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letters to the editor

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Dear Dr. Orosz:

I read your beautifully written Hume lecture (Orosz CG. Immunomythology of transplantation. *Graft* 1: 175-180,1998). There is very little in it that I would want to debate. Although the reductionist approach has served science well since the time of Francis Bacon, you have brilliantly identified its weaknesses. I was especially taken by your sections on phylogenics and scaffolding.

What is your "master switch". Zinkernagel and I defined this in terms of antigen migration and localization (reprint enclosed), rather than by some discrete effector mechanism. Except for emphasis, however, our position (derived exclusively from in vivo observations) and yours make a remarkable fit.

Although we have accepted parasitic microorganisms as the evolutionary drive for development of the immune system, you were wise to imply other possibilities. Having gained the impression somewhere that the pathologist, Metchnikoff, (also an embryologist) suspected a develop-mental role, I tried a year or so ago to obtain confirmation from Metchnikoff's biographer, Fred Tauber (Metchnikoff and the Origins of Immunology: from metaphor to theory, 1991 Oxford University Press); Tauber did not think so.

However, about 30 years ago, Grobstein summarized evidence of mesenchymal -> epithelial signaling as an essential control in the shaping of tissues and organs (Grobstein C: Mechanisms of Organogenetic Tissue Interaction. *National Cancer Institute Monograph* 26:279-299, 1967). Unmistakable molecular evidence of this has come from the German laboratories of Birchmeier (see Sonnenbert et al: Scatter factor/ hepatocyte growth factor and its receptor, the c-met tyrosine kinase, can mediate a signal exchange between mesenchymal and epithelia during mouse development. *J Cell Biology* 123:223-235, 1993).

It is no surprise that a number of "homeobox" (regulatory) genes have been described in the last 3 or 4 years (e.g. see B. Hentsch et al: H1x homeobox gene is essential for an inductive tissue interaction that drives expansion of embryonic liver and gut. *Genes & Development* 10:70-79, 1995) that appear to regulate the organogenesis of upper limbs, lower limbs, thoracic organs and the hepato-intestinal complex. If the anlage of the immune system is involved, this might be called "non-immunologic" function of the immune system components that you hinted at. It could be a semantic distinction.

Liver regeneration, following partial hepatic resection or transplantation of "small for recipient size" allografts is a good example of recapitulation in adult life of organogenesis and organ shaping. I began a search more than 35 years ago for the molecules controlling this and ended up with a family of eight hepatotrophic and two antihepatotrophic factors, all of which also affect immune function. All, including the product of the ALR gene that we discovered, profoundly influence regeneration, and several are now formally classified as cytokines (e.g. hepatocyte growth factor).

Along potentially related lines, Rinkevich (an oceanographer from Haifa) who frequently collaborates with Irving Weissman [Stanford], has been studying a pro-vertebrate (tunicate) which in ontogeny goes through an abortive notochord phase. The tunicate has a single MHC haplotype (called "a fusibility/histocompatibility locus") that controls the fusion and establishment of a common circulation (analogous to Owen's Freemartin cattle) that is necessary for the formation of the jellyfish colonies that are necessary for their unpleasant community survival (they plague sea-side beaches).

Rather than being defensive against parasites, their primitive immune system (with its adaptive qualities) seems geared more to ensure species homogeneity. However, even if the immune system served quite different (from anti-parasitic) purposes at the beginning of evolution, failure to evolve further to meet the defensive needs would have doomed the increasingly complex multicellular organisms to prompt extinction. Thus, these roles (i.e. growth and antimicroorganism) are not mutually exclusive.

In any event, your paper in *Graft* is going to spark much discussion. I will be interested to see where you go from here. You might want to peruse the book by John Horgan which attempts to debunk the chaos and complexity concepts ("The End of Science", John Horgan, Broadway Books 1997). In contrast, "The Frontiers of Complexity" by Peter Coveney and Roger Highfield is sterling defense of complexity ideas (Ballantine Books, Random House, 1995). The recent book by former editor in chief of *Nature* (John Maddox, Random House, 1996) is another example. A review of the Maddox book is in the January 10, 1999 *New York Times*.

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Dear Dr. Starzl:

Thank you for your letter of January 7th, and for the kind compliments that it contained regarding the content of the Hume Lecture. I "came out of the closet" with the publication of the lecture in *Graft*. I no longer live a double life as I continue to develop my nonconventional views on the immunobiology of transplantation. I feel both liberated and exhilarated by the intellectual risk of stepping beyond the limits of conventional thought. While this is an experience that you have had many times during your career , it is all quite new to me.

The breadth of the comments in your scholarly letter will require considerable thought on my part, but I would like to address one or two issues that your letter has already raised in my mind. You asked about a "master switch." I cannot answer this question without defining the context of the biologic process that I envision. I have followed the conceptual developments that you have provided in papers co-authored with Rolf Zinkernagel, and I believe that there exists a fundamental difference between our conceptualizations and my own. Your views are decidedly T cell-centric, and reminiscent of the Gershonian orchestra in which all members of both the orchestra and the audience are focused intently on the venerable conductor, the T cell. The curtain rises in the presence of antigen, the performance is over when the antigen has been eradicated, and everyone goes home to await another performance.

