Autoimmunity to hsp65 and the Immunologic Paradigm*

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Editor's Introduction

Dr. Irun Cohen's review of autoimmunity 8 years ago in volume 29 of this series introduced what was then a dawning concept, namely that autoimmunity is primarily physiological and only when aberrant is it pathological. Since then, an avalanche of studies with the powerful tools of recombinant DNA technology has put autoimmunity research on a molecular basis. T-cell receptors and their ligands have been structurally defined in the quest to decipher the complex process of self and nonself recognition. Dr. Cohen reminds us that lymphocyte recognition of antigens should trigger a reflex attack on antigen-bearing cells, whether self or foreign. Burnet's clonal selection theory by which T cells capable of recognition of self antigens are deleted before birth, is, however, only one of several possible explanations of immunologic self-tolerance. Other mechanisms include active suppression of the process, one which for a long time we have called anergy (and still do). What Dr. Cohen now specifically reviews is his recent work and that of others on autoimmunity related to the heat shock protein hsp65. This 65-kd protein antigen produced by heat-stressed cells of all species, bacterial and animal, appears to be intimately involved in autoimmune disease in man and mouse, because suppression of autoimmune responses to hsp can abort autoimmune injury. The impact of heat-shock protein immunology on current concepts of suppression of autoimmunity is the subject of Dr. Cohen's brilliant avant garde review.

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The Reflex Paradigm of Immunity

In most walks of life, unfulfilled expectations are a misfortune; in science, they can be providential. Expectations are preconceived notions. Their

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frustration by controlled observation is an opportunity to discard an inadequate idea and draw a better mental approximation of nature. Orthodox ideas about autoimmunity rest on the premise, described by Burnet in his theory of clonal selection,[1] that the etiology of autoimmune disease is self-recognition: the binding of lymphocyte receptors (or antibodies) to self-antigens. Recognition is central to immunology. Clonal selection posits that the immune response to an antigen is triggered by the fit between a preformed lymphocyte receptor and an antigen (the receptor's ligand). The consequences of recognition, to use a neurologic metaphor, are as inexorable as a reflex[2]: sensory input (reception of the antigen signal) automatically leads to an effector output (the immune attack mediated by activated lymphocytes and antibodies). Recognition, according to notions of classic clonal selection, is both the means and the aim of the immune system. The job of the system is to distinguish between self and foreign. The foreign, upon recognition, is designated for attack. The self obviously must not be attacked. Since recognition induces reflex attack, the self is best protected by not being recognized, i.e., by being a nonstimulus.

In a simple reflex, the strength of the output often is linked to the strength of the input: foreign antigens elicit a strong protective response when they are quantitatively present in high concentration and intrinsically immunogenic. How can self-antigens avoid being recognized? Clearly, self-antigens that float freely in body fluids or are attached to exposed surfaces are accessible for recognition. Moreover, even those molecules that exist in the interiors of cells are processed and brought to the surface as peptide epitopes in the clasp of major histocompatibility complex (MHC) gene products.[3] So, for self-antigens not to be recognized, the logic of clonal selection requires that there be no receptors in the system capable of recognizing them. Burnet reasoned that the immune system might rid itself of self-recognizing lymphocytes at an early stage in lymphocyte maturation when contact with antigen induces programmed death.[4] Self-antigens, he proposed, might contact developing lymphocytes and kill the self-recognizers. The lymphocytes with receptors for foreign antigens mature safe from antigen contact and meet their antigens late, when contact induces reflex activation.

