The danger theory: 25 years later.

(Adapted from a submission to Frontiers Immunology in 2013)
(The original was squeezed into a 2000 word essay to avoid publication costs. Here it is slightly expanded but still in pithy style.)

In their critique of the "danger" theory, Pradeu and Cooper (1) raised a number of issues. Here, I give my opinion – that the "danger" model should be integrated into the framework of tissue homeostasis. The conclusions are arguably tautologous so my style is conclusory. Readers must remain critical of my explanations and wary of the metaphors.

First, some definitions

- the adaptive immune system (T-cells, B-cells and antibodies) uses a family of polymorphic receptors that are generated by rearranging immunoglobulin superfamily genes. I use Jerne's "paratope" to encompass this receptor family. The size of a paratope spans 15-22 amino acids. It binds to a corresponding epitope, usually part of a molecule (most often protein). A paratope is an inverted match of the size, shape and physico-chemical properties of its corresponding epitope, so that the two interlock.
- "self" signifies cooperating-whole-cells that are descendants from one zygote.

History

The consensus view of the immune system in the 1980s was that:

- It is a microbe hunting, chasing and killing system
- It eliminates lymphocytes with self reactive paratopes (*in utero* and epitope by epitope). The remaining lymphocytes are primed for aggression. They are clonally expanded when they actually encounter nonself epitopes.
- It observes a horror autotoxicus to self molecules; self reactivity is physiologically forbidden.
- The immune "universe" is lymphocentric; lymphocytes are in executive command and control.
- The innate system is an evolutionary remnant, eclipsed by a far superior adaptive system.

Janeway (2) challenged this lymphocentric view. In 1989 he proposed:

- The immune system evolved specifically to recognise and respond to infectious micro-organisms.
- Innate immunity initiates all immune responses.
- Using linguistic gymnastics, he proposed:
  - aggressive adaptive responses are promoted only in the presence of PAMPs.
  - Self/nonself discrimination remains unchanged in his new conception.

Matzinger (3) published her "danger" theory in 1994.

- She did not address innate immunity in any depth.
- She proposed that:
  - cell damage, rather than nonself, is the primer for (aggressive) adaptive immunity
  - apoptosis protects against immune aggression
  - "bad cell-death" (whole-self-cells implied) leads to immune aggression
  - damaged tissues release alarm signals.

My opinion

Polly Matzinger correctly challenged the hegemony of self-epitope/nonself-epitope models. But, has the "danger" theory rested on its conceptual laurels? Has it openly modified its language and broadened its scope? Is it integrating into a wider perspective? In particular, has it fully embraced innate immunity and inflammation? Retrospectively, "danger" is an inappropriate metaphor for danger classification requires a proactive, intelligent classification system. "Damage" is a better metaphor: cell damage, death and its resulting debris reactively trigger aggressive-adaptive-immune responses. Matzinger embraces "damage" in the mechanism but not in the title. She has not jettisoned "danger"; doing so would improve the model.

Claims that "danger" is analogous to a Copernican revolution are tenuous whilst it focuses principally on how aggressive adaptive immunity is primed. Copernicus transposed the geocentric firmament into a heliocentric planetary system. This made better sense of its elements (sun, planets, moons, comets, stars). "Danger" does not rearrange relationships between immune elements. However, the parallels with a Copernican revolution are stunning once we adopt a phago-centric view of the "immune universe" and even more so when we embrace an inflammo-centric perspective. The adaptive immune system is now slave to

inflammation. It “remembers” a snapshot of the debris generated by pathogenic stimuli (e.g., apoptotic cells, necrosing cells, spilt cytoplasm and interlopers).

All debris is ingested, cleared and processed. All the epitopes thus generated are taken by phagocytes (particularly APCs) to local lymph nodes and presented to lymphocytes. Lymphocytes with matching paratopes are clonally expanded. They can then either aggravate or suppress inflammation when the epitope is re-encountered. The chosen polarisation emulates the inflammatory/non-inflammatory milieu that predominated when the APCs ingested and processed their debris. This inflammatory milieu does not reflect any intrinsic quality of the epitope and can change independently of it.

