The following is a concatenation of Xenix files that I created on my work computer and I have used the file date to indicate their title/source.

See <19930718_lymphocyte_commitment> (below) as an example.

I have avoided identifying the people and journals in these reproductions. I have substituted many instances of "which" for "that" and corrected some spelling mistakes. Otherwise these are faithful to the originals.

19910101_tw

Dear Dr W,

I wonder if you would be prepared to look at this article entitled "Morphostasis and Immunity" and see if you feel there is any value in the concept?

You know me better as a local GP than as a writer of hypotheses on immunity. I have had a fascination for this subject over the last 14 years. It began with an interest in the neurological complications of Behcet's syndrome. I wanted to get a feel for what what causing this disease. It soon became obvious to me that I was going to have to learn some immunology and also look at a variety of other diseases to see how each was structured and responded to age and immune status. The immunological interest has, to a very large degree, taken over.

I've touted these ideas around in less mature form for some time. In particular, the article on Behcet's syndrome has been seen by several people who consider it to be too hypothetical. I've included a copy simply to demonstrate that my allegation that MS and neurological-BS are potentially "linked" is not an idea presented without considerable thought and reading. Please do not waste time looking through this. Since the assumptions eventually feed back to enhance the morphostasis hypothesis, it reinforces my own conviction that there is some value in these ruminations.

I recently saw your two articles, in which you suggest that auto-immunity might be the unfortunate result of a physiological response rather than a totally inappropriate immune phenomenon. I felt that you might, therefore, not be too hostile to my presumptive intrusion into an expert field: particularly as the hypothesis fulfils the premise that immune auto-aggression (auto-rejection) is a normal process.

My increasingly busy life (and committment!) as a GP mean that I am able to spend less and less time on a subject that demands reading and knowledge over an exceptionally wide and expanding stage. I have grown resigned to the idea that my efforts may have no other reward or recognition than that they were good fun. But!!..... I still have this feeling that it's a pity other people might not get round, at the very least, to considering seeing things from my point of view.

I'm still trying to tidy up the references and get up to date sources BUT I cannot do this rapidly with only occasional days in the library, so my apologies that the reference lists may seem incomplete or inappropriately old at times.

If asked for a short paragraph to justify WHY you should spend any of your valuable time reading this, this would be my reply:

This concept welds morphogenesis, morphostasis, inflammation, phagocytosis and amnestic immunity into a well integrated continuum. It offers a pretty clear explanation for the prevalence and pathophysiology of three broad groups of disease (auto-rejective, cancer and infections). It offers a neat explanation for the precipitate evolutionary emergence of the amnestic immune system around the era that protochordates and vertebrates originated.

Yours

19910401_tw

My apologies for hounding you. Have you managed to spare some time to look at the hypothesis I sent you? I was considering sending it off to the Journal of Theoretical Biology though I would rather wait for some criticism first. Don't worry if you cannot afford time to look at it but I'd be grateful if you would let me know.

Once again, I have been through it searching out the flowery language and the non-sequiturs so there is a more readable version should you prefer it.

19910501_tw

Thank you for your reply. I wondered about invading your privacy with yet another version but have decided, since the English in this has been made deliberately plainer, you may as well have access to it when you do find the time. The content is little changed.

19910519_tw

19th May 1991

Dear T,

Many thanks for taking the time and trouble to come along and talk to me last Wednesday night. I hope you didn't get too annoyed with my undisciplined mind.

I know you are busy and may well not find time for a while to think on this but I have enclosed an article on invertebrate immunity that discusses at length the idea of specific recognition by phagocytes. It fails to address the problem of the "generation of phagocyte specifity" in a ZDC and to conceive that the affected cell itself, or its immediate neighbours, might identify its own "sickness" rather than leave this identification to the immune cells. The authors look on the loss of (healthy) self identity as entirely passive.

One thing that may well not have come across clearly to you is the analogy I made on the development of the vertebrate eye. Superficially it looks far too complex to have evolved gradually but its evolution can be traced back in graduated developments from some very rudimentary light receptors. There is an analogous problem in the immune system. How do you develop, in the space of a very short evolutionary time, a complicated and sophisticated anamnestic immune system (I have spelt it right ONCE in the article!) at the moment of the evolution of the branchiates. This conundrum has taxed many people. The system would seem to be next to useless until an extensive repertoire is possible. IF, however, the genes encoding diversity of receptors began their evolutionary life in order to select a single ZDC specific receptor (generation of specificity to allow specific phagocyte recognition) then this can develop right from a two receptor choice through to a million, and more, receptor choice in gentle graduations. Flip it around into the generation of diversity about the time the branchiates emerged (and a thymus) and you have the rudiments of a highly versatile anamnestic immune system.

The idea that intense immuno-inflammatory activity "saturates" and hence leads to local surveillance depression in intensely inflammatory lesions, came from clinical features and particularly the behaviour of pathogens like TB and syphilis. Syphilis is a far greater mimic of the BS syndrome complex than is TB, which you would soon realise if you looked hard at it. So the need for a focal switch off (focal anergy) arrived, in concept, as a clinical necessity. You seemed surprised at what I said about staph boils - the attached extract from a book reinforces the concept that bacteria DO use this "saturation" effect as a cloak for unbridled activity.

The section on "WHERE DO LYMPHOCYTES AND ANTIBODIES FIT IN" is, recently written, badly explained and needs further reading and revamping. I will do this. The principle that it is a simply permittive rather than a highly directive system is what needs to be emphasised. Similarly, "MORPHOSTATIC EVOLUTION" could be better presented.

STUDY LEAVE

I've enclosed the note about DoH sponsered study leave, though a sticky problem with this is that it leaves me somewhat out of pocket. You could help to support me, if you felt able, by emphasising the value of the study I have already done and the probability that it will be of benefit to myself and, possibly, others if I had further leave to pursue these ideas. A word with Bob Thomas in the postgraduate centre would secure the support needed.

Yours sincerely,

19911015_ec

15th October 1991

Dear Dr (*should have been Professor*) C,

I really don't know what to do with this article. I’m probably deluding myself that there is either basis or value in the ideas presented. Those people who have seen earlier versions don't seem to see significant value in it. I'm more or less totally isolated from academic immunology. My interest in the subject stemmed from trying to understand what was causing Behçet's syndrome (the essential reason for playing with these ideas). There is a long article on this that I have only alluded to in this article - it's not published. It struck me that you would be in a position to see value in the concept if it is there. I can no longer find time out of my busy professional life as a general medical practitioner to persue these ideas in anything more than a superficial way (much as I'd like to do nothing but that). I'm beginning to believe that I have no ideas that the world of immunology wants or needs. But! I can't help feeling this is too "round" and predictive to be total rubbish. I'm hoping that you might see just a glimmer of benefit in this way of conceiving the immunological process.

I hope you don't mind me imposing on you.

Yours sincerely,

19911126_ec

26th November 1991

Dear Professor C,

Thank you for your reply.

I chose you because of your interest and influence in immune phylogeny. Also you are in position to broadcast this locally or generally if you considered it had value.

The enclosed article is a synopsis of Part 1 of the article I sent in October. Referencing will be more difficult here and may simply evolve into a reading list based on the numbered sections. I have tried to be more direct. I hope that I have stripped it down to the bare bones. I consider that its content must belong either in the trash can or it is important. I don't believe that there can be much intermediate ground. If it's the second, it NEEDS broadcasting. I am not unduly concerned how this might be done nor is my main goal to grab the credit. I have found myself academically isolated and, consequently, impotent at interesting anyone in this idea. I simply hope that the concept rather than I will be taken seriously.