An alternative to clonal deletion as the mechanism controlling autoimmunity can be derived from the evidence that nonresponsiveness of lymphocytes to some foreign antigens might be caused by active suppression.[5] Tolerance to self-antigens too might be realized by suppressor lymphocytes that regulate the activities of self-recognizing effector lymphocytes. However, unlike helper T cells, suppressor T cells for specific antigens have evaded cloning and characterization and, therefore, many investigators have been led to doubt their existence.[6]

Recently, experiments have shown that T cells with receptors for certain antigens present in the thymus actually are deleted.[7] In addition, T cells also have been shown to become unresponsive, or anergic, upon contact with antigens outside of the thymus.[8] The mechanisms of clonal deletion and clonal anergy can be viewed together as the experimental vindication

of the idea that self-tolerance may arise from the death or functional inactivation of self-recognizing lymphocytes.[6] A collection of papers on the subject under the heading "Tolerance in the Immune System" appears in volume 248 of the 1990 issue of *Science,* pages 1335 to 1393.

The reflex paradigm of the immune response can be phrased thus: molecules that are recognized by immunologically competent cells are operationally defined as foreign. Conversely, the self is operationally defined as that which is not recognized by immunologically competent cells. The mechanisms responsible for creating this difference between the self and the foreign are clonal deletion and anergy. Hence, the sensory world perceived by the immune system is fashioned out of the visible (the foreign) and the invisible (the self); the visible constitute the subjects of recognition and the invisible form the ground (Fig 1).

Autoimmune diseases develop, according to this way of thinking, when self-antigens that should be invisible become visible through the illegitimate emergence of competent self-recognizing lymphocytes.[4] The transformation of ground into subject triggers reflex attack. A number of expectations about autoimmunity arise from this reflex paradigm of the immune response:

1. Self-recognition should not occur in healthy persons.
2. Antigens that resemble self-molecules should be poor immunogens.
3. Autoimmune reactions develop by accident and should have no bias toward any particular set of self-antigens.

This chapter has two objectives: to review new information about the immunology and autoimmunology of a molecule recently discovered to be a specific antigen and to ask whether this information fits the reflex paradigm of immunity. The specific antigen is the 65-kd heat shock protein (hsp65), and what we are learning about its immunology appears to contradict our expectations. But nature was interested in hsp65 before she invented lymphocytes, and we shall first consider the physiology of hsp65 before we explore its immunology.

A Molecular Chaperone: hsp65

Molecules of hsp constitute a family of proteins divided into subfamilies named for their approximate molecular masses in kilodaltons: hsp90, hsp70, hsp65 (also termed hsp60), and other, lower–molecular-weight proteins.[9] Members of each hsp subfamily are produced by widely different creatures. Nevertheless, they are characterized by high degrees of sequence homology. Molecules of hsp are among the most conserved proteins in evolution. The human and bacterial versions of hsp65 are identical in about 50% of their amino acid residues,[10] despite their divergence from a common ancestor cell at the dawn of life. This remarkable conservation implies that hsp molecules must be essential for the survival of all cells,

