign looking (unusual) epitopes. Every disease that leads to cell damage will induce auto-rejection. Since heat shock proteins are responsible for chaperoning disrupted proteins through the cell, they are frequently presented as potential epitopes in UHS presentations.

TABLE 8



EXAMPLES
(a) Saprophyte
(b) Simple epithelial commensal
(c) Staphylococci and streptococci
(d) Tuberculosis and syphilis
(e)-(f) Multiple sclerosis and sero-negative arthritis

MORPHOSTATIC EVOLUTION

It is now easier to see how the morphostatic system may have evolved. Here is the probable path of the evolution of ZDCs from simple multicellulates to mammals.

(a) In the beginning, all cells in the colony express equally marked phagocytic behaviour.

(b) SELF is established by making holes in the membranes of apposing cells and lining them up to create gap junctions. Cells learn, early on, to allow the uncoordinated, bigger, higgledy piggledy insertion of leaky holes into organisms which fail to demonstrate the membrane LIGANDs used as a focus for the tidy construction of gap junctions.

(c) Cells now divide into phagocytes and soma. They selectively improve the specificity and efficiency of cell junction construction by facilitating and amplifying their construction at the site of cell LIGAND/RECEPTOR interaction. The resulting gap junctional plates are more "transparent" and more specific about where they form. They develop:

SOMA LIGAND(s) - for recognition by resident scaffolders.
PHAGOCYTE LIGAND(s) - for recognition by itinerant scavengers.

(d) Dedicated scavengers (phagocytes) now evolve. They refine this cooperative gap-junctional communication with self and the runaway, leaky hole attack of non-self. The molecules used to do the second will eventually evolve into what we now recognise as the complement components. It is possible that the two construction cascades are related but become independent early in evolution. At this stage the complement components are only secreted locally by phagocytes and their action is directed entirely at membranes. It is a long time before these components are co-opted into a humeral system and very much later that they are co-opted to interact with antibodies (probably an adaptation of specific Mhc recognition).

(e) A "vascular" system now evolves, locking out phagocytes till required. The alternative complement cascade can now be "humeralised" so that circulating C3 can mark clearly foreign organisms to make them more readily identifiable when they meet a phagocyte.

(f) There is now a progressive evolution and expansion of somatic LIGANDs leading to increased tissue compartmentalisation. Phagocytes are derived from a lineage which lies "outside" the three main germ layers so they may

be exploiting this sorting tendency as they infiltrate somatic tissues: it is as if they are able to "clamber" over every other cell type.

(g) Ig supergene like LIGANDs develop to act as a focus on which to grow highly specific gap junctional plates and create developmental compartments. The genes specifying these molecules can now be copied then altered by a "mix and match" process to generate a set of LIGANDs which have a great variability within a herd (primordial Mhc genes). These pleomorphic LIGANDs will now act as the final arbiters of healthy self in each individual. Over many meiotic generations, they have eventually evolved into Mhc Class I LIGANDs. Newly developed scavenger cells (Tnk precursors) may now be able, when required, to co-operate with any somatic cell that displays self specific LIGANDs and observe a horror autotoxicus to it. These new scavengers need a mechanism to produce and/or select self specific RECEPTORs unique to each ZDC. This must be done post-meiotically over a number of mitotic generations - the "generation of specificity". This possibly coincides with the evolution of amniotic molecules which are involved in HS/OTHS discrimination or its modulation These include HSP70, TNF, complement components and the 21-hydroxylases.

(h) By inverting the "generator of specificity" into the "generator of diversity" lymphocytic cells (Tc like) can evolve which are able to recognise and attack cells who's Class I ligands have been altered in the presenting cell by the attachment of a peptide which may make them look like an allotype. This new function depends on the duplication and transposition of the gene which produces the heat shock protein peptide pincer mechanism and bringing this to lie next an the Ig superfamily domain to produce the ancestor of a Class I Mhc gene (Flajnik et al, 1991). These primordial Tc cells first develop to recognise Mhc "Class-I-like" allotypes and then peptide/Class I combinations. They were probably preceded by cells capable of recognising beta-2-microglobulin: hence, the eventual elaboration around this molecule. Sometime between now and the evolution of free antibodies, the so called "alternative" complement pathway is extended into the "classical" pathway. C1 might be specialised for short range triggering of high density, single surface LIGAND/RECEPTOR complexes so that hole construction is now restricted to the target membrane rather than to a coordinated construction in apposing membranes.

(j) The stage is now set to allow the evolution of TH1 cells. Class II Mhc ligands evolve: the "intention" is to process short representative peptides from cellular debris picked up by phagocytes at inflammatory sites. These are then externalised as a Class II/debris combination ready for the attention of uncommitted T-cells. The "generator of diversity" can now be enrolled into memorising the inflammatory context of these processed epitopes. On re-encountering the processed epitope these T-cells can rapidly attract large numbers of phagocytes to the site and "angrify" them: inflammation now has a memory. Note that only a very limited set of cells - APCs, phagocytes and a few others - can present these combinant epitopes so this amplification of the inflammatory cascade can only start after OTHS has been processed.

(k) The need to instruct T-cells to tolerate healthy soma epitopes has to evolve simultaneously with Tc and TH1 cells. T-cells capable of recognising healthy self epitopes are mostly decommissioned. This may be a co-operative process (Th/Ts cooperation akin to Th/B-cell co-operation). Whatever, aggression is averted by having them "mopped up" by Ts commitment. This happens because these epitopes are more likely to be met in a non-inflammatory context. However, uncommitted self specific T-cells continue to be released from the thymus and can become recruited into

aggression. Aggression to self epitopes will be most likely to be induced and permitted when the inflammatory process is prolonged and foreign epitopes are sparse. Tolerance might be amplified by Ts cell clonal expansion and, perhaps, the release of anti-inflammatory agents at the site of epitope re-encounter. Like TH2 and B-cell interaction, helper and suppressor epitopes tend not to overlap, suggesting a similar co-operative mechanism.

(m) Last of all, TH2 cells can now be incorporated into the system to prime the B-cell system and lead to freely circulating antibodies. The B-cells are also derived from a scavenger cell. This is designed to secrete large quantities of free, circulating antibody. Antibodies help by opsonising organisms (preparing them as a "meal" for phagocytes). The classical complement cascade is now optimised to work within the vascular system and to interact with antibody tagged antigen. This system has proved invaluable as a pre-emptive defence.

THE ADVANTAGES OF THIS PERCEPTION

By now I hope that you will be aware that all this suggests a clear path in self/non-self discrimination. Its beginnings can be seen in simple animals like sponges, which demonstrate differential cell reaggregation (for they, too, have gap junctions) and it proceeds through to the complex mammalian immune system. In this respect, it is interesting to read that differential sorting is, in embryos, a direct consequence of CAM expression (Takeichi, 1990). The reasons why embryonic cells sort according to tissues rather than according to species is that their CAMs have remained highly conserved across widely separated species.

Let me tabulate the advantages of this way of perceiving the process:

1) Seamless integration from embryonic development to anamnestic immunity.
2) The innate and the acquired immune system are no longer seen as fundamentally disparate entities. They are fused into a seamless whole.
3) A clearer understanding of preferential alloreactivity by T cells.
4) A clear evolutionary progression from organisms with no cellular differentiation, through simple organisms with phagocytes, then the evolution of a retinue of specialised cells all derived from the primitive scavenger. A "logical progression" would start with Tnk like cells, go to Tc like cells, then TH1 like cells, then TH2 like cells and finally B cells.
5) A far clearer perception of the cancerous process (not detailed here but there is good evidence that gap-junctional communication is involved (Yamasaki et al., 1988, Yamasaki 1990).
6) The potential to explain the process of aging (Kelley et al., 1979, Peacock & Campisi, 1991).
7) It all makes good biological sense. Indeed, it integrates so many biological, developmental and immunological mechanisms into a continuous whole that it begins to hold out the promise of a "grand unification theory".

SUMMARY

I have proposed reshaping the perception of immunity to encompass the broader principle of MORPHOSTASIS. The loss of healthy self is sensed and expressed by the malfunctioning cell itself or, at furthest, emanates from the membrane doublet where contact is established between this cell and its immediate neighbours. This "foul" is broadcast by the release of inflammatory mediators. These invite phagocytes into the area to assess the local population. Phagocytes (and perhaps Tnk cells) then attack those cells with which they fail to become electrically continuous. The time they have to make this connection varies with the "anger" of the phagocytes. Phagocytes now present

cell debris to lymphocytes in local lymph nodes. The epitopes which are most strange to the lymphocytes are selected to act as the pegs on which to hang a greatly accelerated inflammatory infiltration on any subsequent encounter of these epitopes.

I have also proposed redefining the concept of "horror autotoxicus": it is established by successful cell to cell communication. Both somatic and scavenger cells use this mechanism. The concept of immunological surveillance is simultaneously redefined. But now surveillance is for any malfunctioning cell and not just for neoplasia. The evolution of a thymus dependent lymphocytic system with memory may have occurred at the expense of an increased prevalence of cancer, for intense focal suppression of surveillance now occurs whenever a strong positive feedback leads to an exaggerated attack on self epitopes. This then permits a tumour cell compartment to reach a critical mass beyond which surveillance fails (Yamasaki, 1990).

This explanation undoubtedly contains errors and I am sure many of the more specific assumptions will prove to have been far too simplistic. For example, the immune system has gathered a great number of refinements throughout its evolution including various specialised phagocytes and permanently resident, non-itinerant antigen presenting cells: little has been said about these. However, I am confident that the "flavour" of the concept is essentially correct and the hypothesis will prove to be a useful framework for refinement. It should now be clear that the breaking of cellular junctions is probably an important event which leads on to the declaration of an OTHS "foul". There are a number of close similarities between the insertion of gap junctions into self cell membranes and the insertion of complement membrane attack complexes into invaders. If it could be shown that there is a continuing or a distant relationship between their respective insertion mechanisms, then it would be reasonable to assume that HS is, indeed, sensed by the speed with which both somatic cells and scavenger cells establish an electrical continuum with those cells that they encounter.

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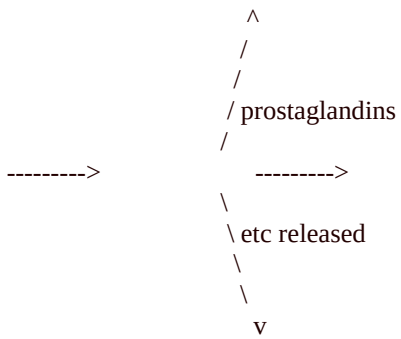
THE OVERALL PROCESS



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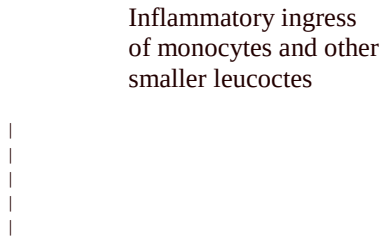

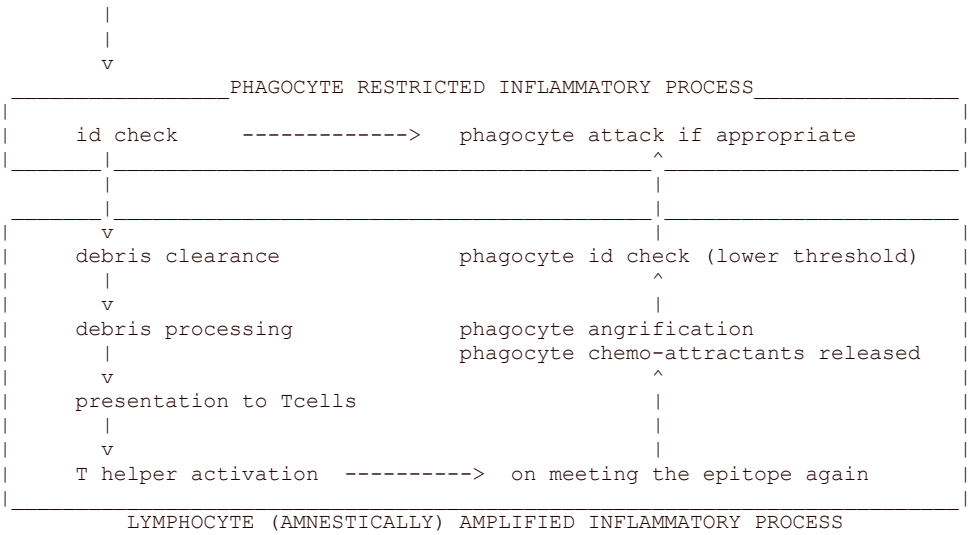

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HYPOTHESIS

Morphostasis is tissue homeostasis. Tissue form is stable whilst cells remain in intimate contact by intercellular junctions. This enables joined cells to establish various degrees of electrical and metabolic synchronisation and it promotes cooperation. Synchronisation is greatest when the cytoplasms are in direct continuity through gap junctions or synctial structures. The specificity of the molecular mechanisms that lead to cell adhesion, coupling and connective tissue scaffolding, in effect, give cells a <healthy self (HS)> identity. Similarly, the loss of <HS identity> is accompanied by dismantling of the connective tissue scaffold and cell undocking. Self cells monitor each others' identity. When a cell becomes sick it recognises its own disorder and abandons <HS identity>. It can shut down the channels that join its cytoplasm with those of adjacent cells and then detach its membrane from them in a process called apoptosis. This leads to tidy, elected cell death. Adjacent cells and phagocytes ingest apoptotic cells before they burst. This induces T-cell tolerance. Necrosis is an untidy form of cell death. Such dying cells burst and spill their contents, so releasing inflammatory cytokines. These, in turn, trigger aggressive anamnestic immune reponses which accelerate the identification and elimination of other cells resembling those that previously evoked an inflammation. Once order is restored, adjacent healthy cells duplicate and replenish lost cells.

CAM	=	cell adhesion molecule
GJ	=	gap junction
HS	=	healthy self
ICJ	=	intercellular junction
Ig	=	immunoglobulin
IgSF	=	Ig superfamily
N-CAM	=	Neural CAM
OTHS	=	other than healthy self
UHS	=	unhealthy self
ZDC	=	zygote derived colony

INTRODUCTION

Brevity demands a synoptic style so here I present my perception of the immune process.

Zygote derived colony (ZDC). Every animal is a colony derived from a single

zygote cell. Each ZDC cell needs some way of preferring its own kind as neighbours and inhibiting the growth of foreign cells or organisms in its vicinity. This is helped by using selective CAMS which lead to the construction of ICJs, a scaffold of connective tissues and electrical/metabolic synchronisation1,2.

The self aware cell. Each animal cell is a self assessing unit, able to survey its own behaviour and function. When it malfunctions, it senses this abnormality and notifies other cells that something is wrong (by various cytokines, changes in surface markers and by breaking junctional communication). A sick cell can sacrifice itself by apoptosis3,4,5: its calcium level rises, it rounds up and its GJ are closed before these and other ICJs are disassembled. Apoptotic cells are phagocytosed by adjacent cells or phagocytes before their membranes burst.

Healthy self (cell) / other than healthy self (cell) discrimination. All metazoan animals make this discrimination. What differs from organism to organism is the sophistication with which it is embellished6.

Morphostasis7. Tissue homeostasis can be maintained by:-
(a) displaying markers on the membranes of HS cells which identify them as HS.
(b) discriminating OTHS cells from HS cells by the absence of HS identity.
(c) attacking and removing OTHS cells (UHS and foreign cells/organisms).
(d) replacing lost UHS cells with fresh HS cells (resurgent morphogenesis).

GAP JUNCTIONS

The cytoplasms of static cell populations are often joined through GJs8. These channels are shut down when a cell becomes sick9,10,11. A rise in intracellular calcium initiates GJ closure8. GJ channels are then disassembled during apoptosis3,4.

The whole embryo is electrically connected through GJs and this establishes the boundaries of <self>12. It contains sub-compartments where member cells are joined by plaques of GJs which have high permeability. They are surrounded by a layer of cells with lower permeability and these define the compartment borders. They correspond with developmental compartments. N-CAM promotes the construction of highly permeable GJ plaques13.

Thus, a consensus sequence in N-CAM, resembling the Ig constant region, may have evolved to spawn multiple, highly permeable GJs much as the complement C1,C2,C4,C3 cascade spawns multiple well formed MACs. If so, the C7,8,&9 genes have either evolved from connexon genes or they have highjacked the mechanism which encourages the construction of highly permeable channels, inverting it into an attack mechanism. Note that leaky holes will lead to a rise in intracellular calcium and so close GJ channels.

APOPTOSIS, NECROSIS and INFLAMMATION

Successful self surveillance leads to apoptosis and elective suicide. This mechanism deals with many sick cells. It has failed when cells die by lysis. Then, membranes rupture, their contents are spilled, eicosanoids are released and inflammation is promoted. Inflammation provokes aggressive T-cell responses. Tc cells induce apoptosis in cells which carry markers resembling cells that have previously died and provoked an inflammation. TH1 cells remember the inflammatory context in which they met their epitope. When they reencounter similar peptides they turn up the inflammatory "heat". They do not, themselves, kill: this is left to "angrified" phagocytes which are more particular about what they will accept as <HS identity>.

Peptide debris processed after phagocytosing apoptotic cells promotes T-cell suppression. For example, when a cell dies following a virus infection its debris is processed by adjacent cells and phagocytes. If cell death occurs following successful internal surveillance (apoptosis), tolerance will be promoted to the processed peptide debris. When unsuccessful (eg, lytic death),

inflammation promotes T-cell aggression. Since apoptosis is common, self peptides usually promote suppression and so shrink the pool of self reactive precursor T-cells available to be later recruited to aggression. Also, the threshold at which uncommitted T-cells can be triggered into aggression falls as they age. This further focuses aggression onto strange epitopes.

<HS cells> in an inflammatory area are protected from attack because they still demonstrate <HS identity>. This is a form of horror autotoxicus. Phagocytes from closely related species share similar specificity. Most non-pathogenic organisms are easily identified as non-self. Unless complement is present, bacteria and viruses must rupture a cell and/or disrupt its ICJs to invoke an inflammatory reaction and an anamnestic immune response.

Inflammatory cells need to be restrained from entering healthy tissues until things goes wrong since their intrusion disrupts tissue function. The endothelial cell linings of blood vessels tend to lock out phagocytes until they are invited in. This is done more rigorously in the central nervous system - the blood brain barrier. This is necessary as nervous function relies on the electrical (GJ) disconnection of neurons during their terminal differentiation and the resulting asynchronisation then makes them more susceptible to macrophage attack.

MORPHOSTATIC EVOLUTION

This is the way I suspect that the metazoan system evolved. Note that each new step is an embellishment of the former and all of them remain functional in mammal morphostasis.

(a) Elective cell suicide (apoptosis) is established as a means of protecting the colony.

(b) Electrical/metabolic synchronisation, through ICJs, establishes a sense of self. ICJs are the immediate consequence of cell surface ligand/ligand or ligand/receptor interactions and these molecules are Cell Adhesion Molecules, CAMs1,2. Membrane holes in apposing cells, once paired up, form GJs (similar channels are important in plants14,15). IgSF CAMs (eg, N-CAM) develop later to act as a focus on which to build highly permeable GJ plaques. This "multiplier" mechanism is later adapted to spatter bigger, leaky holes into cells or organisms which do not display features of self (the alternative complement cascade).

(c) The progressive expansion of different somatic CAMs lead to, subordinate, self within self identities and thus tissue specialisation. These define new developmental compartments where the borders are demarcated by a sheet of cells having GJs of low permeability. The cells within the compartment express IgSF CAMs and are joined by highly permeable GJ plaques. Cell sorting is dependent on CAM expression, particularly cadherins1,2.

(d) Animal cells now split into dedicated phagocytes and soma.

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SOMA LIGAND(s)        - for recognition by resident scaffolders.
PHAGOCYTE LIGAND(s)   - for recognition by itinerant scavengers.
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Dedicated phagocytes evolve. They refine both their cooperative ICJ communication with self cells and the attack system which inserts leaky holes into non-self cells: the latter will become the complement system.

Phagocytes assess self by making ICJs with underlying cells. This leads to a degree of electrical/metabolic synchronisation. The specificity of this ICJ connection is at least species wide and recognises <selfness> which may be shared with closely related species. The phagocyte uropod establishes ICJ connections with an underlying cell and then reaches out lamellipodial fingers to examine adjacent cells/organisms for

synchronisation. Capacitatively induced potential differences may trigger attacks. Other recognition strategies can also be used (eg, recognising surface markers which are invariably bacterial in origin).

(e) A "vascular" system evolves which is able to lock out most phagocytes till required and an inflammatory mechanism is established. The alternative complement cascade is now "humoralised" so that circulating C3 can mark clearly foreign organisms and make them more readily identifiable when they meet a phagocyte.

(f) The specificity and diversity of N-CAM ligand interaction is achieved by a process of alternative RNA splicing1. N-CAM like genes are now adapted to produce multiple different ligand specificities within a herd rather than within a ZDC. These are the ancestors of the Mhc class I genes and will act as cell surface "flags" to advertise a more personalised HS status. These new genes are joined by another duplicated and transposed gene to produce Class I like Mhc genes16. This gene encodes a pincer mechanism like the HSC70 heat shock proteins (these look after "sick" proteins)

These new identity ligands are recognised by a new cell (the ancestor of Tnk cells) which has evolved from phagocytes. This attacks membranes in general but observes a horror autotoxicus to any cell/organism that displays self specific ligands17. These Tnk like scavengers need a mechanism to produce and/or select self specific receptors unique to each ZDC. This must be done, after meiosis, over a number of mitotic generations - the "generation of specificity".

CELL TYPES AND MODES OF ACTION

Cell type	Receptors disabled	Receptors enabled	Normal state	Triggered state
Primitive scavenger (Tnk like precursor)	non pure self	pure self	aggressive	passive (horror autotox-icosis)
	GENERATOR OF SPECIFITY			
Tc cell	pure self	non pure self	passive	aggressive
	GENERATOR OF DIVERSITY			

To achieve this diversity in ligand recognition, a mechanism was required to produce many different receptors from which an appropriately specific receptor could be selected - "the generator of specificity". It is from this that the antibody genes have subsequently evolved. molecules that are involved in HS/OTHS discrimination or its modulation. These include the TAP genes, HSP70, TNF, complement components (C2, Bf and C4) and the 21-hydroxylases.

(h) Both the complexity and the repertoire of this mechanism for generating and selecting specific receptors is able to evolve gradually. The inversion of its function can lead to a mechanism able to recognise and attack non-pure self. Thus Tc like cells could evolve to recognise and, when appropriate, attack cells whose Class I ligands had been altered by the intended attachment of peptides to the pincer mechanism.

Class I mechanism: now, short, representative peptides from cellular debris picked up by phagocytes at inflammatory sites are processed. These for the attention of uncommitted T-cells. The "generator of diversity" is now enrolled into creating a system to memorise the inflammatory context in which these processed epitopes were first encountered. On

re-encountering the processed epitope, these T-cells are programmed to attract large numbers of phagocytes to the site and "angrify" them. This gives inflammation a memory. The "angrified" phagocytes still have to sort HS from OTHS but their threshold for regarding a cell as OTHS is lowered. Tc and TH1 cells are not involved in assessing <selfness>. They are primed by other cells, particularly phagocytes, to remember the inflammatory context in which their epitopes were presented to them when they became committed.

(k) Antibodies can now be launched as "icing on the cake". They help by opsonising organisms. The alternative complement cascade is now adapted to be triggered by C1,2,&4. C1,2,&4 evolve from the components which are triggered by N-CAM to spawn GJ plaques.

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HYPOTHESIS

Morphostasis is tissue homeostasis. Tissue form remains stable whilst cells are in intimate contact by intercellular junctions. This enables joined cells to establish various degrees of electrical and metabolic synchronisation and it promotes cooperation. Synchronisation is greatest when the cytoplasms are in direct continuity through gap junctions or synctial structures. The specificity of the molecular mechanisms that lead to cell adhesion, coupling and connective tissue scaffolding, in effect, give cells a <healthy self (HS)> identity. Similarly, the loss of <HS identity> is accompanied by dismantling of the connective tissue scaffold and cell undocking. Self cells monitor each others' identity. When a cell becomes sick it senses its own disorder and abandons <HS identity>. It can shut down the channels that join its cytoplasm with those of adjacent cells and then detach its membrane from them in a process called apoptosis. This leads to tidy, elected cell death. Adjacent cells and phagocytes ingest apoptotic cells before they burst. The processed peptides induce T-cell tolerance. Necrosis is an untidy form of cell death. Such dying cells burst and spill their contents, so releasing inflammatory cytokines. These processed peptides trigger aggressive anamnestic immune reponses which accelerate the identification and elimination of cells which carry markers previously encountered on cells that have died and provoked an inflammation. Once order is restored, adjacent healthy cells duplicate and replenish lost cells.

CAM	=	cell adhesion molecule
GJ	=	gap junction
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OTHS	=	other than healthy self
UHS	=	unhealthy self
ZDC	=	zygote derived colony

INTRODUCTION

In 1963 the Lancet published an hypothesis, "The role of lymphoid tissue in morphostasis"1. In this article Burwell made the comment that "immunology still awaits incorporating into the general pattern of biology" and suggested that immune function had an important role to play in morphostasis. Morphostasis is defined as the "steady state condition which maintains a particular (tissue) pattern". It seems to me that immunology is still perceived as a discrete and clearly demarcated system. In this article I hope

to show how morphostasis should be regarded as the origin and continuing drive of immune function and how it is the cornerstone of metazoan existence. I believe that this hypothesis is fully compatible with experimental fact.

The following points set the scene. A morphostatic system must interface with these biological systems:

1) Intracellular and molecular biology
2) Cell to cell communication and cooperation (gap junctions in particular)
3) Embryo - development from zygote to mature animal
   - evolution from simple metazoans to mammals
4) The general scheme of morphostasis including
   - the surveillance for sick cells
   - cell and animal senescence2
   - malignancy
   - the changing susceptibility to various diseases with aging
   - the renewal of sick cells and tissues
5) Basic pathological mechanisms
6) Immunity - innate
   - anamnestic
   - immune ontogeny
   - immune phylogeny (from simple metazoans to mammals)3
   - shed some light on plant defence4,5

Brevity demands a synoptic style so I shall not explore the rationale for proposing a new perspective. What follows is my perception of the process and its elements are not necessarily statements of accepted fact. The bibliography has been chosen to provide an investigative trail, with many of the articles providing further sources of reference.

THE ZYGOTE DERIVED COLONY (ZDC)

Every animal is a colony derived from a single cell, the zygote. No cell in the ZDC has functional capabilities that are not potentially present in the zygote's genes or cytoplasm. Each ZDC cell needs some way of preferring its own kind as neighbours and inhibiting the growth of foreign cells or organisms in its vicinity. This is helped by using selective CAMs which lead to the construction of ICJs, a scaffold of connective tissues and electrical/metabolic synchronisation6,7.

THE SOPHISTICATION OF SINGLE CELLS: THE SELF AWARE CELL

Each animal cell is a self assessing unit, capable of surveilling its own behaviour and function. It does this both internally and with respect to its neighbours. The cell has a variety of internal checkpoint controls. These are particularly well defined in the growth cycle. When an animal cell malfunctions, it senses the abnormality and notifies other cells that something has gone wrong (by various cytokines, alterations in cell surface markers and by breaking junctional communication). A sick cell can elect to sacrifice itself by apoptosis8,9,10: its calcium level rises, it rounds up and its GJs are closed before these and other ICJs are disassembled. Apoptotic cells are phagocytosed by adjacent cells or phagocytes before their membranes burst.

HEALTHY SELF (CELL) / OTHER THAN HEALTHY SELF (CELL) DISCRIMINATION

All metazoan animals are able to make this discrimination. What differs from organisms to organism is the sophistication with which it is embellished. It reaches a high level of sophistication in mammals. Every embellishment of the morphostatic system, including anamnestic immunity, requires an <UHS cell> to "advertise" its presence.

MORPHOSTASIS Tissue homeostasis can be maintained by:
(a) displaying "flags" on the membranes of HS cells which mark them as HS.
(b) recognising OTHS cells on the basis of absent HS markers (<HS identity>).
(c) attacking and removing OTHS cells (UHS and foreign cells/organisms).
(d) replacing lost UHS cells with fresh HS cells (resurgent morphogenesis).

IN SUMMARY

Identity - healthy ZDC cells display identity markers (these double up as "docking" molecules which lead to ICJs and a connective tissue scaffolding).

Self surveillance - cells are able to sense <UHS> status.

Altruism - cells are able to opt for apoptosis (suicide).

Neighbour surveillance - cells are able to sense a neighbour's appropriateness.

Sick cells - either declare their own presence or are recognised as such by their neighbours. These include damaged cells, dying cells, aging cells, genetically damaged cells, malignant cells, infected cells and other sick cells.

GAP JUNCTIONS

The cytoplasms of static cell populations are often joined through GJs11. These channels are shut down when a cell becomes sick12,13,14. A rise in intracellular calcium initiates GJ closure11. GJ channels are then disassembled during apoptosis.

The whole embryo is electrically connected through GJs and this establishes the boundaries of <self>15. Within this electrically continuous <self> there are sub-compartments in which member cells are joined by plaques of GJs which have higher permeability. They are surrounded by a layer of cells with GJs of lower permeability and these define the compartment borders. They correspond with developmental compartments. N-CAM promotes the construction of highly permeable GJ plaques16. Three possible explanations spring to mind: these plaques contain more GJs; the component GJs are bigger; construction is more efficient and there is a higher yield of good junctions.

I would like to propose that the consensus sequence motif of N-CAM, which resembles the Ig constant region, evolved in order to spawn multiple, highly permeable GJs much as the complement C1,C2,C4,C3 cascade spawns multiple well formed MACs around Ig constant regions. If so, the C7,8,&9 genes have either evolved from connexon genes or they have highjacked the mechanism which encourages the construction of highly permeable channels, inverting it into an attack mechanism. Note these points: (1) C9 inserts itself into membranes without C3-C8 amplification but this is inefficient; (2) leaky holes lead to a rise in intracellular calcium and so close GJ channels; (3) the connective tissue origin of C1q.

APOPTOSIS, NECROSIS and INFLAMMATION

Successful self surveillance leads to apoptosis and elective suicide. This mechanism deals with many, if not most, sick cells. It has failed when cells die by necrosis. Then, membranes rupture, their contents are spilled and inflammation is promoted. Inflammation provokes aggressive T-cell responses. When sick cells rupture, they release a characteristic set of cytokines, particularly eicosanoids. These are the messengers that notify adjacent somatic and inflammatory cells that something serious is amiss. In consequence, Tc cells induce apoptosis in cells which carry markers resembling cells that have previously died and provoked an inflammation. TH1 cells remember the inflammatory context in which they met their epitope. When they reencounter similar peptides they turn up the inflammatory "heat". They do not, themselves, kill: this is left to "angrified" phagocytes which become more particular about what they will accept as <HS identity>.

When peptide debris is processed after phagocytosing apoptotic cells, it promotes T-cell suppression. For example, when a cell dies following a virus infection its debris is processed by adjacent cells and phagocytes. If cell death occurs following successful internal surveillance (apoptosis), tolerance will be promoted to presented peptide debris and this will include viral peptide. When unsuccessful (eg, lytic or necrotic death), inflammation will promote T-cell aggression to presented peptides: and this will include self peptides. However, since apoptosis is such a common process, self peptides have previously promoted suppression and so shrunk any pools of self reactive precursor T-cells available to be recruited into aggression. Also, the threshold at which uncommitted T-cells are triggered into aggression falls as they age. This further focuses aggression onto strange epitopes.

<HS cells> in an inflammatory area are protected from self attack because they still demonstrate <HS identity>. I contend that this is the real horror autotoxicus. Phagocytes from closely related species share similar specificity. Most non-pathogenic organisms are easily identified as non-self. Unless complement is present, bacteria and viruses must rupture a cell and/or disrupt its ICJs to invoke an inflammatory reaction and trigger an anamnestic immune response. Some dedicated pathogens appear to have evolved mechanisms to heighten inflammation in order to create themselves the niche they need to survive (eg, TB).

Inflammatory cells need to be restrained from entering healthy tissues until things goes wrong since their intrusion disrupts tissue function. The endothelial cell linings of blood vessels tend to lock out phagocytes until they are invited in. This is done more rigorously in the central nervous system - the blood brain barrier. This is necessary as nervous function relies on the electrical (GJ) disconnection of neurons during their terminal differentiation and the resulting (functional) asynchronisation then makes them more susceptible to macrophage attack (note how traumatic paraplegia is ameliorated with steroids). This need for segregation is likely to be important in the origin of the vascular system and inflammatory regulation.

MORPHOSTATIC EVOLUTION

This is the way I suspect that the metazoan system evolved. Note that each new step is an embellishment of the former and all of them remain functional in mammal morphostasis.

(a) Elective cell suicide (apoptosis) is established as a means of protecting the colony (also seen in plants4).

(b) The interaction of CAMs, ICJs and the extracellular matrix gives cells a sense of "belonging". The consequent electrical/metabolic synchronisation, through ICJs, establishes <HS identity>. ICJs are the immediate consequence of cell surface ligand/ligand or ligand/receptor interactions and these molecules are Cell Adhesion Molecules, CAMs6,7. Once paired up, membrane holes in apposing cells form GJs (similar channels are important in plants4,5). IgSF CAMs (eg, N-CAM) develop later to act as a focus on which to build highly permeable GJ plaques. This "multiplier" mechanism will later be adapted to spatter bigger, leaky holes into cells or organisms which do not display features of self (the alternative complement cascade). A complement like cascade mechanism similar to the Bb/C3b et seq sequence evolves as the general agent which recognises cell membranes. In the presence of self markers it leads to GJs and in their absence, to attack.

(c) The progressive expansion of different somatic CAMs lead to subordinate, self within self identities and thus tissue specialisation. These define new developmental compartments where the borders are demarcated by a sheet of cells having GJs of low permeability. The cells within the compartment express IgSF CAMs and are joined by highly permeable GJ plaques. Note

that cell sorting is dependent on CAM expression, particularly cadherins6,7. Homoeotic gene expression has also been noted to change at these compartment boundaries17.

(d) Animal cells split into dedicated phagocytes and soma. The soma abandons most of its capacity for wandering and aggression. The scavengers abandon most of their capacity for extensive connective tissue scaffolding.

SOMA LIGAND(s) - for recognition by resident scaffolders.
PHAGOCYTE LIGAND(s) - for recognition by itinerant scavengers.

Dedicated phagocytes evolve. They refine both their cooperative ICJ communication with self cells and the attack system which inserts leaky holes into non-self cells: the latter will eventually lead to the complement system.

Phagocytes are derived from a cell lineage which lies outside the three main germ layers so they may, when they infiltrate somatic tissues, be demonstrating a property akin to the sorting tendency of disaggregated cells: they appear to be able to clamber over all other cell types and envelope them.

Phagocytes assess one aspect of self by making ICJs with underlying cells. This leads to a degree of electrical/metabolic synchronisation. The specificity of this ICJ connection is at least species wide and recognises <selfness> which may be shared with closely related species. First the phagocyte uropod establishes ICJ connections with an underlying cell and then it reaches out lamellipodial fingers to test the synchronisation of adjacent cells/organisms with the uropod attached cell. Capacitatively induced potential differences may be the trigger for an attack. The phagocyte uses other strategies like recognising apoptotic cells and, perhaps, surface markers which are invariably bacterial in origin. Note these points: (1) C9 has a thrombospondin motif which is used, in other circumstances, to recognise apoptotic cells; (2) basement membranes maintain physical barriers between tissues and help to minimise the area of cell membrane contact between different compartments.

(e) A "vascular" system evolves which is able to lock out most phagocytes till required and an inflammatory mechanism is established. The alternative complement cascade is now "humoralised" so that circulating C3 can mark clearly foreign organisms and make them more readily identifiable when they are met by a phagocyte.

(f) The specificity and diversity of N-CAM ligand interaction is achieved by a process of alternative RNA splicing6. N-CAM like genes are now adapted to produce multiple different ligand specificities within a herd rather than within a ZDC. These are the ancestors of the Mhc class I genes and will act as cell surface "flags" to advertise a more personalised HS status.

TABLE 1

Cell types and modes of action

Cell	Receptors	Receptors	Normal	Triggered

type	disabled	enabled	state	state
Primitive scavenger (Tnk like precursor)	non pure self GENERATOR OF SPECIFITY	pure self	aggressive	passive (horror autotox- icosis)
Tc cell	pure self GENERATOR OF DIVERSITY	non pure self	passive	aggressive

These new identity ligands are recognised by a new cell (the ancestor of Tnk cells) which has evolved from phagocytes. This attacks organism membranes in general (Nb that the complement Bb/C3b complex has the same function) but observes a horror autotoxicus to any cell/organism that displays self specific ligands19. These Tnk like scavengers need a mechanism to produce and/or select self specific receptors unique to each ZDC. This must be done, after meiosis, over a number of mitotic generations - the "generation of specificity".

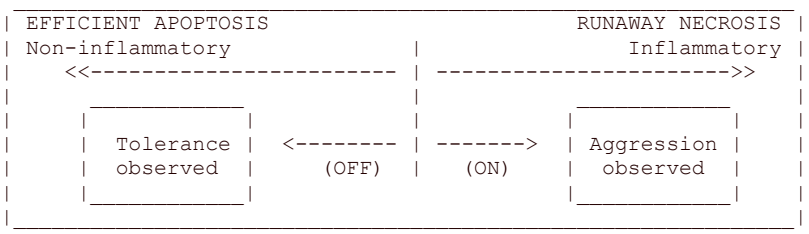
To achieve this diversity in ligand recognition, a mechanism was required to produce many different receptors from which an appropriately specific receptor could be selected - "the generator of specificity". It is from this that the antibody genes have subsequently evolved. Horror autotoxicosis needs redefinition: only <HS cells> are protected by it. Selection in Tnk cells may be by alternative RNA splicing.
molecules that are involved in HS/OTHS discrimination or its modulation. These include HSP70, TNF, complement components (C2, Bf and C4) and the 21-hydroxylases20 and the TAP genes are close by.

(h) Both the complexity and the repertoire of this mechanism for generating and selecting specific receptors is able to evolve gradually. The inversion of its function can lead to a mechanism able to recognise and attack non-pure self (Tc function). At some stage, perhaps with the advent of Tc cells, the identity genes are joined by another duplicated and transposed gene to produce Class I like Mhc genes18. This gene encodes a pincer mechanism like the HSC70 heat shock proteins (these look after "sick" proteins). Thus Ts and Tc like cells could evolve to recognise and, when appropriate, tolerate or attack cells whose Class I ligands had been altered by the intended attachment of peptides to the pincer mechanism.
mechanism evolves from the Class I mechanism: now, short, representative peptides from cellular debris processed by phagocytes after apoptosis or at inflammatory sites are processed. These are then externalised as a uncommitted T-cells. The "generator of diversity" is now enrolled into creating a system to memorise the inflammatory/non-inflammatory context in which these processed epitopes were first encountered. If it was inflammatory, on re-encountering the processed epitope, these T-cells are programmed to attract large numbers of phagocytes to the site and "angrify" them. This gives inflammation a memory. The "angrified" phagocytes still have to sort HS from OTHS but their threshold for regarding a cell as OTHS is lowered. Tc and TH1 cells are not, therefore, involved in assessing <selfness>. They are primed by other cells, particularly phagocytes, to remember the inflammatory/non-inflammatory context in which their epitopes were presented to them when they became committed (ie, lytic/apoptotic discrimination).

(k) The system of tolerance needs to evolve hand in hand with aggression. Even though apoptotic cells fragment, each particle retains an intact membrane and all are tidily phagocytosed by adjacent cells or phagocytes.

The peptides processed in consequence need and should not activate Tc or TH1 cells: rather, tolerance is desirable. However, cells which rupture and spill their contents have not been identified by the surveillance/apoptosis mechanism and pose a threat. They release eicosanoids and other cytokines which provoke inflammation and this then leads to the activation of Tc and TH1 cells.

TABLE 2
THE BINARY COMMITMENT OF INDIVIDUAL LYMPHOCYTES
depending on how the peptide is presented



So, uncommitted T-cells are sensitive to the inflammatory cytokines or non-inflammatory environment they sense when they meet their respective epitope. They become committed accordingly. Self antigens are copious and are regularly encountered in the course of efficient apoptosis. The majority of precursor T-cells with paratopes recognising processed apoptotic debris (the majority of which is self peptide) will be "mopped up" into a commitment to suppression (tolerance). These T-cells will either be decommissioned or primed to inhibit inflammation on epitope re-encounter. However, uncommitted T-cells with paratopes specific for self Ags continue to be released from the bone marrow and they may be primed rather than filtered in the thymus (where enhanced apoptosis removes many self reactive lymphocytes). At least a proportion of these may become committed to aggression if the inflammatory process is prolonged and foreign epitopes, which accelerate its resolution, are sparse. This system is probably enhanced by the simple expedient of allowing the threshold at which aggression can be triggered to fall as precuror T-cells age. This focuses aggression onto strange epitopes.

(l) The antibody system can now be launched as "icing on the cake". TH1 cells can be adapted to TH2 function and these in turn used to co-operate with B-cells. The B-cells evolve to secrete large quantities of circulating antibody. Antibodies help by opsonising organisms. The alternative complement cascade is now adapted to be triggered by C1,2,&4. These have evolved from the ancestral components which are used by N-CAM to spawn GJ plaques. The antibody system is optimised to work within the vascular system. It can interfere with any intended function of the Ag and tag it for enhanced phagocyte attention and attack. This system has proven to be invaluable as a pre-emptive defence. (I have presumed antibodies have developed late because it makes current sense. However, there may have been a function which encouraged the early or simultaneous emergence of B-cells to produce IgM like free antibodies.)

