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Morphostasis: an evolving perspective

Abstract

In an earlier article, I proposed a pathway by which morphostasis (tissue homeostasis) may have evolved. It began in single-celled organisms and culminated in the mammalian immune system. This evolutionary path is now traced from its source - the intracellular surveillance within an isolated cell of its own internal health. Morphostasis sequentially incorporates heat shock proteins, apoptosis, cell adhesion molecules, complement components, gap junctions, phagocytes, natural killer cells, cytotoxic T-cells, helper cells and antibodies. I propose that the sequence leading to the insertion of gap junctions is an ancestor of the complement attack sequence. Although contentious, this deduction is intriguing, since numerous, minimal clues support the proposition. The broad hypothesis emphasizes a theme that may prove to be a useful framework on which to hang a better understanding of immunology and embryology. It highlights points where a concentrated research effort may rapidly advance our understanding of both.

Introduction

Lymphocytes and, in particular, anamnesis dominate current perspectives of immunology. An earlier article, 'Morphostasis and immunity' *(1)*, challenged the propriety of this emphasis. Here I concentrate on the evolution of the morphostatic system, since 'Nothing in biology makes sense except in the light of evolution', Dobzhansky *(2)*. Readers should note that what follows is my current surmise. They must satisfy themselves that any of my deductions *(original says inductions)* are valid - for there are ideas here that border on conjecture. Many will need modification but, since they are pointing to an increasingly clear overall theme, I believe they warrant an airing.

The Tautologies

This article contains statements that are tautologous - *i.e.*, self-evident truths. Current ideology may give them lip service but it does not always remember to reward them with a commensurate emphasis.

- Every animal is and functions as a colony of cells. This colony develops from a single cell and forms the zygote-derived colony (ZDC). Colonial behaviour rules metazoan function.
- Genetic information (*i.e.*, phenotypic potential) can be lost but not gained as cells differentiate.
- Every ZDC cell monitors its own internal well being. It notifies its neighbours of sickness.
- All metazoans discriminate healthy-self-cells (HS) from other-than-healthy-self-cells (OTHS). The mammalian immune system revolves around this.
- The majority of irretrievably sick cells shut themselves down in a controlled way. They aim to destroy their own contents, particularly their own and any invader's genes.
- Morphogenesis and morphostasis rely on gap junctional intercellular communication (GJIC).
- "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 *(3)*. Immune function evolved and still revolves around phagocytes. Other immune cells are subservient to them. They enhance phagocyte efficiency.
- Tissue homeostasis (morphostasis) is maintained by:
    - displaying HS markers on the membranes of HS cells;
    - recognizing OTHS cells by absent HS markers or present UHS (unhealthy self) markers;
    - attacking and removing OTHS cells (UHS and foreign cells or organisms);
    - replacing lost UHS cells with fresh HS cells (resurgent morphogenesis).

Other-than-healthy-self-cells further definition

Since 'Morphostasis and immunity' *(1)* was written, the concept of OTHS has been refined. It consists of:

- self-identified sick cells undergoing apoptosis (low danger); these are probably advertised by
    - inside out membrane lipids
    - thrombospondin and other surface motifs
    - other specific apoptosis identifiers;
- stressed cells that need to be monitored carefully by other cells (possibly dangerous);
- disordered cells where the onset has been too sudden or they failed to effect a controlled shut down (dangerous);
- non-ZDC cells (probably dangerous and almost always expendable); they
    - can be recognized by the possession of various surface peptidoglycans
    - can be identified using receptors like the mannose binding lectin (protein)
    - are, characteristically, neither electrically nor metabolic ally coupled through gap junctions (GJs)

The probable sequence of evolutiouary events

The sequence of events described below may have led to the mammalian morphostatic system. Note that each step is an embellishment of a former step, and all remain functional in mammal morphostasis. Each is a new 'shell' that is superimposed over and is complementary to the former shell.