Protagonists sometimes regard “danger” as an intrinsic quality of an epitope and they anticipate that it is established epitope by epitope. This is a conceptual millstone, inherited from self-epitope/nonself-epitope perceptions. The committed T-cell independently acquires its memory of general “damage” and specific “debris”. This pairing can change on subsequent responses to presented epitopes. This change to aggression or suppression depends upon the release of fresh precursor T-cells (from the marrow) that are able to be committed to aggression (ie, the pool of available naive T-cells is only committed into tolerance or aggression when paratope specific T-cells encounter their designate epitope – as happens during apoptosis of self tissues or during a damaging event. Debris from apoptosing self cells will favour tolerance and debris from damaged self cells, particularly in the presence of strange debris, will favour aggression).

Very large numbers of “self” cells die as they become compromised by age or other dysfunction. These generally enter a programme of controlled cell shutdown (of which, apoptosis is the dominant one). Apoptotic debris litters extracellular spaces; it is “silently” cleared by phagocytes (both amateur and professional). The professionals (e.g., APCs and macrophages) process this debris into peptides that they then carry off to present to lymphocytes – cradled in the peptide groove of a self Mhc molecule. When lymphocyte paratopes “recognise” these apoptotic-debris-epitopes (as Mhc+peptide agglomerates) they are programmed, when the respective epitopes are re-encountered, to exaggerate a tolerant milieu. Massive apoptosis occurring in the foetal thymus is likely to generate many T-regs (4). These apoptosing cells probably represent that majority of precursor lymphocytes that do not spontaneously generate receptors able to recognise self-Mhc molecules. These melt away in large numbers. Their debris almost certainly leads to a commitment into T-reg thymocytes (thymic lymphocytes). If catastrophic death does occur in the thymus and it encounters strange epitopes (self-Mhc+peptide) - within the thymic environment – then these should be capable of triggering an aggressive T-cell commitment.

“Housekeeping” throughout the animal body generates enormous quantities of apoptotic “self” debris – particularly at night, in synchrony with the circadian rhythm. This constant deluge melts away with minimal or no inflammation. Necrotic death (characterised by spilt cytoplasm) incites intense inflammation and aggressive immune activation. Once we regard adaptive immunity as a subservient mechanism, controlled by phagocyte lineages (APCs, macrophages etc), then it becomes clear that the mammalian inflammatory system (dominated by phagocytes) has partially separated its initiation phase (using APCs) from its execution phase (the inflammatory invasion by an army of phagocytes). Adaptive immunity now bridges the two and can accelerate, exaggerate and focalise inflammation (an innate immune process) on fresh encounters of memorised debris. This exaggeration includes T-reg suppression of inflammation to appropriately-controlled-shutdown-debris. The signature of a pathogenic stimulus is probably recorded as a suite of epitopes; this is a testable prediction. There should be intensifying inflammation as more members of this suite coincide in a re-encounter. “Self” apoptotic debris swamps other forms of debris; it favours tolerance and this is hard to overturn as lymphocytes with “self” reactive paratopes will be exhaustively committed into tolerance. A later aggressive T-cell activation will only occur if there is protracted “self”-cell-debris and angry inflammation. Eventually, the bone marrow will release precursor lymphocytes with appropriate paratopes and the previous exhaustion can be overcome. Thus, repeated injections of adjuvant, mixed with pulverised tissues, eventually provoke auto-aggression (eg, experimental allergic arthritis, encephalitis, ophthamitis, etc). This is incompatible with traditional perceptions of horror autotoxicus.

So what assortment of debris is encountered? In order of prevalence, this includes:

- Apoptosing “self” cells (senescent, abnormal, infected; these are rapidly and silently removed); this is safe, controlled, cell-shutdown.
- Apoptotic bodies can overwhelm the system’s capacity to remove them
    - When there are too many
    - When the clearance mechanisms are flawed

- Catastrophic cell death
  - Is common in trauma. Rarely, this trauma may be too abrupt to set off internal alarm responses (note how laser scalpels cause minimal inflammation).
  - Necrosis (following virus infection, heat, radiation, ischaemia, trauma)
- Other than healthy-zygote-derived-cells. This includes microbes. Complement and MAMPs help to identify many of these.