CLINICAL CONSEQUENCES

There is insufficient space here for a detailed elaboration so here is a whistle stop tour:

(1) ANERGY. This term has acquired several meanings but here I am referring to the loss of delayed type hypersensitivity responsiveness that occur in

diseases like TB and cancer. Because the T-helper system is capable of training its aggressive attention on self antigens when clearly strange antigen is sparse (eg, adjuvant arthritis), the immune system has to have a failsafe cut-out mechanism. This shuts off phagocyte aggression when the tissue destruction becomes too fierce. The effect is dominantly focal though there is a systemic spillover effect. It impairs focal surveillance by phagocytes.

(2) PATHOGENS. Non-pathogens are easily identified and eliminated except when there is focal impairment of surveillance (anergy). Pathogens need to devise means of breaching the morphostatic defence. They do so by mimicking, blocking and fooling identity mechanisms21. Tuberculosis, in particular, deliberately invokes intense inflammation, causing extensive auto-rejection. It then flourishes in a resulting focus of phagocyte impotence.

(3) AUTO-REJECTION. The result of all this is that any disease which evokes cell necrosis and an inflammatory response develops an element of T-cell augmented auto-rejection. It inevitably consists of a mixture which varies from an attack directed almost exclusively at the pathogen (usually leading to mild inflammation) to an attack directed almost entirely at self (often highly inflammatory): the latter occurs when organisms or cells provoke prolonged inflammation but do not provide or present clearly foreign looking (unusual) epitopes. Every disease that leads to cell damage will induce auto-rejection, even if this goes no further than apoptosis. Since heat shock proteins are responsible for chaperoning disrupted proteins through the cell, they are frequently presented as epitopes in UHS presentations.

Auto-rejection rumbles along at a low level all the time. When inflammation is prolonged and no clearly foreign epitopes are present to bring it to a conclusion, precursor T-cells specific for self Ags may be progressively recruited into aggressive action. These intensify local inflammation and so enhance tissue rejection. This appears to be what happens in adjuvant arthritis.

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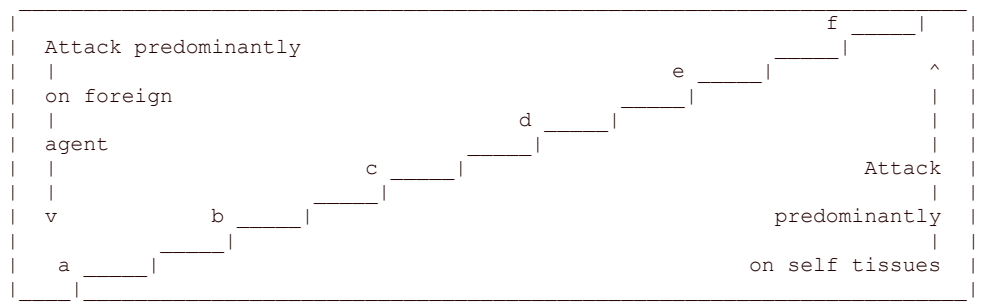
The stepped progression of attack on self



EXAMPLES

(a) Saprophyte

(b) Simple epithelial commensal

(c) Staphylococci and streptococci

(d) Tuberculosis and syphilis

(e)-(f) Multiple sclerosis and sero-negative arthritis

(4) CANCER. GJ communication between normal and cancerous cells is disrupted22. There are two broad groups. The first are cancer cells which only communicate with their own kind and make no communication with adjacent normal cells. These are relatively less aggressive and invade

locally rather than metastasize distantly. The other group contain cells which also cease to communicate with each other. They are immortal cell lines which have escaped from the usual Hayflick restriction of (about) 50 doublings. (Note that as cells age they become progressively poorer communicators through GJs2 and that they eventually elect to cease reproducing.) These cancers metastasize haematogenously to distant sites. Phorbol esters, which are cancer promoters, stabilise cells which would otherwise elect for apoptosis. The depression of focal surveillance that occurs in the wake of lymphocyte amplified auto-rejection is at least partially responsible for allowing malignant cells to escape detection and elimination. The final event that leads to immortalisation of the cancer cell line is probably the loss of the ability to effect apoptosis (through the p53 mechanism) when internal surveillance indicates it is appropriate.

CONCLUSION

The general principles of morphostasis are discussed. I have made a committed assumption that GJs are the most important ICJs in maintaining HS identity. Other ICJs may contribute a larger part than I have credited here. If well founded, the hypothesis should prove to be a useful framework for a more focused investigation of the biochemical processes of morphostasis.

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HYPOTHESIS

Morphostasis is tissue homeostasis. Tissue form remains stable whilst cells are in intimate contact by intercellular junctions. This enables joined cells to establish various degrees of electrical and metabolic synchronisation and it promotes cooperation. Synchronisation is greatest when the cytoplasms are in direct continuity through gap junctions or synctial structures. The specificity of the molecular mechanisms that lead to cell adhesion, coupling and connective tissue scaffolding, in effect, give cells a <healthy self (HS)> identity. Similarly, the loss of <HS identity> is accompanied by dismantling of the connective tissue scaffold and cell undocking. Self cells monitor each others' identity. When a cell becomes sick it senses its own disorder and abandons <HS identity>. It can shut down the channels that join its cytoplasm with those of adjacent cells and then detach its membrane from them in a process called apoptosis. This leads to tidy, elected cell death. Adjacent cells and phagocytes ingest apoptotic cells before they burst. The processed peptides induce T-cell tolerance. Necrosis is an untidy form of cell death. Such dying cells burst and spill their contents, so releasing inflammatory cytokines. These processed peptides trigger aggressive anamnestic immune reponses which accelerate the identification and elimination of cells which carry markers previously encountered on cells that have died and provoked an inflammation. Once order is restored, adjacent healthy cells duplicate and replenish lost cells.

CAM	=	cell adhesion molecule
GJ	=	gap junction
HS	=	healthy self
ICJ	=	intercellular junction
Ig	=	immunoglobulin
IgSF	=	Ig superfamily
N-CAM	=	Neural CAM
OTHS	=	other than healthy self
UHS	=	unhealthy self
ZDC	=	zygote derived colony

INTRODUCTION

In 1963 the Lancet published an hypothesis, "The role of lymphoid tissue in morphostasis"1. In this article Burwell made the comment that "immunology still awaits incorporating into the general pattern of biology" and suggested that immune function had an important role to play in morphostasis. Morphostasis is defined as the "steady state condition which maintains a particular (tissue) pattern". It seems to me that immunology is still perceived as a discrete and clearly demarcated system. In this article I hope to show how morphostasis should be regarded as the origin and continuing drive of immune function and how it is the cornerstone of metazoan existence. I believe that this hypothesis is fully compatible with experimental fact.

The following points set the scene. A morphostatic system must interface with these biological systems:

1) Intracellular and molecular biology
2) Cell to cell communication and cooperation (gap junctions in particular)
3) Embryo - development from zygote to mature animal
   - evolution from simple metazoans to mammals
4) The general scheme of morphostasis including
   - the surveillance for sick cells
   - cell and animal senescence2
   - malignancy
   - the changing susceptibility to various diseases with aging
   - the renewal of sick cells and tissues
5) Basic pathological mechanisms
6) Immunity - innate
   - anamnestic
   - immune ontogeny
   - immune phylogeny (from simple metazoans to mammals)3
   - shed some light on plant defence4,5

Brevity demands a synoptic style so I shall not explore the rationale for proposing a new perspective. What follows is my perception of the process and its elements are not necessarily statements of accepted fact. The bibliography has been chosen to provide an investigative trail, with many of the articles providing further sources of reference.

THE ZYGOTE DERIVED COLONY (ZDC)

Every animal is a colony derived from a single cell, the zygote. No cell in the ZDC has functional capabilities that are not potentially present in the zygote's genes or cytoplasm. Each ZDC cell needs some way of preferring its own kind as neighbours and inhibiting the growth of foreign cells or organisms in its vicinity. This is helped by using selective CAMs which lead to the construction of ICJs, a scaffold of connective tissues and electrical/metabolic synchronisation6,7.

THE SOPHISTICATION OF SINGLE CELLS: THE SELF AWARE CELL

Each animal cell is a self assessing unit, capable of surveilling its own behaviour and function. It does this both internally and with respect to its neighbours. The cell has a variety of internal checkpoint controls. These are particularly well defined in the growth cycle. When an animal cell malfunctions, it senses the abnormality and notifies other cells that something has gone wrong (by various cytokines, alterations in cell surface markers and by breaking junctional communication). A sick cell can elect to sacrifice itself by apoptosis8,9,10: its calcium level rises, it rounds up and its GJs

are closed before these and other ICJs are disassembled. Apoptotic cells are phagocytosed by adjacent cells or phagocytes before their membranes burst.

HEALTHY SELF (CELL) / OTHER THAN HEALTHY SELF (CELL) DISCRIMINATION

All metazoan animals are able to make this discrimination. What differs from organisms to organism is the sophistication with which it is embellished. It reaches a high level of sophistication in mammals. Every embellishment of the morphostatic system, including anamnestic immunity, requires an <UHS cell> to "advertise" its presence.

MORPHOSTASIS Tissue homeostasis can be maintained by:
(a) displaying "flags" on the membranes of HS cells which mark them as HS.
(b) recognising OTHS cells on the basis of absent HS markers (<HS identity>).
(c) attacking and removing OTHS cells (UHS and foreign cells/organisms).
(d) replacing lost UHS cells with fresh HS cells (resurgent morphogenesis).

IN SUMMARY

Identity - healthy ZDC cells display identity markers (these double up as "docking" molecules which lead to ICJs and a connective tissue scaffolding).
Self surveillance - cells are able to sense <UHS> status.
Altruism - cells are able to opt for apoptosis (suicide).
Neighbour surveillance - cells are able to sense a neighbour's appropriateness.
Sick cells - either declare their own presence or are recognised as such by their neighbours. These include damaged cells, dying cells, aging cells, genetically damaged cells, malignant cells, infected cells and other sick cells.

GAP JUNCTIONS

The cytoplasms of static cell populations are often joined through GJs11. These channels are shut down when a cell becomes sick12,13,14. A rise in intracellular calcium initiates GJ closure11. GJ channels are then disassembled during apoptosis.

The whole embryo is electrically connected through GJs and this establishes the boundaries of <self>15. Within this electrically continuous <self> there are sub-compartments in which member cells are joined by plaques of GJs which have higher permeability. They are surrounded by a layer of cells with GJs of lower permeability and these define the compartment borders. They correspond with developmental compartments. N-CAM promotes the construction of highly permeable GJ plaques16. Three possible explanations spring to mind: these plaques contain more GJs; the component GJs are bigger; construction is more efficient and there is a higher yield of good junctions.

I would like to propose that the consensus sequence motif of N-CAM, which resembles the Ig constant region, evolved in order to spawn multiple, highly permeable GJs much as the complement C1,C2,C4,C3 cascade spawns multiple well formed MACs around Ig constant regions. If so, the C7,8,&9 genes have either evolved from connexon genes or they have highjacked the mechanism which encourages the construction of highly permeable channels, inverting it into an attack mechanism. Note these points: (1) C9 inserts itself into membranes without C3-C8 amplification but this is inefficient; (2) leaky holes lead to a rise in intracellular calcium and so close GJ channels; (3) the connective tissue origin of C1q.

APOPTOSIS, NECROSIS and INFLAMMATION

Successful self surveillance leads to apoptosis and elective suicide. This mechanism deals with many, if not most, sick cells. It has failed when cells

die by necrosis. Then, membranes rupture, their contents are spilled and inflammation is promoted. Inflammation provokes aggressive T-cell responses. When sick cells rupture, they release a characteristic set of cytokines, particularly eicosanoids. These are the messengers that notify adjacent somatic and inflammatory cells that something serious is amiss. In consequence, Tc cells induce apoptosis in cells which carry markers resembling cells that have previously died and provoked an inflammation. TH1 cells remember the inflammatory context in which they met their epitope. When they reencounter similar peptides they turn up the inflammatory "heat". They do not, themselves, kill: this is left to "angrified" phagocytes which become more particular about what they will accept as <HS identity>.

When peptide debris is processed after phagocytosing apoptotic cells, it promotes T-cell suppression. For example, when a cell dies following a virus infection its debris is processed by adjacent cells and phagocytes. If cell death occurs following successful internal surveillance (apoptosis), tolerance will be promoted to presented peptide debris and this will include viral peptide. When unsuccessful (eg, lytic or necrotic death), inflammation will promote T-cell aggression to presented peptides: and this will include self peptides. However, since apoptosis is such a common process, self peptides have previously promoted suppression and so shrunk any pools of self reactive precursor T-cells available to be recruited into aggression. Also, the threshold at which uncommitted T-cells are triggered into aggression falls as they age. This further focuses aggression onto strange epitopes.

<HS cells> in an inflammatory area are protected from self attack because they still demonstrate <HS identity>. I contend that this is the real horror autotoxicus. Phagocytes from closely related species share similar specificity. Most non-pathogenic organisms are easily identified as non-self. Unless complement is present, bacteria and viruses must rupture a cell and/or disrupt its ICJs to invoke an inflammatory reaction and trigger an anamnestic immune response. Some dedicated pathogens appear to have evolved mechanisms to heighten inflammation in order to create themselves the niche they need to survive (eg, TB).

Inflammatory cells need to be restrained from entering healthy tissues until things goes wrong since their intrusion disrupts tissue function. The endothelial cell linings of blood vessels tend to lock out phagocytes until they are invited in. This is done more rigorously in the central nervous system - the blood brain barrier. This is necessary as nervous function relies on the electrical (GJ) disconnection of neurons during their terminal differentiation and the resulting (functional) asynchronisation then makes them more susceptible to macrophage attack (note how traumatic paraplegia is ameliorated with steroids). This need for segregation is likely to be important in the origin of the vascular system and inflammatory regulation.

MORPHOSTATIC EVOLUTION

This is the way I suspect that the metazoan system evolved. Note that each new step is an embellishment of the former and all of them remain functional in mammal morphostasis.

(a) Elective cell suicide (apoptosis) is established as a means of protecting the colony (also seen in plants4).

(b) The interaction of CAMs, ICJs and the extracellular matrix gives cells a sense of "belonging". The consequent electrical/metabolic synchronisation, through ICJs, establishes <HS identity>. ICJs are the immediate consequence of cell surface ligand/ligand or ligand/receptor interactions and these molecules are Cell Adhesion Molecules, CAMs6,7. Once paired up, membrane holes in apposing cells form GJs (similar channels are important in plants4,5). IgSF CAMs (eg, N-CAM) develop later to act as a focus on which to build highly permeable GJ plaques. This

"multiplier" mechanism will later be adapted to spatter bigger, leaky holes into cells or organisms which do not display features of self (the alternative complement cascade). A complement like cascade mechanism similar to the Bb/C3b et seq sequence evolves as the general agent which recognises cell membranes. In the presence of self markers it leads to GJs and in their absence, to attack.

(c) The progressive expansion of different somatic CAMs lead to subordinate, self within self identities and thus tissue specialisation. These define new developmental compartments where the borders are demarcated by a sheet of cells having GJs of low permeability. The cells within the compartment express IgSF CAMs and are joined by highly permeable GJ plaques. Note that cell sorting is dependent on CAM expression, particularly cadherins6,7. Homoeotic gene expression has also been noted to change at these compartment boundaries17.

(d) Animal cells split into dedicated phagocytes and soma. The soma abandons most of its capacity for wandering and aggression. The scavengers abandon most of their capacity for extensive connective tissue scaffolding.

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SOMA LIGAND(s)  -  for recognition by resident scaffolders.

PHAGOCYTE LIGAND(s)  -  for recognition by itinerant scavengers.
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Dedicated phagocytes evolve. They refine both their cooperative ICJ communication with self cells and the attack system which inserts leaky holes into non-self cells: the latter will eventually lead to the complement system.

Phagocytes are derived from a cell lineage which lies outside the three main germ layers so they may, when they infiltrate somatic tissues, be demonstrating a property akin to the sorting tendency of disaggregated cells: they appear to be able to clamber over all other cell types and envelope them.

Phagocytes assess one aspect of self by making ICJs with underlying cells. This leads to a degree of electrical/metabolic synchronisation. The specificity of this ICJ connection is at least species wide and recognises <selfness> which may be shared with closely related species. First the phagocyte uropod establishes ICJ connections with an underlying cell and then it reaches out lamellipodial fingers to test the synchronisation of adjacent cells/organisms with the uropod attached cell. Capacitatively induced potential differences may be the trigger for an attack. The phagocyte uses other strategies like recognising apoptotic cells and, perhaps, surface markers which are invariably bacterial in origin. Note these points: (1) C9 has a thrombospondin motif which is used, in other circumstances, to recognise apoptotic cells; (2) basement membranes maintain physical barriers between tissues and help to minimise the area of cell membrane contact between different compartments.

(e) A "vascular" system evolves which is able to lock out most phagocytes till required and an inflammatory mechanism is established. The alternative complement cascade is now "humoralised" so that circulating C3 can mark clearly foreign organisms and make them more readily identifiable when they are met by a phagocyte.

(f) The specificity and diversity of N-CAM ligand interaction is achieved by a process of alternative RNA splicing6. N-CAM like genes are now adapted to produce multiple different ligand specificities within a herd rather than within a ZDC. These are the ancestors of the Mhc class I genes and will act as cell surface "flags" to advertise a more personalised HS status.

TABLE 1

Cell types and modes of action

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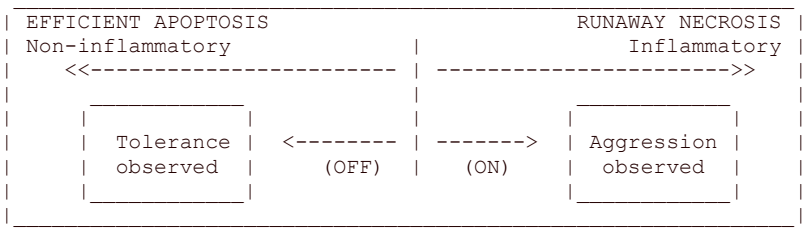

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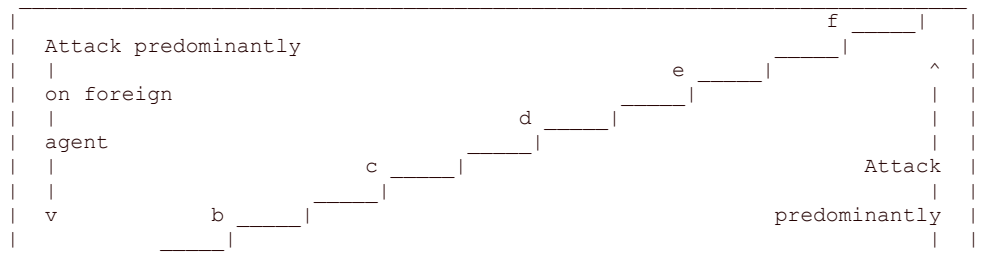

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(4) CANCER. GJ communication between normal and cancerous cells is disrupted22. There are two broad groups. The first are cancer cells which only communicate with their own kind and make no communication with adjacent normal cells. These are relatively less aggressive and invade locally rather than metastasize distantly. The other group contain cells which also cease to communicate with each other. They are immortal cell lines which have escaped from the usual Hayflick restriction of (about) 50 doublings. (Note that as cells age they become progressively poorer communicators through GJs2 and that they eventually elect to cease reproducing.) These cancers metastasize haematogenously to distant sites. Phorbol esters, which are cancer promoters, stabilise cells which would otherwise elect for apoptosis. The depression of focal surveillance that occurs in the wake of lymphocyte amplified auto-rejection is at least partially responsible for allowing malignant cells to escape detection and elimination. The final event that leads to immortalisation of the cancer cell line is probably the loss of the ability to effect apoptosis (through the p53 mechanism) when internal surveillance indicates it is appropriate.

CONCLUSION

The general principles of morphostasis are discussed. I have made a committed assumption that GJs are the most important ICJs in maintaining HS identity. Other ICJs may contribute a larger part than I have credited here. If well founded, the hypothesis should prove to be a useful framework for a more focused investigation of the biochemical processes of morphostasis.

19931230_cl_grab

ANERGY

The fates of individual cells that make up an animal are only important in that neither their death nor their survival should endanger gene propagation, particularly in the herd. (Across the aeons of evolutionary history, those species which fail to maintain a critical "herd mass" founder: the gene pool is all important). So (auto-)rejection of suspect cells is a logical method of housekeeping: cell deficits are, self evidently, renewable by tissue regeneration (a resurgence of morphogenesis). However, if an inflammatory process is particularly strong and there is little if any clearly foreign antigen, lymphocytes are not prevented from mounting an aggressive response to Ags typical of the local tissues (e.g., in burns [21] and adjuvant arthritis [22,23]). The resulting acceleration of tissue turnover could easily get out of hand and lead to extreme tissue destruction (auto-rejection - see below). Auto-antibodies and auto-TH1 reactivity may even be useful in focusing phagocyte attention to specific tissues until a more focused response to foreign Ag has matured (e.g., say, pharyngeal antigen in a viral pharyngitis).

This mechanism for concentrating phagocyte attention risks a positive feedback and, without constraint, it would lead to catastrophic auto-rejection. Failsafe mechanisms must exist which can be brought into play if tissue destruction becomes excessive. This could be controlled at any or all of the following points:-

(a) inhibition of phagocyte ingression (chemotaxis),
(b) inhibition of phagocyte aggression,
(c) inhibition of further aggressive lymphocyte activation,
(d) a tightening of endothelial cell junctions.
(e) encapsulation in a fibrin sheath (fibrocytes later)
(f) promotion of lymphocytic tolerance to typical Ag
(g) production of auto-antibodies to the newly cloned, locally reactive lymphocytes (lymphocytotoxic Abs)

This failsafe is most necessary within and around the affected tissue so we should expect to see it strongly localised. However, a spillover effect may be anticipated, with a systemic depression of delayed type hypersensitivity (the immune mechanism largely responsible for tissue rejection). This may explain, at least in part, why anergy occurs in diseases such as TB and sarcoidosis. There is evidence that anergy is expressed more intensely at a local rather than a systemic level (footnote 3). General references:- [24,25,26,27,28,29].

GROWTH AND REGENERATION

The rate at which generation (growth) and regeneration (mostly repair) can proceed is limited. Since these are essentially similar morphogenetic processes, auto-rejection will result in the temporary suspension of growth. Auto-rejection cannot be allowed to reach the level of intensity in a growing animal that can be permitted in a mature animal. If it does growth will be stunted. That is:-

Generation + Regeneration = a set maximum

Therefore:-

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| generation high ------> regeneration relatively restricted |
| generation low  ------> regeneration relatively unimpaired |
|_____________________________________________________________|
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Put another way, the luxury of extensive auto-rejection, as part of a morphostatic technique, can only be fully afforded in adult animals. Thus, in order to avoid stunting of growth, those mechanisms which initiate and accelerate rejection (of all kinds) need to be less fierce in growing animals than they are in adults: lymphocytes must behave less aggressively and this is probably brought about by moderating the intensity with which APCs stimulate aggressive lymphocytes (APCs = antigen presenting cells) [30,31,31a]. Both CMI (cell mediated immunity) and IgG activity must be dampened (at least, for those IgGs capable of reaching the extracellular spaces even when there is no inflammation). The result of all this is to promote a relative immunological tolerance in very young animals. This impaired capacity to reject (and consequently autoreject) is apparent in the neonate in which the tolerance of grafts is much enhanced: the neonate can also tolerate a level of cerebral ischaemia which, in adults, would cause extensive tissue death (in large part an auto-rejective event). This relative incapacity to auto-reject is also a protection against the dangerous sequelae that follow virus infections (they may even have been a significant driving force to require it). These tend to produce their most severe effects when they first strike in adult life, eg, infectious mononucleosis [32], infectious hepatitis (both often mere URTIs in young children), mumps, chicken pox and measles; and an example from the mouse, lymphochoriomeningitis [33]. The sequelae, arthritis, jaundice, meningitis, orchitis & etc, can be prevented or at least ameliorated by immunosuppressives or steroids. From this point of view, "immunological immaturity" is a misleading term because the infant's immune system is likely to be perfectly adapted for an optimal compromise [newref].

There are certain tissues where extensive auto-destruction could prove disastrous: such an event might seriously impair the ZDC's function and survival. These include the eye and the nervous system. These sites enjoy a so called "immunological privilege". This privilege seems to be achieved, at least in part, by locking out inflammatory cells behind tight endothelial cell junctions: the sparse population of local APCs is probably a direct consequence of this.

AUTO-REJECTIVE DISORDERS

Tissue rejection is largely accomplished by cells and cell mediated mechanisms. Whilst antibodies can affect the course of organ rejection, they cannot, on their own, precipitate it. In contrast, rejection can be provoked with injections of appropriately activated lymphocytes. Once it is apparent that disordered self cells are actively rejected, we are in a position to state the following:

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 ___________________________________________________
| Every disease which leads to an inflammatory reponse will |
| have an auto-rejective element even if this is limited to |
| a mildly increased tissue turnover.                       |
|___________________________________________________________|
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So, there ought to be a group of disorders which are largely auto-rejective and who's pathogenesis is little, if at all, affected by humoral auto-immunity. Since immune function changes through life, the intensity of auto-rejection is likely to be dependent upon age. It will be at its climax in the healthy young adult. The initiation of auto-rejection is suppressed in the very young [30,31,new] and its execution becomes progressively impaired in the elderly

[40]. Thus, a disease which is caused by extensive auto-rejection will be most likely to occur and also to be at its most severe in this central age range (figure 2). One likely cause of such disease is deliberate interference with and mimicry of aspects of the host's identity machinery. Micro-organisms, with their capacity for rapid genetic adaptation, are the most likely offenders. Where micro-organisms develop antigenic determinants close to some element of the host's identity machinery they will appear less foreign and gain easier access to the host's tissues and cytoplasm. Cells which are damaged in consequence of this should still signal malfunction (shout "foul"). However, because there may be a relative scarcity of clearly foreign antigen, the resultant inflammatory reaction will concentrate its enhanced attention on self Ags. Whenever these self Ags are reencountered, an amplified inflammation will ensue and the consequent auto-rejective attack will not necessarily remain confined to the initiating site.

Adjuvant arthritis is of interest because it produces a constellation of disease who's features are similar to those seen in the sero-negative arthritides and sarcoidosis. This experimental disease may be caused because clearly foreign antigen is sparse and the immune response is consequently concentrated upon local tissue antigens (eg, heat shock proteins or other mycobacterial antigens which cross react with the host) (table x). Whipple's disease may be an extreme example of this sort of disease (note the idiosyncratic infection [41,42] and familial aggregation of cases [42,43]).

The bacteria which colonise epithelial surfaces present a special hazard to the colony. It is well recognised that they have the ability to bind selectively to cells at particular epithelial sites [10]. Since they have evolved this specificity it is also highly likely that they have evolved some mimicry of and interference with the host's identity machinery (especially tissue/site be definable from basic principle: compatibility of organ transplants ranges from a common slight compatibility to a rare complete compatibility [13]. When this observation is extrapolated to microbial mimicry, one would expect to find minor mimicry often and extreme mimicry rarely. The seronegative arthritides and their component complications show just this sort of structuring (table 1). Their clinical pattern can be summed up by an axiom:-

The severity of any single patient's disease(*) is inversely proportional to its incidence in the population and directly proportional to the number of components found in association with one another.
(*) - Whether it is an isolated component or a syndrome complex of more than one component.

For example, recurrent aphthous ulceration (RAU) occurs in about 5% of the population, oro-genital ulceration in about 0.5% or less and Behcet's syndrome (BS) in about 0.0001% (in Britain). As the apparent disease in any particular patient is observed to be more severe, so we notice an expanding clinical overlap: more individual components coincide in one patient (table x). The pathogenesis of these disorders should be dominated by cell mediated immune aggression just as it is in non-acute graft rejection [44]: any contribution from circulating antibodies should simply be a bystander phenomenon. The pathological tempo of the individual components is often seen to increase with the severity of the syndrome disorder. Thus, in psoriasis, the prevalence of arthritis and iritis increases greatly in patients who have the exfoliative and the pustular forms of the disease [45]. On the basis of a personal study (in which the prime objective was to review the world literature on neurological

Behcet's syndrome - unpublished) I believe that the meningo-encephalitis of multiple sclerosis should be regarded as the respective isolated component which becomes more severely expressed in the meningo-encephalitis that is encountered in BS (nb., MS is a meningo-encephalitis [46]).

The age incidences of all these disorders are typical [47]. The population incidences of the commoner conditions begin and peak earlier than in the rarer disorders. In the majority of components it is clear that they are constantly modulated by certain events: menstrual exacerbation, second and third trimester quiescence, puerperal exacerbation, stress precipitation and, finally, amelioration of symptoms with steroid and immunosuppressive therapy. (This pattern matches Tnk cell activity and numbers.)

At least two further disorders have features to suggest that they might legitimately be included amongst the (predominantly) auto-rejective disorders. These are sarcoidosis and systemic lupus erythematosis. Both of these demonstrate some clinical overlap with the sero-negative arthritides: and SLE has a similar component structuring. (Nb., high turnover granulomas are a recognised consequence of many cell mediated immune reactions [48]).

CANCER

Broadly speaking it can be surmised that cancer follows:-

(a) a triggering event (induction)
(b) a change in cell behaviour (promotion).
(c) a breakdown in surveillance (progression).

The event which finally trips an affected cell into loss of growth control need not concern us in this article other than to point out that it usually arises in a single cell from which the tumour then develops. A unifying feature is that a normal growth control gene starts being transcribed inappropriately (induction). But let's leave this to one side. I will, instead, focus attention on the reasons for the body's failure to identify the miscreant cell and its progeny (promotion/progression). Before proceeding, note how stark the contrast is between the Hayflick limit of about 50 doublings (in cultures of healthy cells) (footnote 4) and the apparent immortalisation of cell lines derived from many cancers.

GJ communication is clearly important in the evolution of a cancer. Two sorts of cancer are discernable:

(1) The first where inappropriate local CAMs are utilised to make junctional communication and the adjacent, normal cells cannot make satisfactory connection. In this situation, the malignant cells make good communication with each other but not with normal adjacent tissue.

(2) The second sort is where the cell becomes "immortalised". This process is dependant upon cell growth becoming independant of GJ communication (by mutation). Normally, GJ communication becomes progressively inhibited as the number of cell doublings approaches 50 and eventually cell duplication is abolished. Immortalisation frees cells from this constraint but they now operate as independant rather than colony cells. Malignancies which form distant haematogenous metastases are almost invariably of this sort.

The morphostatic surveillance fails when local conditions inhibit its efficiency. The main reason for this is the focal depression of phagocyte activity that seems to be necessary to limit the intense tissue destruction that the lymphocytic system would otherwise be capable of unleashing. Malignant cells which communicate with each other will not be seen as UHS by phagocytes which invade the substance of the tumour. Only at the interface of normal/malignant tissue will they discriminate and then it will be against normal cells if the uropod attaches to a malignant cell or vice versa if the uropod attaches to a healthy cell.

Surveillance in immortalised malignancies is probably suppressed by chemotactic inhibitors which have been induced, originally, during focal auto-rejection but become self perpetuating as attempted rejection of the tumour cells takes over.

Phorbol esters stabilise cell communication and inhibit apoptosis by preventing a rise in intracellular calcium. In so doing, they probably allow an otherwise correctly identified miscreant cell to survive when it should have been eliminated.

Opportunistic infections and cancer should, presumably, be most prevalent when morphostatic surveillance is least effective. The cells making up an animal (there are around 10 to the power 13 of them in man!) are highly regimented and, presumably, intense cell co-operation has to be exercised to maintain such order within the ZDC's tissues. This implies that, by and large, disruptive cells (dead, damaged, dying, mutated and those with disordered growth control) are largely rejected. And, indeed, it has long been clear that phagocytes do recognise these cells and remove them. Our main attention here should be directed solely at those events which lead to the impairment and subsequent failure of surveillance. Focal anergy is likely to be one of these events and may well be the major contributor to the escape of malignant cells from surveillance.

In mammals, this impairment of surveillance should (generally) be at the extremes of life or following prolonged focal auto-rejection and its consequent anergy. In the elderly, the increasing impairment of immunity coupled with the heightened susceptibility of epithelium to various noxiae (and thus auto-rejection) will predispose to a high incidence of carcinomas. Focal anergy on its own (consequent upon intense auto-rejection) may be a major cause of the predilection for certain cancers to strike young adult to middle aged patients (e.g., lymphomas and focal cancers like colonic cancer in ulcerative colitis or testicular tumours following mumps). In the very young there is a relative incapacity to reject tissues. It is worth noting, then, that the predisposition for epithelial cancers found in the elderly is not present in the young. Cancers are relatively common in the very young and there is evidence to suggest that many regress before they reach clinical significance [49]. (Note that, in general, carcinoma-in-situ is far commoner than overt cancer: the abnormal cells tend either to be kept in check or eliminated by lympho-monocytic cells.)

Cancer is characterised by a failure of growth control and the cells affected revert to a form of behaviour more typical of embryonic cells (retrodifferentiation [50]). Using a "reductio ab adsurdam" argument these changes are much more likely to happen when regeneration and/or proliferation are exuberant (eg, T-cells in lymphomas) rather than relatively quiescent (eg, cartiledge, neurones, macrophages). Note that lymphomas are relatively common in the years in which auto-rejection is most intense (16-45yrs) and also note

that, in granulomatous disorders, lymphomas predominate over other cancers perhaps because local tissue regeneration is impaired [51,52].

The rate at which malfunctional cells arise (for any reason) probably increases with age. The net effect of this will be to cause a diffuse increase in the multiple foci of auto-rejection and, consequently, a gradual summation of focal anergy. This will eventually lead to a systemic spillover of this focal effect, a saturation effect. Epithelium is the tissue most exposed to infection, noxiae, regeneration and, in consequence, an increased probability of genetic divergence. Foci of anergy will be very frequent in this tissue form and carcinomas should consequently be more prevalent than sarcomas. Once initiated, cancer will itself lead to auto-rejection and, in turn, increased focal anergy. Thus, it is likely that there exists a critical mass and growth rate above which surveillance is irreparably blocked and the cancerous process becomes self perpetuating [53]. (Macrophages observed in vitro are clearly able to recognise malignant cells as abnormal [54,55].)

Now it is instructive to compare the age incidence profiles of various cancers with those of the auto-rejective disorders. However, before doing so it is important to establish which cancers are likely to flourish in the wake of intense auto-rejection (probable examples are lymphomas and testicular tumours [56,57,58]). These must be recognised as distinct from the commonest form of cancer (carcinoma) which seems to occur most frequently in the wake of age related impairment in immune surveillance. In general, these have a gradually rising incidence with age. Some cancers, particularly mesodermal malignancies, follow an incidence pattern showing a nadir in the middle years. It is interesting to note that the age incidence pattern of acute leukaemia is a complete inversion of the age incidence pattern of the auto-rejective disorders (figure 2). (See [59]).

It should now be clear that the lymphocytic system can have a dichotomous effect on cancer surveillance. It may enhance the focal accumulation of phagocytic cells and thus aid the (auto-)rejection of aberrant cells. However, the more aggressively it does this, the more likely it is to precipitate a suppression of focal rejection in order to avert piecemeal self destruction. Indeed, in those animals that have evolved them, the possession of lymphocytes may have incurred an increased risk of cancer: cancer is relatively uncommon in primitive animals [60,61] and is relatively scarce in congenitally athymic mice [62,63] which have abundant aggressive phagocytes [64] and natural killer cells [65]. It is interesting to note that in the animal kingdom there is an inverse relationship between the capacity to extensively regenerate body form and the prevalence of cancer [66,67]: and that carcinogens may induce supernumerary structures in lower phylae (eg, limbs) [68,69].

Napolitano et al [70] report that tumour cells generally display less class I Mhc Ag at their surface. They draw attention to the fact that the more malignant the tumour is the less class I Ag it expresses. They interpret this as a cause of the malignant behaviour. However, I would interpret this as a cell adjustment going, pari passu, with the loss of HS identity. Macrophages in vitro have little trouble in identifying malignant cells [55]. So, it seems that some quirk is allowing the lymphocytic amplification system to become preoccupied with an inappropriately strong response to the "wrong" tissue Ags: this, in turn, has led to focal auto-aggression and focal anergy. The phagocytes' capacity to eliminate UHS (tumour) cells is thus impaired, permitting a (so far) dormant carcinoma-in-situ to grow to a critical mass where focal anergy will never subside: at this point, the focal impairment of phagocyte activity becomes irreversible and uncontrolled growth of the tumour

proceeds unabated. This is consistent with the finding that tumour cells towards the centre of the tumour have a lower expression of class I Ags than tumour cells towards the outside. At the edges of the tumour, macrophage activity is likely to be much more active and successful in eliminating abnormal cells [55].

INFECTION

Infection can be defined as the survival and proliferation of an organism, not descended from the originating zygote, within the tissues of the ZDC. The colony need only remove these cells if they interfere with its structure or function (though the generality of the "dog eat dog" principle suggests that those that don't interfere will be highly specialised commensals or symbionts). Below I suggest four discrete ways in which surveillance can be overcome:-

(a) The first form of infection occurs when an organism acquires the ability to interfere, agonistically or antagonistically, with the host's machinery for establishing cell identity. Strategies based on species and tissue site identity can be cultured throughout the whole mass (surface mostly!) of a species and over its entire duration as a discrete species. The way in which foetal cells reaggregate into tissues rather than species [8,9] and the success, in nude mice, of skin transplants from distant species [71] suggests that tissue site identities may be broadly similar across widely separated species. A variety of infectious organisms could be interfering with this tissue site identity (eg, streptococci [72] and staphylococci). Others also show a clear species specificity (e.g., mycobacterium TB, bovine TB, avian TB etc, and various plant infections [73]). Interference with individual (Mhc) identities can only be evolved in a short timespan (about 60-70yrs in man) and in a small mass (about 60-70kg of which only a small proportion is actually epithelium). Should close mimicry of personal identity develop, this will facilitate that organism's access to the ZDC's tissues and, once there, there would be a relative lack of clearly foreign antigen to "attack". The resulting inflammatory response will tend to concentrate attention on tissue antigens common to both the organism and the host or just to the host. These self Ags will be selected as anchors for the subsequent lymphocyte accentuated inflammation, so leading to an accelerated rejection of self tissues. This kind of destructive attention to self is probably occurring in adjuvant arthritis [22,23]. This disorder has clinical features closely reminiscent of the sero-negative arthritides and sarcoidosis (table 2). It is likely, therefore, that a highly idiosyncratic form of infection is a factor in the pathophysiology of the "auto-rejective disorders". Such disease could be precipitated by interference with the host's Mhc machinery by the microbe and this will probably have evolved in the lifetime of the animal. In biological systems, things are rarely black or white so the relative blend of the common/consensus and the idiosyncratic/individual response to infection will probably vary in a spectral manner (diag $). (Note that bacteria that manage to invade and survive within the cytoplasm could well pose a greater threat for this form of auto-rejective disease).

[Rejection will always be aimed at whatever is most apparently OTHS. The amount of auto-rejection will increase with the angrification of phagocytes, especially when clearly foreign OTHS is sparse. With the angrification of phagocytes, the threshold of HS expression required to avoid attack will be higher. In consequence, fewer self cells will continue to qualify as immune from self attack.]

(b) A second group of organisms manage to foil surveillance by virtue of their small size and obligate intracellular existence. The organisms of this group

are the viruses. As soon as an infected cell is sufficiently compromised it should signal a malfunction so triggering inflammation and attracting phagocyte attention. This will lead to the activation of appropriate precursor lymphocyte clones. After an interval of 10-14 days a strong amnestic response to various viral*peptide+Mhc antigens will have developed. In the meantime, selected self Ags may be used to anchor an immune accelerated phagocyte accumulation at the affected site whilst waiting for the emergence of a more specific anti-viral activity. (In general, these are "hit and run" infections: they are soon suppressed or cleared from the system and those that persist do so by remaining dormant within cells.)

(c) The third group are the opportunistic infections. Whilst these may interfere with tissue and species identity mechanisms [74] their success is dependent on the depressions of focal surveillance which follow virus infections, burns, surgical incisions and trauma (etc.). Each of these noxiae lead to the auto-rejection of damaged and malfunctioning tissue with subsequent focal anergy [27]. Probable examples of such opportunistic infections include bacterial tonsillitis, otitis, sinusitis, bronchitis and various wound infections.

(d) The last group are organisms which set out to subvert the immune response by deliberately creating a field of intense focal anergy. They do so by maximally stimulating focal inflammation with the object of inducing intense focal auto-rejection. Mycobacterium TB is the example which will be considered here though syphilis is probably another. The properties of such an organism should include:

(1)	poor initial foreign antigenicity
(2)	a strong attraction for macrophages (adjuvant attraction)
(3)	a good resistance to initial destruction as evidenced by prolonged survival within macrophages

The result of these 3 properties is that intense focal inflammation and then auto-rejection is induced. In consequence, there is intense focal anergy and this leads to a field of surveillance impairment in which the bacterium flourishes, feeding upon the cell debris which is left in the wake of this auto-destruction [75,76]. Clinical mimicry of the auto-rejective disorders should be discernible: this, in fact, can be seen and is most noticeable in the middle years, an observation which is in keeping with the auto-rejective disorders (table 3).