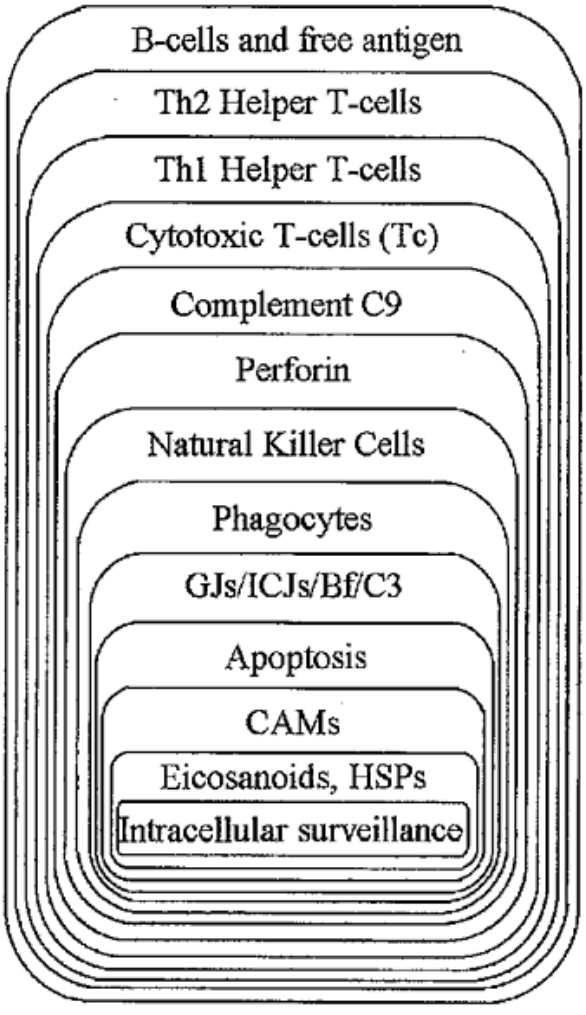

Fig. 1 Morphostasis — the progressive addition of 'shells'.

It is important to note the fundamental centrality and importance of this structure. Shells are built upon - they are neither abandoned nor substituted but remain fully functional (Fig. 1). The genes that code for proteins involved in the lowest shells tend to be conserved across wide phylogenetic boundaries. The further out the shell, the greater, in general, is the tendency for genes to diverge. Perhaps this reflects the growing pressure, with increasing sophistication, to acquire ever more specific identity.

- The sequence starts in single-celled organisms. 'Self' is delimited by the lipid bilayer. In general, anything within this membrane is part of self. HS or UHS is advertised by molecules expressed on the surface membrane. Many of these have evolved to interact with other organisms. Their role includes the recognition of food, potential pathogens, self-species and sexual partners. The lipid bilayer, with all the interactions that are immediately at its disposal (e.g., inositol phospholipids, eicosanoids, trans membrane signalling), occupies the earliest and most central shell of the morphostatic system.
- Cells develop a system of internal self-surveillance to detect damage, infection and dysfunction. They do so long before evolving into multicellular forms. Internal, cell-cycle, checkpoint controls are established. Stress proteins evolve to deal with "sick" proteins and ubiqitins become the cell's garbage collectors. Sick proteins are the product of disordered construction (eg, when forced by viruses) and cell damage (heat shock and other insults). The HSP70 group of proteins work within this system. When sick proteins have been altered beyond redemption, they are channelled into the ubiquitin and proteosome pathways and from there, broken down into short peptides to be transported to the endoplasmic reticulum.
- Apoptosis evolves early - elective death is apparent even in colonial bacteria. A ZDC that depends on its members to propagate its genes can afford to avert danger by sacrificing threatened cells. The controlled shutdown of self cells is an effective defence *(4-6)*. Confined within the lipid bilayer, destructive enzymes digest the cell's contents. Invaders will also be attacked and can only survive if they have evolved protective mechanisms. Apoptosis is progressively refined. Cells shut down GJIC when they become sick. [Ca2+]i regulation and the inositol phospholipids are central to this process *(7)*.
- Unicellular organisms develop homeotic genes. These allow cells to build or rebuild themselves according to alternative, differentiation "blueprints". These govern construction and remodelling and enable cells to pursue alternative roles. Single celled organisms can express just one "blueprint" (or phenotype-set) at any one moment (eg, Mat-alpha-2 repressor in yeast) *(8)*.
- The extracellular matrix (ECM) soon develops. An ECM is formed wherever cells meet. Integrin-like molecules bridge the interface between the cell cytoskeleton and the extracellular matrix *(9)*. Amoebocytes (phagocyte-like cells) use beta-2 integrins to interact with others. With the advent of specialised soma cells, the integrin family is expanded *(9)*.
- Gap junctions (GJs) evolve to enhance functional and locomotor coordination and also cell survival. Tissue form is stable whilst cells maintain intimate contact through intercellular junctions. Joined cells establish electrical and metabolic synchronisation to promote co-operation and survival. This is enhanced when cytoplasms are in direct continuity through GJs and absolute in synctia. Sick cells sense internal disorder and abandon HS identity. First they shut down the channels joining their cytoplasms with other cells, then they "un-dock" their membranes. This process can progress into apoptosis. This is tidy, elected cell death. Self cells monitor each other's identity. Neighbouring cells and phagocytes ingest apoptotic cells before they burst. Necrosis, or lysis, is untidy death: now cells burst, spill their contents and release a battery of inflammatory cytokines.
- Unicellular organisms exchange genes. This depends on sexual, self-self "docking" and on cell adhesion molecules (CAMs). The integrins may be the earliest contributors and have since been adapted for use in multicellular organisms (note how complement iC3b and C3d interact with the alpha-M-beta-2 and alpha-x-beta-2 integrins) *(10)*. In multicellulates, immunoglobulin superfamily (IgSF) CAMs may be the next to evolve. They help in the construction of GJs permeable to Lucifer yellow (LY) *(1)*. Cadherins may help to form a barrier of LY impermeable cells wherever cells with different cadherins come into apposition. This splits the embryo into communication compartments. In plants, cells in plamodesmatal compartments *(11)* express the same homeotic proteins *(12)*. LY permeable blocs of animal cells may also have a synchronised homeotic phenotype *(13)*. The combination of communicating transmembrane junctions and homeotic genes may enable an

organism to enlarge itself whilst still maintaining overall form - converting it from a single celled organism into a cell-mosaic. The advent of cadherins enables the ZDC to build organisms within an organism (tissues and organs). The integrins are now diversified. The beta-4 based integrins evolve to construct basement membranes that separate organs *(9)*. GJs are particularly useful to co-ordinate movement in blocs of cells (*e.g.*, heart, uterus). They also have important functions in development, but these tend to be in pattern formation rather than differentiation.