Metaphorically, we have:
- Tidily packaged apoptotic bodies – "bagged debris": generally, rapidly removed and non inflammatory.
- Bagged debris - but the collection systems may be overwhelmed: so they are potentially inflammatory should they spill their contents.
- The "bags" split when they are left lying around too long: highly inflammatory.
- Catastrophic rupture with exploding cell debris: this is highly inflammatory.
- Rupturing "nonself" cells (micro-organisms included) are also highly inflammatory (eg, the Herxheimer reaction).

All of this debris is collected then transported by APCs through lymph channels to be presented to T- and B-cells. The initial inflammatory/non-inflammatory milieu can now be exaggerated on re-encountering epitopes by putting local innate immune mechanisms on hyper-excited-alert.

I think that some of Polly Matzinger's early explanations were too simplistic.

My reinterpretation of some of these points follows:
- Grafts: Grafts deteriorate even before the transplant is complete; surgeons cut, spill and bruise tissue. There is also anoxic damage. There is an inevitable inflammatory response to damage when circulation is restored. In transplants between identical twins, the post-operative debris processed by APCs contains debris from cells with epitopes that were regularly encountered in the daily deluge of apoptotic cells (before transplantation). Their respective lymphocytes will already have been exhaustively committed to suppression; graft acceptance should prevail. With less compatible grafts, strange antigens are presented in an inflammatory context and they will provoke an aggressive adaptive response and an exaggerated inflammation on re-encounter.
- Allergy: This is probably the misfortune of being a bystander to an intense inflammatory stimulus. The triggered inflammation may have no other association than this with the allergen. Paratope-carrying-cells are triggered by suitable epitopes (allergens) and will be clonally expanded into aggressive immune cells. An allergic sensitisation is most likely if the allergen has not been previously encountered.
- Cancer: Inflammation is a double edged sword having both aggressive and tolerant phases. It is worth remembering that phagocytes view microbes as anciently favoured foodstuff. They also inspect "self"-cells. Suspicious "self" cells can be auto-rejected and removed; this must not get out of control. It needs to be moderately, not excessively, activated. If it does become too intense, then it must be switched off (focal anergy) to prevent wholesale self destruction. That debris provides invaders with a heightened opportunity to persist. Dendritic cells ingest (and macrophages clear) unhealthy-"self"-cells, "nonself" cells (like bacteria) and spilt cell debris ("self" and other-than-"self"). Here, complement is the phagocytes' ally. The resolution phase of inflammation encourages regeneration; there is a need to temporarily switch off phagocyte aggression. This allows stem cells to invade the damaged area (now cleared of debris) without the threat of rejection. Macrophages change from an aggressive M1 phenotype to a regeneration promoting M2 type. Tissue form can return to normal as contact inhibition and normal intercellular junctions are restored. The destructive phase of inflammation reaches a maximum at around 48-72 hours. Resolution is mostly complete within 10-14 days. Cancer stem cells probably fail to switch off proliferation (oncogenic mutations) and so fail to signal "completed". Such miscreant cells would normally be noticed as unhealthy-"self"-cells and deleted. But, when inflammation persists, due to some coincident event, then the anergic phase is extended and cancers stem cells are able to grow to a size at which piecemeal destruction may, itself, start to switch off local surveillance (auto-destruction must not get out of hand). The neo-angiogenesis of wound repair is also anti-inflammatory, by courtesy of its tightened endothelial junctions. The chromosomal "monsters", that characterise the histopathology of cancer, probably survive because of the prolonged focal anergy. It is the (cancer) stem cells, not these monsters, that are the problem cells.