What are my plans? To persuade someone in one of the main fields of immunology (and the best is probably immune phylogeny) to find some very good reasons why this is undoubted rubbish or to take it seriously.

Where do you fit in? I'm not sure that I am sure. I have no mentor. I have no resources beyond a local medical library and my office at home. I would appreciate help and I thought that you, as the editor of a journal on immune phylogeny, might be willing or able to point me in the right direction.

By the way, Part 2 was largely complete before Part 1 was conceived. It was from this direction that I approached the subject.

Yours sincerely,

19911129_ec

29th November 1991

Dear Professor C,

Sorry to keep writing but this version is in better English and probably more easily read and understood. Having written it at short notice I will continue to alter the English and some parts of the explanation over the next 2 weeks. I will give more thought now to the referencing.

Yours sincerely,

19911216_ec

16th December 1991

Dear Professor C,

When you suggested I rewrite the article it gave me the opportunity to do some more reading and thinking and it seems I have been slow to appreciate that Tnk cells probably ARE the inverted Tc cell. The reasoning is outlined in this addendum. Many of the assumptions are too speculative for anything more than rumination. No references are given here but I'll provide them if you wish.

Either this enormous jig-saw puzzle is coming together very quickly or I'm just a crank! I hope you can figure out which statement is correct.

Yours sincerely,

19911221_ec

21st December 1991

Dear Professor C,

I hope that you are not losing patience with me BUT this hypothesis CONTINUES to evolve. A number of points have become clearer. I have corrected some errors and improved the explanations given in the last letter I sent you.

The main reason for writing today is to send you this explanation of how I now think SELF/NON-SELF discrimination is ultimately organised. This was finally synthesised this morning.

I have also made some improvements to the main article I sent you that I will send but only if you request it.

Yours sincerely,

19920324_crit

MY RESPONSE TO THE CRITIQUES: The shorter critique states that the concept of morphostasis has been proposed before. This is a strange comment when I quote the source of the idea. I have just carried out a Medline search over the last 5 years. It quotes three instances of the use of this term. Two did not concern immunology. There was one on lymphoid directed morphostasis in the liver. I don't claim to have invented "morphostasis". I do propose that it is a fundamentally crucial process. It is the PRIME function of inflammatory and other immune cells. There should have been thousands of references to it, not just one. Langman was, in the past, one of the main protagonists of specific phagocytic recognition. The quoted article from Coombe, Ey and Jenkin gives an extensive review of the literature on this idea. Katz and Skidmore (Self recognition and cell communication - I have the photocopy but no reference) got close to what I'm suggesting but they failed to drive the concept home. None of these authors have made the final step to say that self(cell)/non-self(cell) recognition is made by virtually every cell in the body and that immunological self(Ag)/non-self(Ag) reactivity is the consequence, not the source, of self/non-self discrimination. Here is a quote from Edelman (1991) p177 (1b). "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" (ie, morphostasis!!). (See below also).

The second referee's longer critique is more provocative. He has clearly been ruffled by my presumption. However, I regard this response as evidence that he has failed to grasp the implications of what I am suggesting. It seems to me that he is caught in the straight jacket of conventional perception. He is clearly convinced that the final explanation will come only in detailed dissection of intricate mechanisms. To me this is analogous to being given the bits of a smashed watch and then studying the intricacies of each individual part to work out the whole. It will probably get there in the end. However, an alternative is to stand back and, from every possible viewpoint, hypothesise wildly, piece together the broad principles then refine these until the details begin to fit. In particular, this referee seems to be caught in the quagmire conviction that lymphocytes and antibodies are ultimately responsible for distinguishing self from non-self. The fact that they TEND to be aggressive to non-self antigens and TEND to be tolerant of self antigens is consequence rather than source of self(cell)/non-self(cell) discrimination.

I will take his points in order:

1) "Verbiage and unprofessional" style: indubitably NOT the style of most articles on immunology: and I intend that as a criticism. I love to read Jan Klein (hope he wasn't the referee!): plain anglo-saxon and a minimum of jargon where it will suffice! I have consciously and laboriously toiled over this goal. I have introduced no greater technical complexity than is absolutely necessary. Where I have used "jargon" I have listed it in a table. I have invented FOUR new terms, ZDC (zygote derived colony), US (unhealthy self), OTHS (other than healthy self) and the "generator of specificity". I think these are self explanatory: in my view they were vital concepts. My presumption here has clearly annoyed him again. Wild hypothesiser I certainly am. I know this is unfashionable in medicine and biology. BUT, is it a bad thing?

2) "Macrophages rearranging their genome:" This has been a major conundrum. Nowhere is there anything but the merest soupcon of evidence that phagocytes can specifically recognise self Mhc. The need for a cell that does or did this came from the clinical realisation that auto-rejection is a NORMAL, regularly employed process (based on the work I have done leading up to an analysis of neuro-Behçet's syndrome, other sero-negative arthritides and multiple sclerosis). There is no automatic horror autotoxicus to self epitopes where T cells are concerned. When auto-rejection is so general, it must have physiological significance: it must be part of the morphostatic mechanism. Unless it's a Heath Robinson (or in American, Rube Goldberg) system, self/non-self discrimination can't be left to cells that are regularly able to mount an aggressive response to self epitopes. This realisation led directly to the OTHS concept. I have been slow to appreciate that Tnk cells might offer a way out of this conundrum and could be the cells that (originally?) recognise(d) self Mhc (this only occurred to me just before Christmas). Phagocytes, lymphocytes, fibroblasts and platelets are all derived from the same lineage. They are almost certainly all descended from a primitive scavenger. They have simply caricaturised and refined specific properties of this general scavenger to make the mature mammal more versatile.

3) To my mind, this referee now demonstrates that he has not grasped the concept that I have proposed. All the IgSF (Immunoglobulin superfamily) molecules employed right up to and including natural killer cells are acting exclusively as CAMS and CAM receptors. N-CAM and the Cadherin family trigger the immediate construction of membrane holes to form communicating gap junctions. In so doing the adjoined cells become a cooperative synctium. A similar process PROBABLY occurs in phagocytes and Tnk cells (though some other adaptation may bypass the need for gap junctional communication). Cells that fail to make contact by appropriate CAM recognition are given larger, leaky holes but in the receptor membrane alone. This increases their intracellular Ca++ levels and reduces their membrane potential. I half suspect this leads to a capacitive triggering of a membrane action potential in the lamellipodia of the phagocyte or the Tnk membrane. It is of interest that cells that MUST uncouple before they perform their differentiated function (eg, nerve cells and muscle) display large amounts of negatively charged sialic acid on their surfaces. Similarly, macrophages have one of the highest membrane potentials of all cells (I can't at the

moment find the source but I'm sure I have read this). It was only with the advent of Tc cells that receptor/ligand interaction was detached from the CAM function. (It may prove to be that it was even detached in Tnk but, logically, this is neither likely nor necessary.)

Free IgSF molecules are NOT intentionally released at any of these stages. Phagocytes are NOT required to pinocytose various molecules. I doubt that a ubiquitous principle like gap junctional communication is ignored by phagocytes or natural killers and I strongly suspect that, at the phagocyte's podium, it attaches and electrically couples with underlying self tissue. I strongly suspect that this accounts for a major part of self/non-self discrimination by these cells.