In contrast, I prefer a more Copernican view in which the T cell at an inflammatory site is just one of many satellites orbiting around a local sun, the macrophage. The macrophage is but a small part of the immunologic galaxy (including lymphoid organs and other immune response sites) which, in turn, is imbedded in the cosmos of physiology. In this cosmology, tissue inflammation, immunity and repair are phases of a single, seamless process managed by macrophages. These macrophages are adept at using T cells, B cells (Ig), NK cells (and probably many other cell types, including endothelial cells and parenchymal cells), all operating in parallel, to generate accurate information regarding the nature of the evolving problem posed to the immune system at a site of tissue damage or foreign molecule expression. The master switches are the biochemical mechanisms that promote the transitions from one phase to the next during the response process. In this context, antigen engagement by T cells is just another piece of information processed by macrophages during the decision-making process. Thus, TGFβ, for example, acts as a master switch to proceed from one complex array of processes to another, i.e., from immunity to tissue repair. How these switches are thrown is far from clear.

More importantly, I believe that these phenomena constitute only a small part of immune function. In general, I believe that the immune system is a cognitive device that constantly monitors the state of the internal environment. Its job is to make decisions on how best to avoid physiologic disruption and maintain homeostasis while interacting intimately with the external environment. Many (maybe most) times, the decision favors incorporation of foreign molecules into the physiologic meshwork, rather than their eradication. (Simple ignorance of foreign molecules is probably too risky to be a viable choice for the immune system). In places like the gut and respiratory tract, incorporation is probably favored, unless the foreign molecules are obviously disruptive (cause cell malfunction or tissue damage). Indeed, engagement of the eradication mechanisms is inherently dangerous, since these mechanisms also disrupt local physiology. In contrast, when foreign antigens are placed within the skin, they usually evoke the opposite immune decision, eradication. Our primary challenge is to determine how these different immunologic decisions are made. In this context, the ever more sophisticated examination of mechanisms by which antigen is eradicated pales in importance. Nevertheless, if we choose to study the eradication mechanisms, we should not ignore the counterbalancing acceptance mechanisms, nor should we treat them as if they were separate immunologic entities. This series of considerations reflects the point that you made in your letter about tunicates, which use their "MHC" for acceptance responses in the development of jellyfish colonies.

I appreciate the suggestions in your last paragraph regarding suggested readings. Indeed, I have read Horgan's book "The End of Science." In general, I accept his premise as plausible. The role of science in our culture, as well as the way that it is practiced, are clearly changing ("continue to evolve" would be a better way to put it). I found "The Frontiers of Complexity" to be a reasonable introductory work about the many facets of complexity theory, although not one of the best. The best book that I have ever read on the topic was the least technical, Kevin Kelly's "Out of Control." It is fun to read, fascinating and instructional. I have taken the liberty of enclosing a copy with this letter. I hope that you enjoy it as much as I have. I've already reread it twice. I especially like the first 7 chapters which discuss the principles of networked systems, and chapter 14, which describes the fascinating concept of the Borgian library. If you have already read the book, please feel free to pass it on to one of your fellow theoreticians.

Finally, I would like to leave you with one interesting idea. I mentioned earlier that we should learn how the immune system makes decisions. I suggest that important clues may lie in the realm of game theory, a subdiscipline of complexity theory. In the late 1940s, a computer program was developed that allowed a computer to learn how to win at checkers. The computer was not preprogrammed with all possible moves for all possible situations. Rather, it was provided with a few rules that allowed it to evaluate the current (and unpredictable) configuration of the board at each step of play, and choose the most effective move. The principles on which this program was developed are startlingly insightful, and seem quite applicable to the immune system. If you are interested in this ideal, you will find game theory discussed in a book by John Holland called "Emergence: From Chaos to Order" published in 1998 by Addison-Wesley Publishing Company.

Thank you once again for your stimulating letter and your encouraging comments.

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asts hume lecture

May 14th 1998

The Immunomythology of Transplantation:

If you think that you know the answers, you probably don't appreciate the nature of the problem.

Charles G Orosz

I would like to thank the American Society of Transplant Surgeons, and especially its President, Dr. Ronald Ferguson, for the honor of delivering this year's Hume Lecture.

As most of you know, I am a Ph.D. and an Immunologist who adopted transplantation in the early 80's. My obsession with this discipline developed directly from a series of collaborative studies that I undertook with Ron Ferguson regarding the functional activities of cloned T cells when they were under the influence of a newly discovered drug, then called Cyclosporin A.

For perspective, I should tell you that I consider the field of Transplantation to be currently in its mid-to-late adolescence, recognizable as an unique entity but still somewhat immature. In this context, I consider the changes that I referred to a few minutes ago to be but a few of the growing pains that the discipline will experience on its road to full maturity. When I first joined the business, in the early 1980s, Transplantation was in its *early* adolescence. I'd already missed its childhood, the era of first and second generation pioneers whose names are now legendary in the field. I find it quite humbling to note that several of these pioneers, including Sir Peter Medawar, Robert Good, and Thomas Starzl, have given Hume Lectures before me. By the time I arrived on the Transplant scene, the core surgical and therapeutic techniques had already been established, primarily on the basis of clinical empiricism. The role of the transplant investigator was to provide a scientific foundation for these empiricisms. In those days, the primary obstructions to transplant success were infection and rejection, both considered to be immunologic problems. Thus, investigators turned to Immunology as the warehouse of information that would provide solutions to their problems, and, as we all know, Immunology has dominated transplant research ever since.