When tuberculosis occurs outside these middle years it is, accordingly, different in its clinical expression. The lesions now tend to be miliary and disseminated and occur without the same intense tissue destruction. Instead, the pattern now resembles miliary cancer. At the extremes of life TB appears to be acting more like an opportunistic infection. The overall age incidence of TB can, therefore, be regarded as a combination of the auto-rejective and the cancer type age incidence (figure 2).

AUTO-IMMUNE DISORDERS

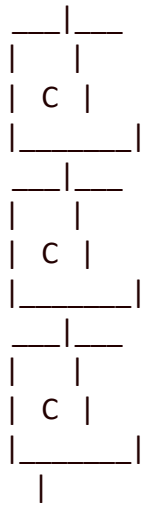
In several previous articles where immune surveillance has been discussed it has been suggested that cancer and auto-immunity might be expected to represent opposite poles of surveillance efficiency. However, the auto-immune title does not automatically imply auto-rejectiion. Rather than being dominantly auto-rejective, these disorders tend to result in one of two disturbances. The first is an interference with functional membrane molecules by the attachment

to them of auto-antibodies (e.g., Graves disease, myaesthenia gravis). The second is a tissue destruction which is centred predominantly around (non-cellular) connective tissues (the "collagenoses") and is apparently exacerbated, if not caused, by excessive auto-antibody production and the widespread deposition of Ab/Ag immune complexes. Here, cell destruction is possibly secondary to the activation of macrophages in the locality of this connective tissue. Towards the end of life auto-immune disorders are relatively more common than the sero-negative arthritides. Their prevalence at these older ages may possibly be exacerbated by a decline in the efficiency with which phagocytes clear tissue debris: this, in turn, could lead to enhanced auto-antibody (immunoglobulin) production (the latter certainly appears to be a feature of many diseases causing widespread anergy, eg sarcoidosis [77]).

19941029_morph_conjec

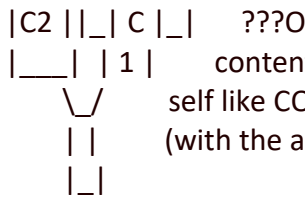
THOUGHTS ON THE EVOLUTION OF HOLE CONSTRUCTION - shamefully conjectural!

CADHERIN CAMs These have no homology with the IgSF family. They perhaps lead to low density GJ formation without extensive plates. (Not having the capacity to "breed" many hole construction centres from one CAM.)

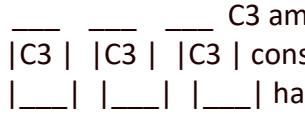


IgSF CAMs - especially N-CAM - may be adapted to creating dense plates of GJs by a cascading mechanism analogous to that seen with Complement C3. These IgSFs are made up of multiple CONSTANT region domains. (C = constant region).

Non-self identification - self protected by C3 inhibitor.

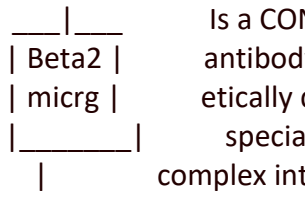


???Originally a self identifier? - Nb the connective tissue content of C1. Definitely adapted for interaction with a self like CONSTANT domain. If so, the conversion to attack (with the advent of antibodies) is a late event.

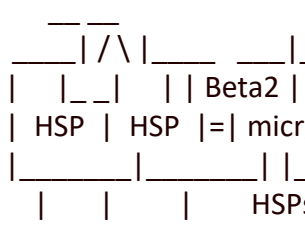


C3 amplification cascade. This lays down a carpet of hole construction centres - this must be analogous to what happens when GJs are laid down.

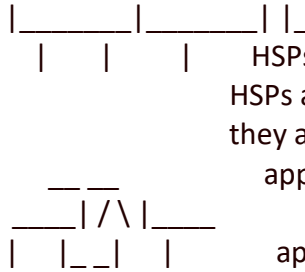
C4 is a specialised C3 like molecule used to link the C1/2 sequence into the conventional membrane attack sequence.



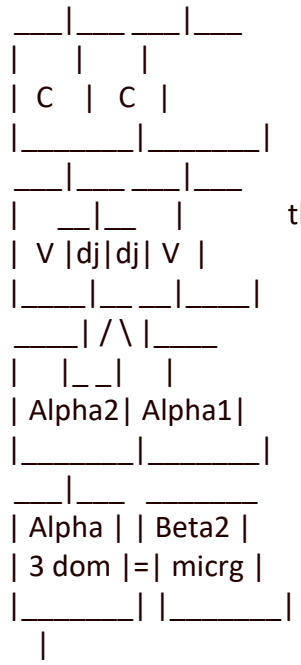
Is a CONSTANT region domain and it can trigger C1 just like antibody constant region domains. It is found in phylogenetically diverse species (eg, earthworm). Perhaps this was specialised to interact with Heat Shock Proteins in a complex intended for recognition.



Perhaps beta-2-microglobulin was adapted to shadow HSPs and form a recognisable complex for phagocytes to recognise. Some specificity of the interaction may have excluded foreign HSPs from being interpreted as self. All because HSPs are involved in damage limitation - where they are, so is danger. The HSP peptide clasp appears to be associated with its function as a protein (re)naturer. At times of stress HSPs appear in profusion. The next step is to hoist the HSP gene onto a CONSTANT region gene so that a Class I like ligand appeared. The V region genes were evolved from CONSTANT region domains to recognise the new HSP like molecule. Initially, when Tnk like cells appeared, they were only looking at the none clasp part of the molecule. With the advent of the Tc cells, the incorporation of the pincer mechanism into the



recognition process was inevitable.



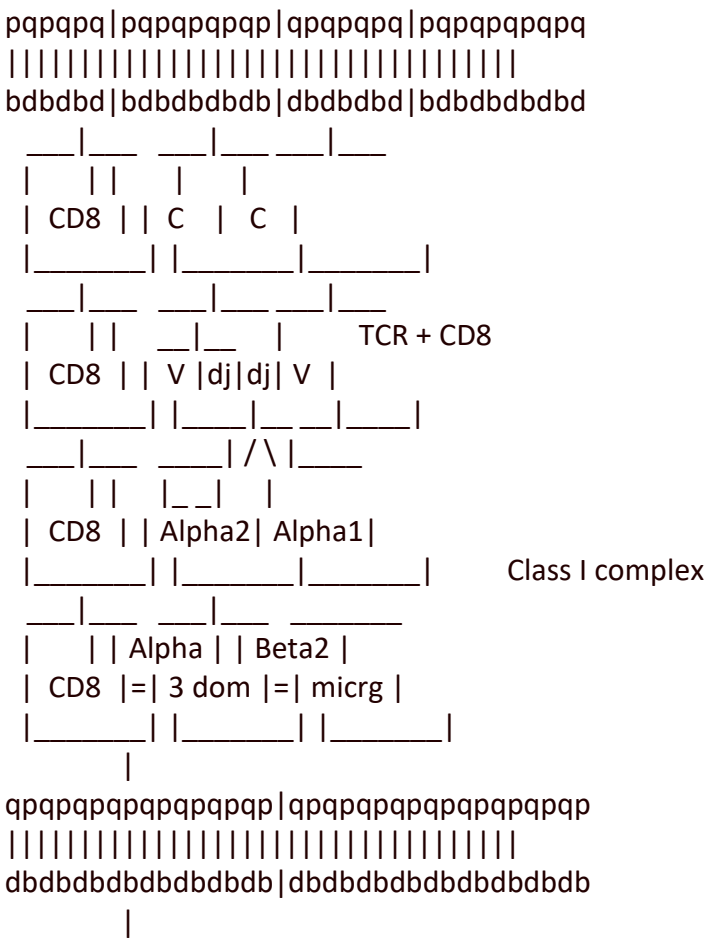
Eventual result of the TcR and Class I Ag interaction. The DJ region evolves to create the extra diversity to recognise the peptide-clasp section of the molecule.

Tnk EVOLUTION - Perhaps Tnk not interested in anything other than the non-clasp region of the HSP molecule (just beta-2-microg/HSP combination to start and Class I to finish). Tnk seems to be most interested in cells expressing HSPs.

Tc INVERSION - ?? looking (in thymus) for cells with binding but not triggering of TCR (T-cell receptor). Non-binding not cultivated and triggering are clonally deleted. This would tend to pick out many "interlopers" who are trying to use mimickry as a means of defence breaching.

ANTIBODY EVOLUTION - Attaching a newly developed "self" CONSTANT domain marker to selected epitopes (antigens) means that the self recognising C1/C2 comple-ment system can be brought in to be adapted to marking these antigens ready to trigger an an effector cascade.

| | |

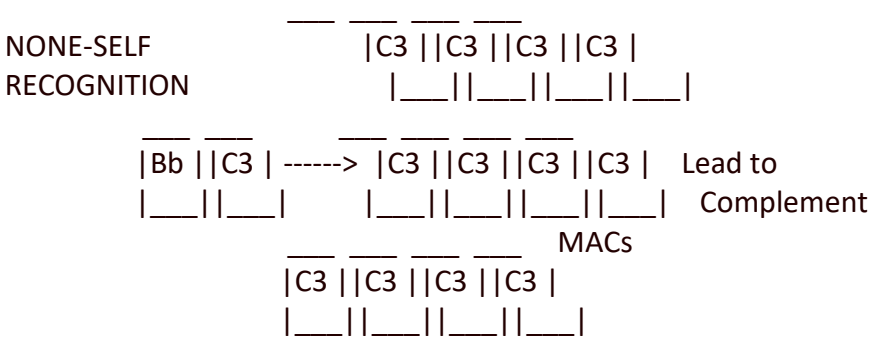


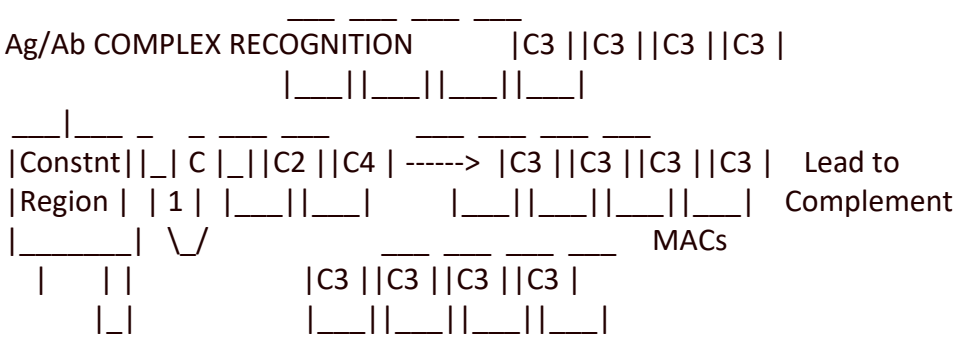
TCR + CD8

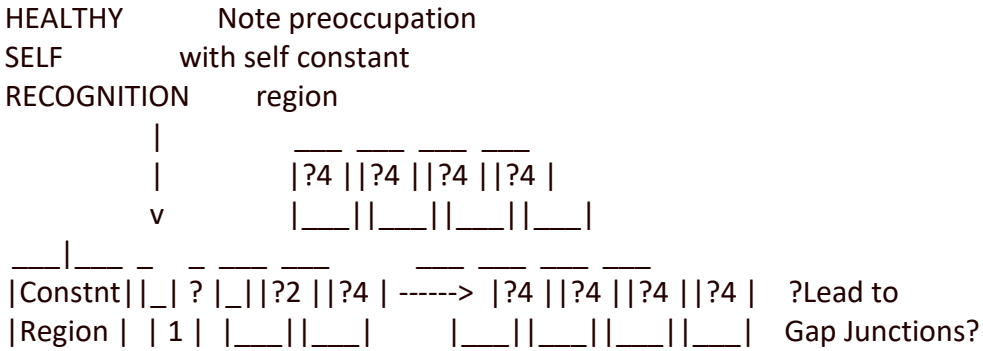
GJ/MAC DIVERGENCE This presumes that there is a relatiionship between GJs and MACs. When did it occur and how completely are their respective insertion mechanisms now duplicated and diverged? Reports of the complete absence of C1, C2 and C4 genes suggest that any molecules like these responsible for GJ insertion must be long since duplicated and that the GJ genes function independantly of MAC insertion genes.

The next set of diagrams indicate what may be happening. C2 (self recognition sequence) triggers the C4 based construction of holes. Could it be that there is a C4 like molecule that spawns hole construction centres, like C3, but constructed more slowly and tidily. The faster they are "zapped" into the membrane the bigger they can be, perhaps? (That might work with the the two kinds of GJs already noted in the text.) The trick in linking the C4 like slower construction for GJs into the faster C3 constuction for MACs would have been to fuse bits of the C4 like gene to

bits of the C3 like gene to form what is now the C4 gene.
This could be something to look for!







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Note connective
tissue nature
of C1 component

19941029_ln

HYPOTHESIS

Morphostasis is tissue homeostasis. Tissue form is stable whilst cells remain in intimate contact by intercellular junctions. This enables joined cells to establish various degrees of electrical and metabolic synchronisation and it promotes cooperation. Synchronisation is greatest when the cytoplasms are in direct continuity through gap junctions or synctial structures. The specificity of the molecular mechanisms that lead to cell adhesion, coupling and connective tissue scaffolding, in effect, give cells a <healthy self (HS)> identity. Similarly, the loss of <HS identity> is accompanied by dismantling of the connective tissue scaffold and cell undocking. Self cells monitor each others' identity. When a cell becomes sick it recognises its own disorder and abandons <HS identity>. It can shut down the channels that join its cytoplasm with those of adjacent cells and then detach its membrane from them in a process called apoptosis. This leads to tidy cell death. Adjacent cells and phagocytes ingest apoptotic cells before they burst. Necrosis is an untidy form of cell death. Such dying cells burst and spill their contents, so releasing inflammatory cytokines. These, in turn, trigger aggressive anamnestic immune reponses which accelerate the identification and elimination of any cells which resemble cells that previously evoked an inflammation. Once order is restored, adjacent healthy cells duplicate and replenish lost cells.

INTRODUCTION

In 1963 the Lancet published an hypothesis, "The role of lymphoid tissue in morphostasis"1. In this article Burwell made the comment that "immunology still awaits incorporating into the general pattern of biology" and suggested that immune function had an important role to play in morphostasis. Morphostasis is defined as the "steady state condition which maintains a particular (tissue) pattern". Immunology is currently perceived as a discrete, clearly demarcated system. In this article I hope to show how morphostasis should be regarded as the origin and continuing drive of the immune system. To be credible, the hypothesis must be compatible with experimental fact and I believe this criterion is met. The morphostatic system must interface with these biological systems:

1) Intracellular and molecular biology
2) Cell to cell communication and cooperation (gap junctions in particular)
3) Embryo - development from zygote to mature animal
- evolution from simple metazoans to mammals
4) The general scheme of morphostasis including
- the surveillance for sick cells
- cell and animal senescence2
- malignancy
- the changing susceptibility to various diseases with aging

- the renewal of sick cells and tissues

5) Basic pathological mechanisms

6) Immunity - innate

- anamnestic
- immune ontogeny
- immune phylogeny (from simple metazoans to mammals)3

- shed some light on plant defence4,5

Brevity demands a synoptic style so I shall not explore the rationale for proposing a new perspective. What follows is my perception of the process and its elements are not necessarily statements of accepted fact. The bibliography has been chosen to provide an investigative trail, with many of the articles providing a further source of reference.

ABBREVIATIONS

Ag	=	antigen
CAM	=	cell adhesion molecule
GJ	=	gap junction
HS	=	healthy self
ICJ	=	intercellular junction
Ig	=	immunoglobulin
N-CAM	=	Neural CAM
OTHS	=	other than healthy self
TNF	=	tumour necrosis factor
UHS	=	unhealthy self
ZDC	=	zygote derived colony

THE ZYGOTE DERIVED COLONY (ZDC)

Every animal is a colony derived from a single cell, the zygote. No cell in the ZDC has functional capabilities that are not potentially present in the zygote's genes or cytoplasm. Every ZDC cell needs some way of preferring its own kind as neighbours and inhibiting the growth of foreign cells or organisms in its vicinity. This is achieved by using selective CAMs which lead to the construction of ICJs, a scaffold of connective tissues and electrical/metabolic synchronisation6,7.

THE SOPHISTICATION OF SINGLE CELLS: THE SELF AWARE CELL

Each animal cell is a self assessing unit, capable of surveilling its own behaviour and function. It does this both internally and with respect to its neighbours. When an animal cell malfunctions, it senses this abnormality and notifies other cells that something has gone wrong (by various cytokines, alterations in cell surface markers and by disruption of junctional communication). A sick cell can sacrifice itself by apoptosis8,9: its calcium level rises, it rounds up and its GJs are closed before these and other ICJs are disassembled. Apoptotic cells are phagocytosed by adjacent cells or phagocytes before their membranes burst.

HEALTHY SELF (CELL) / OTHER THAN HEALTHY SELF (CELL) DISCRIMINATION

All metazoan animals are able to make this discrimination. What differs from organisms to organism is the sophistication with which it is embellished.

It reaches a high level of sophistication in mammals. Every embellishment of the morphostatic system, including anamnestic immunity, requires an <UHS cell> to "advertise" its presence.

MORPHOSTASIS

Tissue homeostasis can be maintained by:
(a) displaying markers on HS cell membranes which identify them as HS.
(b) discriminating OTHS cells from HS cells by the absence of HS identity.
(c) attacking and removing OTHS cells (UHS and foreign cells/organisms).
(d) replacing lost UHS cells with fresh HS cells (resurgent morphogenesis).

IN SUMMARY

Identity - healthy ZDC cells display identity markers (these double up as "docking" molecules which lead to ICJs and a connective tissue scaffolding).
Self surveillance - cells are able to sense <unhealthy self> status.
Altruism - cells are able to opt for apoptosis (suicide).
Neighbour surveillance - cells are able to sense a neighbour's appropriateness.
Sick cells - either declare their own presence or are recognised as such by their neighbours. These include damaged cells, dying cells, aging cells, genetically damaged cells, malignant cells, infected cells and other sick cells.

GAP JUNCTIONAL COMMUNICATION IN HEALTH AND DISEASE

The communication of cell cytoplasms through GJs appears to be a common feature of healthy cells10. These cell to cell channels are shut down if a cell is sick12,13,4. The initial closure of the GJ channels is caused by a rise in intracellular calcium10. Physical disconnection occurs later, often as part of the apoptotic process8,9.
The whole embryo is electrically coupled by GJs and this defines the boundaries of <self>14. Within this electrically continuous <self> are sub-compartments within which member cells are joined by plaques of densely packed and more permeable GJs. The cells so connected constitute a developmental compartment. The permeability of these GJ plaques is greater than the population of GJs which characterise the compartment borders. The expression of N-CAM appears to encourage the spawning of highly permeable plaques15. Three possible explanations spring to mind: these plaques contain more GJs; the componenet GJs are bigger; construction is more efficient and there is a higher yield of good junctions.

This suggests that the consensus sequences of N-CAM, which resemble Ig constant regions, may act as the focus for a cascading multiplier system (akin to the complement C3 mechanism) with one activated consensus sequence spawning hundreds of GJ construction sites. If this proves correct, then the complement attack sequence has probably evolved from it. Either the C7,8,&9 genes have evolved directly from GJ connexon genes or they have highjacked the mechanism to amplify hole construction sites. They have subsequently inverted it into a mechanism to insert leaky holes into membranes. In self cells, leaky holes will cause a rise in intracellular calcium, so closing the GJ channels. Note the connective tissue origin of C1q.

APOPTOSIS, NECROSIS and INFLAMMATION

Successful self surveillance leads to apoptosis and elective suicide. This mechanism deals with many sick cells. It has failed when cells die by necrosis Then, membranes rupture, their contents are spilled and inflammation is promoted. Inflammation provokes aggressive T-cell responses. When sick cells disconnect, their membranes can be disrupted and they may, in consequence, release a variety of cytokines (particularly eicosanoids). These are the

messengers that notify adjacent somatic and inflammatory cells that something is amiss. Tc cells induce apoptosis in cells which carry markers resembling other cells that have previously died and provoked an inflammation. TH1 cells remember the inflammatory context in which they met their epitope. When they reencounter similar peptides they turn up the inflammatory "heat". They do not, themselves, kill: this is left to "angrified" phagocytes which are more fussy about what they tolerate as <HS identity>.

Peptide debris, processed after phagocytosing apoptotic cells, promotes T-cell suppression. For example, when a cell dies following a virus infection its debris is processed by adjacent cells and phagocytes. If cell death occurs following successful internal surveillance (apoptosis), tolerance will be promoted to presented peptide debris. When unsuccessful (eg, necrotic death), inflammation promotes T-cell aggression. Since apoptosis is common, self peptides usually promote suppression and so shrink the pool of self reactive precursor T-cells available to be later recruited to aggression. The threshold at which uncommitted T-cells can be triggered into aggression falls as they age. This further focuses aggression onto strange epitopes.

<HS cells> in an inflammatory area are protected from attack because they demonstrate <HS identity>. This is a form of horror autotoxicus. Phagocytes from closely related species share similar specificity. Most non-pathogenic organisms are easily identified as non-self. In the absence of complement, bacteria and viruses must rupture a cell and/or disrupt its ICJs to invoke an inflammatory reaction and an anamnestic immune response. Some dedicated pathogens appear to have evolved mechanisms to heighten inflammation in order to create themselves the niche they need to survive (eg, TB).

Inflammatory cells need to be restrained from entering healthy tissues until things goes wrong since their intrusion disrupts tissue function. The endothelial cell linings of blood vessels tend to lock out phagocytes until they are invited in. This is done more rigorously in the central nervous system - the blood brain barrier. This is necessary as nervous function relies on the electrical (GJ) disconnection of neurons during their terminal differentiation and asynchronisation then makes them more susceptible to macrophage attack (note how traumatic paraplegia is ameliorated with steroids). This need for segregation is likely to be an important factor in the origin of the vascular system.

MORPHOSTATIC EVOLUTION

This is the way I suspect that the metazoan system evolved.

(a) In the beginning, all cells in the colony are equally able to express somatic and phagocytic behaviour. Elective cell suicide (apoptosis) is established early as a means of protecting the colony.

(b) A colony relies on the electrical synchrony of its cells to provide one important aspect of its sense of self. This synchrony is an immediate consequence of cell surface ligand/ligand or ligand/receptor interactions which lead to the construction of various ICJs (the precedence hypothesis). In particular, holes in the membranes of apposing cells can be paired up to form GJs. The importance of cytoplasmic continuity is evident in plants4. The ligands that enable ICJs to form are Cell Adhesion Molecules (CAMs)6,7. Ig supergene CAMs (eg, N-CAM) develop later to act as a focus on which to encourage the growth of highly permeable GJ plaques. At some stage, cells will adapt this multiplier mechanism to spatter bigger, leaky holes into cells or organisms which fail to display features of self (probably a species specific identity). These will eventually give rise to the alternative complement cascade.

(c) There is now a progressive evolution and expansion of different somatic CAMs (probably cadherins and integrins) leading to (subordinate) self

within self identities and an increased tissue specialisation. These subordinate identities define developmental compartments. The borders of the compartments are demarcated by a sheet of cells having low permeability (even electrically rectifying) GJ communication with each other whilst the block of cells within the compartment also express Ig superfamily CAMs and become interconnected by highly permeable plaques of GJs. The boundaries of certain compartments have been observed to correspond with the boundaries of homoeotic gene expression16.

(d) Thus far, when necessary, all cells have to act as phagocytes. Next, however, the cells of an animal will split into dedicated phagocytes and soma with the soma abandoning most of its capacity for wandering and aggression and scavengers theirs for connective tissue construction.

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SOMA LIGAND(s)       - for recognition by resident scaffolders.
PHAGOCYTE LIGAND(s)  - for recognition by itinerant scavengers.
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Dedicated scavengers (phagocytes) now evolve. They refine the cooperative GJ communication with self and the runaway, leaky hole attack of non-self to form the complement system. At this stage complement components are secreted locally by phagocytes and their action is directed entirely at membranes. It is a long time before these components are co-opted into a humoral system and very much later that they are co-opted to interact with antibodies.

Phagocytes are derived from a cell lineage which lies outside the three main germ layers so they may, when they infiltrate somatic tissues, be demonstrating a property akin to the sorting tendency of disaggregated cells: they behave as if they can clamber over all other cell types and envelope them. Note that cell sorting is dependent on CAM expression, particularly cadherins6,7.

Phagocytes can assess self by making ICJs with underlying cells so leading to (at least) a/c electrical synchrony. The specificity of this connection will be at least species wide and will probably recognise <selfness> which is common to a range of related species. It seems more likely that it is based on a cadherin or integrin rather than an Ig superfamily CAM. The phagocyte uropod makes ICJ connections (perhaps GJs) with an underlying cell and reaches out lamellipodial fingers to examine adjacent cells/organisms for iso-electric synchrony. Asynchrony in the capacitatively induced potential differences are probably able to trigger the attack sequence (perhaps by focal membrane depolarisation). Other strategies to recognise OTHS are almost certainly used in addition (eg, surface markers of unquestionable bacterial origin). Note that basement membranes maintain physical barriers between tissues and help to minimise the area of cell membrane contact between different compartments.

(e) A "vascular" system now evolves, locking out phagocytes till required and an inflammatory mechanism is developed. The alternative complement cascade is now free to be "humoralised" so that circulating C3 can mark clearly foreign organisms to make them more readily identifiable when they meet a phagocyte.

(f) Mammalian N-CAM genes consist of multiple Ig superfamily motifs. The specificity and diversity of N-CAM ligand interaction is achieved by a process of alternative RNA splicing6. New N-CAM like genes now develop by gene duplication and divergence to form multiple different ligands within a ZDC. Copies of these genes can be put to a new use: they can be altered by a "mix and match" process (genetic cross-overs between

chromatids) to generate a set of ligands which use their variability within a herd rather than within a zygote derived clone. These pleomorphic ligands will become the Mhc class I genes and will act as cell surface "flags" to advertise a more personalised HS status: they evolve to create an almost unique identity in each individual. This new gene is soon joined by another duplicated and transposed gene, this time the one that encodes the pincer mechanism of the HSC70 heat shock protein (these look after "sick" proteins). This gene copy is brought to lie next to an Ig superfamily domain, so producing the ancestor of a Class I Mhc gene17.

A new scavenger cell (the ancestor of Tnk cells) must co-evolve and be able, when required, to observe a horror autotoxicus to any cell/organism that displays these self specific ligands18. They were probably preceded by cells capable of recognising an ancestral beta-2-microglobulin: hence, the eventual elaboration around this molecule. These new scavengers need a mechanism to produce and/or select self specific receptors unique to each ZDC. This must be done post-meiotically over a number of mitotic generations - the "generation of specificity".

This simplistic view is useful for appreciating how Tnk activity arose. However, did the incorporation of the pincer mechanism predate or succede the IgSF motif recognition? Succession seems the obvious choice. This could extend a horror autotoxicus based on <pure-self> recognition to one based on <pure-self+healthy-self-peptide> recognition. All this raises a question: does this Mhc like ligand promote ICJ connection of Tnk cells with self cells? Note how lymphotoxin and TNF are selectively damaging to cells not in communication by GJs19. This Tnk like cell will function as outlined in the following table.

TABLE 1

Cell types and modes of action

Cell type	Receptors disabled	Receptors enabled	Normal state	Triggered state
Primitive scavenger (Tnk like precursor)	non pure self	pure self	aggressive	passive (horror autotox-icosis)
	GENERATOR OF SPECIFICITY			
Tc cell	pure self	non pure self	passive	aggressive
	GENERATOR OF DIVERSITY			

To achieve this diversity in ligand recognition, a mechanism was required to produce many different receptors from which an appropriately specific receptor could be selected - "the generator of specificity". It is from this that the antibody genes have subsequently evolved. Horror autotoxicosis needs redefinition: only <HS cells> are protected by it (initially <pure self>, later <self+self-peptide>).

(g) Note that the Class III Mhc region contains a variety of genes encoding molecules that are involved in HS/OTHS discrimination or its modulation. These include HSP70, TNF, complement components (C2, Bf and C4) and the 21-hydroxylases.

(h) Both the complexity and the repertoire of this mechanism for generating and selecting specific receptors is able to evolve gradually. It should be clear from the table that, at some point, inverting its function can

provide a mechanism adapted to recognising and attacking non-pure self. By inverting the "generator of specificity" into the "generator of diversity" lymphocytic cells (Tc like) could evolve which were able to recognise and, when appropriate, attack cells whose Class I ligands had been altered by the (intended) attachment of peptides to the pincer mechanism.

(j) The stage is now set to allow the evolution of TH1 cells. Class II Mhc ligands evolve as extensions of the Class I mechanism: the "intention" is to process short, representative peptides from cellular debris picked up by phagocytes at inflammatory sites. These are then externalised as a <Class II>/<peptide debris> combination ready for the attention of uncommitted T-cells. The "generator of diversity" can now be enrolled into creating a system to memorise the inflammatory context in which these processed epitopes were first encountered. On re-encountering the processed epitope, these T-cells are programmed to attract large numbers of phagocytes to the site and "angrify" them. Inflammation now has a memory. The "angrified" phagocytes still have to sort HS from OTHS but their threshold for regarding a cell as OTHS is lowered. So, Th cells are not involved in assessing <selfness>. They simply accentuate an inflammatory response when they reencounter an epitope formerly met in an inflammatory environment. Only a limited set of cells - APCs, phagocytes, etc - can present combinant epitopes so the amplification of the inflammatory cascade can only start after OTHS has been processed.

(k) A system of tolerance needs to evolve hand in hand with aggression. Even though apoptotic cells fragment, each particle retains an intact membrane and all are tidily phagocytosed by adjacent cells or phagocytes. The peptides processed in consequence need not activate Tc or TH1 cells: rather, tolerance is desirable. Cells which rupture and spill their contents are not disposed of so tidily. They release eicosanoids and other cytokines which provoke inflammation and can activate Tc and TH1 cells.

TABLE 2

THE BINARY COMMITMENT OF INDIVIDUAL LYMPHOCYTES
depending on how the peptide is presented

EFFICIENT APOPTOSIS		RUNAWAY NECROSIS
Non-inflammatory		Inflammatory
<<----------	---------->>	
Tolerance observed	<-------- (OFF)	-------> (ON)	Aggression observed

So, uncommitted T-cells are sensitive to the inflammatory cytokines or non-inflammatory environment they sense when they meet their respective epitope. They become committed accordingly. Self antigens are copious and are often encountered in the course of efficient apoptosis. The majority of precursor T-cells with paratopes recognising processed apoptotic debris (much of it self peptides) will be "mopped up" into a commitment to suppression (tolerance). These T-cells will either be decommissioned or primed to inhibit inflammation on epitope re-encounter. However, uncommitted T-cells with paratopes specific for self Ags continue to be released from the bone marrow and primed in the thymus. At least a proportion of them may become committed to aggression if the inflammatory process is prolonged and foreign epitopes, which accelerate its resolution, are sparse. The system is probably enhanced by the simple

expedient of reducing the threshold at which aggression can be triggered as precuror T-cells age. This concentrates aggression onto strange epitopes. Tolerance could be amplified by Ts cell clonal expansion and/or the release of anti-inflammatory agents at the site of epitope re-encounter.

(l) The antibody system can now be launched as "icing on the cake". TH1 cells can be adapted to TH2 function and these in turn used to co-operate with B-cells. The B-cells evolve to secrete large quantities of circulating antibody. Antibodies help by opsonising organisms. The alternative complement cascade is now adapted to be triggered by C1,2,&4. These have evolved from the ancestral components which are used by N-CAM to spawn GJ plaques. The antibody system is optimised to work within the vascular system. It can interfere with any intended function of the Ag and tag it for enhanced phagocyte attention and attack. This system has proven to be invaluable as a pre-emptive defence.

CLINICAL CONSEQUENCES

There is insufficient space here for a detailed elaboration so here is a whistle stop tour:

(1) ANERGY. This term has acquired several meanings but here I am referring to the loss of delayed type hypersensitivity responsiveness that occur in diseases like TB and cancer. Because the T-helper system is capable of training its aggressive attention on self antigens when clearly strange antigen is sparse (eg, adjuvant arthritis), the immune system has to have a failsafe cut-out mechanism. This shuts off phagocyte aggression when the tissue destruction starts to get too fierce. The effect is dominantly focal though there is a systemic spillover effect. It impairs focal surveillance by phagocytes.

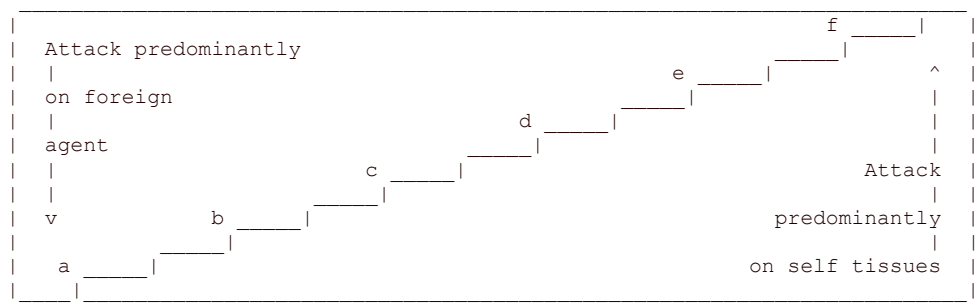
(2) PATHOGENS. Non-pathogens are easily identified and eliminated except when there is focal impairment of surveillance (anergy). Pathogens need to devise means of breaching the morphostatic defence. They do so by mimicking, blocking and fooling identity mechanisms20. Tuberculosis, in particular, deliberately invokes intense inflammation, causing extensive auto-rejection. It then flourishes in a resulting focus of phagocyte impotence.

(3) AUTO-REJECTION. The result of all this is that any disease which evokes cell necrosis and an inflammatory response develops an element of T-cell augmented auto-rejection. It inevitably consists of a mixture which varies from an attack directed almost exclusively at the pathogen (usually leading to mild inflammation) to an attack directed almost entirely at self (often highly inflammatory): the latter occurs when organisms or cells provoke prolonged inflammation but do not provide or present clearly foreign looking (unusual) epitopes. Every disease that leads to cell damage will induce auto-rejection, even if this goes no further than apoptosis. Since heat shock proteins are responsible for chaperoning disrupted proteins through the cell, they are frequently presented as epitopes in UHS presentations.

Auto-rejection rumbles along at a low level all the time. When inflammation is prolonged and no clearly foreign epitopes are present to bring it to a conclusion, precursor T-cells specific for self Ags may be progressively recruited into aggressive action. These intensify local inflammation and so enhance tissue rejection. This appears to be what happens in adjuvant arthritis.

DIAGRAM 1

The stepped progression of attack on self



EXAMPLES
(a) Saprophyte
(b) Simple epithelial commensal
(c) Staphylococci and streptococci
(d) Tuberculosis and syphilis
(e)-(f) Multiple sclerosis and sero-negative arthritis

(4) CANCER. Communication by GJs is disrupted in cancer21. Phorbol esters, which are cancer promoters, stabilise cells which would otherwise elect for apoptosis. The depression of focal surveillance that occurs in the wake of lymphocyte amplified auto-rejection is at least partially responsible for allowing malignant cells to escape detection and elimination.

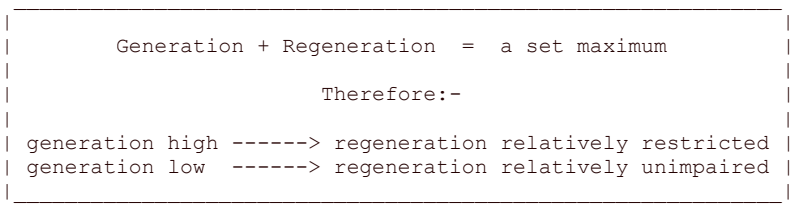
CONCLUSION

The general principles of morphostasis are discussed. I have made a committed assumption that GJs are important in maintaining HS identity. Other ICJs may contribute a larger part than I have credited here. If well founded, the hypothesis should prove to be a useful framework for a more focused investigation of the biochemical processes of morphostasis.

19941029_clinmorph

GROWTH AND REGENERATION

It is inevitable that the rate at which generation (growth) and regeneration (mostly repair) can proceed is limited. Since these are essentially similar morphogenetic processes, auto-rejection as a morphostatic technique cannot be allowed to reach the level of intensity in a growing animal as that which can be permitted in a mature animal. If it does growth will be stunted. That is:-



Put another way, the luxury of extensive auto-rejection, as part of a morphostatic technique, can only be fully afforded in adult animals. Thus, in order to avoid stunting of growth, those mechanisms which initiate and accelerate rejection (of all kinds) need to be less fierce in growing animals than they are in adults: lymphocytes must behave less aggressively and this is

probably brought about by moderating the intensity with which APCs stimulate aggressive lymphocytes (APCs = antigen presenting cells) [30,31,31a]. Both CMI (cell mediated immunity) and IgG activity must be dampened (at least, for those IgGs capable of reaching the extracellular spaces even when there is no inflammation). The result of all this is to promote a relative immunological tolerance in very young animals. This impaired capacity to reject (and consequently autoreject) is apparent in the neonate in which the tolerance of grafts is much enhanced: the neonate can also tolerate a level of cerebral ischaemia which, in adults, would cause extensive tissue death (in large part an auto-rejective event). This relative incapacity to auto-reject is also a protection against the dangerous sequelae that follow virus infections (they may even have been a significant driving force to require it). These tend to produce their most severe effects when they first strike in adult life, eg, infectious mononucleosis [32], infectious hepatitis (both often mere URTIs in young children), mumps, chicken pox and measles; and an example from the mouse, lymphochoriomeningitis [33]. The sequelae, arthritis, jaundice, meningitis, orchitis & etc, can be prevented or at least ameliorated by immunosuppressives or steroids. From this point of view, "immunological immaturity" is a misleading term because the infant's immune system is likely to be perfectly adapted for an optimal compromise [new].

There are certain tissues where extensive auto-destruction could prove disastrous: such an event might seriously impair the ZDC's functionality and survivability. These include the eye and the nervous system. These sites enjoy a so called "immunological privilege". This priviledge seems to be achieved, at least in part, by locking out inflammatory cells behind tight endothelial cell junctions: the sparse population of local APCs is probably a direct consequence of this.

AUTO-REJECTIVE DISORDERS

Tissue rejection is largely accomplished by cells and cell mediated mechanisms. Whilst antibodies can affect the course of organ rejection, they cannot, on their own, precipitate it. In contrast, rejection can be provoked with injections of appropriately activated lymphocytes. Once it is apparent that disordered self cells are actively rejected, we are in a position to state the following:

Every disease which leads to an inflammatory reponse will have an auto-rejective element even if this is limited to a mildly increased tissue turnover.

So, there ought to be a group of disorders which are largely auto-rejective and who's pathogenesis is little, if at all, affected by humoral auto-immunity. Since immune function changes through life, the intensity of auto-rejection is likely to be dependent upon age. It will be at its climax in the healthy young adult. The initiation of auto-rejection is suppressed in the very young [30,31,new] and its execution becomes progressively impaired in the elderly [40]. Thus, a disease which is caused by extensive auto-rejection will be most likely to occur and also to be at its most severe in this central age range (figure 2). One likely cause of such disease is deliberate interference with and mimicry of aspects of the host's identity machinery. Micro-organisms, with their capacity for rapid genetic adaptation, are the most likely offenders. Where micro-organisms develop antigenic determinants close to some element of the host's identity machinery they will appear less foreign and gain easier access to the host's tissues and cytoplasm. Cells which are damaged in consequence of this should still signal malfunction (shout "foul"). However,

because there may be a relative scarcity of clearly foreign antigen, the resultant inflammatory reaction will concentrate its enhanced attention on self Ags. Whenever these self Ags are reencountered, an amplified inflammation will ensue and the consequent auto-rejective attack will not necessarily remain confined to the initiating site.

Adjuvant arthritis is of interest because it produces a constellation of disease who's features are similar to those seen in the sero-negative arthritides and sarcoidosis. This experimental disease may be caused because clearly foreign antigen is sparse and the immune response is consequently concentrated upon local tissue antigens (eg, heat shock proteins or other mycobacterial antigens which cross react with the host) (table x). Whipple's disease may be an extreme example of this sort of disease (note the idiosyncratic infection [41,42] and familial aggregation of cases [42,43]).

The bacteria which colonise epithelial surfaces present a special hazard to the colony. It is well recognised that they have the ability to bind selectively to cells at particular epithelial sites [10]. Since they have evolved this specificity it is also highly likely that they have evolved some mimicry of and interference with the host's identity machinery (especially tissue/site be definable from basic principle: compatibility of organ transplants ranges from a common slight compatibility to a rare complete compatibility [13]. When this observation is extrapolated to microbial mimicry, one would expect to find minor mimicry often and extreme mimicry rarely. The seronegative arthritides and their component complications show just this sort of structuring (table 1). Their clinical pattern can be summed up by an axiom:-

The severity of any single patient's disease(*) is inversely proportional to its incidence in the population and directly proportional to the number of components found in association with one another.
(*) - Whether it is an isolated component or a syndrome complex of more than one component.

