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Covering letter:

Xxx Yyy
The Editor
Zzzz Journal
31st August 1993

Dear Xxx Yyy,
Re MORPHOSTASIS & IMMUNITY
I have been evolving this concept since 1975 and, at least, it has kept me amused! Earlier versions have been sent to other journals which publish hypotheses but these were rejected. The Lancet published Burwell's hypothesis on Morphostasis in 1963 (reference 7).
This may well seem an alien concept to a conventional immunologist but, from my perspective, many facets of what I know and understand begin to fall into place under this skeleton structure. I cannot believe it is all nonsense but I am fully aware that my approach is cavalier in style and that I jump to conclusions which later need modification - but then, that is the way of hypothetical advance.
I am isolated from other people involved in this field so I have had no opportunity to argue the case. This paper is necessarily synoptic. There is a considerable amount of work that has led up to this conclusion and it mainly started in a clinical approach. In case this hypothesis gets further than your return mail basket, I have enclosed more extensive discussion papers. Apart apart from the longer "Morphostasis and Immunity", these are older versions. They might help a reviewer to fill in some flesh on the short, bare bones, presentation. In particular, "Clinical Morphostasis" expands the section called "Clinical Consequences" in the longer "Morphostasis and Immunity". Including this would have made the submitted version too long.
It is more important to me that I should infect other people with this concept than it is that I should see it in print. Unless it is crackpot nonesense, it contains at least a smattering of important concepts. I would be happy to discuss, recompose, shorten or otherwise alter this presentation. Nevertheless, I suspect you'll send it back (crackpot!) return of post.
Yours sincerely,
Jamie CUNLIFFE

Article – I am pretty sure that this was the version submitted (certainly all the conclusions predated this submission).

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.

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 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.

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

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 GENERATOR	OF SPECIFITY	aggressive	passive (horror autotox- icosis)
Tc cell	pure self GENERATOR	non pure self OF DIVERSITY	passive	aggressive

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>).

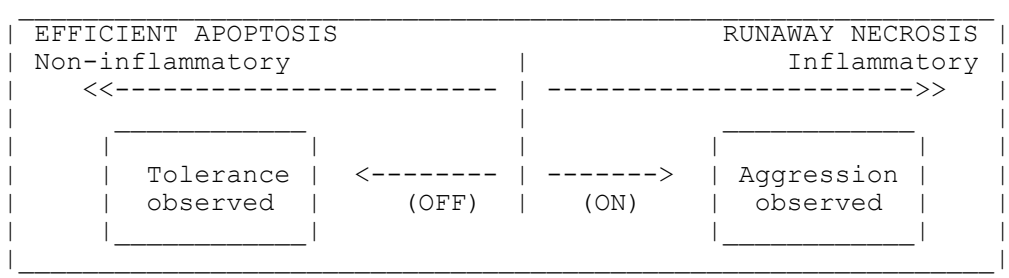
(g) Note that the Class III Mhc region contains a variety of genes encoding molecules that are involved in HS/OTHS discrimination or its modulation. These include HSP70, TNF, complement components (C2, Bf and C4) and the 21-hydroxylases.

(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



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

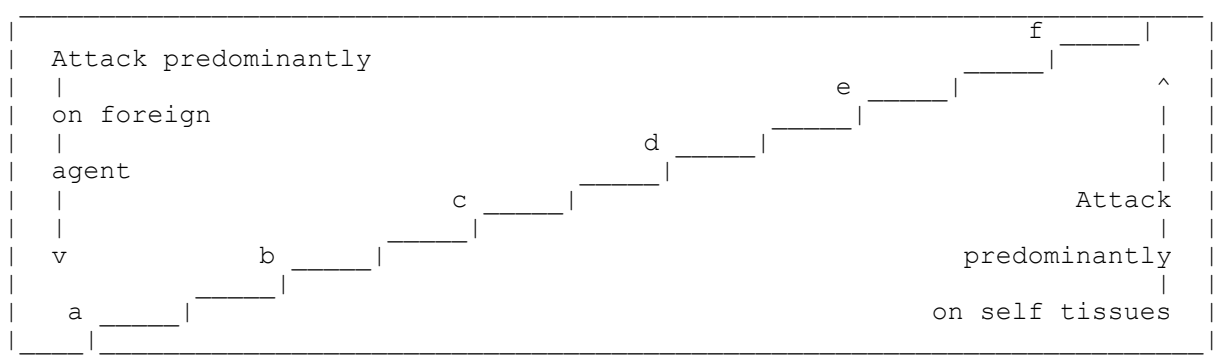
There is insufficient space here for a detailed elaboration so here is a whistle stop tour:

(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.