Interestingly, it is now clear in retrospect, that Immunology, despite its vast scope and current state of complexity, is also a field in its mid-to-late adolescence. In many ways, Immunology and Transplantation are growing up together. In my experience, teenagers can be quite outspoken regarding their opinions on many of life's thornier problems. I often find these opinions to be different, interesting, and sometimes even informative. It seems that teenagers have been endowed with most of the answers, despite the fact that they have yet to fully appreciate the true nature and complexity of the real problems. As you will see from the content of this talk, I believe that this is also true for the two adolescents currently under consideration, Transplanta-tion and Immunology.

In the old days

I like to think of the 20-25 year span that began in the early 80's as the period in transplant research during which I stood "my watch". Thus far on my watch, I have observed with great interest the continuing co-evolution of transplantation and immunology. Both have become much more sophisticated. Both have become more politicized, and both have become more entrepreneurial. Neither look at all like the disciplines I joined almost 20 years ago.

Life as a transplant immunologist was much simpler then. It had become pretty obvious that T cells caused graft rejection, and that they could be divided into three functional subsets: the now-famous CTL, which caused destruction of graft tissues, the equally famous HTL, which somehow assisted in the generation of CTL, and the poor, unfortunate suppressor T cell. While the fame of the CTL and HTL was spreading, suppressor T cells lingered near oblivion until only recently, when they have re-emerged, Phoenix-like, from studies on immunologic tolerance in several rodent models of disease.

In those days, the only known Interleukins were IL1 and IL2. Cyclosporine was a new drug without a reputation. The MLR dominated transplant research, and we relied heavily on it to provide most of the information required to understand the processes of acute rejection. We needed merely to tie up a few loose ends and to assemble this growing body of in vitro information into patterns of immune activity that could be validated in vivo, when and if we got around to it.

There was rampant optimism in the field. Specialty niches had been successfully carved out of both medicine and immunology. The intellectual land rush was on. We knew the key immunologic players, we knew how they worked, and we were learning how to control them. We were rapidly closing in on the answers. Interestingly, we had yet to learn about APCs and their subtypes, TH1 or TH2 cells, and multitudes of cytokines, chemokines and adhesion molecules. The molecular structures of the TcR and of MHC molecules were still unknown. There were no signal transduction pathways, no lymphocyte-endothelial interactions, no antigen processing and presentation pathways, no costimulator mechanisms. There was no molecular immunology, and gene therapy was Star Wars (itself a recent cinematic phenomenon).

In many ways, these were the halcyon days of transplantation. There were only a few immunosuppressive agents to worry about: immuran, prednisone and ALG. Research funding for transplant-related studies was generally easy to get, and huge research laboratories could be built and maintained solely on the basis of continuous NIH funding.

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Renal allografts dominated clinical transplant efforts, and success was measured in simple ways, usually as rates of one year graft survival. Tissue typing labs were loose and-free-wheeling, concerned with serologic identification of new MHC alleles, pre-transplant serologic testing, and whatever else interested them, all of which was financed by the government. Most importantly, there was a tangible esprit-de-corps that united all the clinical and research components of a Transplant Program around the welfare of the individual transplant patient.

Where do we go wrong?

So, what went wrong? Despite almost 50 years of intense clinical and basic research, we still cannot define simple mechanisms of acute allograft rejection. Similarly, the mechanisms of chronic allograft rejection remain highly conjectural, and only recently have they even been addressed by the transplant research community. Pharmacologically-induced allograft acceptance, (I decline to use the argumentative term "tolerance") has been achievable in rodents for more than 15 years, but no coherent mechanism of allograft acceptance has yet been proposed, let alone applied to clinical transplantation. Until recently, progress in experimental xenotransplantation was announced when graft survival was extended by a few minutes to a few hours, and now new mechanisms of rejection seem to appear as soon as the known mechanisms succumb to pharmacologic or genetic control. The question is: *Why have we been so spectacularly unsuccessful at defining the mechanisms of allograft rejection, especially after all this time and effort*? How have the answers to these seemingly simple questions managed to elude us so effectively for so long?

I propose to you that this is the direct result of the fact that we still do not appreciate the true nature of the biologic and medical problems that we face, nor do we understand the true nature of Immunology, the very tool that we rely on to attack these problems.

Pervasive complexity

I believe that one important reason for this lack of understanding is the sheer complexity of immune function as we now know it. In an effort to tie up a few loose ends, we have opened Pandora's box. The TcR turned out to be a multi-molecular complex endowed with the capacity to further cluster with numerous additional cell surface and cytoplasmic molecules. There is an ever-growing number, currently close to 600, of known MHC alleles, each endowed with an individualized peptide binding motif. There are 166 recognized CD molecules, ie., defined cell surface molecules associated with leukocyte differentiation. There are nearly 20 official Interleukins, each with multiple, sometimes- overlapping functions, and each with one or more differentially expressed receptors. There are numerous additional cytokines with immunologic importance, including at least a dozen chemokines and a dozen more tissue growth factors. Each of these also have one or more differentially expressed receptors. Finally, there are multitudes of intercellular adhesion molecules (at least 20 in the integrin family alone) that play major roles in leukocyte mobilization and activation.