I risk being lynched now. Antibodies are icing on the cake. Extremely useful, evidently important but dominantly aimed at preempting the proliferation of blood borne pathogens and pathogens that colonise epi/endothelial surfaces. It's quite 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" (Manning and Turner 1976). We can safely put antibodies to one side until towards the end - which is more or less where they evolved. Looking for the explanation of self/non-self discrimination amongst antibodies is, to me, manifestly Heath Robinson (Rube Goldberg). Saying that "the first step in immune reactivity was the development of a molecule for recognition and response, antibody" seems, to me, an empty comment when Cadherins, N-CAM and other CAMs are already regarded by many as the origin of this recognition and response (01). Lymphocytic cells that bear resemblance to Tnk cells are even seen in invertebrates. Beta-2-microglobulin was around long before antibodies and specific immunity. No! Antibodies are a "recent" adaptation of ancient mechanisms. (They are all the more necessary when you consider that blood is a broth held at 37 deg C with the capacity to disseminate microorganisms all over the body: morphostasis in the blood cannot happen as it does in tissues: special arrangements - the spleen - have to be made.) To me, immune network theories of self/non-self discrimination smack of the need for an "immaculate conception" - the repertoire needs to be complete before it can adequately work or so many stipulations have to be put upon it that it becomes Heath Robinson (Rube Goldberg).

4) "Even the most primitive extant vertebrates produce Ig, but as far as can be discerned, they do not have the various T-cell subsets postulated by the author." I quote above from one of the earliest books on immune phylogeny (Manning and Turner) and I'm not aware that opinions have altered on this. If these cells have not been identified then the question is rather "Why?" than evidence that they never existed.

microscopically dissected this spring in the watch because it is detail not principle. The principle predicts the detail. Start with a simple CAM/CAM interaction like N-CAM. This is recognised in a straight like/like interaction and leads on to immediate GJ communication (the insertion mechanism is, I suspect, akin to the complement cascade with C3 like amplification leading to large patches of apposing membrane holes). Tnk cells probably evolved to recognise a deliberately individualised CAM (Mhc class I precursor). This population of pleomorphic CAMs in the "herd" were developed to ensure a greater individual specificity and acted as a "backstop" check of self identity. They brought with them the "generator of specificity". So, when the generator of diversity arose its SOLE preoccupation was in the recognition of Mhc ligands: NOT a diverse set of non-Mhc epitopes. The evolution of membrane recycling and peptide (pocket) presentation of antigen simply acted to make peptide/self Mhc combinants appear like a set of pleomorphic Mhc antigens that the inverted Tnk like cell (now a Tc like cell) was ready primed to recognise. With the evolution of antibodies the restriction of this specificity to a limited set of Mhc allotypes was bypassed by introducing complex gene rearrangements and somatic hypermutation. [In the beginning (the primitive Tnk like cell), germ line V genes would probably have been sufficient without the necessity for even D-J rearrangement].

6) So too, he seems to have misunderstood the proposed principle of the prime function of T cells and antibodies. The principle is simple - there's no need to dissect detail here: no need to delve into the complexities of peptide pockets, cytokines, suppressor mechanisms and immune networks. T-helper cells and antibodies are largely concerned with heightening inflammation and of bringing cells possessing particular epitopes to the attention of phagocytes (and Tnk cells). Direct killing is NOT the prime function. They are not remembering epitopes just to "kill" them. The precursor lymphocyte logs the context in which it first set eyes on its epitope. If it was inflammatory then at the next encounter it will 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 is that they're 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 focalisation of this response). Tc cells are relatively more independent but even these are only allowed to become aggressive if they have been primed by IL-1 from APCs in an inflammatory encounter. And these, too, encourage a rapid inflammatory response once they start attacking cells.

On the other hand, if the first presentation of the epitope was non-inflammatory, suppression is favoured. Most paratopes specifying self epitopes are confined to the suppressor pool unless they are infrequently exposed or nearly always exposed in inflammatory situations (eg, heat shock proteins). This certainly accords with clinical and experimental experience. This is the broad principle: we can leave the detail to the watch dissectors. It need not concern the principle that suppressor T cells tend to choose slightly different parts of a molecule than helpers. Anyway, this may be a result of cooperative presentation rather than of "self" selection (like Th/B-cells).

7) The author's inversion of B-cell and T-cell function: First I didn't suggest the inversion of B-cell function. ONLY of Tc cell function. The purpose of this inversion is for a Tnk like cell to specifically recognise self Mhc alone (at least 2 possible epitopes in a diploid animal). The term pure self is used to distinguish self from self+x (this formal term obscures the principle). These

paratopes were originally used as CAM receptors to establish communication with self cells. Cells that could not make such communication were attacked by Tnk like cells.

This all sounds arrogant but I'm becoming increasingly confident that this hypothesis can take whatever knocks are thrown at it. Those that do hit hard reveal a misunderstanding that, once corrected, leads to an enhancement of the whole.

Let me tabulate the advantages of this way of perceiving the process: 1) Seamless integration from embryonic development to anamnestic immunity. 2) The innate and the acquired immune system are no longer seen as fundamentally disparate entities. They are fused into a seamless whole. 3) Automatic explanation of preferential alloreactivity by T cells. 4) 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 starting with Tnk like cells, going to Tc like cells, Td like cells, Th like cells and finally B cells. (This progression is logical.) 5) A far clearer perception of the cancerous process (not detailed here but there is good evidence that gap-junctional communication is involved (02)). 6) The potential to explain the process of ageing (03). 7) It all makes excellent biological sense. Indeed, it integrates so many biological, developmental and immunological mechanisms into a continuous whole that it has the rumblings of biology's equivalent of the "grand unification theory" of physics.

There is a strange double-think that pervades immunology when it comes to the subject of the phagocytic recognition of non-self and unhealthy self. Every MEDICAL STUDENT knows that phagocytes recognise dead, damaged, sick and effete cells. Every medical student knows they can recognise foreign organisms [particularly non-(dedicated)-pathogens] and eliminate them. Every text book devotes its statutory (short) introductory opening to the importance of phagocytes and innate immunity: then, almost without fail, each author is seduced, with indecent haste, into an intense dissection of the principles of anamnesis and lymphocyte function. What makes this worse is that the anamnestic immune system is not essential to prepare cells for phagocyte attention. The phagocytic system works well in invertebrates. Self/non-self discrimination works well in invertebrates. Hardly any of the specific points that I have written in the main article could be said to be in any way radically new BUT, the emphases on what's important IS. I am even more convinced now that the conventional perception is flawed.

There can be no doubt that the reason for this tendency to ignore the fundamental importance of phagocytes is a lack of understanding of the mechanisms of self/non-self discrimination by these cells. There is, at the very least, a strong possibility that this may be because no significant research has been carried out on the junctional communication between these cells and underlying somatic cells.

Am I deluding myself? Is all my assumption JUST the mad rambling of a crank? I don't pretend that I am specifically right in all that I have proposed but I KNOW that I'm not wrong in the general "flavour": and this "flavour" is a long way detached from conventional perception and wisdom. THE MOLECULAR MECHANISMS UNDERLYING HS/OTHS DISCRIMINATION

"Morphogenesis is an integral part of a morphostatic system. It is, therefore, reasonable to expect that the component elements of morphostasis will use molecular machinery that is genetically related for they have (presumably) been closely associated through every epoch of metazoan evolution."