For example, recurrent aphthous ulceration (RAU) occurs in about 5% of the population, oro-genital ulceration in about 0.5% or less and Behcet's syndrome (BS) in about 0.0001% (in Britain). As the apparent disease in any particular patient is observed to be more severe, so we notice an expanding clinical overlap: more individual components coincide in one patient (table x). The pathogenesis of these disorders should be dominated by cell mediated immune aggression just as it is in non-acute graft rejection [44]: any contribution from circulating antibodies should simply be a bystander phenomenon. The pathological tempo of the individual components is often seen to increase with the severity of the syndrome disorder. Thus, in psoriasis, the prevalence of arthritis and iritis increases greatly in patients who have the exfoliative and the pustular forms of the disease [45]. On the basis of a personal study (in which the prime objective was to review the world literature on neurological Behcet's syndrome - unpublished) I believe that the meningo-encephalitis of multiple sclerosis should be regarded as the respective isolated component which becomes more severely expressed in the meningo-encephalitis that is encountered in BS (nb., MS is a meningo-encephalitis [46]).

The age incidences of all these disorders are typical [47]. The population incidences of the commoner conditions begin and peak earlier than in the rarer disorders. In the majority of components it is clear that they are constantly modulated by certain events: menstrual exacerbation, second and third

trimester quiescence, puerperal exacerbation, stress precipitation and, finally, amelioration of symptoms with steroid and immunosuppressive therapy. (This pattern matches Tnk cell activity and numbers.)

At least two further disorders have features to suggest that they might legitimately be included amongst the (predominantly) auto-rejective disorders. These are sarcoidosis and systemic lupus erythematosis. Both of these demonstrate some clinical overlap with the sero-negative arthritides: and SLE has a similar component structuring. (Nb., high turnover granulomas are a recognised consequence of many cell mediated immune reactions [48]).

CANCER

Broadly speaking it can be surmised that cancer follows:-

(a) a triggering event (induction)
(b) a change in cell behaviour (promotion).
(c) a breakdown in surveillance (progression).

The event which finally trips an affected cell into loss of growth control need not concern us in this article other than to point out that it usually arises in a single cell from which the tumour then develops. A unifying feature is that a normal growth control gene starts being transcribed inappropriately (induction). But let's leave this to one side. I will, instead, focus attention on the reasons for the body's failure to identify the miscreant cell and its progeny (promotion/progression). Before proceeding, note how stark the contrast is between the Hayflick limit of about 50 doublings (in cultures of healthy cells) (footnote 4) and the apparent immortalisation of cell lines derived from many cancers.

Opportunistic infections and cancer should, presumably, be most prevalent when morphostatic surveillance is least effective. The cells making up an animal (there are around 10 to the power 13 of them in man!) are highly regimented and, presumably, intense cell co-operation has to be exercised to maintain such order within the ZDC's tissues. This implies that, by and large, disruptive cells (dead, damaged, dying, mutated and those with disordered growth control) are largely rejected. And, indeed, it has long been clear that phagocytes do recognise these cells and remove them. Our main attention here should be directed solely at those events which lead to the impairment and subsequent failure of surveillance. Focal anergy is likely to be one of these events and may well be the major contributor to the escape of malignant cells from surveillance.

In mammals, this impairment of surveillance should (generally) be at the extremes of life or following prolonged focal auto-rejection and its consequent anergy. In the elderly, the increasing impairment of immunity coupled with the heightened susceptibility of epithelium to various noxiae (and thus auto-rejection) will predispose to a high incidence of carcinomas. Focal anergy on its own (consequent upon intense auto-rejection) may be a major cause of the predilection for certain cancers to strike young adult to middle aged patients (e.g., lymphomas and focal cancers like colonic cancer in ulcerative colitis or testicular tumours following mumps). In the very young there is a relative incapacity to reject tissues. It is worth noting, then, that the

predisposition for epithelial cancers found in the elderly is not present in the young. Cancers are relatively common in the very young and there is evidence to suggest that many regress before they reach clinical significance [49]. (Note that, in general, carcinoma-in-situ is far commoner than overt cancer: the abnormal cells tend either to be kept in check or eliminated by lympho-monocytic cells.)

Cancer is characterised by a failure of growth control and the cells affected revert to a form of behaviour more typical of embryonic cells (retrodifferentiation [50]). Using a "reductio ab adsurdam" argument these changes are much more likely to happen when regeneration and/or proliferation are exuberant (eg, T-cells in lymphomas) rather than relatively quiescent (eg, cartiledge, neurones, macrophages). Note that lymphomas are relatively common in the years in which auto-rejection is most intense (16-45yrs) and also note that, in granulomatous disorders, lymphomas predominate over other cancers perhaps because local tissue regeneration is impaired [51,52].

The rate at which malfunctional cells arise (for any reason) probably increases with age. The net effect of this will be to cause a diffuse increase in the multiple foci of auto-rejection and, consequently, a gradual summation of focal anergy. This will eventually lead to a systemic spillover of this focal effect, a saturation effect. Epithelium is the tissue most exposed to infection, noxiae, regeneration and, in consequence, an increased probability of genetic divergence. Foci of anergy will be very frequent in this tissue form and carcinomas should consequently be more prevalent than sarcomas. Once initiated, cancer will itself lead to auto-rejection and, in turn, increased focal anergy. Thus, it is likely that there exists a critical mass and growth rate above which surveillance is irreparably blocked and the cancerous process becomes self perpetuating [53]. (Macrophages observed in vitro are clearly able to recognise malignant cells as abnormal [54,55].)

Now it is instructive to compare the age incidence profiles of various cancers with those of the auto-rejective disorders. However, before doing so it is important to establish which cancers are likely to flourish in the wake of intense auto-rejection (probable examples are lymphomas and testicular tumours [56,57,58]). These must be recognised as distinct from the commonest form of cancer (carcinoma) which seems to occur most frequently in the wake of age related impairment in immune surveillance. In general, these have a gradually rising incidence with age. Some cancers, particularly mesodermal malignancies, follow an incidence pattern showing a nadir in the middle years. It is interesting to note that the age incidence pattern of acute leukaemia is a complete inversion of the age incidence pattern of the auto-rejective disorders (figure 2). (See [59]).

It should now be clear that the lymphocytic system can have a dichotomous effect on cancer surveillance. It may enhance the focal accumulation of phagocytic cells and thus aid the (auto-)rejection of aberrant cells. However, the more aggressively it does this, the more likely it is to precipitate a suppression of focal rejection in order to avert piecemeal self destruction. Indeed, in those animals that have evolved them, the possession of lymphocytes may have incurred an increased risk of cancer: cancer is relatively uncommon in primitive animals [60,61] and is relatively scarce in congenitally athymic mice [62,63] which have abundant aggressive phagocytes [64] and natural killer cells [65]. It is interesting to note that in the animal kingdom there is an inverse relationship between the capacity to extensively regenerate body form and the prevalence of cancer [66,67]: and that carcinogens may induce supernumerary structures in lower phylae (eg, limbs) [68,69].

Napolitano et al [70] report that tumour cells generally display less class I Mhc Ag at their surface. They draw attention to the fact that the more malignant the tumour is the less class I Ag it expresses. They interpret this as a cause of the malignant behaviour. However, I would interpret this as a cell adjustment going, pari passu, with the loss of HS identity. Macrophages in vitro have little trouble in identifying malignant cells [55]. So, it seems that some quirk is allowing the lymphocytic amplification system to become preoccupied with an inappropriately strong response to the "wrong" tissue Ags: this, in turn, has led to focal auto-aggression and focal anergy. The phagocytes' capacity to eliminate UHS (tumour) cells is thus impaired, permitting a (so far) dormant carcinoma-in-situ to grow to a critical mass where focal anergy will never subside: at this point, the focal impairment of phagocyte activity becomes irreversible and uncontrolled growth of the tumour proceeds unabated. This is consistent with the finding that tumour cells towards the centre of the tumour have a lower expression of class I Ags than tumour cells towards the outside. At the edges of the tumour, macrophage activity is likely to be much more active and successful in eliminating abnormal cells [55].

INFECTION

Infection can be defined as the survival and proliferation of an organism, not descended from the originating zygote, within the tissues of the ZDC. The colony need only remove these cells if they interfere with its structure or function (though the generality of the "dog eat dog" principle suggests that those that don't interfere will be highly specialised commensals or symbionts). Below I suggest four discrete ways in which surveillance can be overcome:-

(a) The first form of infection occurs when an organism acquires the ability to interfere, agonistically or antagonistically, with the host's machinery for establishing cell identity. Strategies based on species and tissue site identity can be cultured throughout the whole mass (surface mostly!) of a species and over its entire duration as a discrete species. The way in which foetal cells reaggregate into tissues rather than species [8,9] and the success, in nude mice, of skin transplants from distant species [71] suggests that tissue site identities may be broadly similar across widely separated species. A variety of infectious organisms could be interfering with this tissue site identity (eg, streptococci [72] and staphylococci). Others also show a clear species specificity (e.g., mycobacterium TB, bovine TB, avian TB etc, and various plant infections [73]). Interference with individual (Mhc) identities can only be evolved in a short timespan (about 60-70yrs in man) and in a small mass (about 60-70kg of which only a small proportion is actually epithelium). Should close mimicry of personal identity develop, this will facilitate that organism's access to the ZDC's tissues and, once there, there would be a relative lack of clearly foreign antigen to "attack". The resulting inflammatory response will tend to concentrate attention on tissue antigens common to both the organism and the host or just to the host. These self Ags will be selected as anchors for the subsequent lymphocyte accentuated inflammation, so leading to an accelerated rejection of self tissues. This kind of destructive attention to self is probably occurring in adjuvant arthritis [22,23]. This disorder has clinical features closely reminiscent of the sero-negative arthritides and sarcoidosis (table 2). It is likely, therefore, that a highly idiosyncratic form of infection is a factor in the pathophysiology of the "auto-rejective disorders". Such disease could be precipitated by interference with the host's Mhc machinery by the microbe and this will probably have evolved in the lifetime of the animal. In biological

systems, things are rarely black or white so the relative blend of the common/consensus and the idiosyncratic/individual response to infection will probably vary in a spectral manner (diag $). (Note that bacteria that manage to invade and survive within the cytoplasm could well pose a greater threat for this form of auto-rejective disease).

[Rejection will always be aimed at whatever is most apparently OTHS. The amount of auto-rejection will increase with the angrification of phagocytes, especially when clearly foreign OTHS is sparse. With the angrification of phagocytes, the threshold of HS expression required to avoid attack will be higher. In consequence, fewer self cells will continue to qualify as immune from self attack.]

(b) A second group of organisms manage to foil surveillance by virtue of their small size and obligate intracellular existence. The organisms of this group are the viruses. As soon as an infected cell is sufficiently compromised it should signal a malfunction so triggering inflammation and attracting phagocyte attention. This will lead to the activation of appropriate precursor lymphocyte clones. After an interval of 10-14 days a strong amnestic response to various viral*peptide+Mhc antigens will have developed. In the meantime, selected self Ags may be used to anchor an immune accelerated phagocyte accumulation at the affected site whilst waiting for the emergence of a more specific anti-viral activity. (In general, these are "hit and run" infections: they are soon suppressed or cleared from the system and those that persist do so by remaining dormant within cells.)

(c) The third group are the opportunistic infections. Whilst these may interfere with tissue and species identity mechanisms [74] their success is dependent on the depressions of focal surveillance which follow virus infections, burns, surgical incisions and trauma (etc.). Each of these noxiae lead to the auto-rejection of damaged and malfunctioning tissue with subsequent focal anergy [27]. Probable examples of such opportunistic infections include bacterial tonsillitis, otitis, sinusitis, bronchitis and various wound infections.

(d) The last group are organisms which set out to subvert the immune response by deliberately creating a field of intense focal anergy. They do so by maximally stimulating focal inflammation with the object of inducing intense focal auto-rejection. Mycobacterium TB is the example which will be considered here though syphilis is probably another. The properties of such an organism should include:

(1)	poor initial foreign antigenicity
(2)	a strong attraction for macrophages (adjuvant attraction)
(3)	a good resistance to initial destruction as evidenced by prolonged survival within macrophages

The result of these 3 properties is that intense focal inflammation and then auto-rejection is induced. In consequence, there is intense focal anergy and this leads to a field of surveillance impairment in which the bacterium flourishes, feeding upon the cell debris which is left in the wake of this auto-destruction [75,76]. Clinical mimicry of the auto-rejective disorders should be discernible: this, in fact, can be seen and is most noticeable in the middle years, an observation which is in keeping with the auto-rejective disorders (table 3).

When tuberculosis occurs outside these middle years it is, accordingly, different in its clinical expression. The lesions now tend to be miliary and disseminated and occur without the same intense tissue destruction. Instead, the pattern now resembles miliary cancer. At the extremes of life TB appears to be acting more like an opportunistic infection. The overall age incidence of TB can, therefore, be regarded as a combination of the auto-rejective and the cancer type age incidence (figure 2).

AUTO-IMMUNE DISORDERS

In several previous articles where immune surveillance has been discussed it has been suggested that cancer and auto-immunity might be expected to represent opposite poles of surveillance efficiency. However, the auto-immune title does not automatically imply auto-rejectiion. Rather than being dominantly auto-rejective, these disorders tend to result in one of two disturbances. The first is an interference with functional membrane molecules by the attachment to them of auto-antibodies (e.g., Graves disease, myaesthenia gravis). The second is a tissue destruction which is centred predominantly around (non-cellular) connective tissues (the "collagenoses") and is apparently exacerbated, if not caused, by excessive auto-antibody production and the widespread deposition of Ab/Ag immune complexes. Here, cell destruction is possibly secondary to the activation of macrophages in the locality of this connective tissue. Towards the end of life auto-immune disorders are relatively more common than the sero-negative arthritides. Their prevalence at these older ages may possibly be exacerbated by a decline in the efficiency with which phagocytes clear tissue debris: this, in turn, could lead to enhanced auto-antibody (immunoglobulin) production (the latter certainly appears to be a feature of many diseases causing widespread anergy, eg sarcoidosis [77]).

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ANERGY

The fates of individual cells that make up an animal are only important in that neither their death nor their survival should endanger gene propagation, particularly in the herd. (Across the aeons of evolutionary history, those species which fail to maintain a critical "herd mass" founder: the gene pool is all important). So (auto-)rejection of suspect cells is a logical method of housekeeping: cell deficits are, self evidently, renewable by tissue regeneration (a resurgence of morphogenesis). However, if an inflammatory process is particularly strong and there is little if any clearly foreign antigen, lymphocytes are not prevented from mounting an aggressive response to Ags typical of the local tissues (e.g., in burns [21] and adjuvant arthritis [22,23]). The resulting acceleration of tissue turnover could easily get out of hand and lead to extreme tissue destruction (auto-rejection - see below). Auto-antibodies and auto-TH1 reactivity may even be useful in focusing phagocyte attention to specific tissues until a more focused response to foreign Ag has matured (e.g., say, pharyngeal antigen in a viral pharyngitis).

This mechanism for concentrating phagocyte attention risks a positive feedback

and, without constraint, it would lead to catastrophic auto-rejection. Failsafe mechanisms must exist which can be brought into play if tissue destruction becomes excessive. This could be controlled at any or all of the following points:-

(a) inhibition of phagocyte ingression (chemotaxis),
(b) inhibition of phagocyte aggression,
(c) inhibition of further aggressive lymphocyte activation,
(d) a tightening of endothelial cell junctions.
(e) encapsulation in a fibrin sheath (fibrocytes later)
(f) promotion of lymphocytic tolerance to typical Ag
(g) production of auto-antibodies to the newly cloned, locally reactive lymphocytes (lymphocytotoxic Abs)

This failsafe is most necessary within and around the affected tissue so we should expect to see it strongly localised. However, a spillover effect may be anticipated, with a systemic depression of delayed type hypersensitivity (the immune mechanism largely responsible for tissue rejection). This may explain, at least in part, why anergy occurs in diseases such as TB and sarcoidosis. There is evidence that anergy is expressed more intensely at a local rather than a systemic level (footnote 3). General references:- [24,25,26,27,28,29].

GROWTH AND REGENERATION

The rate at which generation (growth) and regeneration (mostly repair) can proceed is limited. Since these are essentially similar morphogenetic processes, auto-rejection will result in the temporary suspension of growth. Auto-rejection cannot be allowed to reach the level of intensity in a growing animal that can be permitted in a mature animal. If it does growth will be stunted. That is:-

Generation + Regeneration = a set maximum
Therefore:-
generation high ------> regeneration relatively restricted
generation low ------> regeneration relatively unimpaired

Put another way, the luxury of extensive auto-rejection, as part of a morphostatic technique, can only be fully afforded in adult animals. Thus, in order to avoid stunting of growth, those mechanisms which initiate and accelerate rejection (of all kinds) need to be less fierce in growing animals than they are in adults: lymphocytes must behave less aggressively and this is probably brought about by moderating the intensity with which APCs stimulate aggressive lymphocytes (APCs = antigen presenting cells) [30,31,31a]. Both CMI (cell mediated immunity) and IgG activity must be dampened (at least, for those IgGs capable of reaching the extracellular spaces even when there is no inflammation). The result of all this is to promote a relative immunological tolerance in very young animals. This impaired capacity to reject (and consequently autoreject) is apparent in the neonate in which the tolerance of grafts is much enhanced: the neonate can also tolerate a level of cerebral ischaemia which, in adults, would cause extensive tissue death (in large part an auto-rejective event). This relative incapacity to auto-reject is also a protection against the dangerous sequelae that follow virus infections (they

may even have been a significant driving force to require it). These tend to produce their most severe effects when they first strike in adult life, eg, infectious mononucleosis [32], infectious hepatitis (both often mere URTIs in young children), mumps, chicken pox and measles; and an example from the mouse, lymphochoriomeningitis [33]. The sequelae, arthritis, jaundice, meningitis, orchitis & etc, can be prevented or at least ameliorated by immunosuppressives or steroids. From this point of view, "immunological immaturity" is a misleading term because the infant's immune system is likely to be perfectly adapted for an optimal compromise [newref].

There are certain tissues where extensive auto-destruction could prove disastrous: such an event might seriously impair the ZDC's function and survival. These include the eye and the nervous system. These sites enjoy a so called "immunological privilege". This privilege seems to be achieved, at least in part, by locking out inflammatory cells behind tight endothelial cell junctions: the sparse population of local APCs is probably a direct consequence of this.

AUTO-REJECTIVE DISORDERS

Tissue rejection is largely accomplished by cells and cell mediated mechanisms. Whilst antibodies can affect the course of organ rejection, they cannot, on their own, precipitate it. In contrast, rejection can be provoked with injections of appropriately activated lymphocytes. Once it is apparent that disordered self cells are actively rejected, we are in a position to state the following:

Every disease which leads to an inflammatory reponse will have an auto-rejective element even if this is limited to a mildly increased tissue turnover.

So, there ought to be a group of disorders which are largely auto-rejective and who's pathogenesis is little, if at all, affected by humoral auto-immunity. Since immune function changes through life, the intensity of auto-rejection is likely to be dependent upon age. It will be at its climax in the healthy young adult. The initiation of auto-rejection is suppressed in the very young [30,31,new] and its execution becomes progressively impaired in the elderly [40]. Thus, a disease which is caused by extensive auto-rejection will be most likely to occur and also to be at its most severe in this central age range (figure 2). One likely cause of such disease is deliberate interference with and mimicry of aspects of the host's identity machinery. Micro-organisms, with their capacity for rapid genetic adaptation, are the most likely offenders. Where micro-organisms develop antigenic determinants close to some element of the host's identity machinery they will appear less foreign and gain easier access to the host's tissues and cytoplasm. Cells which are damaged in consequence of this should still signal malfunction (shout "foul"). However, because there may be a relative scarcity of clearly foreign antigen, the resultant inflammatory reaction will concentrate its enhanced attention on self Ags. Whenever these self Ags are reencountered, an amplified inflammation will ensue and the consequent auto-rejective attack will not necessarily remain confined to the initiating site.

Adjuvant arthritis is of interest because it produces a constellation of disease who's features are similar to those seen in the sero-negative arthritides and sarcoidosis. This experimental disease may be caused because clearly foreign antigen is sparse and the immune response is consequently concentrated upon local tissue antigens (eg, heat shock proteins or other

mycobacterial antigens which cross react with the host) (table x). Whipple's disease may be an extreme example of this sort of disease (note the idiosyncratic infection [41,42] and familial aggregation of cases [42,43]).

The bacteria which colonise epithelial surfaces present a special hazard to the colony. It is well recognised that they have the ability to bind selectively to cells at particular epithelial sites [10]. Since they have evolved this specificity it is also highly likely that they have evolved some mimicry of and interference with the host's identity machinery (especially tissue/site be definable from basic principle: compatibility of organ transplants ranges from a common slight compatibility to a rare complete compatibility [13]. When this observation is extrapolated to microbial mimicry, one would expect to find minor mimicry often and extreme mimicry rarely. The seronegative arthritides and their component complications show just this sort of structuring (table 1). Their clinical pattern can be summed up by an axiom:-

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GJ communication is clearly important in the evolution of a cancer. Two sorts of cancer are discernable:

(1) The first where inappropriate local CAMs are utilised to make junctional communication and the adjacent, normal cells cannot make satisfactory connection. In this situation, the malignant cells make good communication with each other but not with normal adjacent tissue.

(2) The second sort is where the cell becomes "immortalised". This process is dependant upon cell growth becoming independant of GJ communication (by mutation). Normally, GJ communication becomes progressively inhibited as the number of cell doublings approaches 50 and eventually cell duplication is abolished. Immortalisation frees cells from this constraint but they now operate as independant rather than colony cells. Malignancies which form distant haematogenous metastases are almost invariably of this sort.

The morphostatic surveillance fails when local conditions inhibit its efficiency. The main reason for this is the focal depression of phagocyte activity that seems to be necessary to limit the intense tissue destruction that the lymphocytic system would otherwise be capable of unleashing. Malignant cells which communicate with each other will not be seen as UHS by phagocytes which invade the substance of the tumour. Only at the interface of normal/malignant tissue will they discriminate and then it will be against normal cells if the uropod attaches to a malignant cell or vice versa if the uropod attaches to a healthy cell.

Surveillance in immortalised malignancies is probably suppressed by chemotactic inhibitors which have been induced, originally, during focal auto-rejection but become self perpetuating as attempted rejection of the tumour cells takes over.

Phorbol esters stabilise cell communication and inhibit apoptosis by preventing a rise in intracellular calcium. In so doing, they probably allow an otherwise correctly identified miscreant cell to survive when it should have been eliminated.

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Napolitano et al [70] report that tumour cells generally display less class I Mhc Ag at their surface. They draw attention to the fact that the more malignant the tumour is the less class I Ag it expresses. They interpret this as a cause of the malignant behaviour. However, I would interpret this as a cell adjustment going, pari passu, with the loss of HS identity. Macrophages in vitro have little trouble in identifying malignant cells [55]. So, it seems that some quirk is allowing the lymphocytic amplification system to become preoccupied with an inappropriately strong response to the "wrong" tissue Ags: this, in turn, has led to focal auto-aggression and focal anergy. The phagocytes' capacity to eliminate UHS (tumour) cells is thus impaired, permitting a (so far) dormant carcinoma-in-situ to grow to a critical mass where focal anergy will never subside: at this point, the focal impairment of phagocyte activity becomes irreversible and uncontrolled growth of the tumour proceeds unabated. This is consistent with the finding that tumour cells towards the centre of the tumour have a lower expression of class I Ags than tumour cells towards the outside. At the edges of the tumour, macrophage activity is likely to be much more active and successful in eliminating abnormal cells [55].

INFECTION

Infection can be defined as the survival and proliferation of an organism, not descended from the originating zygote, within the tissues of the ZDC. The colony need only remove these cells if they interfere with its structure or function (though the generality of the "dog eat dog" principle suggests that those that don't interfere will be highly specialised commensals or symbionts). Below I suggest four discrete ways in which surveillance can be overcome:-

(a) The first form of infection occurs when an organism acquires the ability to interfere, agonistically or antagonistically, with the host's machinery for establishing cell identity. Strategies based on species and tissue site identity can be cultured throughout the whole mass (surface mostly!) of a species and over its entire duration as a discrete species. The way in which foetal cells reaggregate into tissues rather than species [8,9] and the

success, in nude mice, of skin transplants from distant species [71] suggests that tissue site identities may be broadly similar across widely separated species. A variety of infectious organisms could be interfering with this tissue site identity (eg, streptococci [72] and staphylococci). Others also show a clear species specificity (e.g., mycobacterium TB, bovine TB, avian TB etc, and various plant infections [73]). Interference with individual (Mhc) identities can only be evolved in a short timespan (about 60-70yrs in man) and in a small mass (about 60-70kg of which only a small proportion is actually epithelium). Should close mimicry of personal identity develop, this will facilitate that organism's access to the ZDC's tissues and, once there, there would be a relative lack of clearly foreign antigen to "attack". The resulting inflammatory response will tend to concentrate attention on tissue antigens common to both the organism and the host or just to the host. These self Ags will be selected as anchors for the subsequent lymphocyte accentuated inflammation, so leading to an accelerated rejection of self tissues. This kind of destructive attention to self is probably occurring in adjuvant arthritis [22,23]. This disorder has clinical features closely reminiscent of the sero-negative arthritides and sarcoidosis (table 2). It is likely, therefore, that a highly idiosyncratic form of infection is a factor in the pathophysiology of the "auto-rejective disorders". Such disease could be precipitated by interference with the host's Mhc machinery by the microbe and this will probably have evolved in the lifetime of the animal. In biological systems, things are rarely black or white so the relative blend of the common/consensus and the idiosyncratic/individual response to infection will probably vary in a spectral manner (diag $). (Note that bacteria that manage to invade and survive within the cytoplasm could well pose a greater threat for this form of auto-rejective disease).

[Rejection will always be aimed at whatever is most apparently OTHS. The amount of auto-rejection will increase with the angrification of phagocytes, especially when clearly foreign OTHS is sparse. With the angrification of phagocytes, the threshold of HS expression required to avoid attack will be higher. In consequence, fewer self cells will continue to qualify as immune from self attack.]

(b) A second group of organisms manage to foil surveillance by virtue of their small size and obligate intracellular existence. The organisms of this group are the viruses. As soon as an infected cell is sufficiently compromised it should signal a malfunction so triggering inflammation and attracting phagocyte attention. This will lead to the activation of appropriate precursor lymphocyte clones. After an interval of 10-14 days a strong amnestic response to various viral*peptide+Mhc antigens will have developed. In the meantime, selected self Ags may be used to anchor an immune accelerated phagocyte accumulation at the affected site whilst waiting for the emergence of a more specific anti-viral activity. (In general, these are "hit and run" infections: they are soon suppressed or cleared from the system and those that persist do so by remaining dormant within cells.)

(c) The third group are the opportunistic infections. Whilst these may interfere with tissue and species identity mechanisms [74] their success is dependent on the depressions of focal surveillance which follow virus infections, burns, surgical incisions and trauma (etc.). Each of these noxiae lead to the auto-rejection of damaged and malfunctioning tissue with subsequent focal anergy [27]. Probable examples of such opportunistic infections include bacterial tonsillitis, otitis, sinusitis, bronchitis and various wound infections.

(d) The last group are organisms which set out to subvert the immune response by deliberately creating a field of intense focal anergy. They do so by maximally stimulating focal inflammation with the object of inducing intense focal auto-rejection. Mycobacterium TB is the example which will be considered here though syphilis is probably another. The properties of such an organism should include:

(1) poor initial foreign antigenicity
(2) a strong attraction for macrophages (adjuvant attraction)
(3) a good resistance to initial destruction as evidenced by prolonged survival within macrophages

The result of these 3 properties is that intense focal inflammation and then auto-rejection is induced. In consequence, there is intense focal anergy and this leads to a field of surveillance impairment in which the bacterium flourishes, feeding upon the cell debris which is left in the wake of this auto-destruction [75,76]. Clinical mimicry of the auto-rejective disorders should be discernible: this, in fact, can be seen and is most noticeable in the middle years, an observation which is in keeping with the auto-rejective disorders (table 3).

When tuberculosis occurs outside these middle years it is, accordingly, different in its clinical expression. The lesions now tend to be miliary and disseminated and occur without the same intense tissue destruction. Instead, the pattern now resembles miliary cancer. At the extremes of life TB appears to be acting more like an opportunistic infection. The overall age incidence of TB can, therefore, be regarded as a combination of the auto-rejective and the cancer type age incidence (figure 2).

AUTO-IMMUNE DISORDERS

In several previous articles where immune surveillance has been discussed it has been suggested that cancer and auto-immunity might be expected to represent opposite poles of surveillance efficiency. However, the auto-immune title does not automatically imply auto-rejectiion. Rather than being dominantly auto-rejective, these disorders tend to result in one of two disturbances. The first is an interference with functional membrane molecules by the attachment to them of auto-antibodies (e.g., Graves disease, myaesthenia gravis). The second is a tissue destruction which is centred predominantly around (non-cellular) connective tissues (the "collagenoses") and is apparently exacerbated, if not caused, by excessive auto-antibody production and the widespread deposition of Ab/Ag immune complexes. Here, cell destruction is possibly secondary to the activation of macrophages in the locality of this connective tissue. Towards the end of life auto-immune disorders are relatively more common than the sero-negative arthritides. Their prevalence at these older ages may possibly be exacerbated by a decline in the efficiency with which phagocytes clear tissue debris: this, in turn, could lead to enhanced auto-antibody (immunoglobulin) production (the latter certainly appears to be a feature of many diseases causing widespread anergy, eg sarcoidosis [77]).

19941029_morphmol

(This is an edited version which contained some of the present Morphostasis and Immunity article.)

INTRODUCTION

In this hypothesis I propose a fresh perspective of self/non-self discrimination.

SOME BASIC THOUGHTS ON SELF(CELL)/NON-SELF(CELL) DISCRIMINATION
To set the scene, I would like to emphasise these points:

(1) When the first multicellulates evolved, they needed to recognise and discriminate self-cells from non-self-cells.
(2) We have become preoccupied with self(epitope)/non-self(epitope) discrimination, mainly as a result of the sequence of discoveries in immunology.
(3) In a large proportion of metazoans, lymphocytes are self-evidently not the source of self(cell)/non-self(cell) discrimination: they don't have any.
(4) It should be possible to discern gradualism in the evolution of immunity starting in primitive metazoans and leading on to the sophisticated system found in mammals.
(5) In development, ontogeny frequently appears to retrace phylogeny: whilst this is not an absolute blueprint for evolution, it does provide important clues of how things evolved.

MORPHOSTASIS
Morphostasis is tissue homeostasis (Burwell, 1963) and it is well maintained in all animals. It is a core process, the functional hub of the metazoan universe. It works efficiently because cells monitor their own health and keep constant close communication with appropriate neighbours. Anamnestic immunity is a branch of the morphostatic process: it has evolved to enhance the effectiveness of morphostasis in vertebrates.

An animal is built of a large colony of cells all derived from one zygote cell (a zygote derived colony - ZDC). This colony constructs itself a skeleton of connective tissues which, while relatively inert, gives it great versatility (eg, the bony skeleton).

The critical function in morphostasis is discriminating Healthy-Self (HS) cells from all other cells and organisms (other than healthy self - OTHS cells). OTHS includes both UnHealthy Self (UHS) cells (eg, ectopic, sick, damaged, aging) and clearly foreign cells and/or organisms. Morphostasis was needed from the moment that multicellular animals first evolved. It should be clear that the main need at that time was to develop a unique way of tagging healthy self cells, so enabling them to identify and acknowledge one another, and then to devise mechanisms to abandon this healthy self status when things went wrong.

TABLE 1

| Morphostasis (tissue homeostasis) can be maintained by: |

(a) discriminating OTHS cells from HS cells.
(b) removing OTHS cells (UHS and foreign cells/organisms).
(c) replacing lost UHS cells with fresh HS cells (resurgent morphogenesis).

HEALTHY SELF/OTHER THAN HEALTHY SELF DISCRIMINATION

This hypothesis requires that individual cells must either have a fail-safe internal device for recognising that they have become unhealthy and/or an ability to monitor a neighbouring cell's change in health (probably) by monitoring (appropriate) cell to cell communication. The announcement of an "OTHS foul" can then be issued directly from the affected (somatic) cells. Inflammatory cells (mostly phagocytes) are only invited into the soma at this group's request - a "call" is sent out to fetch the "police". Foreign organisms need not induce an inflammatory response unless they unsuccessfully attempt communication with a HS cell, or force their way between cells (and so disrupt communication), or directly attack a cell and make it sick. Peaceful co-existence is an acceptable state.

Several properties may combine to specify HS (or UHS) identity; remember that one or more of the critical aspects which lead to HS (or UHS) recognition must be abandoned (or adopted) when the cell becomes sick. Here are some possible candidates:-

TABLE 2

(a) Lectins and the recognition of saccharides (eg, sialic acid).
(b) The inhibition of complement attack by proteins released from or displayed on the cell membrane (eg, factor H, DAF, MCP).
(c) Beta-2-microglobulin and Class 1 Mhc ligand expression.
(d) Cell to cell cytoplasmic joining - particularly electrical.
(e) Various cytokines, particularly eicosanoids/prostaglandins.
(f) Heat shock proteins and p53 are likely to be intimately involved in HS/UHS recognition and discrimination.

CELL IDENTITY IN THE EMBRYO AND OTHER SYSTEMS

The cells in an embryo recognise each other through Cell Adhesion Molecules (CAMs) (Edelman, 1986, 1987 & 1988, Edelman & Crossin, 1991, McClay & Ettenson, 1987). At the cell surface, both like/like and ligand/receptor interactions of these CAMs lead to cell adhesion. This adhesion then rapidly progresses on to communication through gap junctions (Keane et al., 1988). These CAMs are of three main types: first, the cadherins, second the integrins and third, a group of CAMs which are members of the immunoglobulin superfamily (IgSF) of which N-CAM is an example. Note that the transfer RNA molecules specifying N-CAM are spliced by cells in a variety of different ways to produce a range of N-CAM phenotypes. Edelman & Crossin (1991) state, "The origin of the entire Ig superfamily from an early N-CAM-like gene precursor has deep implications for the understanding of the role of adhesion in processes that are not concerned with morphogenesis but rather with immune defense, inflammation and repair".

The cells of an embryo are able to recognise appropriate neighbours: they navigate themselves into their designated locations where they meet their intended neighbours. There are many other observations of the specific recognition of cells and self in biology. Here are some specific examples:

TABLE 3

Protozoans recognise and discriminate food and sexual partners
Phagocytes are able to recognise their own pseudopodia and avoid self attack.
Simple multicellulates are known to reject allografts (1)
Plants - pollination is highly selective against self (2)
Reaggregation of disrupted foetal cells (see later) (3)
Bacterial agglutination and conjugation can be highly specific to self and (in pathogens) to target tissues. (4)
Plants - tree roots in a forest often fuse together. This is very frequent when they are from the same individual, not uncommon when they are from the same species and far less frequent when they are from unrelated species. (2)
Molecular recognition is a fundamental biological principle (eg, nuclear enzymes).
Cell homing: eg, lymphocytes and injected marrow cells. (5)

(1) Coombe et al., 1984
(2) Heslop-Harrison, 1988 and Lewis, 1979
(3) Garrod & Nicol, 1981 and Takeichi, 1990
(4) Reissig, 1977
(5) Hemler, 1990

Self recognition could, therefore, be observed in several ways, each becoming progressively more specific to the individual animal:-