To make matters worse, T cell function has become much more complicated. Helper cells can be cytotoxic, sometimes, and both helper and cytotoxic T cells produce cytokines that can help some immune responses and, on occasion, suppress others. $CD4^+$ T cells have been divided into Th0, Th1, Th2, and recently Tr1 functional subsets, with more subsets sure to be identified in both the $CD4^+$ and $CD8^+$ populations. Each of these different T cell subsets produce different arrays of lymphokines. The lymphokines, either individually or in various combinations, act to influence the phenotype and function of their companion leukocytes, and of the local parenchymal cells. In turn, leukocytes and parenchymal cells make differential arrays of chemokines, which, among other things, work in various combinations to induce distinctive patterns of adhesion molecule expression on one other. Chemokine activity is mediated by chemokine receptors, which are displayed, again in various combinations, by many different cell types, under the control, to some degree, of specific lymphokines. In turn, the pattern of lymphokine production by T cells is dependent on the encounter of specific cytokines and/or adhesion molecules during TcR engagement. It should come as no surprise that this vast tangle of known molecular and cellular interactions has not been assembled into a coherent mechanism that explains any of the basic forms of allograft rejection.

What has emerged from the years of study on the mechanisms of allograft rejection, and other immune pathologies in general, is not the anticipated understanding of specific pathologic mechanisms, but an increasing awe at the mind-boggling complexity of immune responses. This complexity is so profound and pervasive that it has become the general practice to actively ignore it. The scope of this complexity is just too broad for the human mind to grasp in its entirety. Thus, sanctioned oversimplification is practiced, just to permit coherent discussion of issues, and the formulation of practical experiments.

I propose that it is this sanctioned oversimplification of immune complexity that has subverted our attempts to understand any of the mechanisms of allograft rejection or allograft tolerance. I believe that complexity is an integral and formative element of the biologic system under study, and attempts to understand the system abstracted from its complexity are, in my opinion, doomed to continued failure. By analogy, I doubt that we could describe a misty waterfall, a raging blizzard or a brilliant rainbow, based solely on the even the most rigorous analyses of the physical structure of individual water molecules. These functions of water are phenomena that emerge only under a complex array of specific physical conditions. As scientists, we have trained ourselves to look down, and not up, to watch where we step, but not where we are going. In some ways, we have been seduced by our own scientific mythology. We have taught ourselves to believe that complex systems function as the sum of their parts, and that an understanding of a system can be achieved by the ever more careful analysis of its dissected components. But what if this premise is incorrect?

Paradigm shift

Why are these biologic systems so incredibly complex? Isn't such complexity error-prone? What, then, is the biologic value that preserves such complexity? Most importantly, how do we, as investigators, deal with the awesome complexity of these biologic systems?

I have been thinking about these questions for some time, and for the remainder of this talk, I would like to discuss some of the answers that I have arrived at. In general, I find these answers to be both exhilarating, because they stir the scientific spirit, and

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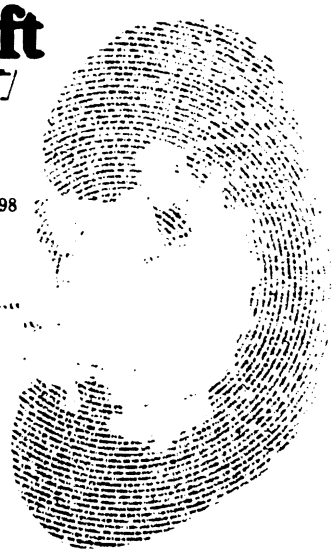

frightening, because they flaunt the well-entrenched and powerful scientific status quo. In short, we do as a few others have done before us when confronted with information that persistently refuses to conform with the accepted pattern of contemporary thought, we develop a new paradigm. And when the conceptual shift is severe enough, we undertake that most creative of human endeavors so elegantly discussed by Thomas Kuhn is his classic treatise, "*The Structure of Scientific Revolutions*". In his words, scientific revolutions occur when "we can no longer evade anomalies that subvert the existing tradition of scientific practice- then begin the extraordinary investigations that lead the profession, at last, to a new set of commitments, a new basis for the practice of science."

I believe that a growing appreciation for biologic complexity has led us to the brink of such a scientific revolution. Further, I believe that the opening events of this revolution, the first of Kuhn's "extraordinary investigations", have already been undertaken. These by a committed group of far-sighted and creative intellectuals working through the Santa Fe Institute to outline the primary features of what they call chaos or complexity theory. Over the last several years, they have begun to apply this theory to a diverse array of biologic, sociologic and economic systems. I will warn you that their viewpoints and arguments can be intellectually enchanting. I also warn you that accepting their arguments will catapult you immediately to the extreme boundaries of scientific thought and procedure, and will change forever the way you perceive the problems that you address in your discipline. Unfortunately, a discussion of the pioneers and of complexity theory, its fascinating conceptual development, is well beyond the scope of this talk. For this, I recommend several excellent books, including *Chaos*, by James Gleick , *The Web of Life*, by Fritjof Capra , and my personal favorite, *Out of Control* by Kevin Kelly .

