

## **Morphostasis and Immunity** (1992 version - unpublished)

### **Abstract**

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

### **Introduction**

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

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

### **The emergence of Self (cell) / Non-Self (cell) discrimination**

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

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

- It should be possible to discern gradual steps in the evolution of immunity starting in primitive metazoans and leading on to the sophisticated system found in mammals. So far, no clear stepwise progression has been elucidated.
- In development, ontogeny frequently appears to retrace phylogeny: whilst this is not an absolute blueprint for evolution, it does provide important clues.

## Morphostasis

Morphostasis is tissue homeostasis (1) and it is well maintained in all animals. It is a core process, the functional hub of the metazoan universe. It works efficiently because cells monitor their own health and keep constant close communication with appropriate neighbours. Anamnestic immunity is a branch of the morphostatic process: it has evolved to enhance the effectiveness of morphostasis in vertebrates.

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

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

Morphostasis (tissue homeostasis) can be maintained by:

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

## Healthy Self / Other Than Healthy Self discrimination

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

Here are some possible candidates:-

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

## Cell identity in the embryo and other systems

The cells in an embryo recognise each other through Cell Adhesion Molecules (CAMs) (2-6). At the cell surface, both like/like and ligand/receptor interactions of these CAMs lead to cell adhesion. This adhesion then rapidly progresses on to communication through gap junctions (7). These CAMs are of three main types: first, the cadherins, second the integrins and third, a group of CAMs that are members of the immunoglobulin superfamily (IgSF) of which N-CAM is an example. Note that the transfer RNA molecules specifying N-CAM are spliced by cells in a variety of different ways to produce a range of N-CAM phenotypes. Edelman & Crossin (5) state, "The origin of the entire Ig superfamily from an early N-CAM-like gene precursor has deep implications for the understanding of the role of adhesion in processes that are not concerned with morphogenesis but rather with immune defense, inflammation and repair". The cells of an embryo are able to recognise appropriate neighbours: they navigate themselves into their designated locations where they meet their intended neighbours. There are many other observations of the specific recognition of cells and self in biology.

Here are some specific examples:

- Protozoans recognise and discriminate food and sexual partners.
- Phagocytes are able to recognise their own pseudopodia and avoid self attack.
- Simple multicellulates are known to reject allografts (8,9)
- Plants - pollination is highly selective against self (10)
- Reaggregation of disrupted foetal cells (see later) (11,12)
- Bacterial agglutination and conjugation can be highly specific to self and (in pathogens) to target tissues. (13)
- Plants - tree roots in a forest often fuse together. This is very frequent when they are from the same individual, not uncommon when they are from the same species and far less frequent when they are from unrelated species (14)
- Molecular recognition is a fundamental biological principle (eg, nuclear enzymes).
- Cell homing: eg, lymphocytes and injected marrow cells (15)
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Self recognition could, therefore, be observed in several ways, each becoming progressively more specific to the individual animal:-

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

## Morphogenesis

Morphogenesis is the process by which tissues and organs are sculptured from a zygote derived colony. It is most obvious in developing embryos: regeneration and repair are achieved by a resurgence of morphogenesis. Since morphogenesis is an integral part of a morphostatic system, it is reasonable to expect that it will share component elements of the same molecular machinery as those used by immune cells and phagocytes. These components have (presumably) been closely associated through every epoch of metazoan evolution. It remains unclear what the complete mechanisms are that lead to embryonic development. However, CAMs (as above) and gap junctions  (16) appear to play critical roles.

## Embryos, CAMs and gap junctions

- Gap junctional communication can be relatively non-specific (crossing species barriers) but it can also be highly selective (as below) (17).
- Gap junctional communication is critical in development. Embryo development fails when GJ communication is disrupted (18).