"Horror Autotoxicus must be built upon the possession of specific and recognisable whole cell properties (very probably expressed at the cell surface): these probably aid the co-operative "docking" of one cell with another."

These quotes come from the original article that you suggested I shorten. Since December I have taken some annual leave to get much needed time in the library and I believe I now understand the process better. The following is a synopsis of this. As you will see, it largely obviates the need for the mental gymnastic of inventing HS and OTHS. No one I have read has suggested an explanation in these terms. It is necessary to be familiar with aspects of gap-junctional communication in development to understand it fully. The following statement is a now a central tenet:

"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 cytoplasmic continuum. 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 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: electrical discontinuity and a lower membrane potential encourage phagocytes to attack. 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 easiest way to proceed is to list the salient points and give a list of supportive references. In all of this I'm treating the whole thing as a jig-saw puzzle. The jig-saw pieces are individual research reports. I contend that there are enough of these already existing to piece together a very nearly complete overall picture. Missing pieces need to be guessed then searched for. Guesses need to be matched against facts and then the dross thrown out. At any point there WILL always be an element of dross though, I suggest, this is mostly concentrated, now, in the more specific assumptions. To clarify this I have written the following notes with:

a) reported fact left unhighlighted
b) probably correct highlighted green

c) guesswork highlighted yellow and
d) wild assumptions highlighted pink.

1) Gap junctional communication is not particularly specific but it appears to be highly selective (a concept well recognised in Ab/Ag interactions)(04).
2) Gap junctional communication is critical in development. Development fails if GJ communication is disrupted (05).
3) When CAMs (cell adhesion molecules) interact with each other or their receptors, this interaction appears to lead directly to gap-junctional communication. CAMs precede GJ insertion and both are necessary for normal development to occur (06a) (06b).
4) Embryos are made up of a number of compartments that have clearly defined boundaries of communication through gap junctions. These correspond with important developmental fields (07). These boundaries also correspond to specific CAM expression (07).
5) The compartments are of two sorts. First, high permeability junctional complexes allowing the free passage of lucifer yellow. I suspect that these enable a large block of cells to be organised by homoeotic genes as if they were one complex cell (eg, look at the complex structure of paramecium). Second, other junctions that allow the free passage of ions or rectifying junctions that occur at communication boundaries (08) (possibly of significance in the way embryonic cells sort, with endoderm to centre and ectoderm to the outside) but are insufficiently large or extensive to allow easy passage of lucifer yellow.
6) N-CAM is not confined to neural tissues. It is expressed strongly and for long periods in neural development. It is expressed more transiently in many other sites. It is a recognised IgSF (Immunoglobulin Super Family) molecule. A number of authors have considered these molecules to be the the probable ancestors of immune IgSF molecules (Edelman is one (01)).
7) The hypothesis is that the scavenger cell developed a CAM receptor molecule to specifically recognise the respective CAM on other self cells (perhaps a beta-2-microglobulin like molecule). It used this as a means of communicating electrically with the underlying self cells (at its podium). A cytoplasmic finger from the scavenger could trigger the phagocyte into aggression if it encountered a cell not in direct electrical communication (via gap junctions through the membrane where it sits on underlying tissues). This may be done by the induction of a capacitative current that then triggers an action potential. Other recognition strategies are used. Changing surface sugars in sick cells is one (loss of the sialic acid residues may increase the capacitive current - sialic acid being negatively charged). The phagocyte probably also has a limited set of receptors for epitopes that are indubitable markers of their non-eucaryotic origin and never occur as part of self. Dedicated pathogens will deliberately avoid displaying these.
8) Now, the beta-2-microglobulin like molecule (see (7)) is gradually found to be inadequate as a backup identity check because various pathogens discover ways of mimicking or interfering with its machinery. This is when a new cell was required (perhaps like the natural killer cell) to recognise a more pleomorphic set of CAMs that are deliberately pleomorphic in a population and more or less unique to each individual. An appropriate set of specific receptors needs to be selected, in embryo, to recognise this unique ligand. These, I contend, are the origin of T cell receptors and they led, by inversion of function, to the cytotoxic T cell. In this vein, note that TNF and lymphotoxin are selectively toxic to cells NOT in gap junctional communication (09).

That leads us on to self/non-self discrimination. It is occurring as part of cell-cell "docking" based on CAM expression. CAM interaction leads to junctional communication, just as it does in embryos and in tissue regeneration (CAMs are reexpressed during periods of regeneration). The proposition here is that phagocytic and Tnk recognition is also achieved through a similar process.

When cells fail to establish communication, membrane reactions begin that lead to the release of a variety of prostaglandins and other cytokines. Similarly, when cells become unhealthy they break junctional communication and become prey to attack by both adjacent cells and inflammatory cells that are (in consequence) called into the area (10). When I first started thinking in these terms, I had found very little literature describing elective suicide and I even looked at plants for evidence of this (the hypersensitivity reaction). However, interest and literature on this have become abundant recently and there are several recent articles, one in Adv Immunology (11), one in the Annual Review of Biology (1991) and the enclosed (very readable) article from the New Scientist. Individual cells DO decide that they are sick and/or redundant. They DO have the the capacity to invite attack by adjacent cells and also to invite phagocytes along to effect their elimination. There is no need to presume that antibodies and lymphocytes are the sole or even the major assessors of healthy self status.

Calcium changes 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 and they are closely tied into the inositol-PO4/DAG/protein-kinase-C network of intracellular second messengers. In this respect, cellular identity and cell health is all tied into proto-oncogene activity and this in turn into gap junctions and communication competence (02). A much clearer understanding of cancer thus seems an imminent prospect.

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. These molecules carry LDL receptor and epidermal growth factor receptor motifs. Any deeper significance of this escapes me at the moment but one important feature is that the receptors they mimic are both endocytosed in clathrin coated pits (like the Mhc molecules themselves).

By now I hope that you will be aware that this suggests a clear path in self/non-self discrimination beginning in sponges, that show differential aggregation (for they, too, have gap junctions), 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 (12). 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 (13).