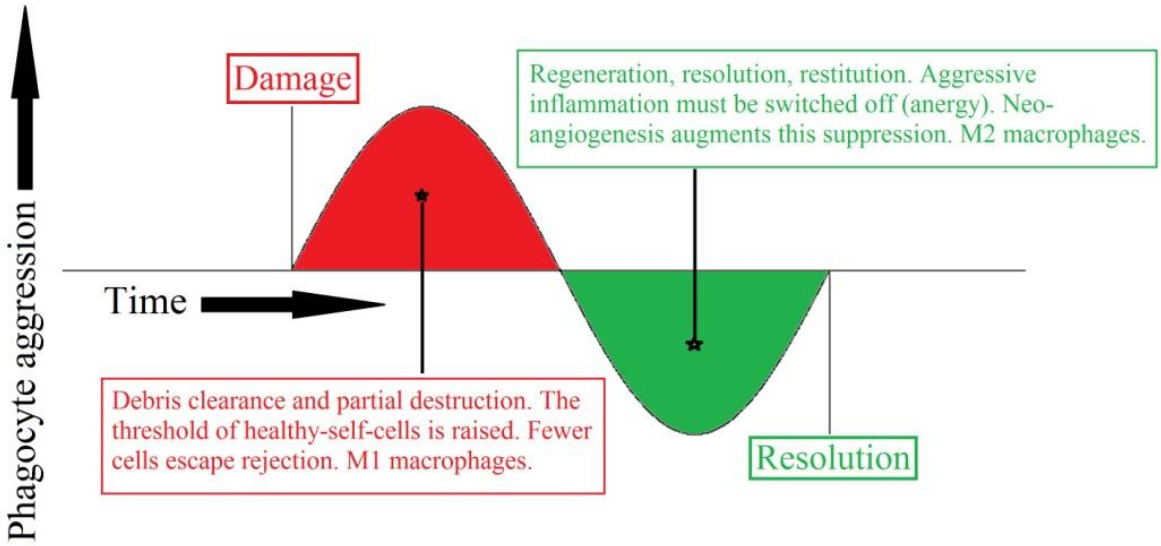

Fig: Idealised representation of the inflammatory sequence
(A more detailed diagram of the process can be found in Reference 7)

More on inflammation

Parenchymal cells can cope with low levels of debris but higher levels require phagocyte assistance. Inflammation is a perivascular invasion and accumulation of phagocytes at a damaged site. It leads to loss of function. Phagocyte invasion is preferentially perivenular. With rising inflammatory intensity, it spreads to include arterioles, then overt arteritis and, eventually, thrombus formation.

Inflammation is increasingly regarded as a tissue homeostatic mechanism. This homeostasis involves cell to cell:

- Recognition on the basis of cell identity – the true source of “self”/ “nonself” discrimination.
- Appropriate (self) recognition triggers cell to cell co-operation; the default state is competition.
- Damaged tissues, once cleared, are regenerated by stem cells that manoeuvre into position to restore function. They need to temporarily sever intercellular junctions in this invasive phase.
- Restitution/resolution; the anergic/locally tolerant phase is terminated and local cells need to restore junctional communication to avert expulsion or attack.

Not new

Pradeu and Cooper (1) mention the “not new” accusation. This is characteristically paraded as a dying convulsion of a failing perspective. *“When a thing was new people said, "It is not true." Later, when its truth became obvious, people said, "Anyway, it is not important," and when its importance could not be denied, people said, "Anyway, it is not new.”* *[William James].*

We will always find that prescient ideas were aired long before a revolution; *“Originality is nothing but judicious imitation” [Voltaire].* Metchnikoff had astounding insights. So did Burnet; his interest in identity primed my thinking

Copernicus told us, in his “De revolutionibus …”, of two philosophers who held heliocentric views 300 yrs BC. Does that imply it was not a Copernican revolution? No. The revolution occurs because an army of researchers begin to realise that they have been shoring up a mis-conceptual skyscraper; they redirect their efforts using radically different and (previously) heretical paradigms. “Immunology” is what is known about immunity. Investigation has generally followed “the path most taken” (5,6) and this has distorted conceptualisation.

In conclusion

It is now clear that:

- complement plays a constructive role in both development and regeneration;
- inflammation is involved in virtually all disease (including obesity, psychiatric illnesses, dementia);
- gut microbes are actively tolerated – even farmed;
- in the CNS, debris clearance is the task of the immune system;

- cancer is disturbed tissue homeostasis; it emerges during periods of prolonged inflammation.

An inflammo-centric view of the immune system has, arguably, become a tautology. How could we ever have conceived that it might be otherwise?

(My publications on tissue homeostasis are listed at www.morphostasis.org.uk/published.htm)

References

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