- Multicellularity evolves and the GJ system with it. Cx43 is the primal GJ connexin. It is the only Cx transcribed in the eight-celled embryo and in macrophages *(14)*. These GJs are LY permeable. A family of other connexins probably evolve from them *(15,16)*. The IgSF CAMs and the cadherins may be related *(17)*. The latter may lead to the construction of GJs that form a thin envelope of LY-impermeable cells (often made using Cx26, Cx31 and Cx32) around an LY-permeable bloc of cells (often made using Cx43 and Cx40). By now, cells must communicate to survive within the ZDC. Each cell must maintain correct form or it will break communication and proceed towards apoptosis. This is achieved against a gradient inclined to disorganization (entropy), so their apparent altruism may be illusory. Note that apoptosis is aborted mitosis and that stress often precipitates either mitosis or apoptosis. Mitosis leads to a break in communication, and high HSP70 expression protects these cells from aggression *(18)*. It is improbable that macrophages (and NK cells) ignore the capacity to communicate with other cells through GJ s, particularly as they express Cx43 genes *(14)* and Cx43 is expressed in the embryo when it has just eight-cells (i.e., one of the earliest 'shells'). Nevertheless, reports of GJ formation by macrophages are sparse: they may be selective about when they communicate. Nature may have needed to invent the construction of membrane holes just once. The cascading complement sequence leads to such holes (MACs). The early stages of this cascade may be capable of forking either towards cooperation *(19)* or towards aggression rather than being exclusively aggressive. Complement regulatory proteins are believed to play an entirely protectiv role but could well be part of the fork towards cooperation. Fig. 2 shows relative sizes and idealized shapes of MACs, T-cell-induced tubules, and GJs.

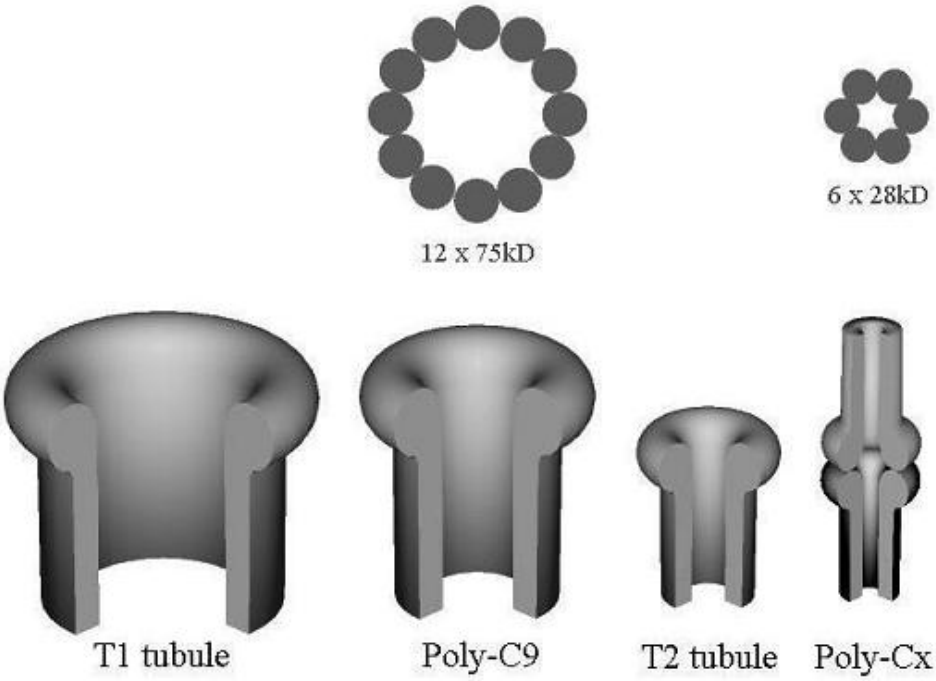

Fig 2. The relative sizes and idealised shapes of complement membrane attack complexes (MACs), NK & T-cell-induced tubules (poly perforins) and gap junctions. Approximate pore diameters: T1 tubules, 150 Angstrom (A), MAC, 100 A; T2 tubules, 50 A; gap junctions, 20 A. Complement components C6, 7, 8, 9 and perforins ahare sequence homology. Connexins and not (obviously) related to the perforin family.