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02 Role of intercellular communication in the control of critical gene expression during multistage carcinogenesis. Yamasaki H, Enomoto K, Fitzgerald DJ, Mesnil M, Katoh F & Hollstein M CELL DIFFERENTIATION, GENES & CANCER Ed Kakunaga T et al IARC Scientific Pubs No 92, ISBN 92 832 11928 Pubs, International Agency for Research on Cancer
03a Development of the aging cell surface Kelley RO, Vogel KG, Crissman HA, Lujan CJ & Skipper BE Exp Cell REsearch 1979 119:127-143
03b Cellular senescence: A Reflection of Normal Growth Control, Differentiation or Aging? Peacocke M & Campisi J J Cellular Biochem 1991 45:147-155
04 Garrod DR, Nicol A "Cell behaviour and molecular mechanisms of cell-cell adhesion." Biol Rev 1981 56:199-244
05 Evidence mounts for the role of gap junctions during development. Green CR BioEssays 1988 8:7-10 (General introduction to GJs and development)
06a Neural Differentiation, NCAM-mediated Adhesion and Gap Junctional Communication in Neuroectoderm. A Study In Vitro. Keane RW, Mehta PP, Rose B, Honig LS, Loewenstein WR and Rutishauser U. Journal Cell Biology 1988 1307-1319 (Linking NCAM expression + gj communication)
06b Regulation of Connexin 43-Mediated Gap Junctional Intercellular Communication by Ca++ in Mouse Epidermal Cells Is Controlled by E-Cadherin Jongen WM, Fitzgerald DJ, Asamoto M, Piccoli C, Slaga TJ, Gros DG, Takeichi M & Yamasaki H J Cell Biol 1991 114:545-555
07 Gap Junctional Communication in the Extraembryonic Tissues of the Gastrulating Mouse Embryo. Kalima GH and Lo CW J Cell Biol 1989 109:3015-3026 (Lucifer yellow compartments and ionic coupling compartments)
08 Formation of hybrid cell-cell channels Werner R, Levine E, Rabadan-Diehl C & Dahl G Proc Nat Acad Sci 1989 86:5380-5384
09a Resistance to the cytolytic action of lymphotoxin and TNF coincides with the presence of gap junctions uniting target cells. Fletcher WH, Shiu WW, Ishida TA, Haviland DL & Ware CF
09b Relationship between tumour cell morphology, gap junctions and susceptibility to cytolysis by tumour necrosis factor. Matthews N & Neale ML Br J Cancer 1989 59:189-193
10a Intercellular communication and tissue growth. II Loewenstein WR and Penn RD J Cell Biol 1967 33:235-242 (Description of communication changes in wounding etc)
10b Gap junctional structure and cell-to-cell coupling regulation: Is there a calmodulin involvement? Peracchia C & Bernardini G Fed Proc 1984 43:2681-2690?+
11 Programmed Cell Death in the Immune System Cohen JJ Advances in immunology 1991 50:55-77+ (General discussion apoptosis etc)
12 & 13 CAMs and cell sorting CAMs and embryonic sorting across species barriers (I have been searching for this reference but can't locate it amongst my photocopies: this discusses the relationship of specific CAMs to cell sorting in disaggregated embryos and also to the fact that cross species similarities in CAMs accounts for selective organ rather than species reaggregation.) This is alluded to in other articles (01 and 16).
14 Growth Factors Modulate Junctional Cell-to-Cell Communication Maldonado PE, Rose B, Loewenstein WR J of Membrane TBiology 1988 106:203-210

Behçet Mophoregulatory molecules Edelman GM Biochemistry 1988 27:3533-3542+

19930206_ProfX

6th February 1993

Dear Professor X,

Re: "Morphostasis & Immunity" Your ref: ####

I spoke with <your secretary> this week who explained that it is taking longer than expected to receive a reply from your referee.

In the six months since I first sent the manuscript I have continued to explore these ideas. I hope you don't mind if I take this opportunity to send this newer version with better language and more refined ideas. Whilst the basic concept is unchanged I have done some fine tuning and added references that add to the overall understanding. I have also added a few sketches that help to see how things might have evolved. Whilst these are too speculative to include in the article they do add some "colour" to the concept of MAC/GJ relationships.

Of particular interest are the references listed below.

Yours

HOMEOTIC GENES AND COMMUNICATION COMPARTMENTS. --Coelho, C.N.D. and Kosher, R.A., (1991). "A gradient of gap junctional communication along the anterior-posterior axis of the developing chick limb bud." Developmental Biology 148:529-535. --Martinez, S., Geijo, E., Sanchez-Vives, M.V. & Gallego, R. "Reduced junctional permeability at interrhomberic boundaries." Development 116:1069-1076 1992 --Risek, B., Klier, F.G. and Gilula, N.B. (1992). "Multiple gap junction genes are utilised during rat skin and hair development." Development 116:639-651.

CANCER --Yamasaki, H., Carcinogenesis. "Gap junctional intercellular communication and carcinogenesis." 11:1051-1058 1990

HEAT SHOCK PROTEIN and CLASS I MOLECULES --Flajnik, M.F., Canel, C., Kramer, J. & Kasahara, M. (1991). "Which came first, MHC class I or class II?" Immumogenetics 33:295-300

19930301tb

6th February 1993

Dear Professor X

Re: "Morphostasis & Immunity" Your ref: ####

I am concerned that I have still not received any communication from you regarding this manuscript. It is now 32 weeks since I sent it and the only feedback I have had was as a result of my telephone call 4 weeks ago to <your secretary>. I am particulary concerned that a rejection now will have resulted in a very substantial delay before I have a chance to submit it elsewhere. I would be very grateful if you could indicate how seriously you are taking this; do you you regard it as cranky nonsense or potentially important - it can't, by its nature, sit in the middle. In view of the delay, I would appreciate some indication of the probability of rejection or acceptance and the progress towards a decision: I would not want to wait much longer before I withdraw it and submit it elsewhere unless I am reassured it's being taken seriously.

Yours

19930309_crit

9th March 1993

Dear Professor X,

Re: "Morphostasis & Immunity" Your ref: ####

Thank you for your reply and the return of my paper.

Please don't be offended if I play devil's advocate and put this point: I do it tongue in cheek. Seven months is an unacceptably long delay to come up with a reflex dismissal of my concept, pronouncing it (as I see it) wrong and worthless. In view of this delay you owe me a favour! I'm going to request two. Please feel free, now, to file this letter to your waste paper basket at any stage you feel fit. I will not anticipate a reply.

" .. tant pis pour les fleurs!" implies that I have swept aside large chunks of known fact to suit my own ideas. Not so! And to suggest otherwise is a clear indication that the referee's emotional rejection of it has exceeded his comprehension of what I have said. He has misinterpretated the presentation. I have only called into question the perception of the facts not their validity. I point out that two important points are missing from current perception that, once appreciated, put everything else in context. The first that every individual cell has the ability to decide and communicate that it is sick (surely a safe bet) and the second that phagocytes are designed to recognise this sickness and do so by noting the absence of healthy self identity (again a pretty safe bet). Current immunological philosophy does not incorporate either of these as "raisons d'etre" in the immune system.

I know that this concept belongs either on the dung heap or it is a critically important perceptual shift. Its very nature dictates that there is no middle ground. I think you all need to be sure I'm a crank before the concept is finally dismissed.

What's wrong with "fervid generalisations" if they are coming up with answers that the current dissective plod will take months or even years to stumble across. The biological and medical community are renounced for disliking broad hypothesis. Nature ran a whole viewpoint article to point out this lack of adventure. Can you be absolutely certain that this is not another instance where conventional perception has been "stick in the mud"? Furthermore, the history of important conceptual shifts suggests they often come from unexpected sources and have often been dismissed at the beginning. I'm sorry that the referee has been offended by its inflammatory style but the points remain. We are missing the point and realising this carries the promise of very important advances in the understanding of immunology, metazoan evolution and embryology. No point in being annoyed if it turns out to be true.

Alright! I accept that the probability is that this will prove to be junk. But it is getting very predictive and I quote two recent instances of this later. The second of these predictions concerns embryology and I have highlighted it so that you can, if you wish, go straight to that section before reading anything else. Please note that I'm a lone "researcher" with no access to experts other than through submission to journals: I don't expect that I have written a "publication ready" article but I have no doubt that I am sitting on an idea of critical importance to all biologists and doctors.

Yours

19930309_critic

MY RESPONSE TO THE REFEREE's COMMENTS *(I am not sure which response was posted – this or the next -19930315_critic)*

It seems to me that this referee has been offended by the essay from the opening paragraphs. I cannot, though, see the evidence that he has properly understood what I am saying and he has certainly misinterpreted it when he implies that I am am throwing aside vast tracts of accepted knowledge to suit my own theory. He is far and away more guilty of prejudice in his reading than I am of the alleged disregard for accepted knowledge. There are a number of assumptions that I see as misinterpretations.