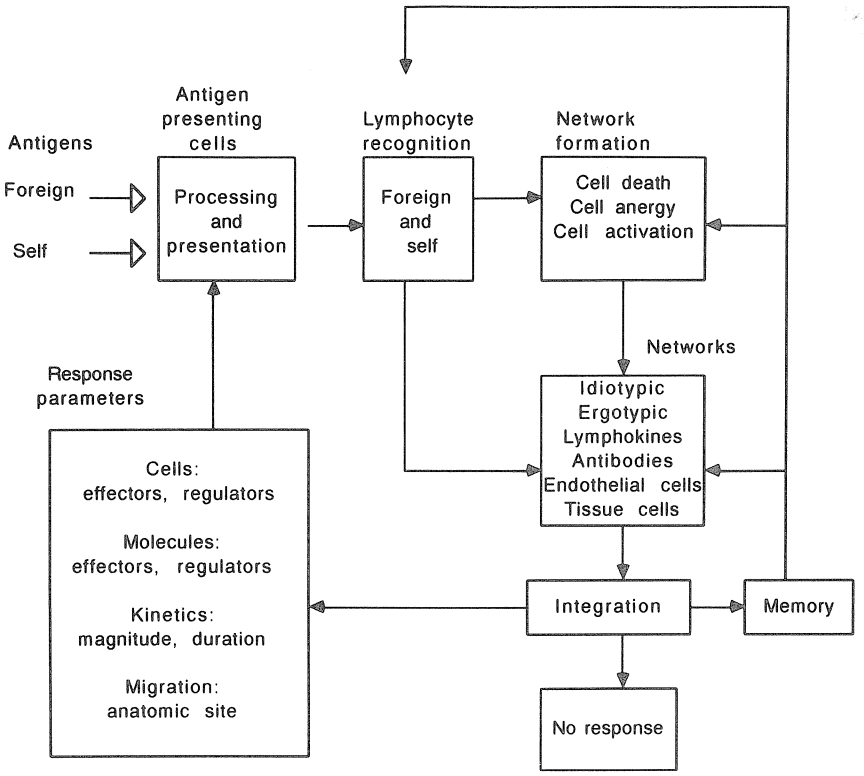

FIG 1.
The reflex paradigm of the immune response. According to this view of the immune system, the immune response is regulated by the act of lymphocyte recognition. A sharp distinction is proposed to exist between the T and B cells that recognize foreign antigens and those that recognize self-antigens. The lymphocytes that recognize self-antigens are either killed or inactivated (anergy) by contact with the self-antigen. In contrast, the lymphocytes that recognize foreign antigens are not inactivated. They constitute the mature repertoire and, by contact with their foreign antigens, become activated to mediate an effector response. Activation also generates memory lymphocytes that feed back into the pool of foreign-recognizers. This scheme shows that antigen-presenting cells, which process the antigens, limit the numbers of antigens available for recognition by lymphocytes (denoted by the transition from *open arrowheads* to *closed arrowheads*).

prokaryotic and eukaryotic. One would expect that the functions of hsp molecules should be transparently clear if these functions were so elemental that the existence of cells without them could not be contrived by nature. Alas, we are only beginning to see a hazy outline of hsp functions. One problem is that the few hsp molecules seem to do too many things.

The term hsp is a misnomer, due to the fact that the hsp family was discovered when their production by cells of fruit flies was noted to be aug-

mented upon heating the cells.[11] It soon became evident that all cells produced hsp molecules when heated. The temperature required to trigger augmented hsp production varies according to the life-style of the cell. Mammalian cells used to living at 37° C respond when exposed to temperatures reached during a high fever, thermophilic bacteria that grow at 65° C respond at 76 to 85° C, and blood parasites such as leishmania produce hsp molecules as they experience the sudden transition from insect vector (about 20° C) to mammalian host (37° C). Thus, the heat shock stimulus can be defined as an elevation of temperature above that to which the molecules currently operating in the particular cell are best adapted.

Heat is not the only inducer of augmented hsp production. Almost any type of metabolic, toxic, or physical stress will do. For this reason, hsp proteins also are called stress proteins.[12] Stress for a multicellular organism usually refers to a perceived threat to survival that elicits a "fight-or-flight" response mediated by the endocrine and nervous systems. Stress at the level of the cell is molecular. Thermal, metabolic, or osmotic stress can produce the unfolding of a cell's molecules called denaturation. An important job of hsp molecules must be to deal with denatured proteins, because hsp molecules are produced in large amounts when cells are threatened by denaturing conditions. It is likely that hsp production is universal, because molecular disorder is a constant feature of cellular life: biology must contend with the laws of thermodynamics.

Disorder is not confined to the disorderly molecule. Unfolding exposes otherwise buried hydrophobic and electrostatic forces so that an unfolded polypeptide can stick to other molecules and in turn denature them. Denaturation of molecules can become epidemic and threaten the whole cell. Molecules of hsp are thought to operate as scavengers that trap disorderly polypeptides and protect the stressed cell from cascading denaturation.[9]

However, unfolded polypeptide chains are generated also during order cellular life independent of stress.[9] Proteins are synthesized as linear polypeptide chains; in other words, proteins are born unfolded. The unfolded polypeptides can fold into well-natured proteins only after synthesis has been completed. Only the whole polypeptide chain furnishes all forces necessary to make the proper intramolecular bridges for shaping mature protein. Molecular disorder (linearity) is a prerequisite for molecular order (folding).