Towards embracing complexity

What I would like to do with my remaining time, is to outline for you an application of complexity theory to transplant immunobiology. I offer this as a plausible alternative to the current working paradigm. As such, it does not contest any of the experimental data accumulated thus far, it merely provides an different perspective for interpretation of these data, ie., an alternative conceptual framework. In an effort to better understand the processes of chronic rejection and tolerance, I have been exploring network theory for the last two-to-three years, and have, in fact, included some discussion of network theory in a few recent review articles. I ask you to remember that this conceptualization is very much a work in progress. I ask you also to view these somewhat radical concepts with an open mind, and to consider how they might, if accurate, change your understanding of important issues in your trade, be it basic re-search, clinical research, or clinical practice in the field of transplantation.

To begin with, it is important to appreciate that the immune system is a special type of networked system. Since the days of Dick Gershon, immunologists have generally accepted the network concept, but few have peered beyond the simple network imagery, and considered the profound implications of a networked immune system. This special type of network has been referred to by Kapra as a complex, adaptive system, defined as an aggregate of diverse elements with extensive cross-connections that displays two special properties: it maintains coherence under change, and it is capable of adaptation and learning. I will discuss how the immune system exhibits each of these properties a little later.

First, I would like to explore complex, adaptive systems a little further. As good examples of complex adaptive systems, think of colonies of ants or bees. (To me, the immune system takes on a completely different character when I compare leukocytes to ants). The colony is a collection of autonomous members, each acting individually, insect-like, according to a set of internal rules, and to signals provided by the local environment. Each member is connected to each other in a peer network. This network functions effectively to perform tasks without central authority or control. Rather, actions are guided by a web-like, non-linear causality of peers influencing peers. In such a system, the individual elements have no recognized individuality, and although their behavior can be complex and unpredictable, all act in a coordinated fashion to maintain the critical functions of the colony. Such systems are fundamentally *inhuman*. They are careless about the needs or rights of the individuals, who are considered insignificant and expendable for the sake of the colony.

What are the advantages of such a system? There are several. The colony is highly *adaptable*, and although individuals may die or change as a result of new conditions, the colony persists. The colony is *evolvable*, since its adaptability permits the accumulation of change. The colony is *resilient*, since multitudes of individuals, operating redundantly and in parallel, can accommodate small failures of other individuals. Furthermore, the colony supports countless novel possibilities, hidden in the exponential combinations of variously linked individuals. These possibilities provide a reservoir of alternative responses in times of stress. There are, of course, some disadvantages. The colonies operate *inefficiently*, due to redundancy and lack of central control, and they are prone to *non-predictable* outcomes, known in the business as emergent behaviors. For example, we have already discussed some of the emergent behaviors of water. However, the worst disadvantage for the investigator is the fact that networked systems are basically *inscrutable*. Since causality is spread laterally throughout the network, both the triggering stimuli and the specific patterns of the responses are obscured. Does this sound at all familiar?

Systems thinking

Now we come to the core of the paradigm shift, the conflict between *analytic thinking* and *systems thinking*. Analytic thinking, the ever more sophisticated analyses of fundamental constituents, is the cornerstone of modern scientific thought and practice. Systems thinking is its complete antithesis. In systems thinking, the properties of the parts do not necessarily define the properties of the system. Indeed the properties of the system can be understood only in the context of its larger whole, ie., when complexity is intact. Clearly, this concept is foreign to classically-trained scientists, like myself. Interestingly, I find that the concept is not so foreign to

- **Shift from importance of elements to importance of organizational pattern**
  (configuration of relationships is characteristic of a particular system)
- **Patterns cannot be measured or weighed, they must be mapped**
- **System properties arise from the specific configuration of ordered relationships**
- **To appreciate biologic mechanism, need to identify patterns of elements, not just elements themselves**

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physicians, who eventually adopt systems thinking from long empirical experience with the complex adaptive systems represented by the physiologies of their own patients. Small wonder that, historically, there has been some degree of intellectual friction between physicians and scientists.

Let us consider, then, how systems thinking and complexity theory impact transplant-related research. How do these concepts apply to biologic webs, and especially to those biologic webs that operate in transplant recipients?

First, it is clear that the biologic responses to injury, ie., inflammation, immunity and tissue repair, are complex, integrated networks of interacting elements. Because they are networked, they can accommodate error or missing elements. In addition, they provide many alternative response options to a perturbation. This networked interaction design provides a much higher survival value than a linear interaction design, where error or interference at any link can disrupt the entire system. Nevertheless, it is interesting to note that virtually all of our current depictions of immune response pathways are displayed as maps of linear interactions.

If networks provide so many options, how, then are networks controlled? This is the core problem with complexity theory. In general, I believe that control of networked systems stems not from the manipulation of selected network elements, but from the shepherding of network response patterns. I submit that there are, in fact, several effective approaches to network control. These are best understood within the context of basic network principles, of which there are four that bear discussion. I will refer to these principles as Phylogenic Layering, Parallel Processing, Dynamic Engagement and Variable Connectivity.