TABLE 4

(a) Tissue type recognition (eg, embryo cell recognition)

```
|                                                                    |
| (b) Species recognition (eg, gamete recognition)                   |
|                                                                    |
| (c) Self ZDC recognition (ie, cells of the individual zygote       |
|     derived clone.  Useful as a "back stop" check of self)         |
|____________________________________________________________________|
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MORPHOGENESIS

Morphogenesis is the process by which tissues and organs are sculptured from a zygote derived colony. It is most obvious in developing embryos: regeneration and repair are achieved by a resurgence of morphogenesis. Since morphogenesis is an integral part of a morphostatic system, it is reasonable to expect that it will share component elements of the same molecular machinery as those used by immune cells and phagocytes. These components have (presumably) been closely associated through every epoch of metazoan evolution. It remains unclear what the complete mechanisms are which lead to embryonic development. However, CAMs (as above) and gap junctions (Green, 1988) appear to play critical roles.

EMBRYOS, CAMs AND GAP JUNCTIONS

1) Gap junctional communication can be relatively non-specific (crossing species barriers) but it can also be highly selective (as below) (Kalima and Lo, 1989).
2) Gap junctional communication is critical in development. Embryo development fails when GJ communication is disrupted (Guthrie & Gilula, 1989).
3) When CAMs (cell adhesion molecules) interact with each other or their receptors, the ensuing cell adhesion appears to lead directly to gap-junctional communication. CAM interaction precedes GJ insertion and both are necessary for normal development (Jongen et al., 1991).
4) Embryos are made up of a number of compartments. Communication through gap junctions is constricted at their boundaries. These compartments correspond to important developmental fields (Kalima & Lo, 1989). They also correspond to fields of specific CAM expression (Keane et al., 1988) and homeotic gene expression (Coelho & Kosher 1991, Risek et al, 1992, Martinez et al, 1992).
5) The gap junctions in these compartments are of two sorts (Kalima & Lo, 1989). First, there are high permeability junctions joining each cell within a compartment. These allow the free passage of larger molecules: lucifer yellow is used to demonstrate this. I suspect that this "open" communication enables a block of cells to be organised, as if it was a single block of cytoplasm (a super-cell) . This may be under the control of the appropriate compartmental homoeotic genes (look at the complex structure of paramecium to see how structuring this block might work - the open cytoplasm of multinucleated drosophila eggs is similar). Second, there are more restrictive junctions which join the cells at the boundaries of these "open" compartments. These only allow small molecules to diffuse (eg, ions) so they are either insufficiently large or insufficiently extensive to allow lucifer yellow to diffuse freely. These junctions allow ions to pass in either both or just one direction. The second sort are rectifying and they correspond to junctions formed from hybrid connexons (Werner et al., 1989, Barrio et al., 1991). This directionality may be of significance in the way that embryonic cells sort, with endoderm to centre and ectoderm to the outside.
6) Despite its name, N-CAM is not confined to neural tissues. Whilst it is expressed strongly and for long periods in neural development, it is also expressed, more transiently, in other sites. It is a recognised IgSF member (Immunoglobulin Super Family). A number of authors have considered these

IgSF CAMs to be the probable ancestors of immunoglobulins, T-cell receptors and histocompatibility antigens.

When embryo cells are disaggregated and allowed to resettle, they reaggregate into tissue layers, ectoderm to the outside, mesoderm to the middle, then endoderm to the centre (Garrod & Nicol, 1981 and refs). When embryonic cells from two mammalian species are mixed, they reaggregate into tissue type rather than species type and this appears to be because the genes which specify the various CAMs are highly conserved across the species barriers (Takeichi, 1990).

MEMBRANE HOLES

It is now possible to make a stab at the general principle which governs HS/OTHS discrimination. I suspect it goes something like this:-

"SELF is established by making holes in the membranes of apposing cells and lining them up to create gap junctions. This allows cells to become electrically coupled and so to act as an electrical and, probably, a metabolic synctium. This ability to couple membranes dates back to the very earliest multicellulates. It relies on the controlled, ordered, simultaneous, adjacent membrane insertion of membrane holes. Cells learned, from the start, to encourage the uncoordinated, bigger, higgledy piggledy insertion of leaky holes into organisms which fail to demonstrate the membrane LIGANDs used as a focus for the tidy construction of gap junctions: the resulting electrical discontinuity and a lower membrane potential leads to an attack by scavengers. Unhealthy self cells can elect to be rejected by uncoupling from adjacent cells then dropping their membrane potential: they can also abandon the membrane LIGANDs which specify self. The mechanisms for constructing leaky holes (complement MACs) may, therefore, be distantly related to the mechanisms for constructing gap junctions."

HORROR AUTOTOXICUS & MORPHOSTASIS

One result of relying on self(cell) recognition is that "horror autotoxicus" (HA - the horror of attacking self) will probably have evolved long before lymphocytes and their memory for previously encountered antigens (anamnesis). However, this HA must be based upon the possession of specific and recognisable cell surface markers ("flags"): these probably aid the cooperative "docking" of one cell with another. Furthermore, because infection, cell damage, mutation, aging, genetic errors and other cell disturbances can also be assumed to be ancient problems, cells of the ZDC probably learned, early on, to observe "horror autotoxicus" to HS cells whilst rejecting, or sometimes just ignoring, OTHS (unhealthy self [UHS] and clearly foreign cells/organisms).

This interpretation of "horror autotoxicus" differs from the classic one in which lymphocytes are deemed to be denied the right to attack self epitopes. In this new interpretation, lymphocyte aggression towards self epitopes is neither denied nor necessarily avoided. However, as will become apparent, once such auto-aggression has arisen, it will decay unless other circumstances actively sustain it.

PHAGOCYTES and DOUBLE-THINK

There is a strange double-think that pervades immunology when it comes to the importance and centrality of phagocytes and the recognition of non-self and/or unhealthy self. Every medical student learns that phagocytes recognise dead, damaged, sick and effete cells. They also learn that phagocytes can recognise foreign organisms and eliminate them (particularly non-dedicated-pathogens).

Every text book devotes its statutory (short) introductory opening to the critical importance of phagocytes and innate immunity: then, almost without fail and with what I regard as indecent haste, authors are seduced into an intense dissection of the principles of anamnesis and lymphocyte function. What makes this more bizarre is that the anamnestic immune system isn't essential to prepare cells for phagocyte attention. The phagocytic system works well, even if slowly, in invertebrates: and so does self/non-self discrimination.

There cannot be much doubt that the reason for this tendency to overlook the fundamental centrality of phagocytes is, first, a relative lack of understanding of the mechanisms of self/non-self discrimination by these cells and, second, the intense acceleration of the inflammatory process induced by lymphocytes. This greatly enhances the efficiency with which OTHS is removed and it has led us, for a long time, to regard lymphocytes as masters rather than servants of the system. There is, at the very least, a possibility that CAM interaction and junctional communication, between phagocytes and underlying somatic cells, may be the most important factor in (inflammatory) HS cell recognition. Furthermore, we have been preoccupied in looking for evidence of non-self recognition rather than healthy self recognition.

INFLAMMATION

Metazoans have evolved an ancient and virtually universal defence mechanism in which somatic tissues become infiltrated with scavenger cells (mostly phagocytes) whenever required. These scavengers are clearly capable of recognising most foreign organisms, particularly those which are not dedicated pathogens. And, in the vast mass of animal life, they appear to do so without the aid of cells which have the ability to "remember" epitopes. They also remove aging and disordered self cells. In fact, their behaviour is ideally suited to eliminating OTHS. I propose two things:

(a) In all complex metazoans, the discrimination of OTHS from HS by phagocytes remains a central and crucial morphostatic process.

(b) All other immune processes are geared to accelerate, accentuate and maximise the discrimination of OTHS from HS by phagocytes. In consequence, the efficiency with which OTHS is removed is greatly enhanced.

Even so (as you will see later) HS/OTHS discrimination does not begin in phagocytes but in somatic cells. It is the consequence of general cell recognition and communication. Inflammation is only established when somatic cells "decide" that they cannot cope alone and "invite" these scavengers in. Static somatic cells are attached to each other at cell junctions. Their cytoplasms are joined by gap junctions (except in those cells whose mature function depends on electrical excitability). When membrane junctions are split apart the disruptions in the cell membranes probably lead to the release of various eicosanoids (prostaglandins etc). This announcement of an OTHS event, by somatic cells, results in an inflammatory reaction. Chemical messengers released at the OTHS site encourage the ingress of phagocytes through the endothelial cell linings of local post-capillary venules. Phagocytes now invade the OTHS site. They begin assessing cells on the basis of their HS status. Note that in electrically excitable cells, like neurones, their terminal differentiation requires that they uncouple from each other: it is left to unusually tightly bound endothelial cells to restrict the ingress of scavenger cells and thus reduce the susceptibility of these tissues to inflammation.

Thus far, the basic process is the same for almost every, if not all, animal species. At this point, vertebrates enrol a new mechanism. Debris from local tissues is processed by phagocytes (or phagocyte related cells) and it is then presented, in local lymph nodes, to the anamnestic immune system as short representative peptides in combination with class II antigens. The aim is to select representative Class II/peptide epitopes and then arrange to retain a memory of them and their inflammatory environment so that, on their next encounter (which must, incidentally, follow phagocyte/APC processing), this inflammatory environment can be rapidly and potently reproduced and, more often than not, exaggerated. This anamnestic response is under the full command of the morphostatic process and, in particular, largely under the control of phagocytes.

MIMICRY

Because morphostasis has always relied on self recognition, dedicated pathogens need to use mimicry (or more subtle interferences with identity molecule expression and recognition) to gain access to and persist in the soma (eg, Murphy, 1993, Chakraborty, 1988, Vanderplank, 1982, Yoshino & Boswell 1986). Every animal needs to stay one step ahead of its competition. Constant pressure is exerted to expand the variety of identity molecules available within a species (pleomorphism). Somatic cells appear to recognise each other by developmental ligands (cell adhesion molecules, CAMs). When embryonic cells from two mammalian species are disaggregated, mixed together and allowed to settle, they segregate into tissue type and not into species. Somatic ligands have probably needed to stay constant over countless meiotic generations. This makes them a sitting duck for determined pathogens. So, somatic cells need a "back stop" identity to be used as a second check when things go wrong (phagocyte based and, perhaps, also Mhc Class 1 based (Versteeg, 1992)). And until they do go wrong, inflammatory cells can be confined to the vascular system, locked out behind tight endothelial cell junctions until invited in. Note that "loss of function" is a cardinal feature of the inflammatory process.

UNHEALTHY SELF ACTIONS: APOPTOSIS AND SELF SACRIFICE

When cells fail to establish communication, membrane reactions probably begin which lead to the release of a variety of eicosanoids and other cytokines (Bach, 1988). Similarly, when cells become unhealthy they break junctional communication and become prey to attack by both adjacent cells and the inflammatory cells which are (in consequence) called into the area (Loewenstein & Penn, 1967). When I first started thinking about self(cell) surveillance, I found scant literature describing elective suicide and I even looked at plants for evidence of this (the hypersensitivity reaction (Prusky, 1988, Fritig et al., 1987). However, interest and literature on this subject have become abundant recently (Bowen & Lockshin, 1981, Cohen, 1991, Ellis et al., 1991, Young, 1992). In synthesis, individual cells do decide that they are sick and/or redundant. They do have the capacity to invite attack by adjacent cells and also to invite phagocytes along to have themselves removed. There is no need to presume that antibodies and lymphocytes are responsible for the primary assessment of (healthy) self status.

Changes in the concentration of calcium ions within the cell are all important in this election for "disposal by consensus". Ca++ ions act as second messengers for a variety of cell processes including apoptosis, nuclear division, growth factor stimulation: they are closely tied into the inositol-PO4/DAG/protein-kinase-C network of intracellular second messengers (Hollywood, 1991, Evans & Graham 1990): and high Ca++ ion concentrations close down the gap junction channels between cells. In this respect, cellular

identity and cell health is all tied into proto-oncogene activity and this in turn into gap junction formation and communication competence (Yamasaki et al, 1988, Yamasaki 1990).

When cells are attacked by C9 or perforin, they are made leaky, their cytoplasmic membrane potential falls and Ca++ ions are allowed into the cell.

THE GENERATION OF SPECIFICITY

A major problem in understanding the evolution of anamnestic immunity is how such a complex system erupted onto the evolutionary scene, so suddenly and so completely, in the vertebrates. One posible explanation is that it evolved, not as a generator of receptor diversity but as a generator of receptor specificity. The table below shows how a scavenger cell could be programmed only to cooperate with self cells which display ligands unique to that single ZDC. The specification of such a scavenger is an exact inversion of the specification of the cytotoxic T cell. Even a repertoire of receptors as few as two would be useful in specificity whereas, in diversity, it is difficult to see how any useful function could have evolved until there was a large repertoire of possible receptors. With a system which develops on the basis of specificity, there would be ample time to develop an extensive repertoire of possible receptors before being precipitously "flipped around" to service a generator of diversity. Note that "pure self" is used to indicate unaltered, self Class I Mhc antigens.

TABLE 5

Cell type	Receptors disabled	Receptors enabled	Normal state	Triggered state
Scavenger (Tnk like)	non pure self	pure self	aggressive	passive
	GENERATOR OF SPECIFITY			
Tc cell	pure self	non pure self	passive	aggressive
	GENERATOR OF DIVERSITY			

There are two possibilities. First, that the ancestors of the T cell receptor may have been used to recognise tissue CAM ligands: this could be the origin of the V gene segments (Allison & Havrin, 1991). Secondly, a descendant of the simple scavenger (phagocyte) may have evolved to recognise a set of pleomorphic CAM like markers which were specifically evolved in a population for them to be used as a back stop identity check unique to each ZDC. Developmental CAMs seem to remain constant over countless generations and this is reflected in the way embryonic cells from different species reaggregate as germ layers and tissues rather than species. The "back stop" CAM like ligand (the precursor of the Class I Mhc antigens) could deliberately borrow bits and bobs from these developmental CAMs to form a unique looking ligand by using a genetic mix and match process.

There seems to be little likelihood that phagocytes are able to rearrange their genome to form specific receptors. And there is no substantive evidence that they can selectively cooperate with cells carrying self Mhc antigens. Natural killer cells, however, might be such a candidate, particularly if they are composed of two populations: one with a lower specificity - perhaps based

on beta-2-microglobulin expression - and another with highly specific receptors for self. They were first identified because F1 Tnk cells attacked parental cells (unlike the classical transplantation laws). This would be consistent with specific (cooperative) recognition. These cells also preferentially attack cells expressing low levels of Class I antigen and beta-2-microglobulin. It seems that, at most, only a proportion of Tnk cells rearrange their receptor genes. (See Trinchieri, 1989 and Versteeg, 1992).

Phagocytes, lymphocytes, fibroblasts and platelets are all derived from the same stem cell. They have almost certainly all evolved from a primitive, ancestral scavenger. Each cell type seems to have caricaturised some specific property of this general scavenger and refined it in order to make the mature mammal's repertoire of responses more versatile. This adds weight to the proposition that a phagocyte like or derived cell might, at one stage, have evolved to have the ability to select/rearrange its genes so that it could specifically recognise healthy self ligands (Mhc "Class-I-like" ligands). The self receptors would have to be selected, in embryo, to be specific to each individual.

One possibility is that, now the lymphocyte system has evolved, this has been so successful that it has largely obviated the need for a scavenger to rearrange its genes to uniquely recognise self. There might even be a positive advantage in achieving the apparent recognition of HS(cells) by inverting the cooperative recognition of self cells into an attack on non-self(epitopes) by Tc lymphocytes. This can be achieved by the clonal elimination of any lymphocyte capable of reacting with "pure self" Class 1 ligands.

Note that complement activity is very much in the style of a horror autotoxicus, with healthy self being protected from attack by inhibitors: and also that phagocytes synthesise enough of all but the terminal components to attack undesirable cells without the aid of circulating complement.

SOMA/SCAVENGER SEGREGATION

I have already alluded to soma/scavenger segregation. The important point to grasp is that somatic cells can and do deal adequately with a fair proportion of OTHS (Young, 1992). Provided the accumulation of OTHS is mild and the local cells can both recognise any loss of HS identity and discriminate foreign organisms from HS, then there is little need for a back stop identity check. HS here is established by displaying appropriate tissue CAMs which lead on to the establishment of a "synctial" communication through GJs. However, when UHS or foreign organisms fail to appear sufficiently OTHS to the local cells, then tissue damage will probably ensue as the foreign cells or UHS cells start to gain the upper hand. It is at this stage that scavengers are "invited" in and this is done by a fail-safe device (the eicosanoid system - prostaglandins etc). These scavengers then establish HS status by employing a "back stop" check on identity. If there is a scavenger which formally recognises HS Class 1 status then this would give the system a highly specific way of recognising self once invoked (eg, the Tnk cell (Versteeg, 1992)).

Inflammatory cells invade and disrupt the normal structure of tissues and this invasion leads to loss of function. They are undesirable intruders in healthy tissues except in small numbers. Hence they need to be kept largely locked out, behind a tightly bound cylindrical pavement of endothelial cells lining the blood vessel walls. This need for segregation is almost certainly the origin of the vascular system. The subsequent recruitment of the vascular system into distributing other "freight" has meant that phagocytes and their evolvents have become adapted to such tasks as encapsulating the inflammatory

process (by clotting factors and platelets), distributing fats in the blood (phagocytes), anamnestic immunity (lymphocytes) and transporting oxygen (red cells).

Now it is possible to add some concluding comments to the six points introduced earlier in the section "EMBRYOS, CAMs AND GAP JUNCTIONS":

7) In this hypothesis I have suggested that scavenger cells (phagocytes mostly) use a CAM receptor molecule to latch onto a respective CAM on self cells. The base of a phagocyte (uropod) remains attached to the underlying tissues. This base probably maintains electrical contact with the underlying cells through GJs. The cytoplasmic fingers of a phagocyte (the lamellipod) constantly probe forward. If these fingers encounter a cell which is not in electrical continuity, the scavenger could be triggered into aggression by the capacitative current which flows as the membranes come close together. This could, in turn, trigger an action potential to arm the cytoplasmic finger of the scavenger cell. Additional recognition strategies may be employed. The changing of surface sugars in sick cells is one (loss of the negatively charged sialic acid residues may increase the capacitive current above the triggering threshold). The phagocyte may well have a limited "hit list" of receptors which recognise markers which are indubitable evidence of their non-eucaryotic origin and which would, therefore, never be found as part of self. Dedicated pathogens will, of course, studiously avoid displaying these.
8) Now, the original self CAM may gradually be found to be inadequate as a back stop identity check because various pathogens discover ways of mimicking or interfering with its machinery. At this stage, a new cell is required (perhaps similar to the natural killer cell) which can recognise a more pleomorphic set of CAMs that are deliberately individualised in each animal of a population and more or less unique to each ZDC. An appropriate set of specific receptors would have to be selected, in embryo, to recognise these unique ligands. These, I contend, may be the close ancestors the T cell receptor which led, by inversion of function, to the cytotoxic T cell. In this vein, note that tumour necrosis factor and lymphotoxin are selectively toxic to cells which are not communicating through gap junctions (Fletcher et al., 1987, Matthews & Neale 1989).

ANAMNESTIC AMPLIFICATION

So, what is the function of lymphocytes: what are they doing? An individual lymphocyte is simply following orders from an antigen presenting cell or phagocyte (in conjunction with an unhealthy somatic cell in the case of Tc cells). This instructs it to attach either an aggressive or a suppressive action to its paratope and to act accordingly on its next encounter with its respective epitope. Direct killing is not the prime function in either delayed type hypersensitivity T-cells (TH1) or helper T-cells (TH2). They are not remembering epitopes for the prime purpose of "killing" them. The precursor lymphocyte logs the context in which it first "sets eyes" on its epitope. If it was inflammatory then at the next encounter it will attempt to recreate a rapid and potent inflammatory response rather than wait for the "cell damage -> cytokine -> inflammation" cascade to build up. "Tipped off" inflammatory cells can then settle down much more quickly and aggressively to their phylogenetically ancient task of sorting HS from OTHS. The main difference now is that these phagocytes are doing it much more quickly and with better targeting. But, they are also doing it more hamhandedly - they'll "bash" anything that looks remotely suspicious (hence the need to focalise this response). Tc cells are relatively more independent and kill directly but even these are only allowed to become aggressive if they have first been

primed by IL-1 released from APCs during an inflammatory encounter. And these, too, encourage a rapid inflammatory response once they start attacking target cells.

Somatic cells probably show some specificity for the epitopes that they present for Tc cell priming. The peptides that they present in combination with Class I antigens have probably been shepherded through the cell by its garbage minders, the ubiquitins. Even leaving this aside, it is still easy to imagine how self/non-self selectivity can occur. When T-cells are released from the thymus they are already committed in specificity (ie, they are committed to recognising a specific epitope) but, they are not committed in activity (aggression or suppression). It is only when they meet their respective epitope that this commitment is made. Self epitopes are, in general, encountered frequently and the first encounter (in embryo) is nearly always in a "healthy self" (non-inflammatory) environment. So tolerance is generally favoured for those lymphocytes which recognise self molecules. Few self specific T-cells will remain uncommitted for more than a brief period while there is a relatively large pool of the relevant self epitope waiting to be encountered.

On the other hand, because only small quantities of a foreign or strange epitope are infrequently met in the body, most T-cells capable of recognising them will remain uncommitted until they meet the epitope, as part of OTHS, in an inflammatory encounter: aggression will be favoured. Furthermore, it seems that it is easier to provoke old rather than young precursor lymphocytes into aggression. This further concentrates the aggressive response onto those epitopes that are most strange to the body. No veto need be imposed on T-cells to prevent them becoming aggressive to self epitopes (except for "pure self" Mhc ligands - these must be clonally disabled). Indeed, epitopes from tissues that are usually hidden behind tight endothelial cell junctions (like the eye and brain), and are infrequently encountered, are more likely to provoke aggression as there will be a larger pool of uncommitted T-cells available. They are, consequently, more inclined to provoke an aggressive response when they are exposed during periods of intense inflammation. (Lymphocytes which have a paratope for recognising certain self Mhc/peptides are clonally deleted in the thymus: this deletion follows the disintegration of self cells in the thymic medulla.)

The bone marrow constantly produces new uncommitted T-cells. So, whenever clearly foreign epitopes are sparse and inflammation is intense and prolonged, attention can gradually turn to self epitopes (eg, as in tuberculosis). In summary, inflammatory acceleration is most likely to develop to clearly foreign (strange) epitopes and a "healthy soma tolerance" most likely to develop to self (frequently encountered) epitopes.

The overall effect is that lymphocytes remember the "inflammatory" or "healthy soma" context in which they first meet their respective epitope (and become committed); and they aim to recreate and caricaturise this memorised inflammatory or non-inflammatory milieu at the next encounter. Whenever TH1 cells provoke an inflammatory response they call large numbers of phagocytes (& Tnk cells?) to the epitope site. These are then switched into a heightened state of "anger". However, phagocytes (& Tnk cells?) still have to discriminate HS from OTHS but now, the threshold at which aggression is considered is greatly reduced. Cells expressing a relatively low level of "HS identity" are now likely to be attacked. This amplification of the inflammatory response by lymphocytes has the potential to escalate catastrophically. It can slip into a loop of strong positive feedback,

particularly when the epitope is an abundant self Ag. When the local auto-rejective response becomes excessive, it must be down-regulated otherwise things will get disastrously out of hand. This could be done in a number of ways and these may account for many instances of clinical anergy (Dwyer, 1984, Meakins, 1988, Meakins & Christou, 1979, Normann et al., 1981, Ninneman, 1981):

TABLE 6

(a) inhibition of phagocyte ingression (chemotaxis)
(b) inhibition of phagocyte aggression
(c) inhibition of further aggressive lymphocyte activation
(d) a tightening of endothelial cell junctions
(e) encapsulation in a fibrin sheath (fibrocytes later)
(f) promotion of lymphocytic tolerance to typical Ag
(g) production of auto-antibodies to the newly cloned, locally reactive lymphocytes (lymphocytotoxic Abs)

TABLE 7

THE FOUR PRINCIPAL MODES OF EPITOPE PRESENTATION

	OTHER THAN HEALTHY SELF CONTEXT	HEALTHY SELF CONTEXT
SOMATIC CELL	Tc activation G (Class I Mhc)	Ts activation G (Direct??)
PHAGOCYTIC CELL	TH1 & TH2 activation (Class II Mhc)	Ts activation (Like T/B cell co-operation? Th/Ts)

AUTO-REJECTION

Tissue rejection is largely accomplished by cell mediated mechanisms. Antibodies are generally bystanders. Similarly, the auto-rejection of abnormal cells will be accomplished predominantly by cell mediated immune mechanisms (eg, in various forms of necrosis like burns and infarction). There is one important inference to be made from examining the structure of the sero-negative arthritides and particularly Behcet's syndrome (based on a personal study). This is that auto-rejective disease covers a wide spectrum of prevalence and severity. The mildest components are VERY common, suggesting that auto-rejection is a normal process. This leads on to the conclusion that there is no automatic horror autotoxicus to self epitopes where T cells are concerned. When auto-rejection is so general, it has to have physiological as well as pathological significance: it must be a functioning element of the morphostatic mechanism.

ANTIBODIES - ICING ON THE CAKE

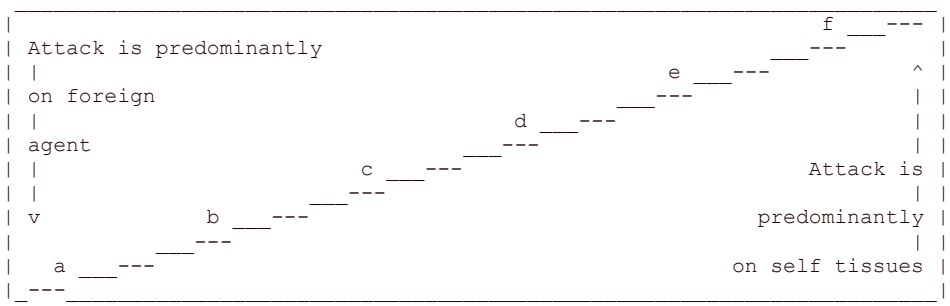
Antibodies are icing on the cake. Extremely useful, evidently important but dominantly aimed at pre-empting the proliferation of blood borne pathogens and pathogens which colonise epi/endothelial surfaces. It's clear that the role

of antibodies in tissue rejection (and hence auto-rejection) is minor if not minimal. The vast mass of animal life copes well without them. "Cell-mediated immunity clearly precedes humeral antibody production in phylogeny" (Manning and Turner, 1976 also emphasised by Cooper, 1982). We can safely put antibodies to one side until towards the end - which is more or less where they evolved. It appears to me that, to bother looking amongst antibodies for an explanation of how self/non-self discrimination evolved, would be manifestly Heath Robinson (or Rube Goldberg!). In this vein, it is worth noting that the spleen may be specifically adapted to make the best of the difficult job of maintaining morphostasis in the suspension of cells circulating in the highly mobile plasma.

THE CLINICAL IMPLICATIONS

The result of all this is that any disease which evokes an inflammatory response has an element of auto-rejection. It inevitably consists of a mixture which varies from an attack directed almost exclusively at the pathogen (usually leading to mild inflammation) to an attack directed almost entirely at self (often highly inflammatory): the latter occurs when organisms or cells provoke prolonged inflammation but do not provide or present clearly foreign looking (unusual) epitopes. Every disease that leads to cell damage will induce auto-rejection. Since heat shock proteins are responsible for chaperoning disrupted proteins through the cell, they are frequently presented as potential epitopes in UHS presentations.

TABLE 8



EXAMPLES

(a) Saprophyte
(b) Simple epithelial commensal
(c) Staphylococci and streptococci
(d) Tuberculosis and syphilis
(e)-(f) Multiple sclerosis and sero-negative arthritis

MORPHOSTATIC EVOLUTION

(As Morphostasis and immunity)

THE ADVANTAGES OF THIS PERCEPTION

By now I hope that you will be aware that all this suggests a clear path in self/non-self discrimination. Its beginnings can be seen in simple animals like sponges, which demonstrate differential cell reaggregation (for they, too, have gap junctions) and it proceeds through to the complex mammalian immune system. In this respect, it is interesting to read that differential

sorting is, in embryos, a direct consequence of CAM expression (Takeichi, 1990). The reasons why embryonic cells sort according to tissues rather than according to species is that their CAMs have remained highly conserved across widely separated species.

1) Seamless integration from embryonic development to anamnestic immunity.
2) The innate and the acquired immune system are no longer seen as fundamentally disparate entities. They are fused into a seamless whole.
3) A clearer understanding of preferential alloreactivity by T cells.
4) A clear evolutionary progression from organisms with no cellular differentiation, through simple organisms with phagocytes, then the evolution of a retinue of specialised cells all derived from the primitive scavenger. A "logical progression" would start with Tnk like cells, go to Tc like cells, then TH1 like cells, then TH2 like cells and finally B cells.
5) A far clearer perception of the cancerous process (not detailed here but there is good evidence that gap-junctional communication is involved (Yamasaki et al., 1988, Yamasaki 1990).
6) The potential to explain the process of aging (Kelley et al., 1979, Peacock & Campisi, 1991).
7) It seems a good common sense explanation.

SUMMARY

I have proposed reshaping the perception of immunity to encompass the broader principle of MORPHOSTASIS. The loss of healthy self is sensed and expressed by the malfunctioning cell itself or, at furthest, emanates from the membrane doublet where contact is established between this cell and its immediate neighbours. This "foul" is broadcast by the release of inflammatory mediators. These invite phagocytes into the area to assess the local population. Phagocytes (and perhaps Tnk cells) then attack those cells with which they fail to become electrically continuous. The time they have to make this connection varies with the "anger" of the phagocytes. Phagocytes now present cell debris to lymphocytes in local lymph nodes. The epitopes which are most strange to the lymphocytes are selected to act as the pegs on which to hang a greatly accelerated inflammatory infiltration on any subsequent encounter of these epitopes.

I have also proposed redefining the concept of "horror autotoxicus": it is established by successful cell to cell communication. Both somatic and scavenger cells use this mechanism. The concept of immunological surveillance is simultaneously redefined. But now surveillance is for any malfunctioning cell and not just for neoplasia. The evolution of a thymus dependent lymphocytic system with memory may have occurred at the expense of an increased prevalence of cancer, for intense focal suppression of surveillance now occurs whenever a strong positive feedback leads to an exaggerated attack on self epitopes. This then permits a tumour cell compartment to reach a critical mass beyond which surveillance fails (Yamasaki, 1990).

There is little doubt that this explanation contains errors and I am sure some of the more specific assumptions will prove to have been far too simplistic. For example, the immune system has gathered a great number of refinements throughout its evolution including various specialised phagocytes and permanently resident, non-itinerant antigen presenting cells: little has been said about these. However, I am confident that the "flavour" of the concept is essentially correct and the hypothesis will prove to be a useful framework for refinement. It should now be clear that the breaking of cellular junctions is

probably an important event which leads on to the declaration of an OTHS "foul". There are a number of close similarities between the insertion of gap junctions into self cell membranes and the insertion of complement membrane attack complexes into invaders. If it could be shown that there is a continuing or a distant relationship between their respective insertion mechanisms, then it would be reasonable to assume that HS is, indeed, sensed by the speed with which both somatic cells and scavenger cells establish an electrical continuum with those cells that they encounter.

19941029_morphos

"There is only one constant element in immunity, whether innate or acquired, and that is phagocytosis. The extension and importance of this factor can no longer be denied."

Elie METCHNIKOFF 1905 [1]

"Immunology is an invention of the devil, who is making it up as he goes along because he's not too clear about this stuff either.". . . . ."Besides, immunology is what we North Americans call a Rube Goldberg system, referring to old cartoons about how to turn on the light, for example: you trip over a footstool, thus startling the cat, who bumps into the kitchen door, which swings shut, knocking over a chair that hits the light switch . . . you get the idea. There has to be an easier way."

Janice Hopkins TANNE 1990 [2]

INTRODUCTION

I would like you to share my thoughts on the function of the immune system. It is a synthesis which you may find preposterous, particularly as it comes from someone so remote from academic immunology. However! Could it be that our current perspective is confusing? And that there is a far better one that can make the whole process much clearer? Currently, the functions of inflammation, phagocytes, accessory cells, lymphocytes, antibodies, and all their paraphernalia seem fragmentary and diverse rather than coordinated and integral. This may simply be because we are looking at it all from the wrong angle. At the very least I hope, that when you have read through this, I will have introduced some new concepts that will lead you to reconsider your current perception of the immune process.

It would be a long winded process if I tried to describe the route by which I reached this synthesis. So, corners will be cut, sparse attempt will be made to justify some statements* and this presentation (a challenge to conventional perspective) will often be relying on its final roundness. I will begin with a brief rumination on the nature of immunity in single celled organisms and then consider the principles that are likely to have evolved on the way towards the mammalian immune system: the latter has the sophisticated capacity to remember antigens which it has previously encountered (anamnesis).

*(It would be intrusive on style and too cumbersome to justify every presumption as it appears in the text. Instead it has been written in an authoratitive and conclusive style. The reader MUST look to the references and his own knowledge before he accepts the validity of any statement in this article.)

PART 1: THE GROUND RULES

SINGLE CELL IMMUNITY & EARLY ANIMAL IMMUNITY

We can assume, with some certainty, that viral and bacterial infections evolved long before the advent of animals constructed from colonies of cells. Similarly, we can assume that single cells evolved appropriate defences long before these colonies (metazoans) appeared. Independant single cells will almost certainly have evolved methods of dealing with and, where possible, pre-empting infection. They will also have evolved ways of repairing and regenerating damaged cytoplasm and membrane.

With the advent of animals constructed from a colony of cells it probably proved to be an imperative that the individual cells belonging to a colony should behave in an impeccably cooperative manner. In this colony of cells (all derived from a single zygote, the Zygote Derived Colony - ZDC), misbehaved or dangerously altered cells would need to be rapidly eliminated from the colony or else risk serious disruption. The only cells to be tolerated would be "Healthy Self cells" (subsequently abbreviated HS). The converse is equally true - all "Other Than Healthy Self" (OTHS) cells are undesirables: they must be efficiently removed. OTHS includes both unhealthy self (UHS) and non-self (NS). In the early stages of metazoan evolution it would not be practicable to learn and remember the individual identities of all cells or proteins which could, potentially, be OTHS. It would be far more expedient for self cells to be uniquely "labelled" and then to arrange for dead, damaged, dying, infected, mutated and otherwise disordered cells to develop a mechanism to abandon this property (footnote 1). I propose that this discrimination remains the basis upon which the mammalian immune system is still run. This implies that disordered self cells should lose their healthy self status and in so doing, be sacrificed for the benefit of the colony. A sacrificial system such as this is, possibly, observed in plants (footnote 2): argument, however, persists as to whether the response is reactive or truly preemptive.

INFLAMMATION

We know that even in the simplest of metazoans (eg the sponges) there are dedicated scavenger cells (phagocytes) which roam the colony [3,4]. It is well recognised that these cells remove foreign organisms and foreign bodies as well as a variety of abnormal self cells (effete, aging, damaged infected and malignant cells). The mechanisms by which they do this remain largely obscure but they are clearly effective at eliminating OTHS. Inflammatory mediators are equally ubiquitous: they are released whenever a cell membrane is disrupted and they attract roaming phagocytes to home in on the event [5]. Thus, the initial identifiers of OTHS are, in all probability, not phagocytes. Whilst phagocytes do attack OTHS once within the affected tissues, OTHS is probably identified by the affected cell itself and is influenced by that cell's interaction with its immediate neighbours (or more precisely, HS exists whilst a membrane doublet maintains a satisfactory communication between adjacent cytoplasms). Its presence is broadcast by the release of inflammatory mediators which encourage an influx of phagocytes. In the vast majority if not all animals, immunity revolves around the identification and elimination of OTHS by phagocytes. In this article I will be suggesting that this continues to be the prime mechanism even in mammals: lymphocytes and antibodies are only engaged to heighten the efficiency of this discrimination.

CELL TO CELL RECOGNITION

It is well established that vertebrate cells can recognise one another. The ability to recognise fellow cells is not unique to complex animals. Even as far afield as protozoans, appropriate organisms are recognised as food whilst sexual partners are recognised in a co-operative fashion. An individual cell

which possesses phagocytic ability has no problem recognising its own cytoplasm when its own pseudopodia encounter itself. In each animal, the cells derived from a single zygote (the ZDC) must have some means of recognising one another. Indeed, a number of simple multicellular animals have been shown to reject allografts, even when these grafts are from close neighbours [3,6]. In general animals and plants rely on broad (species) identities to recognise suitable sexual partners but even so, these encounters can be highly selective and specific (eg, pollination [7]). In animals which have several tissue types (e.g., hydra upwards) there is evidence of tissue site recognition (somatic recognition). In practice, like tissues preferentially aggregate together. Foetal cells do so across broad species barriers: they segregate into tissue type layers - endoderm to centre, ectoderm to the outside [8,9]. Numerous examples of cell recognition are reported. For instance, it occurs in bacterial agglutination and conjugation [10], in slime moulds [11], in sponges [3,6], in other primitive multicellulates [3,6], in plants [12], in vertebrates and in homeotherms (these are the most thoroughly investigated examples) [13]. (Molecular) recognition is a fundamental biological principle. It is evident in enzymes and restriction endonucleases. It occurs in single cell animals: they reject transplanted organelles from foreign cells [6]. In embryogenesis there is a constant recognition of cell position and destination and the selective reaggregation of previously disaggregated organ cells points clearly to an ability to recognise tissue type [8,9]. Further examples of recognition are seen during cell homing: one example is the way injected marrow cells search out the marrow and another is the way that plasma cells, entering the circulation from gut associated lymphoid tissue, home back to the gut wall [14,15,16].

Self recognition could, therefore, be observed in several ways, each becoming progressively more specific to the individual animal:-

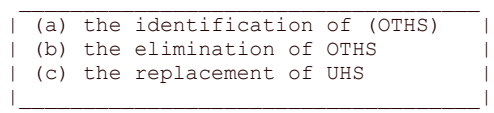
(a) tissue type recognition (germ layers and organs)
(b) species recognition (sexual)
(c) self recognition (ie, cells of the individual zygote derived clone)

HORROR AUTOTOXICUS & MORPHOSTASIS

The result of this reliance on self recognition is that "horror autotoxicus" (HA - the horror of attacking self) will probably have evolved long before lymphocytes and their memory of previously encountered antigens (anamnesis). However, this HA must be built upon the possession of specific and recognisable whole cell properties (very probably expressed at the cell surface): these probably aid the co-operative "docking" of one cell with another. Furthermore, because infection, cell damage, mutation, aging, genetic errors and other cell disturbances can also be assumed to be ancient problems, cells of the ZDC probably learned, early on, to observe "horror autotoxicus" to HS whilst rejecting or ignoring OTHS (unhealthy self [UHS] and clearly foreign cells/organisms). This concept of "horror autotoxicus" differs radically from the classic one in which lymphocytes are deemed to be "denied" the right to attack self antigens. In this interpretation, lymphocyte aggression towards self antigens is neither denied nor necessarily avoided. However, once such auto-aggression has arisen, the system encourages it to decay (see below). This decay is rapid unless some circumstance actively sustains it.

Now, the structure and function of body tissues can be maintained first by identifying and eliminating OTHS and then by regenerating any deficit caused by the rejection of UHS. This process of tissue homeostasis (MORPHOSTASIS

[17,18]) can be summarised thus:-



Before any system can control its morphological form it must first create it. The embryological events which lead to an adult animal (and also the subsequent replacement of unhealthy self (UHS)) can be described as MORPHOGENESIS. Morphogenesis is an integral part of a morphostatic system. It is, therefore, reasonable to expect that the component elements of morphostasis will use molecular machinery which is genetically related for they have (presumably) been closely associated through every epoch of metazoan evolution.

Mammalian cell adhesins (eg NCAM) have been known for a long time but it is only recently, with the isolation of their genes, that it has become apparent that at least some of these proteins are related to the immunoglobulin supergene family [19]. This suggests that histocompatibility antigens and the immunoglobulin system have evolved from simple cell adhesins (and thus cell cell recognition.

MORPHOSTATIC EVOLUTION

Now let's make some guesses about the evolution of tissue differentiation (diag 1). The animal prototypes from which metazoans evolved are the protozoa. Such cells have phagocytic (scavenger) behaviour but they can also behave cooperatively: they can distinguish between self (so their own pseudopodia don't attack self membrane), potential sexual partners and the general nature of food (organic properties - probably on the basis of membrane or cell/bacterium wall properties). Put colloquially, they can make a "self", "mate" or "meal" discrimination.

It is important to remember the colonial nature of all multicelluates. A mammal is essentially a colony of zygote derived clonal cells which have built themselves a scaffold of various connective tissues. This scaffold allows the colony (animal) to become highly versatile and the whole structure ultimately acts as the platform from which the originating zygote propagates its genome. This "platform" also encompasses individual, herd and social behaviour.

The first level of animal complexity was probably a structureless colony in which there was no cell differentiation and where the cells simply adopted a gregarious behaviour. To start with there was probably no specialisation of cells into phagocytes or soma (all cells would have kept strongly phagocytic functions). However, when this specialisation did occur at least two identities were possible: soma/soma recognition and phagocyte/all self recognition. Where is the point in splitting the general recognition of self into somatic and phagocytic? Well, it would certainly free somatic cells from the (itinerant) chore of "cleaning up" so leaving them to concentrate their identity systems on tissue building and the subsequent construction of a connective tissue skeleton.

Once healthy self is uniquely labelled in the colony, foreign organisms can be easily identified unless they evolve a way of interfering with or mimicking the self identity code. This means that there will always have been pressure in a "herd", or at least within a species, to produce a variability (pleomorphism) in the molecules signalling identity in individual ZDCs. Individual

variability, however, would be an embarrassment to the soma. Here, constant identity over many successive generations is more important (this is reflected in the way that foetal cells from different species, once disaggregated and mixed up, preferentially reaggregate as tissues rather than species). We can now envisage the situation, in evolving animals, where cell RECEPTOR/LIGAND evolution was separated into phagocyte and soma based mechanisms. For phagocytes to recognise healthy self in a highly specific way, particularly if this identity is specific to a single individual, they would need to select an appropriate RECEPTOR from a diverse repertoire of RECEPTORS, most of which will be rejected, and these would have to be generated over the course of a number of successive mitotic divisions (for they would need to evolve in the early stages of an animal's lifetime). Somatic cells, however, would need to select their appropriate RECEPTOR from a diverse repertoire generated over many successive meiotic divisions (and, unlike the former, the LIGAND and RECEPTOR could be the same, ie, the molecule may be able to recognise itself). Having made this division, somatic identity can be relied upon until OTHS is identified within single cells or by their neighbours (membrane disruptions alone are enough to set off the inflammatory process). The subsequent ingress of inflammatory cells should shift the identity check from one that relies solely on somatic identity to one that is policed by phagocyte sensed identity.