- The interaction of CAMs, ICJs and the ECM gives cells a sense of 'belonging' *(20)*. In effect, the specificity of the mechanisms that lead to cell adhesion, coupling and connective tissue scaffolding gives cells a HS identity. When HS identity is lost, the ECM scaffold is dismantled and the cell 'undocks' from its neighbours. HS identity is responsible for the selective reaggregation that occurs

after embryonic cells are disrupted. Electrical and metabolic synchronization, established through GJs, enhances HS identity. GJ formation is the immediate sequel to cell surface CAM interactions. Once paired up, membrane holes in apposing cells form GJs (similar channels are important in plants *(21)*). IgSF CAMs (e.g., N-CAM) develop to act as a focus on which to build LY-permeable GJ plaques *(1,22)* (e.g., GJs made of Cx43 or Cx40). This 'multiplier' mechanism will later be adapted to spatter bigger, leaky holes into cells or organisms that do not display features of self (the alternative complement cascade). A complement-like cascade mechanism similar to the Bb/C3b et seq cascade evolves as the general agent that identifies a cell membrane. In the presence of self markers it forks towards GJs and, in their absence, towards attack.

- The cell relies on its lipid bilayer to delimit the boundaries of its 'self'. This membrane is able to isolate the cytoplasm both electrically and metabolically from its surroundings. GJs appear early in the evolution of metazoans. They join blocs of cells and allow each bloc to behave as a single, large cell. So, electrical and metabolic synchrony between cells is recruited as the strategy to extend the boundaries of self to encompass the whole ZDC. Cells that differentiate terminally may withdraw GJs to gain function but, at times of stress, they will need to re-establish synchrony to signal HS identity. CAM genes are conserved across species boundaries, so dedicated pathogens will regard them as 'sitting duck' targets. The need soon arises for interspecies and interpersonal variation leading to subsets of CAM identity within the ZDC *(23)*.
- Note that cell sorting is dependent on CAM expression, particularly cadherins. The progressive expansion of different cadherins leads to subordinate, self-within-self identities and thus tissue specialization. The cadherins may be responsible for defining developmental compartments *(24)* by sheets of cells having GJs with altered permeability (possibly where GJs made of mixed beta-connexins form) *(25,26)*. Cells within this envelope express IgSF-CAMs like N-CAM. These CAMs encourage the rapid and efficient insertion of LY-permeable GJ s into apposing membranes *(1,22)*. These form a sub-bloc of highly communicative cells *(22)* that act as a synctial 'super-cell'. Cx43 is probably the commonest connexin forming LY-permeable junctions. Each sub-bloc probably expresses a particular 'blueprint' that is controlled by particular (sets of) homeotic genes. Homoeotic gene expression has been noted to change at compartment boundaries *(13)*. Homeotic genes appear to have control of connexin expression rather than vice versa *(27)*. I have surmised that the construction of GJs is enhanced using a CI/C3-like amplifying and seeding process *(1)*. I guess that factor B, or a molecule like it, is critical for the construction of (at least) LY-permeable GJs. This construction has been enhanced using an amplification process developed around (ancestral) molecules related to CI/C3. It is possible that a cascade similar to C3-C6 (up to C8 even) evolved as the mechanism for spawning multiple construction sites for cooperative GJs. If this is the case then only the final steps in the perforin/C9 mechanism have evolved to attack membranes. It is clear that N-CAM genes are alternatively spliced, and this probably leads to greater specificity of its interactions. N-CAM carries a number of IgSF motifs (similar to immunoglobulin C regions (Ig-C). The Ig-C regions of immunoglobulins activate the classical complement cascade. Like the Ig-C region, beta-2-microglobulin (b-2-m, also identified in the earthworm) is able to bind Cl. So, it seems, there is a primitive role for b-2-m and also, by implication for the classical complement cascade. Is this just for attack? or can it also be for cooperation? This is easily testable if factor B is involved in Cx43 insertion.
- Animal cells split into dedicated phagocytes and soma. The soma acquire a greater potential for enhanced connective tissue scaffolding (probably integrin based) and abandon most of their capacity for wandering and aggression. Scavengers can concentrate on the latter while forfeiting the advantages of this new potential (hence their limited set of integrins).
    - Phagocyte ligand(s) - for recognition by itinerant scavengers
    - Soma ligand(s) - for recognition by resident scaffolders.
- A dedicated phagocyte lineage evolves. Its cells refine both cooperative ICJ communication with self cell, and the attack system that inserts leaky holes into non-self cells: the latter will eventually lead to the mammalian complement system. Phagocytes are derived from a cell lineage that 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 are able to clamber over all other cell types (possibly related to beta-2-based integrins and to their restricted use of just Cx43). One aspect of selfness is established as phagocytes make GJs with underlying cells (using Cx43) (14).