Moreover, even mature, well-folded proteins become unfolded physiologically, for example, when transported across membranes.[9] through the membrane, the unfolded polypeptide must refold to the protein's functional shape. It is believed that hsp molecules play a role in unfolding and refolding required for protein transport.

To summarize, hsp molecules probably function physiologically at baseline levels in protein synthesis, assembly, and transport. In addition, molecules are produced in augmented amounts to combat the denaturation produced by stress. Because the binding of hsp molecules to unfolded polypeptides prevents dangerous liaisons with other molecules, this binding has been called the chaperone function.[9] Hence, hsp65

molecules also are named chaperonins. The idea of the hsp molecular chaperone is attractive; it explains the universal importance of hsp molecules and hence their conservation throughout evolution.

The molecular questions are being investigated now. How can the few chaperonins interact with the thousands of different unfolded or denatured proteins? What is the canonical signal that marks molecules for hsp intervention? What do chaperonins recognize? How do chaperonins bind, and how does binding effect both unfolding and folding? How does the cell sense stress so as to express augmented hsp production? Beyond the basic molecular biology of chaperonins, their likely role in the pathophysiology of disease awaits discovery. The subject of their immunology,[12, 13] to which we shall turn now, only touches the surface of what is in store for the physician.

A Proving Ground for Self-Tolerance

The hsp65 molecule confronts the immune system with a problem in distinguishing between self and foreign. Because of preservation throughout evolution, the hsp65 molecules of all cells, prokaryotes and eukaryotes, are identical in some highly conserved stretches of their amino acid sequence and dissimilar in other variable stretches. Hence, the hsp65 molecule of any invading bacterium or higher parasite confronts the host with a combination of both self and foreign epitopes.[14] Can the immune system respond to such a hybrid between self and foreign? Which hsp65 epitopes are recognized? How is the danger of autoimmunity controlled? These questions can be explored by investigating immunity to hsp65 in three contexts: immune responses to overt infection, natural immunity in the healthy, and immunity in individuals afflicted with autoimmune disease.

hsp65 Is a Dominant Bacterial Antigen

Antigenic dominance refers to the capacity of a particular molecule to arouse a strong immune response in the face of competing antigenic molecules. Infection with bacteria or other cellular parasites exposes the immune system to a large number of invader antigens simultaneously. Which antigens draw immunologic attention? The clonal selection paradigm predicts that the dominant antigen would be the one with the greatest number of foreign epitopes capable of engaging the greatest number of lymphocyte clones. The hsp65 molecule, studded with self-epitopes, would not be expected to attract much attention.

Despite this reasoning, Table 1 shows that the hsp65 molecules of many different infectious agents are dominant antigens. Humans responding to mycobacteria, enteric bacteria, or higher parasites mount major responses to hsp65 molecules.[15] In mice immunized with whole mycobacteria, 20% of all the T cells aroused to respond are directed to hsp65.[16] The hsp65 molecule has been called the common bacterial antigen.[17]

TABLE 1.
Immunologic Dominance of hsp65 in Microbial Immunity*

Immune Response to hsp65	
Pathogens	**Diseases**
Borrelia burgdorferi	Lyme disease
Chlamydia trachomatis	Trachoma
Coxiella burnetii	Q fever
Legionella pneumophila	Legionnaires' disease
Mycobacterium leprae	Leprosy
Mycobacterium tuberculosis	Tuberculosis
Treponema pallidum	Syphilis

*The hsp65 molecules of the above microbes are the targets of immune responses. Reference list appears in reference 15.