We now have a situation where soma and phagocytes rely on different recognition strategies. There is no need to have tissues constantly pervaded by phagocytes. "Foul" is called by somatic cells which release inflammatory mediators in response to a significant OTHS event. Provided that an efficient delivery system is available, phagocytes can be kept locked out of the soma until things go wrong. This probably had an important influence on the evolution and emergence of vascular systems and endothelial cell behaviour.

The specialisation of tissues, based on the possession of unique tissue identities, can begin at the point where cell lines divide into scavengers (phagocytes) and soma. The genes coding for the LIGANDs associated with somatic cells can duplicate on the chromosome and then diverge, allowing a gradual diversification of RECEPTOR/LIGAND interaction (eg specifying endoderm, mesoderm, neuroderm and ectoderm). Tissue homeostasis (morphostasis) can now be regulated and sensed through appropriate interactions with these LIGANDs. Once somatic cells fail to interact normally, they will abandon healthy self identity and inflammatory mediators will be released. These call in phagocytes which then locate and remove those cells with altered identity (disrupted communication probably going hand in hand with this altered identity). This change will be assessed by phagocytes on the basis of LIGANDs unique to the individual (or at least relatively so and in contrast to LIGANDs unique to the tissue: LIGANDs specifying the latter will need to be kept constant throughout the species and it seems that they may even be largely conserved across broad species barriers). It is clear that the early part of the alternative complement cascade results in membrane changes which the phagocytes interpret histocompatibility genes in a wide range of vertebrates (man, mouse frog and others - in fact they are sandwiched between them in both man and mice!). Since class I genes may have arisen as a refinement of the phagocyte recognition system (see later) their continued genetic linkage is noteworthy.

In animals which have a vascular system, scavenger cells (phagocytes) are kept locked out of the soma, behind endothelial cells and their tight junctions, until such time as a local inflammatory event invites a scavenger cell invasion.

Once soma and phagocytes have parted company, the identity system can evolve

further. There is no longer any reason for the somatic LIGANDs (and their RECEPTORs) to remain genetically linked to the scavenger LIGANDs unless there is a positive reason for this to happen. Such pressure could be envisaged if the diversity that is used to specify tissue identities, and which has been developed over LONG periods of time and over countless generations, is borrowed, by a mix and match process, in order to individualise personal animal identities.

In this way, tissue identities will have developed over millions of (meiotic) generations and will, at least in part, have contributed to species drift. These identities can be largely conserved across broad groups of species (note how disrupted foetal cells from two mammalian species reaggregate - not into species but into tissue types [8,9]).

Appropriate LIGANDS, specific to an indivual, must be expressed on all (static*) cells of the zygote derived colony (ZDC) to allow scavenger cells to recognise healthy self cells. The RECEPTOR selected to recognise this LIGAND will be used only by scavenger cells or a related subset of cells delegated to take on the recognition of healthy self (see below). (*Red cells are not normally still for long enough to enable phagocytes to remove them except when they have leaked into tissues - eg, in a bruise.)

MIMICRY

At this point I would like to digress a moment to consider the pressures exerted on these identity systems by foreign organisms. In healthy animals it is manifestly clear that OTHS is efficiently eliminated. Death and the consequent rapid onset of decay is evidence of this: dead tissues suddenly become highly susceptible to bacterial and fungal attack. The invasion of the living ZDC can only be achieved if foreign organisms are either highly evolved pathogens or if the invasion occurs in the wake of a physiological suppression of local rejection processes. Regular pathogens (eg human viruses, syphilis, tuberculosis and streptococcal infections) clearly use sophisticated and highly developed systems to exploit chinks in the morphostatic armour. This is made apparent by the species and/or organ specificity (and dependency) shown by many of these pathogens, particularly viruses. Inevitably, this means that the strategies used by pathogens to fool the identity machinery will, most often, be aimed at LIGAND/RECEPTOR mechanisms (so involving histocompatibility Ags, beta-2-microglobulin, complement components and developmental LIGANDs). The armament used by these organisms will almost certainly include mimicry and other mechanisms which can interfere with identity and its recognition. The constancy of the somatic LIGAND/RECEPTOR machinery across broad species barriers makes it a sitting duck for pathogen mimicry. The second tier of identity checking, based on phagocyte and/or lymphocyte sensed LIGANDs, relies upon more individualised RECEPTORs and so enables HS to be more precisely discriminated from OTHS. The ultimate aim of any species must, therefore, be to refine this personalised identity checker so that, in each individual animal, it is sufficiently unique to protect the herd. The specificity of this identity check could remain the immediate property of the phagocyte or be largely delegated to a subset of cells subservient to phagocytes (this is probably what has happened with Tc cells in mammals). (Evidence for mimicry is found in a variety of animals ( ) and plants (see footnote). Refs M1-n.

Tc CELL INVERSION

launched the mammalian amnestic immune system. Imagine what would happen if the function of the Tc cell was inverted (table 2). It should be clear from this table that the lymphocytic system could have developed from an inversion of phagocyte recognition of self. This would be a neat explanation of how the

system of lymphocytes and anamnesis (memory of previously encountered antigens) has evolved so suddenly and so completely in the vertebrates. It would have begun life not as the generator of RECEPTOR diversity but as the generator of RECEPTOR specificity.

Cell type	Receptors deleted	Receptors selected	Normal state	Triggered state
Scavenger	non self	pure self	aggressive	passive
Tc cell	pure self	non self	passive	aggressive

Should you doubt the likelihood of this, then recall the close ontogenetic relationship of lymphocytes and phagocytes - their myeloid origins - and remember how ontogeny frequently retraces phylogeny.

So, where is this highly specific phagocyte based recognition in mammals? If it was there to launch the lymphocytic system it would appear that it has now largely atrophied. There is patchy evidence to suggest that mammalian phagocytes may be capable of such self recognition but it is sparse and uncertain. The alternative is, of course, that the lymphocytic system has been such a successful innovation that this complicated recognition of self by phagocytes has become redundant. Once the Tc cell evolved, the highly specific identification of self by phagocytes may have become superfluous: despite the fact that the whole process is inverted in lymphocytes, the net effect may have been to produce an system more or less equivalent to specific recognition by phagocytes. This would have left the phagocytes to revert to a reliance on their more primitive complement machinery for (healthy) self identification: if this is the case, phagocytes are still relied upon to discriminate HS from OTHS after the lymphocytic system has enhanced the concentration of "angry" phagocytes at the inflammatory site. So, unhealthy self may be expressed, at least partially, by a failure to switch off the promiscuous membrane attack which characterises the alternative complement pathway. Nevertheless, it is still possible that there is an unidentified subset of phagocytes which are able to recognise HS on the basis of class I Mhc self antigens.

In an animal using this principle of specific, phagocytic recognition, appropriate phagocyte RECEPTORs would need to be selected at an early stage in development from a deliberately diverse set of RECEPTORs (much as T cells in the thymus are selected for alloantibodies and self+x). Embryonic development, within the protected confines of an egg, offers a suitable environment for such selection, locking out infection until such time as the system is mature enough to cope with it (the penalty paid for such protection is a prolonged susceptibility to predators - it is unable to use actively evasive behaviour).

To summarise, OTHS is identified by somatic cells (probably a membrane event) and its removal is accomplished by scavenger cells (phagocytes) which are summoned to the site by inflammatory mediators. Lymphocytes, mainly of the Th and Td variety, act to amplify and accelerate the accumulation of phagocytes and then to "angrify" them. This they do when they come across antigens which they have previously encountered during another inflammatory event (these antigens will be predominantly foreign - see below). This accumulation of "angrified" phagocytes still have to make a final decision of whether to leave

cells alone or to attack them and they will do so on the basis of HS identity.

Neither HS identity nor phagocyte aggressiveness are likely to be absolute "all" or "nothing" properties. This is already evident in phagocytes where aggression is increased by lymphokines, the Fc fragments of Ab/Ag complexes and various complement components. Healthy self identity is also likely to be graded: it should be at its strongest in young and healthy cells whilst weaker in aging or malfunctioning cells. Thus, while the fate of a cell may depend upon its own (or its neighbour's) perception of its health, the threshold at which rejection occurs will ultimately depend upon phagocyte aggressiveness. So, self cell rejection will increase with rising phagocyte aggression and decrease with the intensity of healthy self identity.

WHERE DO LYMPHOCYTES AND ANTIBODIES FIT IN?
to help phagocytes identify OTHS. The first point to make is that lymphocytes are capable of caricaturing the morphostatic system's treatment of previously encountered epitopes whenever they are re-encountered. If the epitope was previously met in a healthy, somatically stable and non-inflamed site then tolerance will be encouraged. Conversely, when an epitope was previously met in an intitially aggressive environment with marked inflammation, then it enhances and accelerates an aggressive phagocyte response at any site where the epitope is re-encountered. It does this by releasing agents which attract phagocytes to the site and then angrifies them. Equally, suppressor cells can suppress this aggression. Phagocyte ingression and aggression can be upregulated and downregulated according to the proportion of aggressor to suppressor cells being triggered as lymphocytes arrive to encounter their respective epitopes at any particular site. In a population of lymphocytes which have affinity for a particular antigen, the balance of aggression to suppression will depend upon the context in which the epitope is usually met (particularly first met) and the final grading will sit somewhere along the following scale:

OTHS PRESENTATION	HS PRESENTATION
Associated with an injurious or useless cell or situation	<<----------->> Associated with a harmless or useful cell or situation
(Ag processed by APCs then presented to paratope)	(Ag directly presented to the paratope without APC processing)
(INFLAMMATORY) Th Td	(NON-INFLAMMATORY) Ts

Until a lymphocyte first meets its appropriate epitope it remains uncommitted to aggression or suppression. The context of this first encounter seems to have a profound influence upon the subsequent committment to aggression or suppression for T cells are then committed to Th, Td or Ts activity. Immature and very young lymphocytes are relatively reluctant to be committed to Th or Td activity compared to more mature lymphocytes. The longer they have remained uncommitted, the more easily they can be triggered into aggression. Antigens encountered predominantly in a non-inflammatory context tend to favour coversion to Ts and those encountered in an inflammatory context favour conversion to Td and Th activity. Thus, common antigens like self are least likely to commit lymphocytes to aggression and strange antigens from newly encountered foreign organisms are most likely to do so. However, even if the antigen is totally strange, unless it is met in a strongly inflammatory

context, it will fail to evoke an aggressive response. And vice versa: if a common self antigen is presented long enough in a strongly inflammatory context, newly formed uncommitted lymphocytes will be progressively recruited into aggression (the older T cells which met this antigen prior to this inflammatory event will have been largely committed to suppression).

So, uncommitted lymphocytes aquire their paratope (binding site specificity of the antibody) spontaneously: these cells then circulate until they meet an appropriate epitope (binding site on the antigen). When individual lymphocytes meet their respective epitope they become totally committed to either suppression or aggression. But, in a whole animal there is a population of committed and uncommitted paratopes many of which may have affinity for one particular epitope. The net effect is that there will be a gradation from suppression to aggression to each epitope and this may change as new precursor cells commit themselves. This grading tends to be set by the context in which the epitope is usually met. When a particular epitope is encountered predominantly in a HS context, tolerance will be favoured, and when encountered predominantly in an OTHS (inflammatory) context then aggression will be favoured. The result can be better seen in a table of favoured responses:-

	HS PRESENTATION	OTHS PRESENTATION
OFTEN ENCOUNTERED	Suppression	Equivocal
RARELY ENCOUNTERED	Equivocal	Aggression

Where strange (foreign) antigens are reencountered during a fresh OTHS event, they will provoke a greatly amplified and accelerated phagocytic attack, thanks to the T cell amnestic system. Most self antigens, however, are usually encountered without significant inflammatory activity (particularly in embryo) and they are generally well tolerated. Common self antigens thus favour tolerance and unusual, strange antigens aggression. Since it is most likely that uncommitted, self specific lymphocytes will meet their respective self epitopes in a HS context, there will be few, if any, uncommitted lymphocytes with this affinity which remain for long in circulation and therefore available for committment to aggression. Thus the natural balence will be to favour Ts activation of self paratopes and Th/Td activation of those paratopes which recognise "stranger" epitopes (Ags). However, antigens from immunologically privileged sites are usually "hidden" behind tight endothelial cell junctions so they are more likely to be regarded as "strange" when they are exposed during spells of unusually intense inflammation (eg, sympathetic ophthalmia [20]). Foreign Ag, on the other hand, is most likely to be met in an OTHS context and the first encounter will, nearly always, provoke an aggressive response. The T-cell system thus favours the selection of the most strange Ag as a trigger for aggression and the most commonly presented Healthy Self antigens as the anchors for suppression. Note that phagocytes (and other APCs) are ideally placed to act as the commanders of other "immune cell troops". Mhc products) they are still needed to give Tc cells their "kick" into aggression through IL-1.

When aggressive lymphocytes or immunoglobulins meet and interact with their appropriate epitope, they release factors which speed up and focus the accumulation of phagocytes and then switch these cells into an "angry" mode. Even when inflammation is accelerated by Th/Td lymphocytes and antibodies, the

final decision of whether to attack or leave self cells (which are marked with an appropriate epitope/antigen) should, for the most part, still be a decision for the phagocyte and this decision should remain influenced by the HS status of the marked cells. However, the fact that these phagocytes have been substantially "angrified" means that the criterion for acceptable HS is much stricter: so as macrophages become angrier, progressively fewer borderline HS cells will escape attack. Even so, a differential aggression, maximal to OTHS and minimal to HS, should still minimise undesirable auto-rejection. Tc cells are able to attack cells independently of phagocytes but, due to the nature of their original activation, they are attacking cells which are already clearly established (by phagocytes) as a threat.

ANERGY

The fates of individual cells that make up an animal are only important in that neither their death nor their survival should endanger gene propagation, particularly in the herd. (Across the aeons of evolutionary history, those species which fail to maintain a critical "herd mass" founder: the gene pool is all important). So the (auto-)rejection of suspect cells is a logical method of housekeeping: cell deficits are, self evidently, renewable by tissue regeneration (a resurgence of morphogenesis). However, if an inflammatory process is particularly strong and there is little if any clearly foreign antigen, lymphocytes are not prevented from mounting an aggressive response to Ags typical of the local tissues (e.g., in burns [21] and adjuvant arthritis [22,23]). The resulting acceleration of tissue turnover could easily get out of hand and lead to extreme tissue destruction (auto-rejection - see below). Auto-antibodies and auto-Th/Td (T-helper and DTH) reactivity may even be useful in focusing phagocyte attention to specific tissues until a more specific response to foreign Ag has matured (e.g., say, pharyngeal antigen in a viral pharyngitis).

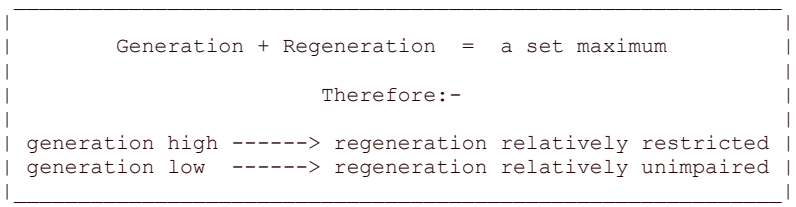
This mechanism for concentrating phagocyte attention is a positive feedback and, without constraint, it could lead to catastrophic auto-rejection. Failsafe mechanisms must exist which can be brought into play if tissue destruction becomes excessive. This could happen at any or all of the following points:-

(a) inhibition of phagocyte ingression (chemotaxis),
(b) inhibition of phagocyte aggression,
(c) inhibition of further aggressive lymphocyte activation,
(d) a tightening of endothelial cell junctions.
(e) encapsulation in a fibrin sheath (fibrocytes later)
(f) promotion of lymphocytic tolerance to typical Ag
(g) production of auto-antibodies to the newly cloned, locally reactive lymphocytes (lymphocytotoxic Abs)

This failsafe is most necessary within and around the affected tissue so we should expect to see it strongly localised. However, a spillover effect may be anticipated, with systemic depression of delayed type hypersensitivity (the immune mechanism largely responsible for tissue rejection). This may explain, at least in part, why anergy occurs in diseases such as TB and sarcoidosis. There is evidence that anergy is expressed more intensely at a local rather than a systemic level (footnote 3). General references:- [24,25,26,27,28,29].

It is inevitable that the rate at which generation and regeneration (growth!) can proceed is limited. Since these are essentially similar (morphogenetic)

processes, auto-rejection in growing animals cannot be allowed to reach the levels of intensity that are permitted in mature animals or growth will become stunted. That is:-



Put another way, the luxury of extensive auto-rejection, as part of a morphostatic technique, can only be fully afforded in adult animals. Thus, in order to avoid stunting of growth, those mechanisms which initiate and accelerate rejection (of all kinds) need to be less fierce in growing animals than they are in adults: lymphocytes must behave less aggressively and this is probably brought about by moderating the intensity with which APCs stimulate aggressive lymphocytes (APCs = antigen presenting cells) [30,31]. Both CMI (cell mediated immunity) and IgG activity must be dampened (at least, for those IgGs capable of reaching the extracellular spaces even when there is no inflammation). The result of all this is to promote a relative immunological tolerance in very young animals. This impaired capacity to reject (and consequently autoreject) is apparent in the neonate in which the tolerance of grafts is much enhanced: the neonate can also tolerate a level of cerebral ischaemia which, in adults, would cause extensive tissue death (in large part an auto-rejective event). This relative incapacity to auto-reject is also a protection against the dangerous sequelae that follow virus infections (they may even have been a significant driving force to require it). These tend to produce their most severe effects when they first strike in adult life (e.g., infectious mononucleosis [32], infectious hepatitis [both often mere URTIs in young children], mumps, chicken pox and measles; and an example from the mouse, lymphochoriomeningitis [33]). The sequelae (arthritis, jaundice, meningitis, orchitis etc) can be prevented or at least ameliorated by immunosuppressives or steroids. From this point of view, "immunological immaturity" is a misleading term because the infant's immune system is likely to be perfectly adapted for an optimal compromise.

SUMMARY

In summary, the concept of "horror autotoxicus" has been redefined and, hopefully, rejuvenated. So, incidentally, has the concept of immunological surveillance [34,35,36,37,38,39]: but this surveillance is for all malfunctioning cells and not just for neoplasia. Indeed the lymphocytic system may have evolved at the expense of an increased prevalence of cancer (see below).

The morphostatic process usually starts within the soma. Local tissue damage or infiltration with infectious agents results in disrupted cell-cell contact and communication. The affected cells use an instantaneous rise in Ca++ to rapidly switch off local communication, then round up and detach from their neighbours. This detachment disrupts membranes which then release various arachidonates: these, in turn, precipitate an inflammatory cascade. Phagocytes and dendritic cells now process the various cellular and foreign debris and present representative peptides (& etc!) to the immune system. As explained above, in the context of this inflammatory presentation, an aggressive response will be favoured. Uncommitted lymphocytes with appropriate

paratopes will now lead to T-helper or T-DTH rather than T-suppressor lymphocytes. Since most self epitopes have previously been encountered in a healthy self context, T cells with paratopes able to recognise them will, for the most part, be already committed to tolerance. The result will be an immune response heavily loaded towards responding to new and unusual epitopes in the (phagocyte processed) OTHS debris. Where auto-reactivity arises, the effector cells tend to be channeled back to the original inflammatory site [15,16] and could be used, physiologically, to enhance the focal inflammatory reaction and tissue clearance. Once attracted to the inflammatory site, phagocytes STILL exert a HS/OTHS discrimination though the criterion for acceptable HS is now much more strict. After the event the balence of tolerance/aggression to self Ags can be returned towards tolerance.

There are certain tissues where extensive auto-destruction could prove disastrous: such an event might seriously impair the ZDC's functionality and survivability. These include the eye and the nervous system. These sites enjoy a so called "immunological privilege". This priviledge seems to be achieved, at least in part, by locking out inflammatory cells behind tight endothelial cell junctions: the sparse population of local APCs is probably a direct consequence of this.

The remainder of this article will consider the way that these basic principles can be extrapolated to account for the pattern of selected groups of disorders.

PART 2: THE CLINICAL CONSEQUENCES.

AUTO-REJECTIVE DISORDERS

Tissue rejection is largely mediated by cell mediated immunity. Whilst antibodies can affect the course of organ rejection, they cannot, on their own, precipitate it. Rejection can, however, be induced with injections of appropriately activated lymphocytes. If we accept the principle that disordered self cells are actively rejected we are now in a position to state the following:

> Virtually all non metabolic disease should have an auto-rejective element even if this is limited to a mildly increased tissue turnover.

So, there ought to be a group of disorders which are largely auto-rejective and who's pathogenesis is little, if at all, affected by humoral auto-immunity. One consequence of the discussion in Part 1 of this hypothesis is that the intensity of auto-rejection is likely to be dependent upon age. It will be at its highest potential in the healthy young adult. The initiation of auto-rejection is suppressed in the very young [30,31] and its execution becomes progressively impaired in the elderly [40]. Thus, a disease which is caused by extensive auto-rejection will be most likely to occur and also to be at its most severe in this central age range (figure 2). One likely cause of such disease is deliberate interference with and mimicry of aspects of the

host's identity machinery. Micro-organisms, with their capacity for rapid genetic adaptation, are the most likely offenders. Where micro-organisms achieve an antigenic profile close to the host's identity they will appear less foreign and gain easier access to the host's tissues and cytoplasm. Cells which are damaged in consequence of this should still signal malfunction (shout "foul"). However, because there is a relative scarcity of clearly foreign antigen, the resultant inflammatory reaction will concentrate its enhanced attention on self Ags. Accelerated auto-rejection will ensue and the attack will not necessarily remain confined to the initiating site.

Adjuvant arthritis is of interest because it produces a constellation of disease who's features are similar to those seen in the sero-negative arthritides and sarcoidosis. This experimental disease may be caused because clearly foreign antigen is sparse and the immune response is consequently concentrated upon local tissue antigens (eg, heat shock proteins) or mycobacterial antigens which cross react with the host (table x). Whipple's disease may be an extreme example of this sort of disease (note the idiosyncratic infection [41,42] and familial aggregation of cases [42,43]).

The bacteria which colonise epithelial surfaces present a special hazard to the colony. It is well recognised that they have the ability to bind selectively to cells at particular epithelial sites [10]. Since they have evolved this specificity it is also highly likely that they have evolved some mimicry of and interference with the host's identity machinery (especially tissue/site be definable from basic principle: compatibility of organ transplants ranges from a common slight compatibility to a rare complete compatibility [13]. When this observation is extrapolated to microbial mimicry, one would expect to find minor mimicry often and extreme mimicry rarely. The seronegative arthritides and their component complications show just this sort of structuring (table 1). Their clinical pattern can be summed up by an axiom:-

The severity of any single patient's disease(*) is inversely proportional to its incidence in the population and directly proportional to the number of components found in association with one another.
((*)- whether it is an isolated component or a syndrome complex of more than one component.)

For example, recurrent aphthous ulceration (RAU) occurs in about 5% of the population, oro-genital ulceration in about 0.5% or less and Behcet's syndrome (BS) in about 0.0001% (in Britain). As the apparent disease in any particular patient is observed to be more severe, so we notice an expanding clinical overlap: more individual components coincide in one patient (table x). The pathogenesis of these disorders should be dominated by cell mediated immune aggression just as it is in non-acute graft rejection [44]: any contribution from circulating antibodies should simply be a bystander phenomenon. The pathological tempo of the individual components is often seen to increase with the severity of the (syndrome) disorder. Thus, in psoriasis, the prevalence of arthritis and iritis increases greatly in patients who have the exfoliative and the pustular forms of the disease [45]. On the basis of a personal study (in which the prime objective was to review the world literature on neurological Behcet's syndrome - unpublished) I believe that the meningo-encephalitis of multiple sclerosis should be regarded as an isolated component equivalent of

the severer meningo-encephalitis that is encountered in BS (nb., MS is a meningo-encephalitis [46]).

The age incidences of all these disorders are typical [47]. The population incidence of the commoner conditions begins and peaks earlier than in the rarer disorders. In the majority of components it is evident that they are constantly modulated by certain events: menstrual exacerbation, second and third trimester quiescence, puerperal exacerbation, stress precipitation and, finally, amelioration of symptoms with steroid and immunosuppressive therapy.

At least two further disorders have features to suggest that they might legitimately be included amongst the (predominantly) auto-rejective disorders. These are sarcoidosis and systemic lupus erythematosis. Both of these demonstrate some clinical overlap with the sero-negative arthritides and SLE has a similar component structuring. (Nb., high turnover granulomas are a recognised consequence of many cell mediated immune reactions [48]).

CANCER

Broadly speaking it can be surmised that cancer follows:-

(a) a triggering event (induction)
(b) a breakdown in surveillance (promotion).

The event which eventually trips an affected cell into loss of growth control need not concern us in this article other than to point out that it usually arises in a single cell from which the tumour then develops. A unifying feature is that a normal growth control gene starts being transcribed inappropriately (induction). But let's leave this to one side. I will, instead, focus attention on the reasons for the body's failure to identify the miscreant cell and its progeny (promotion). Before proceeding, note how stark the contrast is between the Hayflick limit of about 50 doublings in cultures of healthy cells (footnote 4) and the apparent immortalisation of cell lines derived from cancers.

Opportunistic infections and cancer should, presumably, be most prevalent when morphostatic surveillance is least effective. The cells making up an animal (there are around 10 to the power 13 of them in man!) are highly regimented and, presumably, intense cell co-operation has to be exercised to maintain such order within the ZDC's tissues. This implies that, by and large, disruptive cells (dead, damaged, dying, mutated and those with disordered growth control) are largely rejected. And, indeed, it has long been clear that phagocytes do recognise these cells and remove them. Our main attention here should be directed solely at those events which lead to the impairment and subsequent failure of surveillance. Focal anergy is likely to be one of these events and may well be the major contributor to the escape of malignant cells from surveillance.

In mammals, this impairment of surveillance should (generally) be at the extremes of life or following prolonged focal auto-rejection and its consequent anergy. In the elderly, the increasing impairment of immunity coupled with the

heightened susceptibility of epithelium to various noxiae (and thus auto-rejection) will predispose to a high incidence of carcinomas. Focal anergy on its own (consequent upon intense auto-rejection) may be a major cause of the predilection for certain cancers to strike young adult to middle aged patients (e.g., lymphomas and focal cancers like colonic cancer in ulcerative colitis). In the very young there is a relative incapacity to reject tissues and, because auto-rejection is tardily initiated in this group, it is worth noting that there is not the equivalent predisposition to epithelial cancers such as is seen in the elderly. Cancers are relatively common in the very young and there is evidence to suggest that many regress before they reach clinical significance [49]. (Note that, in general, carcinoma-in-situ is far commoner than overt cancer: the abnormal cells tend either to be kept in check or eliminated by lympho-monocytic cells.)

Cancer is characterised by a failure of growth control and the cells affected revert to a form of behaviour more typical of embryonic cells (retrodifferentiation [50]). These changes, it seems to me, are much more likely to happen when regeneration and/or proliferation are exuberant (eg, T-cells in lymphomas) rather than in quiescent tissue (eg, cartiledge, neurones). Note that lymphomas are relatively common in the years in which auto-rejection is most intense (16-45yrs) and also note that, in granulomatous disorders, lymphomas predominate over other cancers perhaps because local tissue regeneration is impaired [51,52].

The rate at which cells become malfunctional (for any reason) probably increases with age. The net effect of this will be to cause a diffuse increase in the multiple foci of auto-rejection and, consequently, a gradual summation of focal anergy. This will eventually lead to a systemic spillover of this focal effect, a saturation effect. Epithelium is the tissue most exposed to infection, noxiae, regeneration and, in consequence, an increased probability of genetic divergence. Foci of anergy will be very frequent in this tissue form and carcinomas should consequently be more prevalent than sarcomas. Once initiated, cancer will itself lead to auto-rejection and, in turn, increased focal anergy. Thus, it is likely that there exists a critical mass and growth rate above which surveillance is irreparably blocked and the cancerous process becomes self perpetuating [53]. (Macrophages observed in vitro are clearly able to recognise malignant cells [54,55].)

Now it is instructive to compare the age incidence profiles of various cancers with those of the auto-rejective disorders. However, before doing so it is important to establish which cancers are likely to flourish in the wake of intense auto-rejection (probable examples are lymphomas and testicular tumours [56,57,58]). These must be recognised as distinct from the commonest form of cancer (carcinoma) which seems to occur most frequently in the wake of age related impairment in immune surveillance. In general, these have a gradually rising incidence with age. Some cancers, particularly mesothelial malignancies, follow an incidence pattern showing a nadir in the middle years. It is interesting to note that the age incidence pattern of acute leukaemia is a complete inversion of the age incidence pattern of the auto-rejective disorders (figure 2). (See [59]).

It should now be clear that the lymphocytic system can have a dichotomous effect on cancer surveillance. It may enhance the focal accumulation of phagocytic cells and thus aid the (auto-)rejection of aberrant cells. However, the more aggressively it does this, the more likely it is to precipitate a suppression of focal rejection in order to avert piecemeal self destruction. Indeed, in those animals that have evolved them, the possession of lymphocytes

may have incurred an increased risk of cancer: cancer is relatively uncommon in primitive animals [60,61] and is relatively scarce in congenitally athymic mice [62,63] which have abundant aggressive phagocytes [64] and natural killer cells [65]. It is interesting to note that in the animal kingdom there is an inverse relationship between the capacity to extensively regenerate body form and the prevalence of cancer [66,67]: and that carcinogens may induce supernumerary structures in lower phylae (eg, limbs) [68,69].

Napolitano et al [70] report that tumour cells generally display less class I Mhc Ag at their surface. They draw attention to the fact that the more malignant the tumour is the less class I Ag it expresses. They interpret this as a cause of the malignant behaviour. However, I would interpret this as a cell adjustment going, pari passu, with the loss of HS identity. Macrophages in vitro have little trouble in identifying malignant cells [55]. So, it seems that some quirk is allowing the lymphocytic amplification system to become preoccupied with an inappropriately strong response to the "wrong" tissue Ags: this, in turn, has led to focal auto-aggression and focal anergy. The phagocytes' capacity to eliminate UHS (tumour) cells is thus impaired, permitting a (so far) dormant carcinoma-in-situ to grow to a critical mass. At this point, the focal impairment of phagocyte activity becomes irreversible with uncontrolled growth of the tumour proceding unabated. This is consistent with the finding that tumour cells towards the centre of the tumour have a lower expression of class I Ags than tumour cells towards the outside. Here, macrophage activity is likely to be less impaired and capable of eliminating many more abnormal cells [55].

INFECTION

Infection can be defined as the survival and proliferation of an organism, not descended from the originating zygote, within the tissues of the ZDC. The colony need only remove these cells if they interfere with its structure or function (though the generality of the "dog eat dog" principle suggests that those that don't interfere will be highly specialised commensals or symbionts). Below I suggest four discrete ways in which surveillance can be overcome:-

(a) The first form of infection occurs when an organism acquires the ability to interfere, agonistically or antagonistically, with the host's machinery for establishing cell identity. Strategies based on species and tissue site identity can be cultured throughout the whole mass (surface mostly!) of a species and over its entire duration as a discrete species. The way in which foetal cells reaggregate into tissues rather than species [8,9] and the success, in nude mice, of skin transplants from distant species [71] suggests that tissue site identities may be broadly similar across widely separated species. A variety of infectious organisms could be interfering with this tissue site identity (eg, streptococci [72] and staphylococci). Others also show a clear species specificity (e.g., mycobacterium TB, bovine TB, avian TB etc, and various plant infections [73]). Interference with individual (Mhc) identities can only be evolved in a short timespan (about 60-70yrs in man) and in a small mass (about 60-70kg of which only a small proportion is actually epithelium). Should close mimicry of personal identity develop, this will facilitate that organism's access to the ZDC's tissues and, once there, there would be a relative lack of clearly foreign antigen to "attack". The resulting inflammatory response will tend to concentrate attention on tissue antigens common to both the organism and the host or just to the host. These self Ags will be selected as anchors for the subsequent lymphocyte accentuated inflammation, so leading to an accelerated rejection of self tissues. This kind of destructive attention to self is probably occurring in adjuvant

arthritis [22,23]. This disorder has clinical features closely reminiscent of the sero-negative arthritides and sarcoidosis (table 2). It is likely, therefore, that a highly idiosyncratic form of infection is a factor in the pathophysiology of the "auto-rejective disorders". Such disease could be precipitated by interference with the host's Mhc machinery by the microbe and this will probably have evolved in the lifetime of the animal. In biological systems, things are rarely black or white so the relative blend of the common/consensus and the idiosyncratic/individual response to infection will probably vary in a spectral manner (diag $). (Note that bacteria that manage to invade and survive within the cytoplasm could well pose a greater threat for this form of auto-rejective disease).

[Rejection will always be aimed at whatever is most apparently OTHS. The amount of auto-rejection will increase with the angrification of phagocytes, especially when clearly foreign OTHS is sparse. With the angrification of phagocytes, the threshold of HS expression required to avoid attack will be higher. In consequence, fewer self cells will continue to qualify as immune from self attack.]

(b) A second group of organisms manage to foil surveillance by virtue of their small size and obligate intracellular existence. The organisms of this group are the viruses. As soon as an infected cell is sufficiently compromised it should signal a malfunction so triggering inflammation and attracting phagocyte attention. This will lead to the activation of appropriate precursor lymphocyte clones. After an interval of 10-14 days a strong amnestic response to viral(+Mhc) antigen will have developed. In the meantime, selected self Ags may be used to anchor an immune accelerated phagocyte accumulation at the affected site whilst waiting for the emergence of a more specific anti-viral activity. (In general, these are "hit and run" infections: they are soon cleared from the system and those that persist do so by remaining dormant within cells.)

(c) The third group are the opportunistic infections. Whilst these may interfere with tissue and species identity mechanisms [74] their success is dependent on the depressions of focal surveillance which follow virus infections, burns, surgical incisions and trauma (etc.). Each of these noxiae lead to the auto-rejection of damaged and malfunctioning tissue with subsequent focal anergy [27]. Probable examples of such opportunistic infections include bacterial tonsillitis, otitis, sinusitis, bronchitis and various wound infections.

(d) The last group are organisms which deliberately set out to subvert the immune response into creating an intense focal anergy. They do so by maximally stimulating focal inflammation with the object of inducing intense focal auto-rejection. Mycobacterium TB is the example which will be considered here though syphilis is probably another. The properties of such an organism should include:

(1)	poor initial foreign antigenicity
(2)	a strong attraction for macrophages (adjuvant attraction)
(3)	a good resistance to initial destruction as evidenced by prolonged survival within macrophages

The result of these 3 properties is that intense focal inflammation and then auto-rejection is induced. In consequence, there is intense focal anergy and

this leads to a field of surveillance impairment in which the bacterium flourishes, feeding upon the cell debris which is left in the wake of this auto-destruction [75,76]. Clinical mimicry of the auto-rejective disorders should be discernible: this, in fact, can be seen and is most noticeable in the middle years, an observation which is in keeping with the auto-rejective disorders (table 3).

When tuberculosis occurs outside these middle years it is, accordingly, different in its clinical expression. The lesions now tend to be miliary and disseminated and occur without the same intense tissue destruction. Instead, the pattern now resembles miliary cancer. At the extremes of life, therefore, TB appears to be acting more like an opportunistic infection. The overall age incidence of TB can, therefore, be regarded as a combination of the auto-rejective and the cancer type age incidence (figure 2).

AUTO-IMMUNE DISORDERS

In several previous articles where immune surveillance has been discussed it has been suggested that cancer and auto-immunity might be expected to represent opposite poles of surveillance efficiency. However, the auto-immune title does not automatically imply auto-rejectiion. Rather than being dominantly auto-rejective, these disorders tend to result in one of two disturbances. The first is an interference with functional membrane molecules by the attachment to them of auto-antibodies (e.g., Graves disease, myaesthenia gravis). The second is a tissue destruction which is centred predominantly around (non-cellular) connective tissues (the "collagenoses") and is apparently exacerbated, if not caused, by excessive auto-antibody production and the widespread deposition of Ab/Ag immune complexes. Here, cell destruction is possibly secondary to the activation of macrophages in the locality of this connective tissue. Towards the end of life auto-immune disorders are relatively more common than the sero-negative arthritides. Their prevalence at these older ages may possibly be exacerbated by a decline in the efficiency with which phagocytes clear tissue debris: this, in turn, could lead to enhanced auto-antibody (immunoglobulin) production (the latter certainly appears to be a feature of many diseases causing widespread anergy, eg sarcoidosis [77]).

CONCLUSION

My synthesis has attempted to re-evaluate various aspects of immunity and has indicated that a broader perspective is gained if it is regarded as an important component of MORPHOSTASIS. Text books on immunology concentrate attention largely upon lymphocytes and antibodies. Consequently, regardless of the authors' actual beliefs, they give rise to the impression that lymphocytes and antibodies are the fulcra about which the mammalian immune system revolves. My synthesis requires that their role is clearly perceived as the servants of phagocytes. The identification of OTHS (first by the soma and then, by invitation, by the phagocytes) becomes the fulcrum of morphostasis. Morphostasis is initiated primarily by somatic cells (or more precisely, by their membranes): these have the capacity to recognise and broadcast the presence of OTHS and do so by invoking an inflammatory cascade. Phagocytes are the central, controlling cells which set in train the restoration of tissue homeostasis.

To summarise:

Morphostasis begins with the identification of Other Than Healthy Self by somatic cells (probably because the membrane doublet between adjacent cytoplasms has ceased to maintain a satisfactory communication): phagocytes are called in to deal with this condition: lymphocytes and their antibodies

are then organised so that they accelerate the phagocytic rejection of OTHS (note that the natural tendency of T cells is towards tolerance - aggression is only provoked when there is inflammation). Auto-rejection is extensively used as the first step in restoring tissue homeostasis. It can range from the simple elimination of aging cells (basal tissue turnover) to the kind of accelerated auto-rejection such as is seen in the sero-negative arthritides and in certain infections (like TB). Organism aging probably occurs because gradual genetic divergences make it progressively more difficult to hold the zygote derived colony together.

Whilst the argument presented is undoubtedly simplistic, I think it will prove to be a useful framework for refinement. The areas which I would most like to see elucidated are: the study of how OTHS is sensed and signalled in the soma: the phylogenetic search for inverted Tc cell function: establishing beyond doubt the predominantly focal nature of anergy: establishing whether or not there are any genetic relationships between gap junction insertion and the insertion of membrane attack complexes (current or past - see footnote 5): and the possibility that "horror autotoxicus" is sensed by the rapidity with which a phagocyte establishes an electrical continuum with the cytoplasm of an adjacent cell (see footnote 5).

4 Footnotes referred to in text

1 Abandonment of HS
2 Sacrificial system in plants
3 Anergy - focal
4 Relationship of MACs to GJs

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"There is only one constant element in immunity, whether innate or acquired, and that is phagocytosis. The extension and importance of this factor can no longer be denied."

Elie METCHNIKOFF 1905 [1]

"Immunology is an invention of the devil, who is making it up as he goes along because he's not too clear about this stuff either.". . . . ."Besides, immunology is what we North Americans call a Rube Goldberg system, referring to old cartoons about how to turn on the light, for example: you trip over a footstool, thus startling the cat, who bumps into the kitchen door, which swings shut, knocking over a chair that hits the light switch . . . you get the idea. There has to be an easier way."

Janice Hopkins TANNE 1990 [2]

INTRODUCTION:
The proposal I am about to make is stark: immunologists are missing the point. Their current perception of the immune process is flawed. Just as astronomers were once confident that the heavens revolved around the earth, so modern immunologists are generally confident that anamnestic immunity and its executors, the lymphocytes, are placed firmly centre stage, at the hub of the mammalian immune universe. In particular, it is current dogma that anamnestic

aggression to non-self(epitopes) and tolerance of self(epitopes) is the source of self(cell)/non-self(cell) discrimination.

describe the way I believe the system works and show how lymphocyte activity is probably the consequence rather than the source of self(cell)/non-self(cell) discrimination.

(1) MORPHOSTASIS:
Morphostasis is tissue homeostasis: it is manifestly efficient in all animals. This is the core function, the true centre of the metazoan universe. It is built upon cell to cell recognition and communication. Anamnestic immunity is but a branch of the morphostatic process and it has evolved to enhance morphostatic efficiency in vertebrates.

An animal is built from a large colony of cells all derived from one zygotic cell (a zygote derived colony - ZDC). This colony constructs itself a relatively inert skeleton of connective tissues which allows it a greatly enhanced versatility. The critical process in morphostasis is to discriminate Healthy Self (HS) cells from Other Than Healthy Self (OTHS) cells. OTHS includes both Unhealthy Self (UHS) cells and clearly foreign organisms. Morphostasis was needed from the moment that multicellular animal forms first evolved. It should be clear that the main need at that time was to develop a unique way of allowing healthy self cells to acknowledge each other and then of devising a means of abandoning this healthy self status when things went wrong.

Morphostasis (tissue homeostasis) can be maintained by:

(a) discriminating OTHS cells from HS cells.
(b) removing OTHS cells (UHS and foreign cells/organisms).
(c) replacing lost UHS cells with fresh HS cells (resurgent morphogenesis).

(2) HEALTHY SELF/OTHER THAN HEALTHY SELF DISCRIMINATION:
This hypothesis requires that individual cells MUST either have a fail-safe internal device for recognising that they have become unhealthy OR an ability to monitor a neighbouring cell's change in health (probably) by monitoring cell to cell communication. The announcement of an "OTHS foul" comes directly from an affected group of somatic cells. Inflammatory cells (mostly phagocytes) are only invited into the area at this group's request - a "call" is sent out to fetch the "police". Foreign organisms need not induce an inflammatory response UNLESS they unsuccessfully attempt communication with a HS cell, OR force their way between cells (and so disrupt communication), OR directly attack a cell and make it sick.

Several mechanisms may combine to contribute to HS identity; remember that one or more of the critical aspects which lead to HS recognition must be abandoned when the cell becomes sick:

(a) Lectins and the recognition of saccharides (eg, sialic acid).
(b) The inhibition of complement attack by proteins released from

| |
| or displayed on the cell membrane (eg, factor H, DAF, MCP). |
| |
| (c) Beta-2-microglobulin and Class 1 Mhc ligand expression. |
| |
| (d) Cell to cell cytoplasmic joining - particularly electrical. |
|_____|

(3) INFLAMMATION:

The infiltration of somatic tissues by inflammatory cells is a ancient and virtually universal metazoan defence mechanism. These cells are clearly able to recognise most organisms (particularly those which are not dedicated pathogens) and, in the vast mass of animal life, they appear to do so without the aid of memory cells. They also remove aging and disordered self cells. In fact, they are ideally adapted to deal with OTHS. I propose that the prime function of the lymphocytic system (which evolved later) was to accelerate and accentuate the inflammatory process and, in turn, make the removal of OTHS by phagocytes more efficient. The discrimination of HS from OTHS by phagocytes remains a central and critical immune process. But HS/OTHS discrimination probably starts in general cell to cell communication.

Static (somatic) cells are attached to each other by several types of cell junction. Their cytoplasms are joined by gap junctions (GJs - except in those cells who's function depends on electrical excitability). When membrane junctions are split apart the disruptions in the cell membranes inevitably lead to the release of various eicosanoids (prostaglandins etc). This announcement of an OTHS event by somatic cells results in an inflammatory reaction (in tissues with few GJs, inflammation is less pronounced). Chemical messengers released at the OTHS site encourage the ingress of phagocytes (in mammals, through the endothelial cell linings of local post-capillary venules). Phagocytes now invade the OTHS site. They begin assessing cells on the basis of their HS status. Thus far, the basic process is the same for almost every, if not all, animal species. At this point, vertebrates enroll a new mechanism. Debris from local tissues is processed by phagocytes (or phagocyte related cells) and it is then presented, in local lymph nodes, to the anamnestic immune system as short representative peptides. The aim is to select representative epitopes and to retain a memory of them and their inflammatory environment so that, on their next encounter, this inflammatory environment can be rapidly and potently reproduced. This anamnestic response is under the full command of the morphostatic process and, in particular, largely under the control of phagocytes.

(4) THE GENERATION OF SPECIFICITY:

This hypothesis requires that (at the very least) a scavenger cell existed in the ancestry of modern vertebrates which was able to recognise a self cell on the basis that it expressed self Mhc "Class-I-like" ligands and, in so doing, it observed a "horror autotoxicus" to that self cell. This cell may still exist (a possible candidate is the natural killer lymphocyte - Tnk). This scavenger would have had a natural tendency to attack cell like structures UNLESS they could prove that they were healthy self cells. (Note that the result of complement component activity is very much in this style, with healthy self being "immune": and also that phagocytes synthesise enough of all but the terminal components to attack cells.) This putative cell would be naturally aggressive to all cellular structures and only switched into

non-aggressiveness by the presence of appropriate "Class-I-like" ligands. This action is an inversion of the activity of the Tc cell. Both phagocytes and lymphocytes are derived from marrow stem cells. They are closely related, adding weight to the proposition that a phagocyte like or derived cell might, at one stage, have evolved to have the ability to select/rearrange its genes so that it could specifically recognise healthy self ligands (Mhc "Class-I-like" ligands: note that N-CAM RNA is selected and rearranged).

Cell type	Receptors disabled	Receptors enabled	Normal state	Triggered state
Scavenger	non pure self	pure self	aggressive	passive
	GENERATOR OF SPECIFITY			
Tc cell	pure self	non pure self	passive	aggressive
	GENERATOR OF DIVERSITY			

This would neatly explain how the anamnestic immune system appears to have erupted onto the evolutionary scene so suddenly and so completely in the vertebrates. Even a repertoire of receptors as few as two would be useful in the generation of specificity whereas a large repertoire seems almost a "sine qua non" for effective T-cell functioning. So, RECEPTOR genes would have had ample time to expand their repertoire before being precipitously "flipped" around for use by an anamnestic immune system.

So why are there virtually no reports to suggest that a scavenger can still specifically recognise self cells on the basis of Class I Mhc ligands? Well, it may be that the lymphocyte based system has been so successful that it has largely obviated the need for a scavenger to rearrange its genes and the system relies on the more primitive phagocytic assessment of HS cells (see (6) below); there might even be a positive advantage in achieving the apparent recognition of HS(cells) by inverting the action into an attack on non-self(epitopes) by Tc lymphocytes (achieved by the clonal elimination of any lymphocyte capable of reacting with "pure self" Class 1 ligands); OR natural killer T-cells (Tnk) are the delegated scavengers which check that somatic cells possess Class I HS ligands (hence enabled/disabled rather that selected/deleted). A final possibility is that we are failing to observe specific recognition even though it exists.

Natural killer cells could certainly fulfil this function. They were first identified because F1 Tnk cells attacked parental cells (quite unlike the classical transplantation laws). These cells also preferentially attack cells expressing low levels of Class I antigen and beta-2-microglobulin. However, it seems that, at most, only a proportion of them rearange their receptor genes. This might imply that they either use different receptors to Tc cells, or, perhaps, most Tnk cells exercise a low specificity recognition (eg, to beta-2-microglobulin alone). Whatever, the observed properties of Tnk cells

are at least partially consistent with the expected functions of an inverted Tc cell.

(5) MIMICRY:
Because morphostatic systems have always relied on self recognition, dedicated pathogens have had to use mimicry (or more subtle interferences with identity molecule expression and recognition) to gain access to and persist in the soma. Every animal needs to stay one step ahead of its competition. Constant pressure is exerted to expand the variety of identity molecules available within a species (pleomorphism). Somatic cells appear to recognise each other by developmental ligands (cell adhesion molecules, CAMs). When embryonic cells from two mammalian species are disaggregated, mixed together and allowed to settle, they segregate into tissue type and not into species. Somatic ligands have probably needed to stay constant over countless meiotic generations. This makes them a sitting duck for determined pathogens. So, somatic cells need a backstop identity to be used as a second check when things go wrong (phagocyte based and Mhc Class 1 based). And until they do go wrong, inflammatory cells can be confined to the vascular system, locked out behind tight endothelial cell junctions until invited in. (Note that "loss of function" is a cardinal feature of the inflammatory process.) Some cell ligands (eg, N-CAM) are acknowledged members of the immunoglobulin supergene family and may even have been the originators of this family.

(6) ANAMNESTIC AMPLIFICATION:
So, what are lymphocytes doing? When T-cells are released from the thymus they are already committed in specificity (ie, they are committed to recognising a specific epitope). But, they are not committed in activity (aggression or suppression). It is only when they meet their respective epitope that they commit themselves. Self epitopes are, in general, encountered frequently and nearly always first in a "healthy self" (non-inflammatory) environment. So tolerance is generally favoured for those lymphocytes which recognise self molecules. Few self specific T-cells will remain uncommitted for more than a brief period while there is a relatively large pool of the relevant self epitope waiting to be encountered. On the other hand, because only small quantities of foreign or strange epitope are met, infrequently, in the body, most T-cells capable of recognising them will remain uncommitted until they meet the epitope in an inflammatory encounter. Inevitably, they are most often met in an inflammatory context and aggression is favoured. Furthermore, it seems that it may be easier to provoke older precursor lymphocytes into aggression. This further concentrates the aggressive response onto those epitopes that are most strange to the body. No veto is imposed on T-cells to prevent them becoming aggressive to self epitopes (except for "pure self" Mhc ligands - these are clonally disabled). Indeed, epitopes that are usually hidden behind tight endothelial cell junctions (like the eye and brain) are infrequently encountered and a larger pool of uncommitted T-cells is likely to be available. They are, consequently, more inclined to provoke an aggressive response when they are exposed during periods of intense inflammation. The thymus constantly produces new uncommitted T-cells. So, whenever clearly foreign epitopes are sparse and inflammation is intense, attention will gradually turn to self epitopes (eg tuberculosis). In summary, aggression is most likely to develop to clearly foreign (strange) epitopes and tolerance most likely to develop to self (frequently encountered) epitopes.

The overall effect is that lymphocytes remember the inflammatory or non-inflammatory context in which they first meet their respective epitope (and become committed); and they aim to recreate and caricaturise this memorised inflammatory milieu at the next encounter. Whenever Td cells provoke an

inflammatory response they call large numbers of phagocytes (& Tnk cells?) to the epitope site. These are then switched into a heightened state of "anger". However, phagocytes (& Tnk cells?) STILL have to discriminate HS from OTHS. But now, the threshold at which aggression is considered is greatly reduced. Cells expressing a relatively low level of "HS identity" are now likely to be attacked. This amplification of the inflammatory response by lymphocytes has the potential to escalate catastrophically. It can slip into a strong positive feedback loop, particularly when the epitope is an abundant self Ag. When the local auto-rejective response becomes excessive, it must be down-regulated otherwise things will get disastrously out of hand. This could be done in a number of ways and these may account for many instances of anergy:

(a) inhibition of phagocyte ingression (chemotaxis)
(b) inhibition of phagocyte aggression
(c) inhibition of further aggressive lymphocyte activation
(d) a tightening of endothelial cell junctions
(e) encapsulation in a fibrin sheath (fibrocytes later)
(f) promotion of lymphocytic tolerance to typical Ag
(g) production of auto-antibodies to the newly cloned, locally reactive lymphocytes (lymphocytotoxic Abs)

(7) MORPHOSTATIC EVOLUTION:
It is now easier to see how the morphostatic system may have evolved. It has been suggested that CAMs belonging to the immunoglobulin supergene family may have appeared early in the history of cell cooperation. If this proves to be the case then there is a clear path in the development of the morphostatic system from early multicellulates to man. Remember that ontogeny frequently retraces phylogeny. Though this trend cannot be regarded as an absolute blueprint for the evolutionary process, it is a useful pointer. Cell to cell recognition in embryos is likely to point towards HS/OTHS discrimination in the adult mammal. Imagine taking a journey through evolution:

EVOLUTION OF ZDCs from SIMPLE MULTICELLULATES to MAMMALS

(a) In the beginning, all cells in the colony express equally marked phagocytic behaviour.

(b) "SELF is established by making holes in the membranes of apposing cells and lining them up to create gap junctions. This allows cells to become electrically coupled and so to act as an electrical and, probably, a cytoplasmic continuum. This ability to couple membranes dates back to the very earliest multicellulates. It relies on the controlled, ordered, simultaneous adjacent membrane insertion of membrane holes. Cells learn, early on, to allow the uncoordinated, bigger, higgledy piggledy insertion of leaky holes into organisms which fail to demonstrate the membrane LIGANDs used as a focus for the tidy construction of gap junctions: electrical discontinuity and a lower membrane potential invite phagocytosis. Unhealthy self cells can elect to be rejected by uncoupling themselves and dropping their membrane potential: they also learn to abandon their membrane (self) LIGANDs."

(c) Cells now divide into phagocytes and soma. They selectively improve the

specificity and efficiency of cell junction construction by facilitating and amplifying their construction at the site of cell LIGAND/RECEPTOR interaction. The resultant gap junctions are (perhaps) larger and more specific. They develop:

somatic LIGAND(s) - for recognition by resident scaffolders.
phagocyte LIGAND(s) - for recognition by itinerant scavengers.

(d) Dedicated phagocytes now evolve. They refine this cooperative gap-junctional communication with self and the runaway, leaky hole attack of non-self. The molecules used to do the second evolve into what we now recognise as the complement components. It is possible that these two construction cascades are related but become independant early in evolution. At this stage the complement components are secreted locally by phagocytes and their action is directed entirely at membranes. It is only much later that these components are co-opted into a humeral system and very much later that they are co-opted to interact with antibodies (probably an adaptation of specific Mhc recognition).

(e) A "vascular" system now evolves, locking out phagocytes till required. The alternative complement cascade can now be "humeralised" so that circulating C3 can mark clearly foreign organisms so that they can be more readily identified when they meet a phagocyte.

(f) There is now a progressive evolution and expansion of somatic LIGANDs leading to increased tissue compartmentalisation.

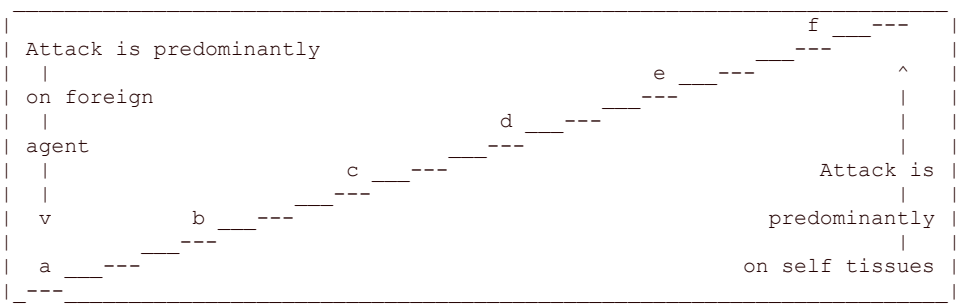
(g) Ig supergene like LIGANDs develop to act as a focus on which to grow highly specific gap junctional plates and create developmental compartments. The genes specifying these molecules are now copied then altered by a "mix and match" process to generate one set of LIGANDs which have a great variability within a herd. These pleomorphic LIGANDs now act as the final arbiters of healthy self in each individual. Over many meiotic generations, they have evolved into Mhc Class I LIGANDs. Newly developed scavenger cells are now able, when required, to electrically couple with any somatic cell that displays self specific LIGANDs and observe a horror autotoxicus to it. These scavengers need a mechanism to produce and/or select self specific RECEPTORs unique to each ZDC. This must be done post-meiotically over a number of mitotic generations - the "generation of specificity". (This possibly coincides with the appearance of "eggs".) These scavengers resemble natural killer cells.

(h) By inverting the "generator of specificity" into the "generator of diversity" lymphocytic cells evolve which are able to recognise and attack cells who's Class I ligands have been altered. It is well recognised now that viruses and other intracellular pathogens interfere (by attachement) with ligand/receptor machinery. If these altered Class I ligands are processed, leaving representative peptides attached, viral particles in association with self Mhc can be remembered then, on their next encounter, attacked by an inverted scavenger (?Tnk). These are the equivalent of Tc cells and recognise Mhc "Class-I-like" ligands. Sometime between now and the evolution of free antibodies, the so called "alternative" complement pathway is extended into the "classical" pathway. C1 might be specialised for short range triggering of high density, single surface LIGAND/RECEPTOR complexes so that hole construction is now restricted to the target membrane rather than to a coordinated construction in apposing membranes. ligands evolve: the "intention" is to present these on the inner surface

of phagocyte lysosomes where they are allowed to interact with cellular peptide debris picked up by phagocytes at inflammatory sites. These are of uncommitted T-cells. The "generator of diversity" can now be enrolled into memorising the inflammatory context of these epitopes. On re-encountering the epitope these T-cells can now rapidly attract large numbers of phagocytes to the site and "angrify" them: inflammation now has a memory. (Note that only a very limited set of cells - APCs, phagocytes and a few others - can present the combinant epitopes so this amplification of the inflammatory cascade can only start after OTHS has been processed.)

(k) The capacity to develop T-cell tolerance has to evolve simultaneously with Tc and Td cells. T-cells capable of recognising self epitopes are mostly decommissioned. This may be a co-operative process (Td/Ts cooperation akin to Th/B-cell co-operation). Whatever, agression is averted by having them "mopped up" by Ts commitment. This happens because these epitopes are more likely to be met in a non-inflammatory context. However, self specific T-cells continue to be released from the thymus and can become available for aggression. Aggression to self epitopes will be most likely to be induced and permitted when the inflammatory process is prolonged and foreign epitopes are sparse. Tolerance might be amplified by Ts cell clonal expansion and, perhaps, the release of anti-inflammatory agents at the site of epitope re-encounter. (Like Th and B-cell interaction, helper and suppressor epitopes tend not to overlap, suggesting a co-operative mechanism: it may also reflect the preferential attention of Tc and Td cells to allotypes.)

(l) The result of all this is that any disease which evokes an inflammatory response has an element of auto-rejection. It inevitably consists of a varying mixture of attack directed exclusively at the pathogen (usually leading to mild inflammation) and attack directed almost entirely at self (often highly inflammatory): the latter occurs when organisms or cells provoke prolonged inflammation but do not provide or present clearly foreign looking (unusual) epitopes. Every disease that leads to cell damage will be also be accompanied by auto-rejection.



EXAMPLES
(a) Saprophyte
(b) Simple epithelial commensal
(c) Staphylococci and streptococci
(d) Tuberculosis and syphilis
(e)-(f) Multiple sclerosis and sero-negative arthritis

(m) Last of all, Th cells can now be enrolled into the system to create the B-cell system and freely circulating antibodies. The B-cells are also

derived from a scavenger cell but designed, now, to secrete large quantities of circulating antibody. Antibodies help by opsonising organisms (preparing them as a "meal" for phagocytes). The classical complement cascade is now optimised to work within the vascular system and to interact with antibody tagged antigen. This system has proved invaluable as a preemptive defence.

SUMMARY:
The perception of immunity has been reshaped to encompass the broader principle of MORPHOSTASIS. The loss of healthy self is sensed and expressed by the malfunctioning cell itself or emanates from the site at which it makes contact with its immediate neighbours. This "foul" is broadcast by the release of inflammatory mediators. These invite phagocytes into the area to assess local cells. Phagocytes (and Tnk cells) then attack those cells with which they fail to become electrically contiguous. The time they have to make this connection varies with the "anger" of the phagocytes. Now phagocytes present cell debris to lymphocytes in local lymph nodes. The most foreign "looking" epitopes are selected to act as the pegs on which to hang a greatly accelerated inflammatory ingress on any subsequent encounter of these epitopes.

The concept of "horror autotoxicus" is now redefined and it is seen to be dependant on successful cell to cell communication. Both somatic and scavenger cells use this mechanism. The concept of immunological surveillance is also redefined. But now this surveillance is for any malfunctioning cell and not just for neoplasia. The evolution of a thymus dependant (anamnestic) lymphocytic system may have occurred at the expense of an increased prevalence of cancer, for intense focal suppression of surveillance now occurs whenever a strong positive feedback leads to an exaggerated attack on self epitopes.

This explanation is undoubtedly simplistic and will prove to be inaccurate in a number of its more specific assumptions. Also, the immune system has gathered a great number of refinements throughout its evolution including various specialised phagocytes and permanently resident, non-itinerant antigen presenting cells: little has been said about these. However, I suggest that the "flavour" of the concept is essentially correct and the hypothesis will serve as a useful framework for refinement.

It should now be clear that the breaking of cellular junctions is probably an important event which leads to the declaration of an OTHS "foul". There are a number of close similarities between the insertion of gap junctions into self cell membranes and the insertion of complement membrane attack complexes into invaders. If it could be shown that there is a continuing or a distant relationship between their respective insertion mechanisms, then it would be reasonable to assume that HS is sensed by the speed with which both somatic cells and scavenger cells establish an electrical continuum with those cells that they encounter.

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HYPOTHESIS
Morphostasis is tissue homeostasis. Tissue form remains stable whilst cells are in intimate contact by intercellular junctions. This enables joined cells to establish various degrees of electrical and metabolic synchronisation and it

promotes cooperation. Synchronisation is greatest when the cytoplasms are in direct continuity through gap junctions or synctial structures. The specificity of the molecular mechanisms that lead to cell adhesion, coupling and connective tissue scaffolding, in effect, give cells a <healthy self (HS)> identity. Similarly, the loss of <HS identity> is accompanied by dismantling of the connective tissue scaffold and cell undocking. Self cells monitor each others' identity. When a cell becomes sick it senses its own disorder and abandons <HS identity>. It can shut down the channels that join its cytoplasm with those of adjacent cells and then detach its membrane from them in a process called apoptosis. This leads to tidy, elected cell death. Adjacent cells and phagocytes ingest apoptotic cells before they burst. The processed peptides induce T-cell tolerance. Necrosis is an untidy form of cell death. Such dying cells burst and spill their contents, so releasing inflammatory cytokines. These processed peptides trigger aggressive anamnestic immune reponses which accelerate the identification and elimination of cells which carry markers previously encountered on cells that have died and provoked an inflammation. Once order is restored, adjacent healthy cells duplicate and replenish lost cells.

ABBREVIATIONS

CAM	=	cell adhesion molecule
GJ	=	gap junction
HS	=	healthy self
ICJ	=	intercellular junction
Ig	=	immunoglobulin
IgSF	=	Ig superfamily
N-CAM	=	Neural CAM
OTHS	=	other than healthy self
UHS	=	unhealthy self
ZDC	=	zygote derived colony

INTRODUCTION

In 1963 the Lancet published an hypothesis, "The role of lymphoid tissue in morphostasis"1. In this article Burwell made the comment that "immunology F
still awaits incorporating into the general pattern of biology" and suggested that immune function had an important role to play in morphostasis. Morphostasis is defined as the "steady state condition which maintains a particular (tissue) pattern". It seems to me that immunology is still perceived as a discrete and clearly demarcated system. In this article I hope to show how morphostasis should be regarded as the origin and continuing drive of immune function and how it is the cornerstone of metazoan existence. I believe that this hypothesis is fully compatible with experimental fact.

The following points set the scene. A morphostatic system must interface with these biological systems:

1) Intracellular and molecular biology
2) Cell to cell communication and cooperation (gap junctions in particular)
3) Embryo - development from zygote to mature animal
   - evolution from simple metazoans to mammals
4) The general scheme of morphostasis including
   - the surveillance for sick cells
   - cell and animal senescence2
- malignancy
   - the changing susceptibility to various diseases with aging
   - the renewal of sick cells and tissues
5) Basic pathological mechanisms

6) Immunity - innate
- anamnestic
- immune ontogeny
- immune phylogeny (from simple metazoans to mammals)3
- shed some light on plant defence4,5

Brevity demands a synoptic style so I shall not explore the rationale for proposing a new perspective. What follows is my perception of the process and its elements are not necessarily statements of accepted fact. The bibliography has been chosen to provide an investigative trail, with many of the articles providing further sources of reference.

THE ZYGOTE DERIVED COLONY (ZDC)

Every animal is a colony derived from a single cell, the zygote. No cell in the ZDC has functional capabilities that are not potentially present in the zygote's genes or cytoplasm. Each ZDC cell needs some way of preferring its own kind as neighbours and inhibiting the growth of foreign cells or organisms in its vicinity. This is helped by using selective CAMs which lead to the construction of ICJs, a scaffold of connective tissues and electrical/metabolic synchronisation6,7.

THE SOPHISTICATION OF SINGLE CELLS: THE SELF AWARE CELL

Each animal cell is a self assessing unit, capable of surveilling its own behaviour and function. It does this both internally and with respect to its neighbours. The cell has a variety of internal checkpoint controls. These are particularly well defined in the growth cycle. When an animal cell malfunctions, it senses the abnormality and notifies other cells that something has gone wrong (by various cytokines, alterations in cell surface markers and by breaking junctional communication). A sick cell can elect to sacrifice itself by apoptosis8,9,10: its calcium level rises, it rounds up and its GJs are closed before these and other ICJs are disassembled. Apoptotic cells are phagocytosed by adjacent cells or phagocytes before their membranes burst.

HEALTHY SELF (CELL) / OTHER THAN HEALTHY SELF (CELL) DISCRIMINATION

All metazoan animals are able to make this discrimination. What differs from organisms to organism is the sophistication with which it is embellished. It reaches a high level of sophistication in mammals. Every embellishment of the morphostatic system, including anamnestic immunity, requires an <UHS cell> to "advertise" its presence.

MORPHOSTASIS Tissue homeostasis can be maintained by:
(a) displaying "flags" on the membranes of HS cells which mark them as HS.
(b) recognising OTHS cells on the basis of absent HS markers (<HS identity>).
(c) attacking and removing OTHS cells (UHS and foreign cells/organisms).
(d) replacing lost UHS cells with fresh HS cells (resurgent morphogenesis).

IN SUMMARY

Identity - healthy ZDC cells display identity markers (these double up as "docking" molecules which lead to ICJs and a connective tissue scaffolding).

Self surveillance - cells are able to sense <UHS> status.

Altruism - cells are able to opt for apoptosis (suicide).

Neighbour surveillance - cells are able to sense a neighbour's appropriateness.

Sick cells - either declare their own presence or are recognised as such by their neighbours. These include damaged cells, dying cells, aging cells, genetically damaged cells, malignant cells, infected cells and other sick cells.

GAP JUNCTIONS

The cytoplasms of static cell populations are often joined through GJs11. These channels are shut down when a cell becomes sick12,13,14. A rise in intracellular calcium initiates GJ closure11. GJ channels are then disassembled during apoptosis.

The whole embryo is electrically connected through GJs and this establishes the boundaries of <self>15. Within this electrically continuous <self> there are sub-compartments in which member cells are joined by plaques of GJs which have higher permeability. They are surrounded by a layer of cells with GJs of lower permeability and these define the compartment borders. They correspond with developmental compartments. N-CAM promotes the construction of highly permeable GJ plaques16. Three possible explanations spring to mind: these plaques contain more GJs; the component GJs are bigger; construction is more efficient and there is a higher yield of good junctions.

I would like to propose that the consensus sequence motif of N-CAM, which resembles the Ig constant region, evolved in order to spawn multiple, highly permeable GJs much as the complement C1,C2,C4,C3 cascade spawns multiple well formed MACs around Ig constant regions. If so, the C7,8,&9 genes have either evolved from connexon genes or they have highjacked the mechanism which encourages the construction of highly permeable channels, inverting it into an attack mechanism. Note these points: (1) C9 inserts itself into membranes without C3-C8 amplification but this is inefficient; (2) leaky holes lead to a rise in intracellular calcium and so close GJ channels; (3) the connective tissue origin of C1q.

APOPTOSIS, NECROSIS and INFLAMMATION

Successful self surveillance leads to apoptosis and elective suicide. This mechanism deals with many, if not most, sick cells. It has failed when cells die by necrosis. Then, membranes rupture, their contents are spilled and inflammation is promoted. Inflammation provokes aggressive T-cell responses. When sick cells rupture, they release a characteristic set of cytokines, particularly eicosanoids. These are the messengers that notify adjacent somatic and inflammatory cells that something serious is amiss. In consequence, Tc cells induce apoptosis in cells which carry markers resembling cells that have previously died and provoked an inflammation. TH1 cells remember the inflammatory context in which they met their epitope. When they reencounter similar peptides they turn up the inflammatory "heat". They do not, themselves, kill: this is left to "angrified" phagocytes which become more particular about what they will accept as <HS identity>.

When peptide debris is processed after phagocytosing apoptotic cells, it promotes T-cell suppression. For example, when a cell dies following a virus infection its debris is processed by adjacent cells and phagocytes. If cell death occurs following successful internal surveillance (apoptosis), tolerance will be promoted to presented peptide debris and this will include viral peptide. When unsuccessful (eg, lytic or necrotic death), inflammation will promote T-cell aggression to presented peptides: and this will include self peptides. However, since apoptosis is such a common process, self peptides have previously promoted suppression and so shrunk any pools of self reactive precursor T-cells available to be recruited into aggression. Also, the threshold at which uncommitted T-cells are triggered into aggression falls as they age. This further focuses aggression onto strange epitopes.

<HS cells> in an inflammatory area are protected from self attack because they still demonstrate <HS identity>. I contend that this is the real horror autotoxicus. Phagocytes from closely related species share similar specificity. Most non-pathogenic organisms are easily identified as non-self. Unless complement is present, bacteria and viruses must rupture a cell and/or

disrupt its ICJs to invoke an inflammatory reaction and trigger an anamnestic immune response. Some dedicated pathogens appear to have evolved mechanisms to heighten inflammation in order to create themselves the niche they need to survive (eg, TB).

Inflammatory cells need to be restrained from entering healthy tissues until things goes wrong since their intrusion disrupts tissue function. The endothelial cell linings of blood vessels tend to lock out phagocytes until they are invited in. This is done more rigorously in the central nervous system - the blood brain barrier. This is necessary as nervous function relies on the electrical (GJ) disconnection of neurons during their terminal differentiation and the resulting (functional) asynchronisation then makes them more susceptible to macrophage attack (note how traumatic paraplegia is ameliorated with steroids). This need for segregation is likely to be important in the origin of the vascular system and inflammatory regulation.

MORPHOSTATIC EVOLUTION

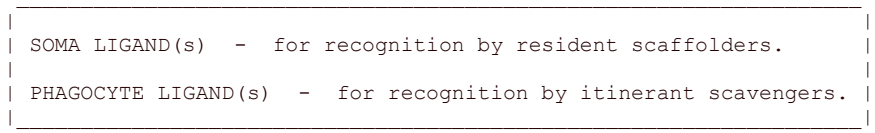
This is the way I suspect that the metazoan system evolved. Note that each new step is an embellishment of the former and all of them remain functional in mammal morphostasis.

(a) Elective cell suicide (apoptosis) is established as a means of protecting the colony (also seen in plants4).

(b) The interaction of CAMs, ICJs and the extracellular matrix gives cells a sense of "belonging". The consequent electrical/metabolic synchronisation, through ICJs, establishes <HS identity>. ICJs are the immediate consequence of cell surface ligand/ligand or ligand/receptor interactions and these molecules are Cell Adhesion Molecules, CAMs6,7. Once paired up, membrane holes in apposing cells form GJs (similar channels are important in plants4,5). IgSF CAMs (eg, N-CAM) develop later to act as a focus on which to build highly permeable GJ plaques. This "multiplier" mechanism will later be adapted to spatter bigger, leaky holes into cells or organisms which do not display features of self (the alternative complement cascade). A complement like cascade mechanism similar to the Bb/C3b et seq sequence evolves as the general agent which recognises cell membranes. In the presence of self markers it leads to GJs and in their absence, to attack.

(c) The progressive expansion of different somatic CAMs lead to subordinate, self within self identities and thus tissue specialisation. These define new developmental compartments where the borders are demarcated by a sheet of cells having GJs of low permeability. The cells within the compartment express IgSF CAMs and are joined by highly permeable GJ plaques. Note that cell sorting is dependent on CAM expression, particularly cadherins6,7. Homoeotic gene expression has also been noted to change at these compartment boundaries17.

(d) Animal cells split into dedicated phagocytes and soma. The soma abandons most of its capacity for wandering and aggression. The scavengers abandon most of their capacity for extensive connective tissue scaffolding.



Dedicated phagocytes evolve. They refine both their cooperative ICJ communication with self cells and the attack system which inserts leaky holes into non-self cells: the latter will eventually lead to the complement system.

Phagocytes are derived from a cell lineage which lies outside the three

main germ layers so they may, when they infiltrate somatic tissues, be demonstrating a property akin to the sorting tendency of disaggregated cells: they appear to be able to clamber over all other cell types and envelope them.

Phagocytes assess one aspect of self by making ICJs with underlying cells. This leads to a degree of electrical/metabolic synchronisation. The specificity of this ICJ connection is at least species wide and recognises <selfness> which may be shared with closely related species. First the phagocyte uropod establishes ICJ connections with an underlying cell and then it reaches out lamellipodial fingers to test the synchronisation of adjacent cells/organisms with the uropod attached cell. Capacitatively induced potential differences may be the trigger for an attack. The phagocyte uses other strategies like recognising apoptotic cells and, perhaps, surface markers which are invariably bacterial in origin. Note these points: (1) C9 has a thrombospondin motif which is used, in other circumstances, to recognise apoptotic cells; (2) basement membranes maintain physical barriers between tissues and help to minimise the area of cell membrane contact between different compartments.

(e) A "vascular" system evolves which is able to lock out most phagocytes till required and an inflammatory mechanism is established. The alternative complement cascade is now "humoralised" so that circulating C3 can mark clearly foreign organisms and make them more readily identifiable when they are met by a phagocyte.

(f) The specificity and diversity of N-CAM ligand interaction is achieved by a process of alternative RNA splicing6. N-CAM like genes are now adapted to produce multiple different ligand specificities within a herd rather than within a ZDC. These are the ancestors of the Mhc class I genes and will act as cell surface "flags" to advertise a more personalised HS status.

TABLE 1

Cell types and modes of action

Cell type	Receptors disabled	Receptors enabled	Normal state	Triggered state
Primitive scavenger (Tnk like precursor)	non pure self GENERATOR OF SPECIFITY	pure self	aggressive	passive (horror autotox- icosis)
Tc cell	pure self GENERATOR OF DIVERSITY	non pure self	passive	aggressive

These new identity ligands are recognised by a new cell (the ancestor of Tnk cells) which has evolved from phagocytes. This attacks organism membranes in general (Nb that the complement Bb/C3b complex has the same function) but observes a horror autotoxicus to any cell/organism that displays self specific ligands19. These Tnk like scavengers need a mechanism to produce and/or select self specific receptors unique to each ZDC. This must be done, after meiosis, over a number of mitotic generations - the "generation of specificity".

To achieve this diversity in ligand recognition, a mechanism was required to produce many different receptors from which an appropriately

specific receptor could be selected - "the generator of specificity". It is from this that the antibody genes have subsequently evolved. Horror autotoxicosis needs redefinition: only <HS cells> are protected by it. Selection in Tnk cells may be by alternative RNA splicing.

(g) Note that the Class III Mhc region contains a variety of genes encoding molecules that are involved in HS/OTHS discrimination or its modulation. These include HSP70, TNF, complement components (C2, Bf and C4) and the 21-hydroxylases20 and the TAP genes are close by.

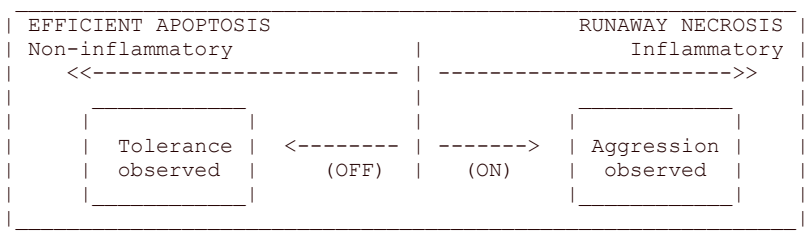
(h) Both the complexity and the repertoire of this mechanism for generating and selecting specific receptors is able to evolve gradually. The inversion of its function can lead to a mechanism able to recognise and attack non-pure self (Tc function). At some stage, perhaps with the advent of Tc cells, the identity genes are joined by another duplicated and transposed gene to produce Class I like Mhc genes18. This gene encodes a pincer mechanism like the HSC70 heat shock proteins (these look after "sick" proteins). Thus Ts and Tc like cells could evolve to recognise and, when appropriate, tolerate or attack cells whose Class I ligands had been altered by the intended attachment of peptides to the pincer mechanism.

(j) TH1 cells evolve by inversion of the Tnk cell function. The Class II Mhc mechanism evolves from the Class I mechanism: now, short, representative peptides from cellular debris processed by phagocytes after apoptosis or at inflammatory sites are processed. These are then externalised as a <Class II>/<peptide debris> combination ready for the attention of uncommitted T-cells. The "generator of diversity" is now enrolled into creating a system to memorise the inflammatory/non-inflammatory context in which these processed epitopes were first encountered. If it was inflammatory, on re-encountering the processed epitope, these T-cells are programmed to attract large numbers of phagocytes to the site and "angrify" them. This gives inflammation a memory. The "angrified" phagocytes still have to sort HS from OTHS but their threshold for regarding a cell as OTHS is lowered. Tc and TH1 cells are not, therefore, involved in assessing <selfness>. They are primed by other cells, particularly phagocytes, to remember the inflammatory/non-inflammatory context in which their epitopes were presented to them when they became committed (ie, lytic/apoptotic discrimination).

(k) The system of tolerance needs to evolve hand in hand with aggression. Even though apoptotic cells fragment, each particle retains an intact membrane and all are tidily phagocytosed by adjacent cells or phagocytes. The peptides processed in consequence need and should not activate Tc or TH1 cells: rather, tolerance is desirable. However, cells which rupture and spill their contents have not been identified by the surveillance/apoptosis mechanism and pose a threat. They release eicosanoids and other cytokines which provoke inflammation and this then leads to the activation of Tc and TH1 cells.

TABLE 2

THE BINARY COMMITMENT OF INDIVIDUAL LYMPHOCYTES
depending on how the peptide is presented



So, uncommitted T-cells are sensitive to the inflammatory cytokines or non-inflammatory environment they sense when they meet their respective epitope. They become committed accordingly. Self antigens are copious and are regularly encountered in the course of efficient apoptosis. The majority of precursor T-cells with paratopes recognising processed apoptotic debris (the majority of which is self peptide) will be "mopped up" into a commitment to suppression (tolerance). These T-cells will either be decommissioned or primed to inhibit inflammation on epitope re-encounter. However, uncommitted T-cells with paratopes specific for self Ags continue to be released from the bone marrow and they may be primed rather than filtered in the thymus (where enhanced apoptosis removes many self reactive lymphocytes). At least a proportion of these may become committed to aggression if the inflammatory process is prolonged and foreign epitopes, which accelerate its resolution, are sparse. This system is probably enhanced by the simple expedient of allowing the threshold at which aggression can be triggered to fall as precuror T-cells age. This focuses aggression onto strange epitopes.

(l) The antibody system can now be launched as "icing on the cake". TH1 cells can be adapted to TH2 function and these in turn used to co-operate with B-cells. The B-cells evolve to secrete large quantities of circulating antibody. Antibodies help by opsonising organisms. The alternative complement cascade is now adapted to be triggered by C1,2,&4. These have evolved from the ancestral components which are used by N-CAM to spawn GJ plaques. The antibody system is optimised to work within the vascular system. It can interfere with any intended function of the Ag and tag it for enhanced phagocyte attention and attack. This system has proven to be invaluable as a pre-emptive defence. (I have presumed antibodies have developed late because it makes current sense. However, there may have been a function which encouraged the early or simultaneous emergence of B-cells to produce IgM like free antibodies.)

CLINICAL CONSEQUENCES

There is insufficient space here for a detailed elaboration so here is a whistle stop tour:

(1) ANERGY. This term has acquired several meanings but here I am referring to the loss of delayed type hypersensitivity responsiveness that occur in

diseases like TB and cancer. Because the T-helper system is capable of training its aggressive attention on self antigens when clearly strange antigen is sparse (eg, adjuvant arthritis), the immune system has to have a failsafe cut-out mechanism. This shuts off phagocyte aggression when the tissue destruction becomes too fierce. The effect is dominantly focal though there is a systemic spillover effect. It impairs focal surveillance by phagocytes.

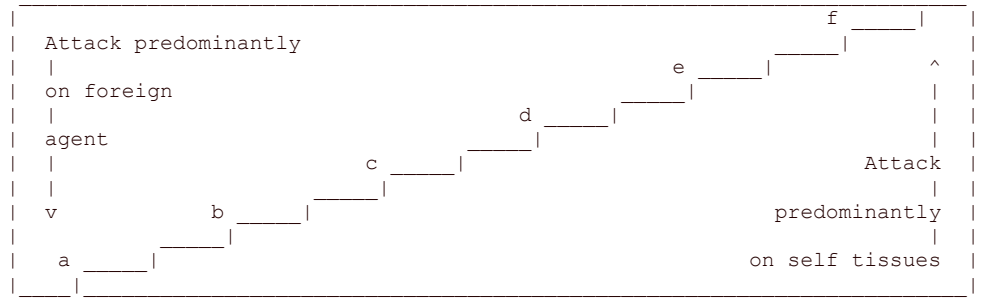
(2) PATHOGENS. Non-pathogens are easily identified and eliminated except when there is focal impairment of surveillance (anergy). Pathogens need to devise means of breaching the morphostatic defence. They do so by mimicking, blocking and fooling identity mechanisms21. Tuberculosis, in particular, deliberately invokes intense inflammation, causing extensive auto-rejection. It then flourishes in a resulting focus of phagocyte impotence.

(3) AUTO-REJECTION. The result of all this is that any disease which evokes cell necrosis and an inflammatory response develops an element of T-cell augmented auto-rejection. It inevitably consists of a mixture which varies from an attack directed almost exclusively at the pathogen (usually leading to mild inflammation) to an attack directed almost entirely at self (often highly inflammatory): the latter occurs when organisms or cells provoke prolonged inflammation but do not provide or present clearly foreign looking (unusual) epitopes. Every disease that leads to cell damage will induce auto-rejection, even if this goes no further than apoptosis. Since heat shock proteins are responsible for chaperoning disrupted proteins through the cell, they are frequently presented as epitopes in UHS presentations.

Auto-rejection rumbles along at a low level all the time. When inflammation is prolonged and no clearly foreign epitopes are present to bring it to a conclusion, precursor T-cells specific for self Ags may be progressively recruited into aggressive action. These intensify local inflammation and so enhance tissue rejection. This appears to be what happens in adjuvant arthritis.

DIAGRAM 1

The stepped progression of attack on self



EXAMPLES

(a) Saprophyte

(b) Simple epithelial commensal

(c) Staphylococci and streptococci
(d) Tuberculosis and syphilis
(e)-(f) Multiple sclerosis and sero-negative arthritis

(4) CANCER. GJ communication between normal and cancerous cells is disrupted22. There are two broad groups. The first are cancer cells which only communicate with their own kind and make no communication with adjacent normal cells. These are relatively less aggressive and invade locally rather than metastasize distantly. The other group contain cells which also cease to communicate with each other. They are immortal cell lines which have escaped from the usual Hayflick restriction of (about) 50 doublings. (Note that as cells age they become progressively poorer communicators through GJs2 and that they eventually elect to cease reproducing.) These cancers metastasize haematogenously to distant sites. Phorbol esters, which are cancer promoters, stabilise cells which would otherwise elect for apoptosis. The depression of focal surveillance that occurs in the wake of lymphocyte amplified auto-rejection is at least partially responsible for allowing malignant cells to escape detection and elimination. The final event that leads to immortalisation of the cancer cell line is probably the loss of the ability to effect apoptosis (through the p53 mechanism) when internal surveillance indicates it is appropriate.

CONCLUSION

The general principles of morphostasis are discussed. I have made a committed assumption that GJs are the most important ICJs in maintaining HS identity. Other ICJs may contribute a larger part than I have credited here. If well founded, the hypothesis should prove to be a useful framework for a more focused investigation of the biochemical processes of morphostasis.

19941029_thbiol

"There is only one constant element in immunity, whether innate or acquired, and that is phagocytosis. The extension and importance of this factor can no longer be denied."

Elie METCHNIKOFF 1905

"Immunology is an invention of the devil, who is making it up as he goes along because he's not too clear about this stuff either.". . . . . "Besides, immunology is what we North Americans call a Rube Goldberg system, referring to old cartoons about how to turn on the light, for example: you trip over a footstool, thus startling the cat, who bumps into the kitchen door, which swings shut, knocking over a chair that hits the light switch . . . you get the idea. There has to be an easier way."

Janice Hopkins TANNE 1990

ABSTRACT

I propose that the current perception of self/non-self discrimination is flawed. Most immunologists consider that lymphocytes are critically responsible for carrying out this discrimination. I propose that self/non-self discrimination is established in a different way and that the role of lymphocytes is one of servitude to the true self(cell)/non-self(cell) discriminator. The latter manipulates lymphocyte activity as a means to focus, caricaturise and amplify its own involvement at the next occurrence of a similar circumstance. All somatic cells are able to sense their neighbour's (healthy) self status. Individual self cells monitor their own health and generate a unique set of "healthy self (HS)" membrane "flags" and cytokines which act as signals to neighbouring HS cells to indicate that cooperation is safe and appropriate. In somatic tissues, minor breaches of HS identity can be dealt with by surrounding HS cells. When tissue damage is excessive, a second, "back stop", identity mechanism is brought to bear by inviting inflammatory cells into the area (mainly phagocytes). These phagocytes then assess local cells for HS status and will attack any cells (or organisms) that fail to exhibit it. Both somatic cells and the phagocytes which carry out this "back stop" check probably use an identity assessment similar to that used by somatic cells as they establish each others' identity when constructing an embryo. Individual helper lymphocytes simply remember the inflammatory or healthy soma context in which their respective epitope was first encountered and then they attempt to caricaturise this inflammatory or healthy soma environment on any fresh encounter. Using various clues, I go on to suggest that healthy self identity is emphasised strongly by groups of cells which are interconnected by gap junctions: these form extensive blocks of tissue which then behave as synctia of electrically and metabolically coupled "super-cells".

INTRODUCTION