This leads to a degree of electrical and metabolic synchronization. The specificity of this GJ connection should be at least species wide. It recognizes a selfness that may be shared by related species. The phagocyte uropod will already be in established GJ connection with an underlying cell whenever it probes adjacent cells or organisms with its lamellipodial fingers to test whether they are synchronized with the uropod-attached cell. The trigger for attack may be the capacitatively induced currents that are generated as their membranes come into apposition. The phagocyte uses additional strategies, e.g. recognizing apoptotic cells and surface markers that are manifestly bacterial.
A recent report describes how neurites search out asynchronous neural cells *(28)*. Neurites may have adopted, adapted and then inverted this property of phagocytes to form neural networks. This action may have begun in either neurites or NK-like cells and then been 'licensed' from one to the other (see below).
A 'vascular' system evolves. Phagocytes can now be inhibited from entering the soma until they are required. An inflammatory cascade is established. At least a part of the alternative complement cascade is 'humoralized' so that circulating C3 can mark clearly-foreign organisms and make them more readily identifiable when they meet a phagocyte.

- I have proposed that macrophages communicate with underlying cells through L Y-permeable GJs. They assess adjacent cells against this 'yardstick' of electrical synchrony. To do so they construct LY-permeable junctions at the site of conjoined N-CAM-like molecules, using Cx43. This interaction has low specificity - GJs can form between unrelated individuals of the same or even related species. Extra specificity is now gained by alternatively splicing N-CAM RNA so that specific ligand-pairs can be used in different locations.
  The next development is the evolution of individual specificity. A new cell evolves to recognize a ligand that is deliberately varied in the population ('herd'). This requires the separation of ligand and receptor function. A family of related but different ligands can now be distributed throughout the herd (pleomorphism), and specific receptors (originally N-CAM-like ligands) for these can be selected in the growing ZDC by alternative RNA splicing. These ligands are the forerunners of Mhc Class I molecules. This receptor cell, derived from the primitive phagocyte line, will later develop into the NK cell and allow a new and personalized assessment of self. NK cells occupy a midway position between macrophages and Tc cells. Like macrophages, they migrate into inflammatory sites. These prototype NK cells deter the evolution of pathogens that could, otherwise, mimic self N-CAM(-like) ligands and so avoid detection and destruction.
  Stressed cells are a danger to the ZDC, as they are often damaged, infected or growth disordered. They have an increased intracellular protein turnover and some of this is not properly folded. The heat shock protein (HSP) system attempts to fix this. When it fails, the protein is ubiquitinated then broken down into peptides, ready for disposal. Mammalian NK cells are selectively non-aggressive to cells that display self Class I Mhc molecules *(20)*. However, Class I molecules on their own are insufficient to protect the cell: they need Class I in association with peptide debris - and early evidence suggests that this peptide debris is derived from self Class I molecules *(30,31)*. So, it looks as though, in quiescent, relatively unstressed self cells, this Class-I-peptide combination is normally dominant and acts to protect cells from NK lysis. When cells become stressed, other peptides displace the protective peptide. Such cells can be encouraged into premature apoptosis when they become isolated (see below).
  So how are appropriately marked cells protected from NK attack? There are several clues. First, cells that are not in GJIC (electrical synchrony) are subject to attack *(32)*. Second, macrophages (probably) use Cx43 to establish synchrony with the uropod base. Third, from the preceding proposition, the complement sequence is able to fork towards either aggression or cooperation, using factor B-like and C3-like molecules. Altogether, these suggest that the conjunction of the NK receptor with the target cell's Class I ligand leads on to the construction of GJs. Protection is necessary for cells in legitimate mitosis, as they often break junctional and ECM communication until mitosis is complete. Tumour necrosis factors (TNF-alpha and -beta) are selectively toxic to cells not in junctional communication *(32)* but a high HSP70 expression renders a cell relatively resistant to lysis by TNF *(18)*. So, NK cells probably pay particular attention to disconnected cells that are processing a lot of peptide debris, gauging whether or not their internal functions are running amuck. One report suggests that the peptide clasp from the Mhc molecule may have originated from a HSP molecule (HSC70-like) *(33)*.