Network regulating mechanisms

The first of these, *phylogenic layering*, is a basic tenant of developmental biology. In essence, new mechanisms are built on top of older, more primitive mechanisms. The primary advantage of this layered pattern is the accumulation of multiple default responses, each of which has a proven track record. In effect, the older mechanisms provide a scaffolding upon which the newer mechanisms can be assembled. Of importance is the fact that the assembly of the newer mechanisms can then be controlled by manipulation of these older scaffolding elements. I believe that this concept explains why George Kupiec-Weglinski can interfere with experimental acute rejection by treating allograft recipients with the CS1 peptide, an integrin-binding fragment of the pro-inflammatory, provisional matrix molecule, fibronectin. Also of importance, the scaffolded assembly system insures the programmed, sequential development of responses. This generates significant order out of apparent chaos. Because of scaffolding, large numbers of inflammatory response components can be stockpiled at inflammatory sites, each to be used only if and when appropriate. Finally, scaffolding permits so-called subsumption control, ie., a master switch that can alter entire response programs. For example, TGFβ generally acts to subvert immune responses, but to promote tissue remodeling responses. This phenomenon has recently gained much attention in several experimental models of immunologic tolerance and chronic allograft rejection.

The second network principle is *parallel processing*. In essence, the system employs all available response mechanisms simultaneously. The actual degree to which each mechanism participates is, of course, governed by a number of variables, including the nature, location and amount of antigen. It is also governed by the nature of the inflammatory factors that are either contributed to the stockpile, or removed, by any of the other response components. However, it is important to note that there is no central control of these responses as they are constructed de novo at a graft site, and that only the presence or absence of antigen influences the propagation of the various response processes. The redundancy of parallel processing makes it somewhat inefficient, but insures its effectiveness. I believe that parallel processing is one reason why IL2 or IFNγ knockout-mice retain the perplexing ability to mount effective graft rejection responses.

The third important network principle is *dynamic engagement*, ie., the continuous arrival and departure (or death) of response participants. In healthy individuals, the immune system has an almost inexhaustible supply of participants which it can throw at any task. This is why clonal deletion is difficult to achieve in adult animals. Graft-reactive lymphocytes are abundant and widely distributed in an allograft recipients, and only a fraction of these become active participants in a given rejection response. The rest remain in reserve at depots throughout the body. Even the most efficient deletion of antigen-activated T cells, unless quite prolonged and systemic, will not result in the clonal deletion of graft-reactive T cells. There are two major advantages to dynamic engagement, a) it allows the immune responses to be inherently self-limiting, and b) it allows the continuous monitoring by the immune system of antigen persistence or elimination. I suggest that dynamic engagement is the context in which most programmed cell death, apoptosis, actually occurs at inflammation sites. The alternative mechanism, leukocyte departure, is presumed to occur, but has been largely overlooked by immunologists. I am not aware of any reports on the length of time that individual lymphocytes actually spend at a site of inflammation. Further, the mechanisms by which lymphocyte might leave these sites are not even under consideration by investigators.

The final, and perhaps most interesting principle of network function is *variable connectivity*. It is intuitively obvious that too little connectivity between network members reduces network options, and too much connectivity locks up the system, so biologic networks must function at some intermediate level of connectivity. In fact, this offers a

Immune Network Principals

1) **Back-up responses**
   - *provides more primative, but effective default responses*
2) **Scaffold requirements (ex. provisional matrix)**
   - *response depends on presence of chaperone components*
3) **Programmed sequential developement**
   - *sequence alteration changes outcome*
   - *resource stockplies allowable*
4) **Subsumption mechanisms: override of earlier responses** - *(vs. TGFβ)*

1) **Panorama of responses governed by nature, location and amout of antigen**
2) **No central control of response pattern** *(presence of absence of antigen controls all)*
3) **Hierarchies of effectiveness and efficiency**
4) **Responses may operate independently, or some may cross regulate others**

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mechanism by which network function can be regulated, ie., by controlling of the degree to which the network's members are interconnected. In some ways, the exact nature of each connection is less important than the overall degree of connectivity among network members. This, again, is systems thinking, rather than analytic thinking.

All networks in complex adaptive systems strive for homeostasis. For each network member, this homeostatic identity is defined in reference to its neighbors. By increasing connectivity after a perturbation, the referencing of the neighbors is enhanced. We are all familiar with many of the immune network connectors. We know them as the pro-inflammatory cytokines, chemokines and adhesion molecules that show up during acute rejection. I suggest that network connectivity is the basic context in which the mind-boggling array of interacting immune and inflammatory mediators actually operate.

Too much connectivity is bad, since hyper connectivity could freeze the network into a state of non-responsiveness and block the re-establishment of homeostasis. I believe that there are a whole series of biologic disconnectors that act to counterbalance the connectors. If so, the maintenance of homeostasis must reflect a constant, dynamic rebalancing of connectors and disconnectors. I believe that we also know many of these immunologic disconnectors, although we rarely think of them as such. Among them are generalized disconnectors like steroids, TGFβ and IL10. There are also specific disconnectors like IL1 receptor antagonist. Ever wonder why there are shed ICAM-1 and VCAM-1 molecules, or shed MHC molecules? What about the multitudes of soluble cytokine receptors, chemokine receptors and growth factor receptors that appear during immune responses? In general, the immune system doesn't seem to like too many free connectors or disconnectors.

There are some interesting additional facets of network connectivity. For example, I view immunosuppressive drugs as artificial disconnectors of the immune network. Perhaps, we should carry this idea further, and learn to harness the natural mechanisms of connector/disconnector production. Because of network considerations, I suggest that we focus not on individual connectors or disconnectors, such as a specific lymphokine, but on the regulators of broad-scale genetic programs, such as the NF-kB system. Alternatively, we could selectively mobilize the natural disconnectors. Perhaps this is the fundamental principle behind the endothelial protective genes described over the last year or two by Christiane Ferran and Wayne Hancock.