Molecular biology has made it technically possible to clone and sequence the genes of antigens recognized by antibodies or T cells. Protein antigens now can be defined by their primary amino acid sequences and not merely by their molecular weight, charge, or mode of preparation. This revolution in microbial immunology has revealed the immunologic dominance of hsp65. It also has revealed the dominance of other microbial antigens that share very high degrees of homology to host molecules, such as the other hsp subfamilies and certain highly conserved essential enzymes. Douglas Young and I have documented and discussed this paradox elsewhere in greater detail.[15] In this article, it need be noted only that microbial antigens selected for major immune responses include protein antigens such as hsp65 that are very much like self to the responding host; the familiar, contrary to the expectations of clonal deletion, is not only recognized but preferred.

Healthy hsp65 Immunity

Because healthy people are commonly in contact with microbial parasites, and because hsp65 is a common bacterial antigen, we would expect healthy people to exhibit immune reactivity to hsp65, but only to epitopes of hsp65 specific for common bacteria. The reflex paradigm of immunity would lead us to predict that healthy people would have no natural immunity to the conserved epitopes of hsp65 shared by human hosts and their parasites. This prediction is not borne out by experimental results. Munk and associates synthesized four peptides of the hsp65 sequence identical or almost identical in humans and mycobacteria and tested the T cell re-

sponses in vitro of healthy people to these peptides: eight of the nine people tested showed T cell immunity.[18] If T cell immunity to self-epitopes of hsp65 can be detected in the healthy, we may conclude that T cells reactive to at least some self-epitopes escape deletion and are not permanently anergic. We may push this line of reasoning forward. If T cell autoimmunity to hsp65 is compatible with health, then either autoimmunity to hsp65 is incapable of causing clinical disease, or there must exist immune mechanisms other than deletion or anergy for regulating the consequences of hsp65 autoimmunity.

hsp65 and Autoimmune Arthritis

My colleagues and I stumbled into the labyrinth of hsp65 autoimmunity while tracking down T cells responsible for adjuvant arthritis, an autoimmune disease of rats induced by immunization to killed *Mycobacterium tuberculosis* organisms. Adjuvant arthritis was described over 30 years ago by Pearson and associates.[19] Whether or not adjuvant arthritis is a good model of human rheumatoid arthritis,[20] it has served as a useful model of progressive inflammation of the joints produced by the immune system. The problem confronting us was to understand the connection between immunity to a bacterial antigen and what appeared to be an autoimmune disease of the joints.

The approach developed in my laboratory for investigating experimental autoimmune diseases is to isolate as long-term lines or clones the autoimmune T cells responsible for producing the particular disease.[21] The key for unlocking the complexities of pathogenesis is to isolate the etiologic agent itself, whether it be a gene, a toxin, a parasite, or an autoimmune lymphocyte. From a rat with adjuvant arthritis, a T cell clone capable of transmitting arthritis was isolated.[22] This clone, called A2b, was found to respond to an antigen present in both *M tuberculosis* and joint cartilage.[23] We could conclude that immunization to the microbe stimulated the rat to develop an immune response to a microbial antigen that looked like a self-antigen of the joints. The arthritis produced by the T cells was the result of antigenic cross-reactivity between host and mycobacterial immunogen.[24]

Progress subsequently was made in identifying the mycobacterial antigen by investigating the reactivity of the arthritogenic T cell clone to mycobacterial antigens that had been genetically engineered into *Escherichia coli*. The target antigen of the arthritogenic T cell clone turned out to be hsp65.[25] The key epitope of hsp65 was identified as the nine amino acids at positions 180 through 188 in the mycobacterial hsp65 sequence.[25] Surprisingly, this part of the hsp65 sequence was in an unconserved segment of the molecule. There was no sequence similarity between the mycobacterial and mammalian epitopes at this site.[10] Thus, the arthritis could not be due to an attack of the T cell clones against the rats' own hsp65 expressed in joints. In agreement with our earlier finding of T cell cross-reactivity between the mycobacterial antigen and cartilage,[23] we noted a slight

homology between the 180 through 188 peptide and a segment of the link protein of cartilage proteoglycan.[26] However, we now know that our T cell clone does not respond in vitro to the proposed peptide sequence of the link protein (unpublished observation, 1990), so the chemical connection between mycobacterial hsp65 and cartilage is yet unresolved.