- When CAMs (cell adhesion molecules) interact with each other or their receptors, the ensuing cell adhesion appears to lead directly to gap-junctional communication. CAM interaction precedes GJ insertion and both are necessary for normal development (19).
- Embryos are made up of a number of compartments. Communication through gap junctions is constricted at their boundaries. These compartments correspond to important developmental fields (17). They also correspond to fields of specific CAM expression (7) and homeotic gene expression (20-22).
- The gap junctions in these compartments are of two sorts (17). First, there are high permeability junctions joining each cell within a compartment. These allow the free passage of larger molecules: lucifer yellow is used to demonstrate this. I suspect that this "open" communication enables a block of cells to be organised, as if it was a single block of cytoplasm (a super-cell). This may be under the control of the appropriate compartmental homoeotic genes (look at the complex structure of paramecium to see how structuring this block might work - the open cytoplasm of multinucleated drosophila eggs is similar). Second, there are more restrictive junctions that join the cells at the boundaries of these "open" compartments. These only allow small molecules to diffuse (eg, ions) so they are either insufficiently large or insufficiently extensive to allow lucifer yellow to diffuse freely. These junctions allow ions to pass in either both or just one direction. The second sort are rectifying and they correspond to junctions formed from hybrid connexons (23-24). This directionality may be of significance in the way that embryonic cells sort, with endoderm to centre and ectoderm to the outside.
- Despite its name, N-CAM is not confined to neural tissues. Whilst it is expressed strongly and for long periods in neural development, it is also expressed, more transiently, in other sites. It is a recognised IgSF member (Immunoglobulin Super Family). A number of authors have considered these IgSF CAMs to be the probable ancestors of immunoglobulins, T-cell receptors and histocompatibility antigens.
- When embryo cells are disaggregated and allowed to resettle, they reaggregate into tissue layers, ectoderm to the outside, mesoderm to the middle, then endoderm to the centre (11). When embryonic cells from two mammalian species are mixed, they reaggregate into tissue type rather than species type and this appears to be because the genes that specify the various CAMs are highly conserved across the species barriers (12).

## Membrane holes

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

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

## Horror autotoxicosis & morphostasis

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

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

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

## Phagocytes and Double-Think

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

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

## Inflammation

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

I propose two things:

- In all complex metazoans, the discrimination of OTHS from HS by phagocytes remains a central and crucial morphostatic process.
- All other immune processes are geared to accelerate, accentuate and maximise the discrimination of OTHS from HS by phagocytes. In consequence, the efficiency with which OTHS is removed is greatly enhanced.

Even so (as you will see later) HS/OTHS discrimination does not begin in phagocytes but in somatic cells. It is the consequence of general cell recognition and communication. Inflammation is only established when somatic cells "decide" that they cannot cope alone and "invite" these scavengers in. Static somatic cells are attached to each other at cell junctions. Their cytoplasms are joined by gap junctions (except in those cells who's mature function depends on electrical excitability). When membrane junctions are split apart the disruptions in the cell membranes probably lead to the release of various eicosanoids (prostaglandins etc). This announcement of an OTHS event, by somatic cells, results in an inflammatory reaction. Chemical messengers released at the OTHS site encourage the ingress of phagocytes through the endothelial cell

linings of local post-capillary venules. Phagocytes now invade the OTHS site. They begin assessing cells on the basis of their HS status. Note that in electrically excitable cells, like neurones, their terminal differentiation requires that they uncouple from each other: it is left to unusually tightly bound endothelial cells to restrict the ingress of scavenger cells and thus reduce the susceptibility of these tissues to inflammation.

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

## Mimicry

Because morphostasis has always relied on self recognition, dedicated pathogens need to use mimicry (or more subtle interferences with identity molecule expression and recognition) to gain access to and persist in the soma (*eg*, 25-28). Every animal needs to stay one step ahead of its competition. Constant pressure is exerted to expand the variety of identity molecules available within a species (pleomorphism). Somatic cells appear to recognise each other by developmental ligands (cell adhesion molecules, CAMs). When embryonic cells from two mammalian species are disaggregated, mixed together and allowed to settle, they segregate into tissue type and not into species. Somatic ligands have probably needed to stay constant over countless meiotic generations. This makes them a sitting duck for determined pathogens. So, somatic cells need a "back stop" identity to be used as a second check when things go wrong (phagocyte based and, perhaps, also Mhc Class 1 based (29)). And until they do go wrong, inflammatory cells can be confined to the vascular system, locked out behind tight endothelial cell junctions until invited in. Note that "loss of function" is a cardinal feature of the inflammatory process.

## Unhealthy Self actions: Apoptosis and Self Sacrifice

When cells fail to establish communication, membrane reactions probably begin that lead to the release of a variety of eicosanoids and other cytokines (30). Similarly, when cells become unhealthy they break junctional communication and become prey to attack by both adjacent cells and the inflammatory cells that are (in consequence) called into the area (31). When I first started thinking about self(cell) surveillance, I found scant literature describing elective suicide and I even looked at plants for evidence of this (the hypersensitivity reaction (32,33)). However, interest and literature on this subject have become abundant recently (34-37). In synthesis, individual cells do decide that they are sick and/or redundant. They do have the capacity to invite attack by adjacent cells and also to invite phagocytes along to have themselves removed. There is no need to presume that antibodies and lymphocytes are responsible for the primary assessment of (healthy) self status.