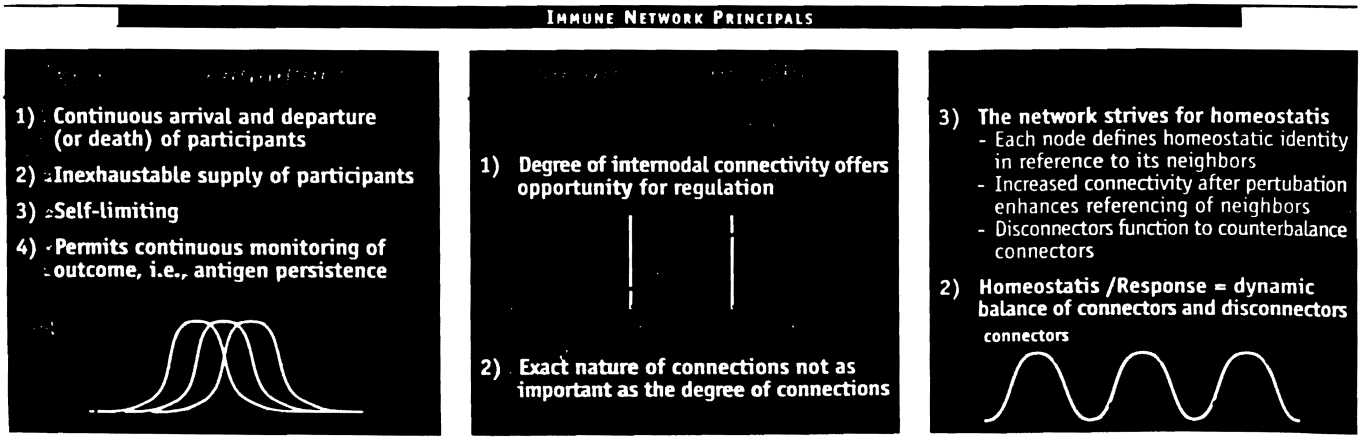
A really interesting application of network connectivity involves immune tolerance. What if the immune system exploits network hyper-connectivity for this purpose? If the immune system decides to accept a new chemical, it must weave it into the existing network fabric. What better way to do this, than to mobilize a paralyzing array of antigen-specific immune responses. Perhaps this is the basic principle behind the phenomenon of infectious tolerance that has been described over the last few years by Herman Waldman.

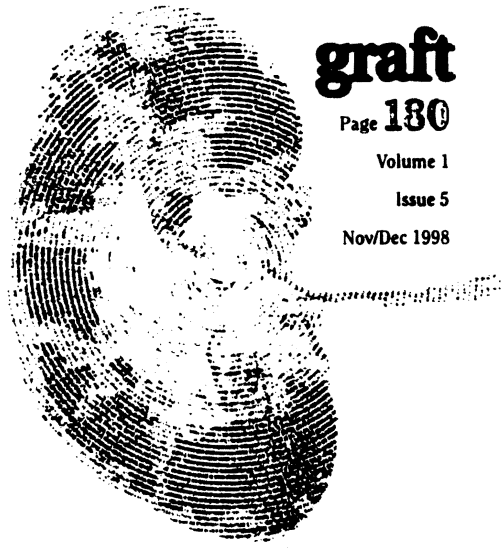
Finally, I suggest that there are both inter- and intra-network connections, ie., that the immune system is a network of networks imbedded within a network of networks. One of those broader networks is systemic physiology. We need to remember that immune responses are, by themselves, perturbations of homeostasis in the cardiovascular system, and that these perturbations have ripple effects that can influence the physiologic functions of tissues and organs. Perhaps this explains Nick Tilney's observations that transient tampering with organ perfusion eventually results in chronic rejection-like vascular remodeling, whether the organ is transplanted or not.

Transplant immunobiology revisited

Aside from these esoteric and somewhat philosophical considerations, network theory and systems thinking have immediate application to current, everyday transplant research. I suggest that an important role of the early pro-inflammatory response is to stockpile components for possible use during subsequent immune and repair responses. Indeed, these responses can proceed only using specific components that have been stockpiled in this manner. For genetic or pharmacologic reasons, the same components are not always stockpiled in different individuals, nor are the components necessarily used, even when they are available. Thus, is should come as no surprise that the time-honored analytic approach of cataloging all the components present at a given immune response site has been relatively uninformative, and probably should be discouraged, or at least de-emphasized. Because of networking, there is probably no single mechanism of acute rejection. Instead, there could be as many different mechanisms as there are pathways through the networks. This accounts for the common observation that the various inbred strains of mice can respond quite differently to contact with the same antigenic stimuli.