"The author feels that immunologists are barking up the wrong tree, with all their emphasis on lymphocytes, receptors, major histocompatibility antigens and so on. Or so he says in no uncertain terms at the beginning of his essay." First, I have not said this. My words were chosen very carefully. It is self(cell)/non-self(cell) discrimination that is not carried out by lymphocytes. I agree that this is hard to swallow when we have been brought up on the assumption that self(epitope)/non-self(epitope) discrimination is the basis of self/non-self discrimination and that convention dictates that this is carried out either by lymphocytes or antibodies. Here is a critical point! Unless you have fully understood the premise that zygote derived cells choose to communicate only with healthy self cells, you will not comprehend the rest. The result of this principle is that there is a tendency for lymphocytes to observe tolerance to self antigens (epitopes) and to encourage aggression (inflammation in fact) at the site of non-self epitopes. This was fully explained in the section "ANAMNESTIC IMMUNITY". The referee has not, apparently, grasped this point or the significance that it is fully compatible with current knowledge.

I do not dismiss any of the known facts about " lymphocyte function, receptors, major histocompatibility antigens and so on". I have simply said that the lymphocyte's principal role is to remember the inflammatory or non-inflammatory context in which it first encountered its respective epitope and became committed. On any subsequent encounter, phagocytic (inflammatory) cells are encouraged to accumulate in a pre-activated state to assess cells on the basis of their healthy self status. It's subtle but VERY important. Tc cells are in a half way house, able to kill directly but passively dependant on phagocytic cell signals to tell them what cells have unhealthy self status before an aggressive activation can take place. Prior encounters with epitopes met in a healthy self context ensure that (in general) Tc and Th cells with receptors for self epitopes are disabled and so unavailable for use to accelerate the inflammatory process.

"Surely, he argues, we should concentrate on other criteria by which health and disease are recorded in animal tissues." With a sarcastic air, this sentence dismisses a very important point. I could point you to a large number of articles that emphasise the ability of individual cells to monitor their internal function. Once we credit individual cells with the ability to monitor their own health status, the subsequent recruitment of appropriate T-cell receptors to aggression or tolerance will follow on the basis of this cell's assessment of its own healthy-self status (a combination of health and correct location). I'm sorry! But to dismiss this point as not being common sense is a foolhardy step: particularly as embryology is replete with examples of cells that decide they are unwanted and proceed to self destruct.

"Epithelia". The referee has managed to mention "epithelia" twice in a paragraph. I have written "epithelial" once and then not even within the general text or in remotely the same context (table 8). I can't even guess where this presumption has its origin. However, he is right that I have made very sparse explanation of where I have conjured up the concept of junctional communication as an indication of health and disease. This was clearly an oversight, brought about because I have had no one before to point out the omission. I have appended some reprint excerpts that qualify this.

".. he urges that signals involved in the formation and failure of (epithelial again!) connectivity are older in phylogeny than components of the immune system." Well! He's certainly showing he's missed a critical concept here. What could be older than CAMs and cell communication? Cell sorting in both sponges (frequently quoted as a primordial representative of self/non-self discrimination) and mammalian embryos is led by CAMs and there is mounting evidence that they lead on to GJ formation. Surely, he is out on a limb here if he doesn't acknowledge that the immune system evolved from an N-CAM like gene? And as CAMS lead on directly to GJ insertion, doesn't that make you pause and wonder if IgSF CAMs have Ig regions that also lead on to membrane hole formation? (See below).

Perhaps the way to progress these ideas is to lay down a challenge. I can set out the bare bones of the hypothesis and, if anyone can prove it to be mortally flawed, then I will capitulate and apologise for my amateur intrusion. However, I contend that the evolutionary gradualism that this hypothesis suggests far supercedes anything that has been previously suggested. And it points clearly to an overall appreciation of the process that is otherwise lacking at the moment. I shall not include that here but would, of course, willingly supply it.

PREDICTION

You are an embryologist (a factor is choosing your journal). You should appreciate the importance of its predictive value in one of the next points. There are two important predictions that literature searches have subsequently supported.

The first was the the idea that Tc function evolved as an inversion of Tnk function. This arose out of the necessity (in the hypothesis) for a cell that recognised self on the basis of specific Mhc Class I identity. This prediction was made in January 1992. Veersteeg's and other articles to support it appeared from July 1992.

The second is embryological. This has only recently (2-3 weeks) fully formed in my mind. There are two major CAMs in development

(1) The cadherins that seem to have great importance in cell sorting. (2) The immunoglobulin superfamily CAMs (like N-CAM). These don't appear to have the same importance in sorting.

There are also two sorts of junction in embryos. First, junctions at compartment borders that display electrical communication but don't allow the free passage of Lucifer yellow (a molecule a little larger than retinoic acid). The second are the junctions within a compartment that allow the "transparent" passage of Lucifer yellow.

I have proposed that the construction mechanism for gap junctions and for complement membrane attack complexes have originated from the same primordial genes (and I'm becoming convinced GJs were first). You need to appreciate that the C9 molecule that forms MACs does not need the complement cascade to construct MACs. However, without it they form much more slowly and are smaller than the complement MACs. More critical, though, is the purpose of the C4/C3 cascade mechanism. It is designed to start MAC construction at the site of 3 or 4 closely situated and activated immunoglobulin constant region genes and then spawn hundreds of closely packed MAC constuction sites. Extrapolating this to the Ig (constant region) like motifs of IgSF CAMs leads to the prediction that high density, Lucifer yellow "transparent" junctions are formed only where N-CAM (or another IgSF CAM) is present.

Armed with this prediction, last night I pulled out all my articles on CAMs and carefully read through them again. Although I have quoted Keane et al's paper in my manuscript, I had not previously appreciated the importance or implications of their study beyond the fact that CAMs lead on to GJs. The findings reported in this paper are fully consistent with this prediction.

It leads on to another predictiion. Note that homoeo-domains are found and probably have a function in unicellular organisms. In the fertilised egg and particularly the multinucleated drosophila egg they define form and function within the cytoplasm so that when compartments form, their fates are already defined. The nature of embryonic compartments and their borders are such that this suggests that (a) electrical continuity through the embryo gives it a sense of "self" (not shared by the trophoblast) and (b) blocks of cells within a compartment are deliberately joined by morphogen "transparent" junctions to permit homoeo genes to map out the morphology of each compartment as if it were a single "super cell".

19930315_critic

TWO FAVOURS!

The first favour I request is this. May I see the full transcript of the criticism(s)? You implied that you might have more than one. At least I can then address the points were I to submit a revision elsewhere.

The second, I suspect, you may have a strict policy to refuse. Can you consider my views on the criticism? Let me state now that authorship is not my prime goal though I would be dishonest if I didn't admit it appeals. I can forego it if that what it takes to make someone listen. I am a solitary worker who has been constantly interested in this subject for 19 years. I have no one to discuss this with. To reach those who might understand it, my only recourse is to submit it to a journal.

MY RESPONSE TO THE REFEREE's COMMENTS

This referee appears to have been offended by the essay from the opening paragraphs. I cannot, though, see the evidence that he has properly comprehended what I am saying and he has certainly misinterpreted it when he implies that I am am throwing aside vast tracts of accepted knowledge to suit my own theory. He is more guilty of prejudice in his reading than I am of the alleged disregard for accepted knowledge. There are a number of assumptions that I see as misinterpretations.