- Note that the Class III Mhc region contains genes that encode molecules able to modulate HS/ OTHS discrimination. These either modify GJIC or the consequences of its presence or absence. These include HSP70 *(18)*, TNF-alpha and -beta (lymphotoxin) *(32)*, complement components (C2, Bf and C4), the 21-hydroxylases and, perhaps, cytotactin. They may be helping NK cells to establish fast GJ s with healthy self cells and, when they cannot, to apply the 'attack if not in electrical continuity' yardstick. Corticosteroids (and sex steroids) modulate GJs - enhancing them in many tissues. Cytotactin is intimately concerned with morphogenesis and can interact directly with IgSF-CAMs *(34)*. Deficiency of factor B is apparently devastating, as there are no reports of affected embryos or individuals.
  The following points suggest that GJIC and the Mhc may be linked. Note that the downregulation of Class I molecule expression (recognised by NK) coincides with the onset of metastasis and, simultaneously, with the loss of GJIC. The possible explanations for this co-incidence include: chance: linked gene promotion/induction/enhancement; even the possibility that Mhc Class I and/or Class III proteins are directly involved in somatic-cell to somatic-cell GJ formation. The listed Class III loci are not in genomic order.
  - **Factor Bf**
    - I have developed the hypothesis that factor B is somehow involved in the specific, cooperative recognition of self. Its aggressive role forks away from cooperation. This leads to an attack on OTHS when appropriate. Note the apparent, absolute lethality of Bf gene deficiency.
  - **C2**
    - A derivative of factor B, adapted to tag antigen with a 'self' marker. It probably proceeds, unforked, to an aggressive attack on membranes.
  - **C4A, C4B**
    - Possibly involved in GJ insertion - speculative
  - **Slp**
    - Sex limited protein
  - **Steroid 21-hydroxylases**
    - Stabilize and enhance GJ communication. Sex steroids modulate GJ activity in sex-specific organs.
  - **Cytotactin**
    - Interacts with the C-like region of N-CAM. Could it be involved in linking the interacting CAMs (ligand-receptor) to the construction of membrane holes? This would encourage the use of the same connexons (half-GJs) in apposing membranes, so leading to higher permeability. (NB, the similar hexabrachion structure to Clq - the genes are not related.)
  - **HsP 70**
    - Protects cells from TNF attack when they are disconnected from surrounding cells - *e.g.*, when in legitimate mitosis
  - **TNF-alpha and -beta**
    - Selectively toxic to cells not in gap junctional communication.
- The NK mechanism is refined. A wide range of allo-receptors evolves, probably just by V gene duplication and divergence. Both the complexity and the repertoire of this mechanism for generating and selecting specific receptors is able to evolve gradually. Once the repertoire is large enough, its function can be inverted, so leading to a mechanism able to recognize and attack all other specificities (Tc function). This suggests an evolutionary pathway to Tc cells. The inversion requires that the GJ-forming cascade be blocked when the ligand-receptor pair are conjoined. Interaction of ligand and receptor must now stimulate the T-cell into action rather than GJ construction. Perhaps the intrathyrnic promotion of precursor T-cells to CD8 and CD4 +ve cells is triggered by GJ formation *(35,36)* at the site of interaction between Mhc-peptide and T-cell receptor (TCR). Non-communicators that lack this nurture should be expected to slip into apoptosis. CD4 and CD8 expression may then block the ligand-receptor pair from triggering the GJ formation cascade. At the same time they donate a transmembrane signalling device (P56lck). The speculative mechanism for the 'spawning' of multiple LY-permeable GJs is shown in Fig. 3.
  Now Tc cells can recognize a variety of epitopes. These can be categorized according to the mode of

death of the target cell when the uncommitted T-cell first meets them. The Ts and Tc cell system can now evolve to recognize cells whose Class I ligands had been altered by the intended attachment of peptides to the pincer mechanism. Cells that carry strange epitopes - typical of other cells that have previously been associated with necrosis - can be encouraged to accelerate into apoptosis. Peptide debris presented by cells that die by apoptosis will suppress the corresponding paratope.
Germline rearrangement of the T-cell receptor DNA must now to be refined by encouraging variety (in the D-J region) so that peptides other than the healthy-self peptide can be recognized. These peptide-Mhc complexes are regarded by the system as allo-Mhc-specificities. Gamma-delta TCRs are not absolutely obliged just to recognize peptide-Mhc complexes that have allo-Mhc-specificity. This reflects a similar freedom enjoyed by Ig genes.