The proposal I am about to make is stark: immunologists are missing the point: their current perception of the immune process is flawed. Just as astronomers were once confident that the heavens revolved around the earth, so modern immunologists are generally confident that anamnestic immunity and its executors, the lymphocytes, are placed firmly centre stage, acting as grand conductors in the (mammalian) immune universe. In particular, it has been an accepted dogma that lymphocytes are the major orchestrators of self/non-self discrimination.

is better regarded as a subservient response to, rather than the active source of, healthy-self(cell)/all-other(cell/organism) discrimination. Few of the component elements of this hypothesis are new. However, the emphasis on how they are perceived is and this new perception leads to a "paradigm shift".

THE EMERGENCE OF SELF(CELL)/NON-SELF(CELL) DISCRIMINATION

To set the scene, I would like to emphasise these points:

(1) When the first multicellulates evolved, they needed to recognise and discriminate self-cells from non-self-cells.
(2) We have become preoccupied with self(epitope)/non-self(epitope) discrimination, mainly as a result of the sequence of discoveries in immunology: this has blinkered our perceptions.
(3) In a large proportion of metazoans, lymphocytes are self-evidently not the source of self(cell)/non-self(cell) discrimination: they don't have any.
(4) It should be possible to discern gradual steps in the evolution of

immunity starting in primitive metazoans and leading on to the sophisticated system found in mammals. So far, no clear stepwise progression has been elucidated.

(5) In development, ontogeny frequently appears to retrace phylogeny: whilst this is not an absolute blueprint for evolution, it does provide important clues.

MORPHOSTASIS

Morphostasis is tissue homeostasis (Burwell, 1963) and it is well maintained in all animals. It is a core process, the functional hub of the metazoan universe. It works efficiently because cells monitor their own health and keep constant close communication with appropriate neighbours. Anamnestic immunity is a branch of the morphostatic process: it has evolved to enhance the effectiveness of morphostasis in vertebrates.

Remember, an animal is built of a large colony of cells all derived from one zygote cell (a zygote derived colony - ZDC). This colony constructs itself a skeleton of connective tissues which, while relatively inert, gives it great versatility (eg, the bony skeleton).

The critical function in morphostasis is discriminating Healthy-Self (HS) cells from all other cells and organisms (other than healthy self - OTHS cells). OTHS includes both UnHealthy Self (UHS) cells (eg, ectopic, sick, damaged, aging) and clearly foreign cells and/or organisms. Morphostasis was needed from the moment that multicellular animals first evolved. It should be clear that the main need at that time was to develop a unique way of tagging healthy self cells, so enabling them to identify and acknowledge one another, and then to devise mechanisms to abandon this healthy self status when things went wrong.

TABLE 1

Morphostasis (tissue homeostasis) can be maintained by:
(a) discriminating OTHS cells from HS cells.
(b) removing OTHS cells (UHS and foreign cells/organisms).
(c) replacing lost UHS cells with fresh HS cells (resurgent morphogenesis).

HEALTHY SELF/OTHER THAN HEALTHY SELF DISCRIMINATION

This hypothesis requires that individual cells must either have a fail-safe internal device for recognising that they have become unhealthy and/or an ability to monitor a neighbouring cell's change in health (probably) by monitoring (appropriate) cell to cell communication. The announcement of an "OTHS foul" can then be issued directly from the affected (somatic) cells. Inflammatory cells (mostly phagocytes) are only invited into the soma at this group's request - a "call" is sent out to fetch the "police". Foreign organisms need not induce an inflammatory response unless they unsuccessfully attempt communication with a HS cell, or force their way between cells (and so disrupt communication), or directly attack a cell and make it sick. Peaceful co-existence is an acceptable state.

Several properties may combine to specify HS (or UHS) identity; remember that one or more of the critical aspects which lead to HS (or UHS) recognition must be abandoned (or adopted) when the cell becomes sick. Here are some possible candidates:-

TABLE 2

(a) Lectins and the recognition of saccharides (eg, sialic acid).
(b) The inhibition of complement attack by proteins released from or displayed on the cell membrane (eg, factor H, DAF, MCP).
(c) Beta-2-microglobulin and Class 1 Mhc ligand expression.
(d) Cell to cell cytoplasmic joining - particularly electrical.
(e) Various cytokines, particularly eicosanoids/prostaglandins.
(f) Heat shock proteins and p53 are likely to be intimately involved in HS/UHS recognition and discrimination.

CELL IDENTITY IN THE EMBRYO AND OTHER SYSTEMS

The cells in an embryo recognise each other through Cell Adhesion Molecules (CAMs) (Edelman, 1986, 1987 & 1988, Edelman & Crossin, 1991, McClay & Ettenson, 1987). At the cell surface, both like/like and ligand/receptor interactions of these CAMs lead to cell adhesion. This adhesion then rapidly progresses on to communication through gap junctions (Keane et al., 1988). These CAMs are of three main types: first, the cadherins, second the integrins and third, a group of CAMs which are members of the immunoglobulin superfamily (IgSF) of which N-CAM is an example. Note that the transfer RNA molecules specifying N-CAM are spliced by cells in a variety of different ways to produce a range of N-CAM phenotypes. Edelman & Crossin (1991) state, "The origin of the entire Ig superfamily from an early N-CAM-like gene precursor has deep implications for the understanding of the role of adhesion in processes that are not concerned with morphogenesis but rather with immune defense, inflammation and repair".

The cells of an embryo are able to recognise appropriate neighbours: they navigate themselves into their designated locations where they meet their intended neighbours. There are many other observations of the specific recognition of cells and self in biology. Here are some specific examples:

TABLE 3

Protozoans recognise and discriminate food and sexual partners
Phagocytes are able to recognise their own pseudopodia and avoid self attack.
Simple multicellulates are known to reject allografts (1)
Plants - pollination is highly selective against self (2)
Reaggregation of disrupted foetal cells (see later) (3)
Bacterial agglutination and conjugation can be highly specific to self and (in pathogens) to target tissues. (4)
Plants - tree roots in a forest often fuse together. This is very frequent when they are from the same individual, not uncommon when they are from the same species and far less frequent when they are from unrelated species. (2)
Molecular recognition is a fundamental biological principle (eg, nuclear enzymes).
Cell homing: eg, lymphocytes and injected marrow cells. (5)

(1) Coombe et al., 1984
(2) Heslop-Harrison, 1988 and Lewis, 1979
(3) Garrod & Nicol, 1981 and Takeichi, 1990
(4) Reissig, 1977
(5) Hemler, 1990

Self recognition could, therefore, be observed in several ways, each becoming progressively more specific to the individual animal:-

TABLE 4

(a)	Tissue type recognition (eg, embryo cell recognition)
(b)	Species recognition (eg, gamete recognition)
(c)	Self ZDC recognition (ie, cells of the individual zygote derived clone. Useful as a "back stop" check of self)

MORPHOGENESIS

Morphogenesis is the process by which tissues and organs are sculptured from a zygote derived colony. It is most obvious in developing embryos: regeneration and repair are achieved by a resurgence of morphogenesis. Since morphogenesis is an integral part of a morphostatic system, it is reasonable to expect that it will share component elements of the same molecular machinery as those used by immune cells and phagocytes. These components have (presumably) been closely associated through every epoch of metazoan evolution. It remains unclear what the complete mechanisms are which lead to embryonic development. However, CAMs (as above) and gap junctions (Green, 1988) appear to play critical roles.

EMBRYOS, CAMs AND GAP JUNCTIONS

1) Gap junctional communication can be relatively non-specific (crossing species barriers) but it can also be highly selective (as below) (Kalima and Lo, 1989).
2) Gap junctional communication is critical in development. Embryo development fails when GJ communication is disrupted (Guthrie & Gilula, 1989).
3) When CAMs (cell adhesion molecules) interact with each other or their receptors, the ensuing cell adhesion appears to lead directly to gap-junctional communication. CAM interaction precedes GJ insertion and both are necessary for normal development (Jongen et al., 1991).
4) Embryos are made up of a number of compartments. Communication through gap junctions is constricted at their boundaries. These compartments correspond to important developmental fields (Kalima & Lo, 1989). They also correspond to fields of specific CAM expression (Keane et al., 1988) and homeotic gene expression (Coelho & Kosher 1991, Risek et al, 1992, Martinez et al, 1992).
5) The gap junctions in these compartments are of two sorts (Kalima & Lo, 1989). First, there are high permeability junctions joining each cell within a compartment. These allow the free passage of larger molecules: lucifer yellow is used to demonstrate this. I suspect that this "open" communication enables a block of cells to be organised, as if it was a single block of cytoplasm (a super-cell) . This may be under the control of the appropriate compartmental homoeotic genes (look at the complex structure of paramecium to see how structuring this block might work - the open cytoplasm of multinucleated drosophila eggs is similar). Second, there are more restrictive junctions which join the cells at the boundaries of these "open" compartments. These only allow small molecules to diffuse

(eg, ions) so they are either insufficiently large or insufficiently extensive to allow lucifer yellow to diffuse freely. These junctions allow ions to pass in either both or just one direction. The second sort are rectifying and they correspond to junctions formed from hybrid connexons (Werner et al., 1989, Barrio et al., 1991). This directionality may be of significance in the way that embryonic cells sort, with endoderm to centre and ectoderm to the outside.

6) Despite its name, N-CAM is not confined to neural tissues. Whilst it is expressed strongly and for long periods in neural development, it is also expressed, more transiently, in other sites. It is a recognised IgSF member (Immunoglobulin Super Family). A number of authors have considered these IgSF CAMs to be the probable ancestors of immunoglobulins, T-cell receptors and histocompatibility antigens.

When embryo cells are disaggregated and allowed to resettle, they reaggregate into tissue layers, ectoderm to the outside, mesoderm to the middle, then endoderm to the centre (Garrod & Nicol, 1981 and refs). When embryonic cells from two mammalian species are mixed, they reaggregate into tissue type rather than species type and this appears to be because the genes which specify the various CAMs are highly conserved across the species barriers (Takeichi, 1990).

MEMBRANE HOLES

It is now possible to make a stab at the general principle which governs HS/OTHS discrimination. I suspect it goes something like this:-

"SELF is established by making holes in the membranes of apposing cells and lining them up to create gap junctions. This allows cells to become electrically coupled and so to act as an electrical and, probably, a metabolic synctium. This ability to couple membranes dates back to the very earliest multicellulates. It relies on the controlled, ordered, simultaneous, adjacent membrane insertion of membrane holes. Cells learned, from the start, to encourage the uncoordinated, bigger, higgledy piggledy insertion of leaky holes into organisms which fail to demonstrate the membrane LIGANDs used as a focus for the tidy construction of gap junctions: the resulting electrical discontinuity and a lower membrane potential leads to an attack by scavengers. Unhealthy self cells can elect to be rejected by uncoupling from adjacent cells then dropping their membrane potential: they can also abandon the membrane LIGANDs which specify self. The mechanisms for constructing leaky holes (complement MACs) may, therefore, be distantly related to the mechanisms for constructing gap junctions."

HORROR AUTOTOXICUS & MORPHOSTASIS

One result of relying on self(cell) recognition is that "horror autotoxicus" (HA - the horror of attacking self) will probably have evolved long before lymphocytes and their memory for previously encountered antigens (anamnesis). However, this HA must be based upon the possession of specific and recognisable cell surface markers ("flags"): these probably aid the cooperative "docking" of one cell with another. Furthermore, because infection, cell damage, mutation, aging, genetic errors and other cell disturbances can also be assumed to be ancient problems, cells of the ZDC probably learned, early on, to observe "horror autotoxicus" to HS cells whilst rejecting, or sometimes just ignoring, OTHS (unhealthy self [UHS] and clearly foreign cells/organisms).

This interpretation of "horror autotoxicus" differs greatly from the classic one in which lymphocytes are deemed to be denied the right to attack self

epitopes. In this new interpretation, lymphocyte aggression towards self epitopes is neither denied nor necessarily avoided. However, as will become apparent, once such auto-aggression has arisen, it will decay unless other circumstances actively sustain it.

PHAGOCYTES and DOUBLE-THINK

There is a strange double-think that pervades immunology when it comes to the importance and centrality of phagocytes and the recognition of non-self and/or unhealthy self. Every medical student learns that phagocytes recognise dead, damaged, sick and effete cells. They also learn that phagocytes can recognise foreign organisms and eliminate them (particularly non-dedicated-pathogens). Every text book devotes its statutory (short) introductory opening to the critical importance of phagocytes and innate immunity: then, almost without fail and with what I regard as indecent haste, authors are seduced into an intense dissection of the principles of anamnesis and lymphocyte function. What makes this more bizarre is that the anamnestic immune system isn't essential to prepare cells for phagocyte attention. The phagocytic system works well, even if slowly, in invertebrates: and so does self/non-self discrimination.

There cannot be much doubt that the reason for this tendency to overlook the fundamental centrality of phagocytes is, first, a relative lack of understanding of the mechanisms of self/non-self discrimination by these cells and, second, the intense acceleration of the inflammatory process induced by lymphocytes. This greatly enhances the efficiency with which OTHS is removed and it has led us, for a long time, to regard lymphocytes as masters rather than servants of the system. There is, at the very least, a possibility that CAM interaction and junctional communication, between phagocytes and underlying somatic cells, may be the most important factor in (inflammatory) HS cell recognition. Furthermore, we have been preoccupied in looking for evidence of non-self recognition rather than healthy self recognition.

INFLAMMATION

Metazoans have evolved this ancient and virtually universal defence mechanism in which somatic tissues become infiltrated with scavenger cells (mostly phagocytes) whenever required. These scavengers are clearly capable of recognising most organisms, particularly those which are not dedicated pathogens. And, in the vast mass of animal life, they appear to do so without the aid of cells which have the ability to "remember" epitopes. They also remove aging and disordered self cells. In fact, their behaviour is ideally suited to eliminating OTHS. I propose two things:

(a) In all complex metazoans, the discrimination of OTHS from HS by phagocytes remains a central and crucial morphostatic process.

(b) All other immune processes are geared to accelerate, accentuate and maximise the discrimination of OTHS from HS by phagocytes. In consequence, the efficiency with which OTHS is removed is greatly enhanced.

Even so (as you will see later) HS/OTHS discrimination does not begin in phagocytes but in somatic cells. It is the consequence of general cell recognition and communication. Inflammation is only established when somatic cells "decide" that they cannot cope alone and "invite" these scavengers in. Static somatic cells are attached to each other at cell junctions. Their cytoplasms are joined by gap junctions (except in those cells who's mature function depends on electrical excitability). When membrane junctions are

split apart the disruptions in the cell membranes probably lead to the release of various eicosanoids (prostaglandins etc). This announcement of an OTHS event, by somatic cells, results in an inflammatory reaction. Chemical messengers released at the OTHS site encourage the ingress of phagocytes through the endothelial cell linings of local post-capillary venules. Phagocytes now invade the OTHS site. They begin assessing cells on the basis of their HS status. Note that in electrically excitable cells, like neurones, their terminal differentiation requires that they uncouple from each other: it is left to unusually tightly bound endothelial cells to restrict the ingress of scavenger cells and thus reduce the susceptibility of these tissues to inflammation.

Thus far, the basic process is the same for almost every, if not all, animal species. At this point, vertebrates enrol a new mechanism. Debris from local tissues is processed by phagocytes (or phagocyte related cells) and it is then presented, in local lymph nodes, to the anamnestic immune system as short memory of them and their inflammatory environment so that, on their next encounter (which must, incidentally, follow phagocyte/APC processing), this inflammatory environment can be rapidly and potently reproduced and, more often than not, exaggerated. This anamnestic response is under the full command of the morphostatic process and, in particular, largely under the control of phagocytes.

MIMICRY

Because morphostasis has always relied on self recognition, dedicated pathogens need to use mimicry (or more subtle interferences with identity molecule expression and recognition) to gain access to and persist in the soma (eg, Lyampert & Danilova, 1975, Chakraborty, 1988, Vanderplank, 1982, Yoshino & Boswell 1986). Every animal needs to stay one step ahead of its competition. Constant pressure is exerted to expand the variety of identity molecules available within a species (pleomorphism). Somatic cells appear to recognise each other by developmental ligands (cell adhesion molecules, CAMs). When embryonic cells from two mammalian species are disaggregated, mixed together and allowed to settle, they segregate into tissue type and not into species. Somatic ligands have probably needed to stay constant over countless meiotic generations. This makes them a sitting duck for determined pathogens. So, somatic cells need a "back stop" identity to be used as a second check when things go wrong (phagocyte based and, perhaps, also Mhc Class 1 based (Versteeg, 1992)). And until they do go wrong, inflammatory cells can be confined to the vascular system, locked out behind tight endothelial cell junctions until invited in. Note that "loss of function" is a cardinal feature of the inflammatory process.

UNHEALTHY SELF ACTIONS: APOPTOSIS AND SELF SACRIFICE

When cells fail to establish communication, membrane reactions probably begin which lead to the release of a variety of eicosanoids and other cytokines (Bach, 1988). Similarly, when cells become unhealthy they break junctional communication and become prey to attack by both adjacent cells and the inflammatory cells which are (in consequence) called into the area (Loewenstein & Penn, 1967). When I first started thinking about self(cell) surveillance, I found scant literature describing elective suicide and I even looked at plants for evidence of this (the hypersensitivity reaction (Prusky, 1988, Fritig et al., 1987). However, interest and literature on this subject have become abundant recently (Bowen & Lockshin, 1981, Cohen, 1991, Ellis et al., 1991, Young, 1992). In synthesis, individual cells do decide that they are sick and/or redundant. They do have the capacity to invite attack by adjacent cells and also to invite phagocytes along to have themselves removed.

There is no need to presume that antibodies and lymphocytes are responsible for the primary assessment of (healthy) self status.

Changes in the concentration of calcium ions within the cell are all important in this election for "disposal by consensus". Ca++ ions act as second messengers for a variety of cell processes including apoptosis, nuclear division, growth factor stimulation: they are closely tied into the inositol-PO4/DAG/protein-kinase-C network of intracellular second messengers (Hollywood, 1991, Evans & Graham 1990): and high Ca++ ion concentrations close down the gap junction channels between cells. In this respect, cellular identity and cell health is all tied into proto-oncogene activity and this in turn into gap junction formation and communication competence (Yamasaki et al, 1988, Yamasaki 1990). Here is the promise of a much clearer understanding of cancer.

When cells are attacked by C9 or perforin, they are made leaky, their cytoplasmic membrane potential falls and Ca++ ions are allowed into the cell. Both these molecules contain sequence motifs similar to the LDL receptor and epidermal growth factor receptor and there may be wider significance in this (see Maldonado et al 1988). One important feature is that both these receptors are endocytosed in clathrin coated pits (like the Mhc molecules themselves).

THE GENERATION OF SPECIFICITY

A major problem in understanding the evolution of anamnestic immunity is how such a complex system erupted onto the evolutionary scene, so suddenly and so completely, in the vertebrates. One explanation is that it evolved, not as a generator of receptor diversity but as a generator of receptor specificity. The table below shows how a scavenger cell could be programmed only to cooperate with self cells which display ligands unique to that single ZDC. The specification of such a scavenger is an exact inversion of the specification of the cytotoxic T cell. Even a repertoire of receptors as few as two would be useful in specificity whereas, in diversity, it is difficult to see how any useful function could have evolved until there was a large repertoire of possible receptors. With a system which develops on the basis of specificity, there would be ample time to develop an extensive repertoire of possible receptors before being precipitously "flipped around" to service a generator of diversity. Note that "pure self" is used to indicate unaltered, self Class I Mhc antigens.

TABLE 5

Cell type	Receptors disabled	Receptors enabled	Normal state	Triggered state
Scavenger	non pure self	pure self	aggressive	passive
	GENERATOR OF SPECIFITY			
Tc cell	pure self	non pure self	passive	aggressive

There are two possibilities. First, that the ancestors of the T cell receptor may have been used to recognise tissue CAM ligands: this could be the origin of the V gene segments (Allison & Havrin, 1991). Secondly, a descendant of the simple scavenger (phagocyte) may have evolved to recognise a set of pleomorphic CAM like markers which were specifically evolved in a population for them to be used as a back stop identity check unique to each ZDC. Developmental CAMs seem to remain constant over countless generations and this is reflected in the way embryonic cells from different species reaggregate as germ layers and tissues rather than species. The "back stop" CAM like ligand (the precursor of the Class I Mhc antigens) could deliberately borrow bits and bobs from these developmental CAMs to form a unique looking ligand by using a genetic mix and match process.

There seems to be little likelihood that phagocytes are able to rearrange their genome to form specific receptors. And there is no substantive evidence that they can selectively cooperate with cells carrying self Mhc antigens. Natural killer cells, however, might be such a candidate, particularly if they are composed of two populations: one with a lower specificity - perhaps based on beta-2-microglobulin expression - and another with highly specific receptors for self. They were first identified because F1 Tnk cells attacked parental cells (unlike the classical transplantation laws). This would be consistent with specific (cooperative) recognition. These cells also preferentially attack cells expressing low levels of Class I antigen and beta-2-microglobulin. It seems that, at most, only a proportion of Tnk cells rearrange their receptor genes. (See Trinchieri, 1989 and Versteeg, 1992).

Phagocytes, lymphocytes, fibroblasts and platelets are all derived from the same stem cell. They have almost certainly all evolved from a primitive, ancestral scavenger. Each cell type seems to have caricaturised some specific property of this general scavenger and refined it in order to make the mature mammal's repertoire of responses more versatile. This adds weight to the proposition that a phagocyte like or derived cell might, at one stage, have evolved to have the ability to select/rearrange its genes so that it could specifically recognise healthy self ligands (Mhc "Class-I-like" ligands). The self receptors would have to be selected, in embryo, to be specific to each individual.

One possibility is that, now the lymphocyte system has evolved, this has been so successful that it has largely obviated the need for a scavenger to rearrange its genes to uniquely recognise self. There might even be a positive advantage in achieving the apparent recognition of HS(cells) by inverting the cooperative recognition of self cells into an attack on non-self(epitopes) by Tc lymphocytes. This can be achieved by the clonal elimination of any lymphocyte capable of reacting with "pure self" Class 1 ligands.

Note that complement activity is very much in the style of a horror autotoxicus, with healthy self being protected from attack by inhibitors: and also that phagocytes synthesise enough of all but the terminal components to attack undesirable cells without the aid of circulating complement.

SOMA/SCAVENGER SEGREGATION

I have already alluded to soma/scavenger segregation. The important point to grasp is that somatic cells can and do deal adequately with a fair proportion

of OTHS (Young, 1992). Provided the accumulation of OTHS is mild and the local cells can both recognise any loss of HS identity and discriminate foreign organisms from HS, then there is little need for a back stop identity check. HS here is established by displaying appropriate tissue CAMs which lead on to the establishment of a "synctial" communication through GJs. However, when UHS or foreign organisms fail to appear sufficiently OTHS to the local cells, then tissue damage will probably ensue as the foreign cells or UHS cells start to gain the upper hand. It is at this stage that scavengers are "invited" in and this is done by a fail-safe device (the eicosanoid system - prostaglandins etc). These scavengers then establish HS status by employing a "back stop" check on identity. If there is a scavenger which formally recognises HS Class 1 status then this would give the system a highly specific way of recognising self once invoked (eg, the Tnk cell (Versteeg, 1992)).

Inflammatory cells invade and disrupt the normal structure of tissues and this invasion leads to loss of function. They are undesirable intruders in healthy tissues except in small numbers. Hence they need to be kept largely locked out, behind a tightly bound cylindrical pavement of endothelial cells lining the blood vessel walls. This need for segregation is almost certainly the origin of the vascular system. The subsequent recruitment of the vascular system into distributing other "freight" has meant that phagocytes and their evolvents have become adapted to such tasks as encapsulating the inflammatory process (by clotting factors and platelets), distributing fats in the blood (phagocytes), anamnestic immunity (lymphocytes) and transporting oxygen (red cells).

Now it is possible to add some concluding comments to the six points introduced earlier in the section "EMBRYOS, CAMs AND GAP JUNCTIONS":

7) In this hypothesis I have suggested that scavenger cells (phagocytes mostly) use a CAM receptor molecule to latch onto a respective CAM on self cells. The base of a phagocyte (uropod) remains attached to the underlying tissues. This base probably maintains electrical contact with the underlying cells through GJs. The cytoplasmic fingers of a phagocyte (the lamellipod) constantly probe forward. If these fingers encounter a cell which is not in electrical continuity, the scavenger could be triggered into aggression by the capacitative current which flows as the membranes come close together. This could, in turn, trigger an action potential to arm the cytoplasmic finger of the scavenger cell. Additional recognition strategies may be employed. The changing of surface sugars in sick cells is one (loss of the negatively charged sialic acid residues may increase the capacitive current above the triggering threshold). The phagocyte may well have a limited "hit list" of receptors which recognise markers which are indubitable evidence of their non-eucaryotic origin and which would, therefore, never be found as part of self. Dedicated pathogens will, of course, studiously avoid displaying these.
8) Now, the original self CAM may gradually be found to be inadequate as a back stop identity check because various pathogens discover ways of mimicking or interfering with its machinery. At this stage, a new cell is required (perhaps similar to the natural killer cell) which can recognise a more pleomorphic set of CAMs that are deliberately individualised in each animal of a population and more or less unique to each ZDC. An appropriate set of specific receptors would have to be selected, in embryo, to recognise these unique ligands. These, I contend, may be the close ancestors the T cell receptor which led, by inversion of function, to the cytotoxic T cell. In this vein, note that tumour necrosis factor and lymphotoxin are selectively toxic to cells which are not communicating

through gap junctions (Fletcher et al., 1987, Matthews & Neale 1989).

ANAMNESTIC AMPLIFICATION

So, what is the function of lymphocytes: what are they doing? An individual lymphocyte is simply following orders from an antigen presenting cell or phagocyte (in conjunction with an unhealthy somatic cell in the case of Tc cells). This instructs it to attach either an aggressive or a suppressive action to its paratope and to act accordingly on its next encounter with its respective epitope. Direct killing is not the prime function in either delayed type hypersensitivity T-cells (TH1) or helper T-cells (TH2). They are not remembering epitopes for the prime purpose of "killing" them. The precursor lymphocyte logs the context in which it first "sets eyes" on its epitope. If it was inflammatory then at the next encounter it will attempt to recreate a rapid and potent inflammatory response rather than wait for the "cell damage -> cytokine -> inflammation" cascade to build up. "Tipped off" inflammatory cells can then settle down much more quickly and aggressively to their phylogenetically ancient task of sorting HS from OTHS. The main difference now is that these phagocytes are doing it much more quickly and with better targeting. But, they are also doing it more hamhandedly - they'll "bash" anything that looks remotely suspicious (hence the need to focalise this response). Tc cells are relatively more independent and kill directly but even these are only allowed to become aggressive if they have first been primed by IL-1 released from APCs during an inflammatory encounter. And these, too, encourage a rapid inflammatory response once they start attacking target cells.

Somatic cells probably show some specificity for the epitopes that they present for Tc cell priming. The peptides that they present in combination with Class I antigens have probably been shepherded through the cell by its garbage minders, the ubiquitins. Even leaving this aside, it is still easy to imagine how self/non-self selectivity can occur. When T-cells are released from the thymus they are already committed in specificity (ie, they are committed to recognising a specific epitope) but, they are not committed in activity (aggression or suppression). It is only when they meet their respective epitope that this commitment is made. Self epitopes are, in general, encountered frequently and the first encounter (in embryo) is nearly always in a "healthy self" (non-inflammatory) environment. So tolerance is generally favoured for those lymphocytes which recognise self molecules. Few self specific T-cells will remain uncommitted for more than a brief period while there is a relatively large pool of the relevant self epitope waiting to be encountered.

On the other hand, because only small quantities of a foreign or strange epitope are infrequently met in the body, most T-cells capable of recognising them will remain uncommitted until they meet the epitope, as part of OTHS, in an inflammatory encounter: aggression will be favoured. Furthermore, it seems that it is easier to provoke old rather than young precursor lymphocytes into aggression. This further concentrates the aggressive response onto those epitopes that are most strange to the body. No veto need be imposed on T-cells to prevent them becoming aggressive to self epitopes (except for "pure self" Mhc ligands - these must be clonally disabled). Indeed, epitopes from tissues that are usually hidden behind tight endothelial cell junctions (like the eye and brain), and are infrequently encountered, are more likely to provoke aggression as there will be a larger pool of uncommitted T-cells available. They are, consequently, more inclined to provoke an aggressive response when they are exposed during periods of intense inflammation. (Lymphocytes which have a paratope for recognising certain self Mhc/peptides

are clonally deleted in the thymus: this deletion follows the disintegration of self cells in the thymic medulla.)

The bone marrow constantly produces new uncommitted T-cells. So, whenever clearly foreign epitopes are sparse and inflammation is intense and prolonged, attention can gradually turn to self epitopes (eg, as in tuberculosis). In summary, inflammatory acceleration is most likely to develop to clearly foreign (strange) epitopes and a "healthy soma tolerance" most likely to develop to self (frequently encountered) epitopes.

The overall effect is that lymphocytes remember the "inflammatory" or "healthy soma" context in which they first meet their respective epitope (and become committed); and they aim to recreate and caricaturise this memorised inflammatory or non-inflammatory milieu at the next encounter. Whenever TH1 cells provoke an inflammatory response they call large numbers of phagocytes (& Tnk cells?) to the epitope site. These are then switched into a heightened state of "anger". However, phagocytes (& Tnk cells?) still have to discriminate HS from OTHS but now, the threshold at which aggression is considered is greatly reduced. Cells expressing a relatively low level of "HS identity" are now likely to be attacked. This amplification of the inflammatory response by lymphocytes has the potential to escalate catastrophically. It can slip into a loop of strong positive feedback, particularly when the epitope is an abundant self Ag. When the local auto-rejective response becomes excessive, it must be down-regulated otherwise things will get disastrously out of hand. This could be done in a number of ways and these may account for many instances of clinical anergy (Dwyer, 1984, Meakins, 1988, Meakins & Christou, 1979, Normann et al., 1981, Ninneman, 1981):

TABLE 6

(a) inhibition of phagocyte ingression (chemotaxis)
(b) inhibition of phagocyte aggression
(c) inhibition of further aggressive lymphocyte activation
(d) a tightening of endothelial cell junctions
(e) encapsulation in a fibrin sheath (fibrocytes later)
(f) promotion of lymphocytic tolerance to typical Ag
(g) production of auto-antibodies to the newly cloned, locally reactive lymphocytes (lymphocytotoxic Abs)

TABLE 7

THE FOUR PRINCIPAL MODES OF EPITOPE PRESENTATION

	OTHER THAN HEALTHY SELF CONTEXT	HEALTHY SELF CONTEXT
SOMATIC CELL	Tc activation (Class I Mhc)	Ts activation (Direct??)
PHAGOCYTIC	TH1 & TH2 activation	Ts activation eration? Th/Ts)

AUTO-REJECTION

Tissue rejection is largely accomplished by cell mediated mechanisms. Antibodies are generally bystanders. Similarly, the auto-rejection of abnormal cells will be accomplished predominantly by cell mediated immune mechanisms (eg, in various forms of necrosis like burns and infarction). There is one important inference to be made from examining the structure of the sero-negative arthritides and particularly Behcet's syndrome (based on a personal study). This is that auto-rejective disease covers a wide spectrum of prevalence and severity. The mildest components are VERY common, suggesting that auto-rejection is a normal process. This leads on to the conclusion that there is no automatic horror autotoxicus to self epitopes where T cells are concerned. When auto-rejection is so general, it has to have physiological as well as pathological significance: it must be a functioning element of the morphostatic mechanism.

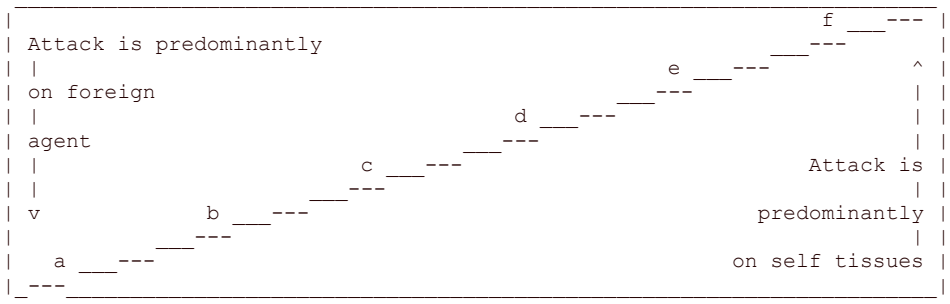
ANTIBODIES - ICING ON THE CAKE

Antibodies are icing on the cake. Extremely useful, evidently important but dominantly aimed at pre-empting the proliferation of blood borne pathogens and pathogens which colonise epi/endothelial surfaces. It's clear that the role of antibodies in tissue rejection (and hence auto-rejection) is minor if not minimal. The vast mass of animal life copes well without them. "Cell-mediated immunity clearly precedes humeral antibody production in phylogeny" (Manning and Turner, 1976 also emphasised by Cooper, 1982). We can safely put antibodies to one side until towards the end - which is more or less where they evolved. It appears to me that, to bother looking amongst antibodies for an explanation of how self/non-self discrimination evolved, would be manifestly Heath Robinson (or Rube Goldberg!). In this vein, it is worth noting that the spleen may be specifically adapted to make the best of the difficult job of maintaining morphostasis in the suspension of cells circulating in the highly mobile plasma.

THE CLINICAL IMPLICATIONS

The result of all this is that any disease which evokes an inflammatory response has an element of auto-rejection. It inevitably consists of a mixture which varies from an attack directed almost exclusively at the pathogen (usually leading to mild inflammation) to an attack directed almost entirely at self (often highly inflammatory): the latter occurs when organisms or cells provoke prolonged inflammation but do not provide or present clearly foreign looking (unusual) epitopes. Every disease that leads to cell damage will induce auto-rejection. Since heat shock proteins are responsible for chaperoning disrupted proteins through the cell, they are frequently presented as potential epitopes in UHS presentations.

TABLE 8



EXAMPLES

(a) Saprophyte

(b) Simple epithelial commensal
(c) Staphylococci and streptococci
(d) Tuberculosis and syphilis
(e)-(f) Multiple sclerosis and sero-negative arthritis

MORPHOSTATIC EVOLUTION

It is now easier to see how the morphostatic system may have evolved. Here is the probable path of the evolution of ZDCs from simple multicellulates to mammals.

(a) In the beginning, all cells in the colony express equally marked phagocytic behaviour.

(b) SELF is established by making holes in the membranes of apposing cells and lining them up to create gap junctions. Cells learn, early on, to allow the uncoordinated, bigger, higgledy piggledy insertion of leaky holes into organisms which fail to demonstrate the membrane LIGANDs used as a focus for the tidy construction of gap junctions.

(c) Cells now divide into phagocytes and soma. They selectively improve the specificity and efficiency of cell junction construction by facilitating and amplifying their construction at the site of cell LIGAND/RECEPTOR interaction. The resulting gap junctional plates are more "transparent" and more specific about where they form. They develop:

SOMA LIGAND(s) - for recognition by resident scaffolders.
PHAGOCYTE LIGAND(s) - for recognition by itinerant scavengers.

(d) Dedicated scavengers (phagocytes) now evolve. They refine this cooperative gap-junctional communication with self and the runaway, leaky hole attack of non-self. The molecules used to do the second will eventually evolve into what we now recognise as the complement components. It is possible that the two construction cascades are related but become independent early in evolution. At this stage the complement components are only secreted locally by phagocytes and their action is directed entirely at membranes. It is a long time before these components are co-opted into a humeral system and very much later that they are co-opted to interact with antibodies (probably an adaptation of specific Mhc recognition).

(e) A "vascular" system now evolves, locking out phagocytes till required. The alternative complement cascade can now be "humeralised" so that circulating C3 can mark clearly foreign organisms to make them more readily identifiable when they meet a phagocyte.

(f) There is now a progressive evolution and expansion of somatic LIGANDs leading to increased tissue compartmentalisation. Phagocytes are derived from a lineage which lies "outside" the three main germ layers so they may be exploiting this sorting tendency as they infiltrate somatic tissues: it is as if they are able to "clamber" over every other cell type.

(g) Ig supergene like LIGANDs develop to act as a focus on which to grow highly specific gap junctional plates and create developmental compartments. The genes specifying these molecules can now be copied then altered by a "mix and match" process to generate a set of LIGANDs which

have a great variability within a herd (primordial Mhc genes). These pleomorphic LIGANDs will now act as the final arbiters of healthy self in each individual. Over many meiotic generations, they have eventually evolved into Mhc Class I LIGANDs. Newly developed scavenger cells (Tnk precursors) may now be able, when required, to co-operate with any somatic cell that displays self specific LIGANDs and observe a horror autotoxicus to it. These new scavengers need a mechanism to produce and/or select self specific RECEPTORs unique to each ZDC. This must be done post-meiotically over a number of mitotic generations - the "generation of specificity". This possibly coincides with the evolution of amniotic molecules which are involved in HS/OTHS discrimination or its modulation These include HSP70, TNF, complement components and the 21-hydroxylases.

(h) By inverting the "generator of specificity" into the "generator of diversity" lymphocytic cells (Tc like) can evolve which are able to recognise and attack cells who's Class I ligands have been altered in the presenting cell by the attachment of a peptide which may make them look like an allotype. This new function depends on the duplication and transposition of the gene which produces the heat shock protein peptide pincer mechanism and bringing this to lie next an the Ig superfamily domain to produce the ancestor of a Class I Mhc gene (Flajnik et al, 1991). These primordial Tc cells first develop to recognise Mhc "Class-I-like" allotypes and then peptide/Class I combinations. They were probably preceded by cells capable of recognising beta-2-microglobulin: hence, the eventual elaboration around this molecule. Sometime between now and the evolution of free antibodies, the so called "alternative" complement pathway is extended into the "classical" pathway. C1 might be specialised for short range triggering of high density, single surface LIGAND/RECEPTOR complexes so that hole construction is now restricted to the target membrane rather than to a coordinated construction in apposing membranes.

ligands evolve: the "intention" is to process short representative peptides from cellular debris picked up by phagocytes at inflammatory for the attention of uncommitted T-cells. The "generator of diversity" can now be enrolled into memorising the inflammatory context of these processed epitopes. On re-encountering the processed epitope these T-cells can rapidly attract large numbers of phagocytes to the site and "angrify" them: inflammation now has a memory. Note that only a very limited set of cells - APCs, phagocytes and a few others - can present these combinant epitopes so this amplification of the inflammatory cascade can only start after OTHS has been processed.

(k) The need to instruct T-cells to tolerate healthy soma epitopes has to evolve simultaneously with Tc and TH1 cells. T-cells capable of recognising healthy self epitopes are mostly decommissioned. This may be a co-operative process (Th/Ts cooperation akin to Th/B-cell co-operation). Whatever, aggression is averted by having them "mopped up" by Ts commitment. This happens because these epitopes are more likely to be met in a non-inflammatory context. However, uncommitted self specific T-cells continue to be released from the thymus and can become recruited into aggression. Aggression to self epitopes will be most likely to be induced and permitted when the inflammatory process is prolonged and foreign epitopes are sparse. Tolerance might be amplified by Ts cell clonal expansion and, perhaps, the release of anti-inflammatory agents at the site of epitope re-encounter. Like TH2 and B-cell interaction, helper and suppressor epitopes tend not to overlap, suggesting a similar co-operative

mechanism.

(m) Last of all, TH2 cells can now be incorporated into the system to prime the B-cell system and lead to freely circulating antibodies. The B-cells are also derived from a scavenger cell. This is designed to secrete large quantities of free, circulating antibody. Antibodies help by opsonising organisms (preparing them as a "meal" for phagocytes). The classical complement cascade is now optimised to work within the vascular system and to interact with antibody tagged antigen. This system has proved invaluable as a pre-emptive defence.

THE ADVANTAGES OF THIS PERCEPTION

By now I hope that you will be aware that all this suggests a clear path in self/non-self discrimination. Its beginnings can be seen in simple animals like sponges, which demonstrate differential cell reaggregation (for they, too, have gap junctions) and it proceeds through to the complex mammalian immune system. In this respect, it is interesting to read that differential sorting is, in embryos, a direct consequence of CAM expression (Takeichi, 1990). The reasons why embryonic cells sort according to tissues rather than according to species is that their CAMs have remained highly conserved across widely separated species.

1) Seamless integration from embryonic development to anamnestic immunity.
2) The innate and the acquired immune system are no longer seen as fundamentally disparate entities. They are fused into a seamless whole.
3) A clearer understanding of preferential alloreactivity by T cells.
4) A clear evolutionary progression from organisms with no cellular differentiation, through simple organisms with phagocytes, then the evolution of a retinue of specialised cells all derived from the primitive scavenger. A "logical progression" would start with Tnk like cells, go to Tc like cells, then TH1 like cells, then TH2 like cells and finally B cells.
5) A far clearer perception of the cancerous process (not detailed here but there is good evidence that gap-junctional communication is involved (Yamasaki et al., 1988, Yamasaki 1990).
6) The potential to explain the process of aging (Kelley et al., 1979, Peacock & Campisi, 1991).
7) It all makes good biological sense. Indeed, it integrates so many biological, developmental and immunological mechanisms into a continuous whole that it begins to hold out the promise of a "grand unification theory".

SUMMARY

I have proposed reshaping the perception of immunity to encompass the broader principle of MORPHOSTASIS. The loss of healthy self is sensed and expressed by the malfunctioning cell itself or, at furthest, emanates from the membrane doublet where contact is established between this cell and its immediate neighbours. This "foul" is broadcast by the release of inflammatory mediators. These invite phagocytes into the area to assess the local population. Phagocytes (and perhaps Tnk cells) then attack those cells with which they fail to become electrically continuous. The time they have to make this connection varies with the "anger" of the phagocytes. Phagocytes now present cell debris to lymphocytes in local lymph nodes. The epitopes which are most strange to the lymphocytes are selected to act as the pegs on which to hang a greatly accelerated inflammatory infiltration on any subsequent encounter of

these epitopes.

I have also proposed redefining the concept of "horror autotoxicus": it is established by successful cell to cell communication. Both somatic and scavenger cells use this mechanism. The concept of immunological surveillance is simultaneously redefined. But now surveillance is for any malfunctioning cell and not just for neoplasia. The evolution of a thymus dependent lymphocytic system with memory may have occurred at the expense of an increased prevalence of cancer, for intense focal suppression of surveillance now occurs whenever a strong positive feedback leads to an exaggerated attack on self epitopes. This then permits a tumour cell compartment to reach a critical mass beyond which surveillance fails (Yamasaki, 1990).

This explanation undoubtedly contains errors and I am sure many of the more specific assumptions will prove to have been far too simplistic. For example, the immune system has gathered a great number of refinements throughout its evolution including various specialised phagocytes and permanently resident, non-itinerant antigen presenting cells: little has been said about these. However, I am confident that the "flavour" of the concept is essentially correct and the hypothesis will prove to be a useful framework for refinement. It should now be clear that the breaking of cellular junctions is probably an important event which leads on to the declaration of an OTHS "foul". There are a number of close similarities between the insertion of gap junctions into self cell membranes and the insertion of complement membrane attack complexes into invaders. If it could be shown that there is a continuing or a distant relationship between their respective insertion mechanisms, then it would be reasonable to assume that HS is, indeed, sensed by the speed with which both somatic cells and scavenger cells establish an electrical continuum with those cells that they encounter.