"The author feels that immunologists are barking up the wrong tree, with all their emphasis on lymphocytes, receptors, major histocompatibility antigens and so on. Or so he says in no uncertain terms at the beginning of his essay." First, I have not said this. My words were chosen very carefully. It is self(cell)/non-self(cell) discrimination that is not carried out by lymphocytes. I agree that this is hard to swallow when we have been brought up on the assumption that self(epitope)/non-self(epitope) discrimination is the basis of self/non-self discrimination and that convention dictates that this is carried out either by lymphocytes or antibodies. Here is a critical point! Unless you have fully understood the premise that zygote derived cells choose to communicate only with healthy self cells, you will not comprehend the rest. In practice, this principle leads to a tendency for lymphocytes to observe tolerance to self antigens (epitopes) and to encourage aggressive inflammation at the site of non-self epitopes. This amplification of the inflammatory response can so extreme that we had, for a long time, come to regard lymphocytes as the prime executors.

This was explained in the section "ANAMNESTIC IMMUNITY". The referee has not, apparently, grasped the significance that this is fully compatible with current knowledge.

I do not dismiss any of the known facts about " lymphocyte function, receptors, major histocompatibility antigens and so on". I have simply said that the lymphocyte's principal role is to remember the inflammatory or non-inflammatory context in which it first encountered its respective epitope and became committed. On any subsequent encounter, phagocytic/inflammatory cells are encouraged to accumulate in a pre-activated state to assess cells on the basis of their healthy self status. It's subtle but VERY important. Tc cells are in a half way house, able to kill cells directly but passively dependant on phagocytic cell signals and the sick cell itself to tell them what is an unhealthy self cell before an aggressive activation can take place. Prior encounters with epitopes met in a healthy self context ensure, in general, that Tc and Th cells with receptors for self epitopes are disabled and so unavailable for use to accelerate the inflammatory process.

"Surely, he argues, we should concentrate on other criteria by which health and disease are recorded in animal tissues." To me this sentence has a sarcastic air and flippantly dismisses a critical concept. It is easy to find large numbers of articles that emphasise the ability of individual cells to monitor their internal function. Once we credit them with the ability to monitor their own health, the subsequent recruitment of appropriate T-cell receptors to aggression or tolerance will follow on the basis of this cell's assessment of its own healthy-self status (a combination of health and correct location). I'm sorry! But to dismiss this point as not being common sense I see as a foolhardy step: particularly as embryology is replete with examples of cells that decide they are unwanted and proceed to self destruct.

"Epithelia". The referee has managed to mention "epithelia" twice in a paragraph. I have written "epithelial" once and then not even within the general text or in remotely the same context (table 8). I'm not sure where this presumption has its origin. However, he is right that I have made very sparse explanation of where I have conjured up the concept of junctional communication as an indication of health and disease. This was clearly an oversight and the ommission reflects the fact that it has not been through peer review. I have appended some reprint excerpts that qualify this and it also needs pointing out that the sequence of events in apoptosis (separation and rounding up) are entirely compatible with this.

".. he urges that signals involved in the formation and failure of (epithelial again!) connectivity are older in phylogeny than components of the immune system." Well! I think this shows that he's missed a critical concept here. What could be older than CAMs and cell communication? Cell sorting in both sponges (frequently quoted as a primordial representative of self/non-self discrimination) and mammalian embryos is led by CAMs and there is mounting evidence that they lead on to GJ formation. Surely, he is out on a limb here if he doesn't acknowledge that the immune system evolved from an N-CAM like gene? And as CAMS lead on directly to GJ insertion, doesn't that make you pause and wonder if the Ig like regions of IgSF CAMs are also designed to amplify membrane hole formation? (See below).

A CHALLENGE

Perhaps the way to progress these ideas is to lay down a challenge. I can set out the bare bones of the hypothesis and, if anyone can prove it to be mortally flawed, then I will capitulate and apologise for my amateur intrusion. However, I contend that the evolutionary gradualism that this hypothesis suggests far supercedes anything that has been previously suggested. And it points clearly to an overall appreciation of the process that is otherwise lacking at the moment. I shall not include that here but would, of course, willingly supply it.

PREDICTION

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I have proposed that the construction mechanism for gap junctions and for complement membrane attack complexes have originated from the same primordial genes (and I'm becoming convinced GJs were first). You need to appreciate that the C9 molecule that forms MACs does not need the complement cascade to construct them. However, without it they form much more slowly and are smaller than C3 dependant MACs. Most critical is the purpose of the C4/C3 cascade mechanism. It is designed to start MAC construction at the site of 3 or 4 closely situated and activated immunoglobulin constant region genes and then spawn

hundreds of closely packed MAC constuction sites. Extrapolating this to the Ig (constant region) like motifs of IgSF CAMs leads to the prediction that high density, Lucifer yellow "transparent" junctions are formed only where N-CAM (or another IgSF CAM) is present.

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This leads on to another predictiion. Note that homoeo-domains are found and probably have a function in unicellular organisms. In the fertilised egg and particularly the multinucleated drosophila egg they define form and function within the cytoplasm so that when compartments form, their fates are already partly defined. The nature of embryonic compartments and their borders suggests that:- (a) electrical continuity through the embryo gives it a sense of "self" (not shared by the trophoblast) and (b) blocks of cells within a compartment are deliberately joined by morphogen "transparent" junctions to permit homoeo genes to map out the morphology of each compartment as if it were a single "super cell".

19930711_y

Dr Y

11th July 1993

Dear Dr Y,

You will probably find that this article is "inventive" but I wonder if the concept has some points of merit. It is hard to find someone willing to explore these ideas so I have decided to target key people who would be in a good position to discern these merits if they exist. I have simultaneously submitted it to "Immunology and Cell Biology" but I suspect the chances of it being approved for publication are slim.

Please do not feel obliged to reply.

Yours

cc Dr E

Dr L

19930711_e

11th July 1993

Dear Dr E

You will probably find that this article is "inventive" but I wonder if the concept has some points of merit. It is hard to find someone willing to explore these ideas so I have decided to target key people who would be in a good position to discern these merits if they exist. I have simultaneously submitted it to "Immunology and Cell Biology" but I suspect the chances of it being approved for publication are slim.

Please do not feel obliged to reply.

Yours

cc Dr Y

Dr L

19930711_l

11th July 1993

Dear Dr L,

You will probably find that this article is "inventive" but I wonder if the concept has some points of merit. It is hard to find someone willing to explore these ideas so I have decided to target key people who would be in a good position to discern these merits if they exist. I have simultaneously submitted it to "Journal_X " but I suspect the chances of it being approved for publication are slim.

Please do not feel obliged to reply.

Yours

cc Dr E

Dr Y

19930711_journal

Editor 11th July 1993

Dear Dr P,

I wonder if there are concepts in this hypothesis that are worth sharing. Will you consider it for possible publication in this or an amended form?

This version is much shrunken and therefore skips across a lot of detail. I have enclosed drafts of two (earlier) articles simply to emphasise that the ideas introduced briefly in Morphostasis and Immunity have been thought through in some detail. This is particularly pertinent to Clinical Morphostasis that contains an appropriate bibliography. It was the clinical hypothesis that led on to Molecular Morphostasis. This followed as it then seemed the logical conclusion.