Changes in the concentration of calcium ions within the cell are all important in this election for "disposal by consensus". Ca++ ions act as second messengers for a variety of cell processes including apoptosis, nuclear division, growth factor stimulation: they are closely tied into the inositol-PO4/DAG/protein-kinase-C network of intracellular second messengers (38,39); and high Ca++ ion concentrations close down the gap junction channels between cells. In this respect, cellular identity and cell health is all tied into proto-oncogene activity and this in turn into gap junction formation and communication competence (40,41). Here is the promise of a much clearer understanding of cancer.

When cells are attacked by C9 or perforin, they are made leaky, their cytoplasmic membrane potential falls and Ca++ ions are allowed into the cell. Both these molecules contain sequence motifs similar to the LDL receptor and epidermal growth factor receptor and there may be wider significance in this (*see* 42). One important feature is that both these receptors are endocytosed in clathrin coated pits (like the Mhc molecules themselves).

## The Generation of Specificity

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

There are two possibilities. First, that the ancestors of the T cell receptor may have been used to recognise tissue CAM ligands: this could be the origin of the V gene segments (43). Secondly, a descendant of the simple scavenger (phagocyte) may have evolved to recognise a set of pleomorphic CAM like markers that were specifically evolved in a population for them to be used as a back stop identity check unique to each ZDC. Developmental CAMs seem to remain constant over countless generations and this is reflected in the way embryonic cells from different species reaggregate as germ layers and tissues rather than species. The "back stop" CAM like ligand (the precursor of the Class I Mhc antigens) could deliberately borrow bits and bobs from these developmental CAMs to form a unique looking ligand by using a genetic mix and match process.

**TABLE 5**

Cell type	Receptors disabled	Receptors enabled	Normal state	Triggered state
Scavenger	Non pure self	Pure self	aggressive	passive
	<b>GENERATOR of SPECIFICITY</b>			
T-cell	Pure self	Non pure self	passive	aggressive
	<b>GENERATOR of DIVERSITY</b>			

There seems to be little likelihood that phagocytes are able to rearrange their genome to form specific receptors. And there is no substantive evidence that they can selectively cooperate with cells carrying self Mhc antigens. Natural killer cells, however, might be such a candidate, particularly if they are composed of two populations: one with a lower specificity - perhaps based on beta-2-microglobulin expression - and another with highly specific receptors for self. They were first identified because F1 NK cells attacked parental cells (unlike the classical transplantation laws). This would be consistent with specific (cooperative) recognition. These cells also preferentially attack cells expressing low levels of Class I antigen and beta-2-microglobulin. It seems that, at most, only a proportion of NK cells rearrange their receptor genes. (See (29,44)).

Phagocytes, lymphocytes, fibroblasts and platelets are all derived from the same stem cell. They have almost certainly all evolved from a primitive, ancestral scavenger. Each cell type seems to have caricaturised some

specific property of this general scavenger and refined it in order to make the mature mammal's repertoire of responses more versatile. This adds weight to the proposition that a phagocyte like or derived cell might, at one stage, have evolved to have the ability to select/rearrange its genes so that it could specifically recognise healthy self ligands (Mhc "Class-I-like" ligands). The self receptors would have to be selected, in embryo, to be specific to each individual.

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

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

## Soma/Scavenger segregation

I have already alluded to soma/scavenger segregation. The important point to grasp is that somatic cells can and do deal adequately with a fair proportion of OTHS (37). Provided the accumulation of OTHS is mild and the local cells can both recognise any loss of HS identity and discriminate foreign organisms from HS, then there is little need for a back stop identity check. HS here is established by displaying appropriate tissue CAMs that lead on to the establishment of a "synctial" communication through GJs. However, when UHS or foreign organisms fail to appear sufficiently OTHS to the local cells, then tissue damage will probably ensue as the foreign cells or UHS cells start to gain the upper hand. It is at this stage that scavengers are "invited" in and this is done by a fail-safe device (the eicosanoid system- prostaglandins etc). These scavengers then establish HS status by employing a "back stop" check on identity. If there is a scavenger that formally recognises HS Class 1 status then this would give the system a highly specific way of recognising self once invoked (eg, the NK cell (29)).