Just because the activities of the damage response networks are complex, doesn't mean that they are random, disordered or chaotic. Quite the contrary, the network functions as an ordered evolution of events, in which complexity merely provides options (I remind you





of the scaffold concept). Since these options are integral to the response process, *the process cannot be accurately studied when complexity is minimized*. This is systems thinking, and it reveals the central problem with in vitro studies. Most in vitro studies strive to minimizing experimental variables, an in so doing they also minimize response options. Not surprisingly, this results in repeatable outcomes. However, a similar outcome may never be observed in vivo, when the intact network is operating. The error lies in the assumption that any response that occurs in vitro will necessarily occur in vivo. In contrast, systems thinking suggests that in vitro studies should start with minimal complexity, to establish baseline cell behavior patterns, and then incrementally add new elements, eventually to reconstruct the actual in vivo conditions. The intent should be to see how the functions of the cells under test are altered by the new, more informative local environment. However, there must always be a reality check. There is no reliable substitute for the original, networked system. Here, more than ever, *in vivo veritas*.

My growing appreciation for the subtleties of networked immunity has made me comfortable a perspective on immune function that differs somewhat from that of my fellow immunologists. For example, I now believe that categorizing T cell subsets by their functional capacities, ie., TH1 vs TH2, is somewhat improper and misleading. I believe that the only reliable subcategories of T cells are CD4 vs. CD8. These markers, which define the type of MHC molecule that the TcR can work with, determine whether the T cell monitors the intracellular or the extracellular environment. Beyond that, I believe that T cell function is highly variable, and depends from moment to moment on the panorama of signals provided by whatever micro environment they happen to occupy, and, to some degree, on their history with specific antigen. These environmental signals are sensed through a massive array of receptors, many of which we now know as CD antigens. I believe that T cells integrate all of this information into discrete responses. It is important to remember that the TcR is but one of a multitude of receptors on T cells, and that, in fact, the TcR is rarely if ever used by most T cells. Yet all T cells routinely change their behavior as they move through different compartments of the body. They do this via mechanisms that are completely independent of TcR function, and are generally ignored by most immunologists.

I believe that every location in the body that can be occupied by T cells is so laden with specific information that the T cells know at all times exactly where they are, what is happening locally, and what, if anything, they are supposed to do about it. This information is continuously provided by vascular cells and parenchymal cells via their displays of cytokines and adhesion molecules. If the TcR is engaged, it merely provides a new consideration for T cells that is factored into its evaluation of the local environment. In general, I think that the TcR is highly over-rated. So, too, in my opinion is the concept of T cell anergy, which I consider to be the penultimate in vitro-fact. I can conceive of no location in the body where a TcR can be engaged in the complete absence of any of the known costimulatory signals.

I believe that our interpretation of CTL function has been fundamentally incorrect. I think that CTL, and T cells in general, play little or no direct role in the destruction of graft tissues. Rather, I believe that they function through cytokines and cell-surface molecules to orchestrate the powerful and dangerous destructive mechanisms mediated by macrophages, NK cells and other leukocyte populations. I believe that CTL, which are known to be efficient at killing other leukocytes, but relatively inefficient at killing parenchymal cells, serve primarily as immune disconnectors at inflammatory sites where T cells have engaged antigen.

I believe that leukocytes are not the only cell types involved in immune responses. Rather, all cells at the site, including parenchymal cells, produce cytokines and adhesion molecules, and thus are actively involved in a local immune response. Evidence for this is widely available. Several cytokines, like IL10 and TGFβ, are known to be made by parenchymal cell types, but this is generally ignored, another case of sanctioned oversimplification. By extension, immunity and physiology are inextricably linked, since parenchymal cells are responsible for the physiologic functions of tissues. I believe that under normal conditions, leukocytes and parenchymal cells use cytokines and adhesion molecules to chit-chat with themselves and with each other. In times of stress, these conversations simply become somewhat more animated. I think that if you could listen to the intercellular conversations that develop at inflammatory sites, it would sound less like the issuance of military commands to the local militia, and more like the frenzied cackle of chickens in the coop when the fox is around.

The end of the beginning

In summary, I find that current experimental observations in transplant immunobiology have become increasingly difficult for me to understand within the context of current immunologic thought. Surprisingly, many of these observation become explainable, and even predictable, when viewed from the perspective of network theory and systems thinking. I have no problem with this. Indeed, paradigm shifts are a natural and important part of the scientific process. However, complexity theory and systems thinking do not represent simple paradigm shifts. They represent a significant philosophic repositioning, a radical departure from accepted pattern of scientific thought. They are the vanguard ideas of a scientific revolution that could change some of the most basic perceptions of our discipline, and perhaps even of our society. They could force the development of entirely new patterns of scientific thought, and new rules by which experiments are designed and implemented. Like other scientific revolutions discussed so thoroughly by Kuhn, this scientific revolution is likely to be painful, and sometimes acrimonious process. There will be many casualties among the revolutionaries, brought down by their inability to convince their peers and the various funding agencies to support their seemingly counter-scientific conceptualizations and their radical methods of experimentation. (I hope that this is not my epitaph.) In the end, however, scientific truth, ie., a more accurate perception of reality, will emerge. Whether or not this reality happens to lie along the path of complexity theory and systems thinking will be determined some time in the 21st century. In the interim, I consider it to be my great good fortune to be a practicing scientist during one of the rarest, and most intellectually exhilarating events in all of science, a scientific revolution.

In closing, I wish to again thank the organizers of this meeting for the opportunity to present these unconventional ideas in so prominent and prestigious a forum. I also want to thank you, the audience, for your patience with my opinions. While I may not be able to provide you with any clear answers, I hope at least, that I will leave you with some useful, new ideas on the nature of the problems.

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