Yours

19930718_lymphocyte_commitment

Xenix file:
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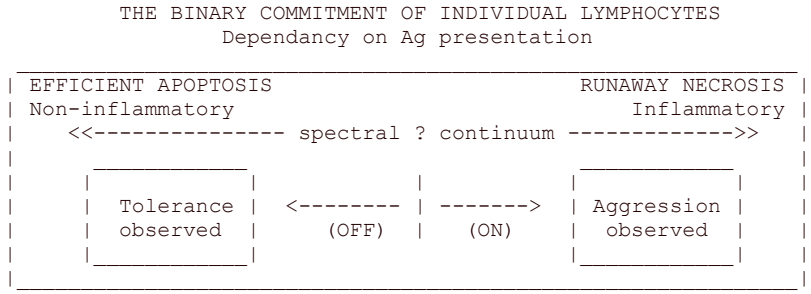

19930718_journal

Dr P
Editor

18th July 1993

Dear Dr P,

Forgive me for writing so soon after submitting the previous documents.

These minor alterations make greater sense of the suggested decision process between tolerance and aggression. The altered text is underlined.

I consider this change in perception sufficiently significant for me to bring it to your attention.

Yours

19930721_journal

Dr P
Editor

25th July 1993

Dear Dr P,

Forgive me for writing so soon after submitting the previous documents but I consider the points raised here are important.

I do not think that I had fully appreciated the significance of how lymphocytes could become committed to aggression or suppression. To correct this I have ammended the text, as underlined, on the original pages 1 and 12. These points may help to make it clearer.

Yours

19930819_la

The Editor (*this was **not** a submission*)
Journal

28th August 1993

Dear Dr X,

Re MORPHOSTASIS & IMMUNITY

This concept has been evolving since 1975. At least it has kept me entertained! I have sent earlier versions to other journals that publish hypotheses but these were rejected.

It will probably seem to be 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 concept. I cannot believe it is all nonsense but I accept that my approach is cavalier.

I am isolated from other people involved in this field so I have had no opportunity to argue the case. What I have written here is necessarily synoptic. There is a lot of work that has led to this conclusion and it mainly started in a clinical approach. I have not enclosed that here but it supports the premise.

I am more interested in infecting someone else with this idea than I am of having it published. I would be happy to discuss, recompose, shorten or otherwise alter this presentation.

Yours

19930819_la

The Editor
Journal

31st August 1993

Dear Dr X,

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 that 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 that 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

19930907_ec

7th September 1993

Dear Professor C,

FIRST - please don't feel obliged to reply.

I have sent this simply because you seemed to express an interest last time and there are changes of some import. It is not submitted for publication. It's here simply to see if I can "infect" you with the idea.

I have touted this idea around several journals since I last wrote to you. The Journal of Theoretical Biology kept it 7 months then rejected it so it was out of circulation a long time. It certainly seems to be "condemned"! I may be wrong, but I got the impression you were not so sure. Hence, here it is again with what I regard as advances in the concept. It's still cavalier and, I'm sure, contains presumptions that will later need modification. But then, that is the way of hypothesis.

One interesting point is that the concept predicted that IgSF CAMs were probably designed to spawn GJs rather like Ig constant region genes and the classical complement pathway spawn MACs. Armed with this, I set out to see if I could find the evidence. The article quoted does support this, suggesting the deduction was justified.

Yours

19931018_ec

30th October 1993

Dear Professor C,

Please don't feel obliged to reply.

Perhaps I'm just a mad crackpot!! But I'm convinced this is not only important but largely right. I've touted it around 4 more journals now. No one seems to appreciate it. I wouldn't be so concerned if it wasn't so clear to me that it is producing increasingly clear support for the earlier assumptions. Either I'm a crackpot or "them out there" are closed minds to an essential paradigm.

I'm afraid you are subject to my missives because I felt that you saw some meat in the idea the first time round.

Throw it straight in the bin if you like! I feel I've got to do something to get the message through to SOMEONE. Meanwhile, I'll try another journal!

Yours sincerely,

Jamie CUNLIFFE

19931106_n

Editor-in-Chief immunology journal (*this was **not** a submission*)

6th November 1993

Dear Dr N,

Re: MORPHOSTASIS and IMMUNITY

I'm at a loss to know what to do with this hypothesis. I think it contains important concepts that everyone should appreciate. So far, I haven't met with much accolade!

Submitting it for publication does not seem to be meeting with much success. One journal kept an earlier version for 7 months before deciding to reject it. This sort of delay is not sensible if it is important. I am now sure that this must seem pretty alien to the conventional immunologist. It reaches conclusions that are probably anathema to people in the field. For instance:

a) Tc and Th cells are not involved in assessing "selfness".

b) Self/non-self discrimination is established by adjacent cells and/or phagocytes (and Tnk cells too).

c) Auto-rejection (loosely the same as auto-immunity) is not avoided by single T-cells , any more than is tolerance of foreign epitopes even though this appears to be what is happening in a large population of T-cells in a mature animal.

Nevertheless, if you were to read carefully and understand what is written here, before dismissing it out of hand, I think you would have to conclude that the concept, at the very least, could work within the framework of what is already known. I think that current perception needs turning on its head.

Now, either I'm an interfering crank (who should leave all this to experts) OR this is something important. OK, it is cavalier in approach and I may be making a number of specific assumptions that will need to be revised. But, that's the way of hypothetical advance. And, anyway, the concept is broadly right. Fresh observations wouldn't hang so well onto the concept if it was all nonsense. It's worth noting these points:

a) The evolving concept predicted the inversion of Tc cell function and that it would probably be fulfilled by Tnk cells.

b) The probability that elective suicide is used by metazoans evolved as a concept before I searched for and found supportive evidence (plant hyperreactivity and apoptosis). c) The concept predicted that N-CAM like ligands should encourage the formation of high permeability gap junctions. The prediction led to a literature search for supportive evidence.

d) The clinical consequencees section, that is expanded in a separate section, Clinical Morphostasis, was largely complete before the concepts in the first part of "Morphostasis and Immunity".

e) It was an interest in the neurology of Behçet's Syndrome that led to the subsequent articles. This article is enclosed to demonstrate that it is is thought through in some detail. It has no list of references for I simply cannot find time to progess it. It is largely unaltered since 1987. The critical concept in here is the expansion of overlapping components and their simultaneous increasing severity.

So, here I have a concept that no-one so far appears to appreciate. What do I do with it? You and your editorial panel are probably some of the best placed professionals to consider whether there is anything in these ideas that other people need to understand. I don't suppose you often receive articles like this, simply asking "Where do I go now?" but then I am isolated from other people who are in a position to be able to criticise it effectively.

Yours

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Dr N

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19931125_e

Dr E

25th November 1993

Dear Dr E,

Re: "MORPHOSTAIS and IMMUNITY"

It is a long time since I sent you the article "Morphostasis and Immunity" and it has evolved considerably since then. If you do find anything of interest in it then this version is more refined.

One thing that needs adding is the possibility that aggressive Tc and TH1 responses may be precipitated, not only by lytic cell death but, by electrical/metabolic asynchronisation (ie, when a phagocyte notices the inspected cell or organism is not in synchrony with adjacent cells).

There is a great deal more detail behind the "Clinical Consequences" section than is provided here. An interest in the neurological complications of Behçet's Syndrome started my interest in this whole subject. It extended into the sero-negative arthritides, cancer and the mechanisms of (dedicated pathogen) infection. "Morphostasis and Immunity" is the culmination of these interests.