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

Now it is possible to add some concluding comments to the six points introduced earlier in the section "Embryos, CAMs and GJs":

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

- Now, the original self CAM may gradually be found to be inadequate as a back stop identity check because various pathogens discover ways of mimicking or interfering with its machinery. At this stage, a new cell is required (perhaps similar to the natural killer cell) that can recognise a more pleomorphic set of CAMs that are deliberately individualised in each animal of a population and more or less unique to each ZDC. An appropriate set of specific receptors would have to be selected, in embryo, to recognise these unique ligands. These, I contend, may be the close ancestors the T cell receptor that led, by inversion of function, to the cytotoxic T cell. In this vein, note that tumour necrosis factor and lymphotoxin are selectively toxic to cells that are not communicating through gap junctions (45,46).

## Anamnestic Amplification

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

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

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

The bone marrow constantly produces new uncommitted T-cells. So, whenever clearly foreign epitopes are sparse and inflammation is intense and prolonged, attention can gradually turn to self epitopes (eg, as in

tuberculosis). In summary, inflammatory acceleration is most likely to develop to clearly foreign (strange) epitopes and a "healthy soma tolerance" most likely to develop to self (frequently encountered) epitopes.

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

This could be done in a number of ways and these may account for many instances of clinical anergy (47-51):

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

## Auto-Rejection

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

## Antibodies - Icing on the Cake

Antibodies are icing on the cake. Extremely useful, evidently important but dominantly aimed at pre-empting the proliferation of blood borne pathogens and pathogens that colonise epi/endothelial surfaces. It's clear that the role of antibodies in tissue rejection (and hence auto-rejection) is minor if not minimal. The vast mass of animal life copes well without them. "Cell-mediated immunity clearly precedes humeral antibody production in phylogeny" (52,53). We can safely put antibodies to one side until towards the end - which is more or less where they evolved. It appears to me that, to bother looking amongst antibodies for an explanation of how self/non-self discrimination evolved, would be manifestly Heath Robinson (or Rube Goldberg!). In this vein, it is worth noting that the spleen may be specifically adapted to make the best of the difficult job of maintaining morphostasis in the suspension of cells circulating in the highly mobile plasma.

**TABLE 7****THE FOUR PRINCIPAL MODES OF EPITOPE PRESENTATION**

	<b>Other Than Healthy Self context</b>	<b>Healthy self context</b>
<b>Somatic</b>	Tc activation	Ts activation - direct?
<b>Phagocytic</b>	Th1 activation	Ts activation - Th/Ts cooperation?

**The Clinical Implications**

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

**Morphostatic Evolution**

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

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

They develop:

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

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

- Dedicated scavengers (phagocytes) now evolve. They refine this cooperative gap-junctional communication with self and the runaway, leaky hole attack of non-self. The molecules used to do the second will eventually evolve into what we now recognise as the complement components. It is possible that the two construction cascades are related but become independent early in evolution. At this stage the complement components are only secreted locally by phagocytes and their action is directed entirely at membranes. It is a long time before these components are co-opted into a humeral system and very much later that they are co-opted to interact with antibodies (probably an adaptation of specific Mhc recognition).
- A "vascular" system now evolves, locking out phagocytes till required. The alternative complement cascade can now be "humeralised" so that circulating C3 can mark clearly foreign organisms to make them more readily identifiable when they meet a phagocyte.

