The danger theory: 21 years later.

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). This mirrors the size, shape and physico-chemical properties of its corresponding paratope.
- "self" signifies cooperating-whole-cells that are descendants of 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 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.

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

- She did not address innate immunity.
- 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

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 it would require a proactive, intelligent classification system. "Damage" is a better metaphor: (cell) damage, death and debris reactively trigger 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 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 phagocentric view of the “immune universe” and even more so when we embrace an “inflammocentric” 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 either aggravate or suppress inflammation when the epitope is re-encountered. This polarisation emulates the inflammatory/non-inflammatory milieu that existed 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 memory of “damage” and “debris” are independently acquired and this combination can change on subsequent presentations of epitopes.

Large numbers of “self” cells die as they become compromised by age or other dysfunction. These generally enter a programme of controlled cell shutdown (apoptosis). 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 epitopes that they then present to lymphocytes. When lymphocyte paratopes “recognise” these apoptotic-debris-epitopes they are programmed, when the respective epitopes are re-encountered, to exaggerate a tolerant milieu. Massive apoptosis occurring in the foetal thymus is no different (4).

“Housekeeping” generates enormous quantities of apoptotic “self” debris. This constant deluge melts away with minimal or no inflammation. Necrotic death, though, 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 has partially separated its initiation phase (by APCs) from its execution phase (inflammatory invasion by phagocytes). Adaptive immunity now bridges the two and can accelerate, exaggerate and focalise inflammation on fresh encounters of memorised 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. 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; rapidly and silently removed); this is safe, controlled, cell-shutdown.
- Apoptotic bodies can overwhelm the system’s capacity to remove them
    - There are too many
    - Clearance mechanisms are flawed
- Catastrophic cell death

    - Trauma. Rarely, this may be too abrupt to set off internal alarm responses (note how laser scalpels cause minimal inflammation).
    - Necrosis (virus infection, heat, radiation, ischaemia, trauma)
- Other than healthy-zygote-derived-cells - microbes included; complement and MAMPs help to identify many of these.

Metaphorically, we have:

- Tidily packaged apoptotic bodies – "bagged debris": rapidly removed and non inflammatory.
- Bagged debris - but the collection systems are overwhelmed: potentially inflammatory.
- The "bags" split when they are left lying around too long: highly inflammatory.
- Catastrophic rupture with exploding cell debris: 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.

Some of Matzinger's early explanations were simplistic.

- Grafts: Grafts deteriorate even before the transplant is complete; surgeons cut, spill and bruise tissue. 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 inflammatory stimulus. The triggering inflammation may have no other association 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 it has not been previously encountered.
- Cancer: Inflammation is a double edged sword. 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). That provides invaders with a heightened opportunity. 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. But, when inflammation persists, due to some coincident event, then the anergic phase is extended and cancers 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.

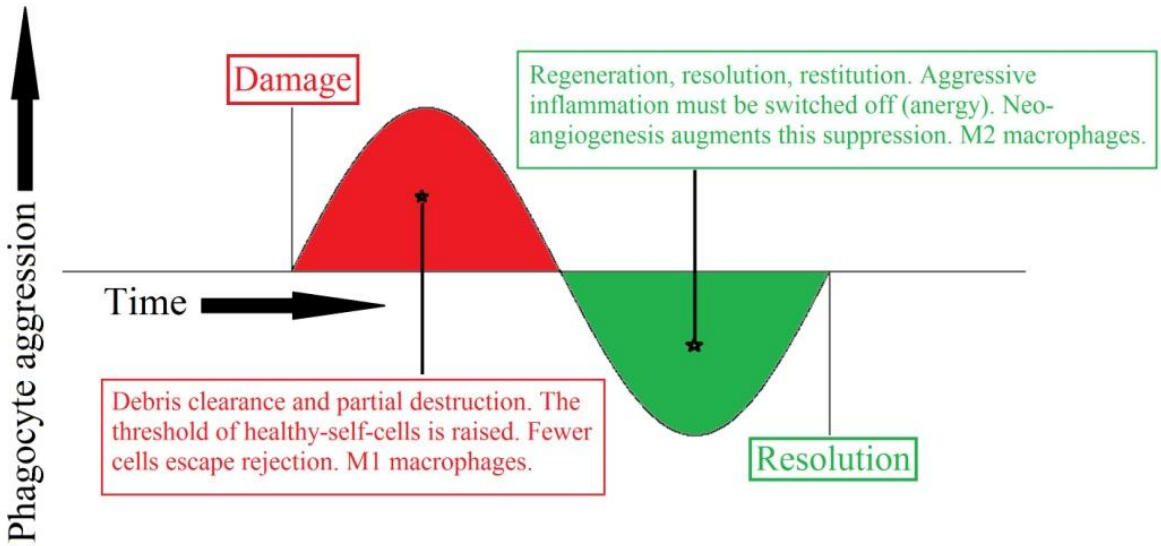

Fig: Idealised representation of the inflammatory sequence

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 source of "self"/ "nonself" discrimination.
- Co-operation, following recognition; the default state is competition.
- Regeneration of damaged tissues as stem cells manoeuvre into position to restore function.
- Restitution/resolution (anergic phase terminated).

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 tells 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 realise that they have been shoring up a misconceptual 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 inflammocentric 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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See notes overleaf:

Alterations from the final (rejected) version but which would have been requested if it had been published. Text highlighted in blue is as submitted in the final version.

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Retrospectively, "danger" is an inappropriate metaphor for it would require a proactive, intelligent classification system.
Retrospectively, "danger" is an obfuscating metaphor.

"Damage" is a better metaphor: (cell) damage, death and debris reactively trigger responses.
"Damage" (reflecting the type of cell death) is better.

##
Claims that "danger" is analogous to a Copernican revolution are tenuous whilst it focuses principally on
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• Regeneration of damaged tissues as stem cells manoeuvre into position to restore function.

• Restitution/resolution (anergic phase terminated).
• Restitution/resolution: anergic phase reversed.

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It is now clear that:

- complement plays a constructive role in both development and regeneration;
- complement has a constructive role in both development and regeneration;
- inflammation is involved in virtually all disease (including obesity, psychiatric illnesses, dementia);
- inflammation is at the root of 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.
- cancer is disturbed tissue homeostasis; it emerges from prolonged inflammation.

Rejection 22/10/2013:
Your manuscript cannot be accepted for publication.
The reason for this decision is:
The quality of the manuscript is substandard and below the generally accepted standards of the community.
The paper has been reviewed by three reviewers. All of them conclude on the confuse *(presumably "confused" meant)* presentation, not in line with common practice. In addition this opinion paper does not appear to include recent understanding.