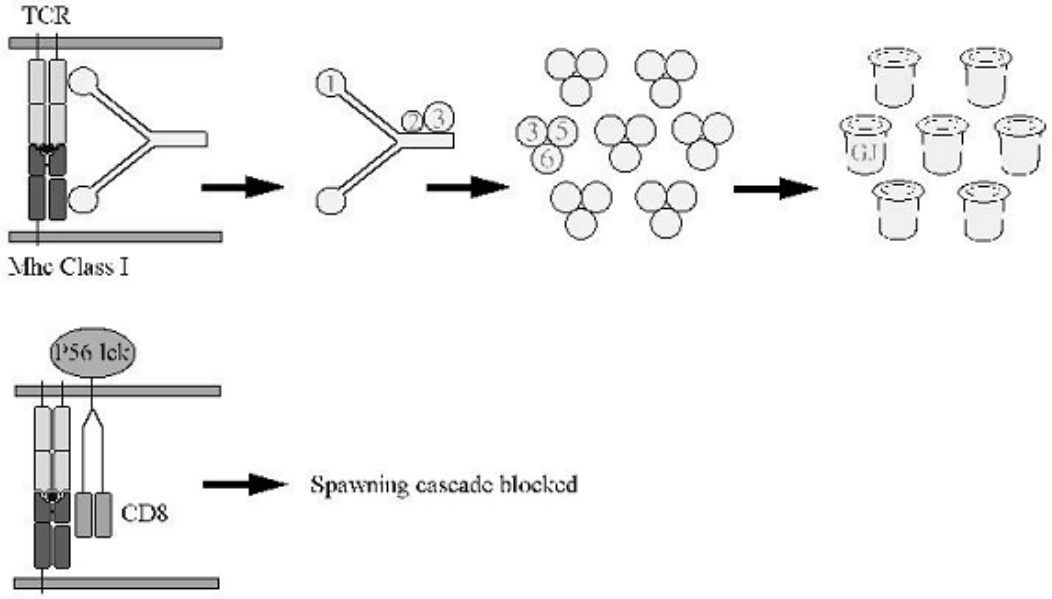

Fig 3: Speculative mechanism for the 'spawning' of multiple LY-permeable gap junctions. AC1q-related molecule is activated when an Mhc ligand and its receptor meet and are well matched. This triggers a sequence of events starting with a C2-like molecule, then a C3- and a C6-like molecule. These lead to an explosion of construction sites and so to multiple GJs. In the thymus, the Tc and Th cells designated to survive may be selected by their ability to construct GJs with thymic epithelial cells. Then their ability to construct GJs could be blocked by CD8 or CD4.

- Both Mhc genes and TCR-V genes (TCRV) code for complementary sets of proteins. Both sets of genes group into families. Genes from different species can be classified into families. They are more related by family than by species. They have probably been maintained as dedicated ligand-receptor pairs (Mhc/TCRVs) over many millennia. In 'Morphostasis and immunity' *(1)* I explored the idea that NK cells evolved to recognize self ligands. Unlike other T-cells, NK cells do not rearrange their TCR genes. Like N-CAM, they may use a system of alternative RNA splicing. The spliced genes might be the same as or a predecessor of the ab TCRVs, much as evolution has duplicated other sets of Ig-like genes (TCR-alpha & TCR-beta to TCR-gamma & TCR-delta to lambda, kappa & heavy Ig genes). NK cells may observe 'horror autotoxicus' by constructing GJs between themselves and their target cells (namely, N-CAM). A recent paper *(37)* may support alternative RNA splicing.
- Thl cells evolve as an extension of Tc cell function. The Class II Mhc mechanism evolves from Class I: now, representative peptides are processed by phagocytes after ingesting cell debris. These are externalized as a Class-II-peptide-debris complex ready for the attention of uncommitted T-cells. The 'generator of diversity' can now be enrolled into creating a system to memorize the inflammatory or non-inflammatory context in which these processed epitopes were encountered. Without controlled shutdown (i.e., without apoptosis), cell death is proinflammatory and Thl cells primed in this situation will, when they re-encounter the processed epitope, attract large numbers of phagocytes to the site and 'angrify' them. This gives inflammation a memory (another tautology). The 'angrified' phagocytes still have to sort HS from OTHS but their threshold for regarding a cell as OTHS is lowered. Neither Tc nor Thl cells are involved in assessing selfness. They are, instead, primed by

other cells, particularly phagocytes, to remember the controlled-shutdown or catastrophic-death context in that their epitopes were presented to them when they first became committed (*i.e.*, apoptotic versus lytic death discrimination) *(1)*.

- Tolerance must evolve with aggression. Though apoptotic cells fragment, each particle is retained in an intact membrane and all are tidily phagocytosed by adjacent cells or phagocytes without inflammation. The cell contents, including intracellular pathogens, will be destroyed by apoptosis unless they evolve mechanisms to survive. Peptides processed in consequence need not - and should not - activate Tc or Thl cells: tolerance is desirable. However, cells that rupture and spill their contents have not been declared 'safe' by the apoptosis-surveillance process. They pose a threat: by releasing eicosanoids and other cytokines they provoke inflammation. This activates Tc and Thl cells.
  So, uncommitted T-cells sense the inflammatory or non-inflammatory context in that they meet their respective epitope and become committed accordingly. Copious self antigens are encountered in widespread apoptosis. Most precursor T-cells, with paratopes recognizing processed apoptotic debris (mostly self peptide), will either be 'mopped up' into a commitment to suppression (tolerance) or clonally deleted. Nevertheless, uncommitted T-cells with paratopes specific for self epitopes continue to be released from the bone marrow. These may be primed rather than clonally deleted in the thymus (where enhanced apoptosis removes lymphocytes able to recognize stressed self). These may become committed to aggression when the inflammatory process is prolonged and foreign epitopes, that accelerate its resolution, are sparse. This system can be enhanced by a simple expedient. As T-cells age, their ability to be committed to aggression is progressively enhanced. This focuses aggression onto strange epitopes.
  The function of precursor T-cells requires them to migrate to and pass through inflammatory nodes. There is a high risk of bystander necrosis in these areas. A protected environment (the thymic medulla) is needed to encourage apoptosis of T-cells in advance of this migration so that tolerance to their epitopes precedes their migration - particularly as they become non-Cx43-communicators on leaving the thymus.
- Cells that burst in a 'panic shutdown' may provoke T-cells more aggressively than cells that burst by trauma. Note that interleukin-converting enzyme (ICE) is a Ced-3 homologue. It releases IL-lbeta intracellularly. When 'spilled', this is pro-inflammatory and stimulates T-cells to become aggressive. The site at which precursor Tc-cells are primed and committed may be the local lymph nodes. If this is the case, then sick cells must detach themselves from their normal tissue position and migrate, in lymph channels, to these nodes. The concept of 'controlled shutdown' versus catastrophic death' discrimination may have been oversimplified in 'Morphostasis and immunity': the Class-I-peptide-presenting cell may need to be alive when it first meets the precursor Tc cell.
  The discrimination of 'controlled shutdown' from 'catastrophic death' also applies to macrophage function. It depends on which class of Mhc antigen presents the peptide. To start, macrophages endocytose OTHS cells. The preferred result is that OTHS cells are destroyed in the macrophages' endosomes. Failing this, the macrophages can carry out a controlled shutdown on the ingested organisms. Macrophages dying by controlled shutdown, as well as committing suicide, will kill intracellular pathogens in the process *(4)*. These two strategies are sufficient to tip the balance of survival in favour of host cells and against invaders. Aggressive Tc responses (to peptide-Class-I complexes) need only be launched against macrophages, or other APCs, when they die catastrophically from infection.
  Next, macrophages pick up debris from adjacent necrosing cells. This debris is encountered along with 'spilled' IL-lbeta that has been generated in a dying cell by ICE. (In controlled shutdown, IL-Ibeta is contained within the dying cell's membrane, which may be why it does not stimulate T-cell aggression.) So, as a dying cell ruptures, it provokes an inflammatory response and, in consequence, a Class II, Thl activation. This will accelerate inflammation on the next encounter.
  The result is a dual attack whenever memorized peptide-Mhc complexes are next encountered. Thl cells will attract large numbers of macrophages to the scene and 'angrify' them. Then, as these activated macrophages start to present peptide-Class-I complexes, they will be encouraged to accelerate towards apoptosis if they are met by Tc cells that have been previously primed to be aggressive on re-encounter of that particular peptide-CIass-I combination.
  This anamnestic amplification of the inflammatory response brings with it a capacity to launch an