- There is now a progressive evolution and expansion of somatic LIGANDs leading to increased tissue compartmentalisation. Phagocytes are derived from a lineage that lies "outside" the three main germ layers so they may be exploiting this sorting tendency as they infiltrate somatic tissues: it is as if they are able to "clamber" over every other cell type.
- Ig supergene like LIGANDs develop to act as a focus on which to grow highly specific gap junctional plates and create developmental compartments. The genes specifying these molecules can now be copied then altered by a "mix and match" process to generate a set of LIGANDs that have a great variability within a herd (primordial Mhc genes). These pleomorphic LIGANDs will now act as the final arbiters of healthy self in each individual. Over many meiotic generations, they have eventually evolved into Mhc Class I LIGANDs. Newly developed scavenger cells (NK precursors) may now be able, when required, to co-operate with any somatic cell that displays self specific LIGANDs and observe a horror autotoxicus to it. These new scavengers need a mechanism to produce and/or select self specific RECEPTORs unique to each ZDC. This must be done post-meiotically over a number of mitotic generations - the "generation of specificity". This possibly coincides with the evolution of amniotic molecules that are involved in HS/OTHS discrimination or its modulation. These include HSP70, TNF, complement components and the 21-hydroxylases.
- By inverting the "generator of specificity" into the "generator of diversity" lymphocytic cells (Tc like) can evolve that are able to recognise and attack cells whose Class I ligands have been altered in the presenting cell by the attachment of a peptide that may make them look like an allotype. This new function depends on the duplication and transposition of the gene that produces the heat shock protein peptide pincer mechanism and bringing this to lie next to the Ig superfamily domain to produce the ancestor of a Class I Mhc gene (54). These primordial Tc cells first develop to recognise Mhc "Class-I-like" allotypes and then peptide/Class I combinations. They were probably preceded by cells capable of recognising beta-2-microglobulin: hence, the eventual elaboration around this molecule. Sometime between now and the evolution of free antibodies, the so called "alternative" complement pathway is extended into the "classical" pathway. C1 might be specialised for short range triggering of high density, single surface LIGAND/RECEPTOR complexes so that hole construction is now restricted to the target membrane rather than to a coordinated construction in apposing membranes.
- The stage is now set to allow the evolution of TH1 cells. Class II Mhc ligands evolve: the "intention" is to process short representative peptides from cellular debris picked up by phagocytes at inflammatory for the attention of uncommitted T-cells. The "generator of diversity" can now be enrolled into memorising the inflammatory context of these processed epitopes. On re-encountering the processed epitope these T-cells can rapidly attract large numbers of phagocytes to the site and "angrify" them: inflammation now has a memory. Note that only a very limited set of cells - APCs, phagocytes and a few others - can present these combinant epitopes so this amplification of the inflammatory cascade can only start after OTHS has been processed.
- The need to instruct T-cells to tolerate healthy soma epitopes has to evolve simultaneously with Tc and TH1 cells. T-cells capable of recognising healthy self epitopes are mostly decommissioned. This may be a co-operative process (Th/Ts cooperation akin to Th/B-cell co-operation). Whatever, aggression is averted by having them "mopped up" by Ts commitment. This happens because these epitopes are more likely to be met in a non-inflammatory context. However, uncommitted self specific T-cells continue to be released from the thymus and can become recruited into aggression. Aggression to self epitopes will be most likely to be induced and permitted when the inflammatory process is prolonged and foreign epitopes are sparse. Tolerance might be amplified by Ts cell clonal expansion and, perhaps, the release of anti-inflammatory agents at the site of epitope re-encounter. Like TH2 and B-cell interaction, helper and suppressor epitopes tend not to overlap, suggesting a similar co-operative mechanism.
- Last of all, TH2 cells can now be incorporated into the system to prime the B-cell system and lead to freely circulating antibodies. The B-cells are also derived from a scavenger cell. This is designed to secrete large quantities of free, circulating antibody. Antibodies help by opsonising organisms (preparing them as a "meal" for phagocytes). The classical complement cascade is now optimised to work within the vascular system and to interact with antibody tagged antigen. This system has proved invaluable as a pre-emptive defence.

## The advantages of this perception

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

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

- Seamless integration from embryonic development to anamnestic immunity.
- The innate and the acquired immune system are no longer seen as fundamentally disparate entities. They are fused into a seamless whole.
- A clearer understanding of preferential alloreactivity by T cells.
- A clear evolutionary progression from organisms with no cellular differentiation, through simple organisms with phagocytes, then the evolution of a retinue of specialised cells all derived from the primitive scavenger. A "logical progression" would start with NK like cells, go to Tc like cells, then TH1 like cells, then TH2 like cells and finally B cells.
- A far clearer perception of the cancerous process (not detailed here but there is good evidence that gap-junctional communication is involved <sup>(40,41)</sup>).
- The potential to explain the process of aging <sup>(55,56)</sup>.
- It all makes good biological sense. Indeed, it integrates so many biological, developmental and immunological mechanisms into a continuous whole that it begins to hold out the promise of a "grand unification theory".

## Summary

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

I have also proposed redefining the concept of "horror autotoxicus": it is established by successful cell to cell communication. Both somatic and scavenger cells use this mechanism. The concept of immunological surveillance is simultaneously redefined. But now surveillance is for any malfunctioning cell and not just for neoplasia. The evolution of a thymus dependent lymphocytic system with memory may have occurred at the expense of an increased prevalence of cancer, for intense focal suppression of surveillance now occurs whenever a strong positive feedback leads to an exaggerated attack on self epitopes. This then permits a tumour cell compartment to reach a critical mass beyond which surveillance fails <sup>(41)</sup>.

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

insertion mechanisms, then it would be reasonable to assume that HS is, indeed, sensed by the speed with which both somatic cells and scavenger cells establish an electrical continuum with those cells that they encounter.

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*Identical to that submitted apart from numerous substitutions of "that" for "which". Also, instances of "T-nk cell" have been replaced with "NK cell" (which is what I meant!). Image links  are recent additions.*