attack focused on healthy self (somatic cell) epitopes. This auto-rejection is a positive inflammatory feedback, driven mainly by Thl cells, and it has the potential to escalate catastrophically. A braking mechanism must be employed to progressively turn off phagocyte aggression as the Th1-accelerated auto-rejection escalates *(38)*. This brake brings a new problem for it leads to the accumulation of tissue debris.

- When Thl activation of phagocytes becomes excessive, anergy is switched on. This inhibits wholesale self-destruction. The aggression of phagocytes is downregulated. However, this leads to the accumulation of tissue debris that must be cleared. Hence Th2 cells, B-cells and the free antibody system evolve to tag this debris in a way that promotes its clearance (C-micron). The system has subsequently elaborated a sophistication of its own (C-delta, C-gamma, C-alpha and C-eta). In mammals, antibody responses now appear to be diverse enough to recognize almost any molecular configuration: but the original function of the generator of specificity was to recognize a limited set of cell surface 'flags', not a virtually limitless number of different epitopes. The alternative complement cascade is adapted to be triggered by Cl, 2 & 4. These have evolved from the ancestral components that are used by N-CAM to spawn GJ plaques. The antibody system is optimized to work within the vascular system. It can interfere with any intended function of the epitope (Ag) and tag it for enhanced phagocyte attention and attack. This has proven to be an invaluable pre-emptive defence.
- Embryonic development and ontogeny may have a function for natural killer - and T-cells before Mhc ligands are transcribed (*e.g.*, gamma-delta TCR receptors), aiding the disposal of redundant tissues during remodelling. The evolution of NK and Tc cells may have roots in this function.
- Placentation may have to await the evolution of a wide repertoire of Tc and Th receptors. NK cell *(originally NK)* activity is downregulated during pregnancy *(39)*, perhaps to protect the foreign foetal graft. It can either be toned down or made less specific to the individual. It can only be afforded if NK cell activity is replaced, by inversion of specificity and action, with a wide repertoire of Tc and Th paratopes. The range of this repertoire must be large enough to approximate a complete coverage of 'all other likely specificities'. Note that the synctiotrophoblast is a synctium and it is, therefore, relatively more resistant to attack by phagocytes and NK cells. The disadvantage of this tissue form is that, when danger appears, it is unable to isolate threatened loci. Only a synctium of cells communicating through GJ s can do this. They isolate sick cells from their immediate neighbours and, where appropriate, go into controlled shutdown *(1)*.

Conclusion

I have explored the probable evolution of morphostasis and conclude that gap junctions (or plasmodesmata in plants) are the cornerstone of metazoan success. While many of the component ideas are speculative, I believe the general theme is crystal clear. The more precise deductions will inevitably require revision. The hypothesis emphasizes the proportions that should be attributed to each part of the system. In particular, it suggests that more investigative effort should be concentrated on GJ physiology. I propose that this hypothesis is a useful framework on which to hang a more focused investigation of the biochemical processes of morphostasis.

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In the original I often used the term "Tnk cells" where I should have written "NK cells". These errors have been corrected in this version.

Many instances of "which" have been changed to "that".

Greek letters have all been replaced by the complete word because of the awkwardness of displaying these letters using HTML.