Our investigations of rat arthritis pointed out a new element for study in human arthritis: does immunity to hsp65 occur in rheumatoid or reactive arthritides? If so, which epitopes are recognized: the 180 through 188 peptide of the mycobacterial hsp65 sequence or other hsp65 epitopes, foreign or self? The experiments done to date clearly show that human arthritis patients manifest T cell responses to hsp65; however, the meaning of the reactivity is obscure. The first investigations indicated that synovial fluid T cells recovered from rheumatoid arthritis joints did respond to hsp65.[27–29] Patients with juvenile rheumatoid arthritis were found to respond to the mycobacterial hsp65 molecule and to its 180 through 188 peptide epitope.[30] However, adult rheumatoid arthritis patients did not show immunity to the 180 through 188 epitope.[30] These differences in immunologic reactivity could result from a real difference in etiology between the juvenile and adult forms of rheumatoid arthritis or they might reflect merely the evolution in time of a single disease entity.

A further complication is the finding that immunity to the mycobacterial hsp65 molecule also accompanies other chronic immunologic diseases, such as scleroderma.[31] Moreover, it was reported that T cell immunity to mycobacterial antigens, including hsp65, may be detected in plural exudates associated with tumors or other causes unrelated to mycobacterial infection.[32] Thus, immunity to mycobacterial hsp65 may accompany various forms of chronic inflammation, not only arthritis.

Another enigma is that many of the T cells responding to mycobacterial hsp65 seem to bear the $\gamma\delta$ T cell receptor for antigen.[33] The function of such T cells is not clear.[34] Perhaps the meaning of hsp65 immunity will become clearer when the target epitopes involved in the different diseases are clarified. Nevertheless, the fact that arthritis can be produced by a rat T cell recognizing a defined epitope of hsp65 suggests that hsp65 immunity may have an intimate relationship to the arthritogenic process, at least in rats. Note, however, that the autoimmune process responsible for the arthritis is not hsp65 autoimmunity but an apparent cross-reactivity between the 180 through 188 peptide of mycobacterial hsp65 and some epitope in joint cartilage.[26] True autoimmunity to a mammalian hsp65 molecule does seem to cause a spontaneous autoimmune disease in mice, i.e., type 1 or insulin-dependent diabetes mellitus (IDDM).

hsp65 and Autoimmune Diabetes

Mice of the nonobese diabetic (NOD) strain spontaneously develop IDDM that mimics the disorder developing in humans.[35] At an early stage in life (4 to 6 weeks of age) NOD mice spontaneously develop inflammation of

the islets. The insulitis persists and progressively damages the beta cells. The cumulative loss of beta cells causes a deficiency of insulin production that becomes manifest as overt diabetes at 4 to 6 months of age. Since T cells can transfer diabetes,[36] it seems likely that the IDDM process involves a T cell response to a specific peptide epitope. Unexpectedly, that epitope turned out to be a fragment of the self-hsp65 molecule.

This observation was made when we employed mycobacterial hsp65, our arthritis antigen, as a "control" antigen for the immune reactivity to insulin that we had been studying in NOD mice.[37] We have discovered since that the most active epitope is not in mycobacterial hsp65, but in the mammalian hsp65 sequence. T cell reactivity to human hsp65 is about fivefold greater than it is to the mycobacterial hsp65.[38]

The evidence implicating human-hsp65 as a target antigen in mouse IDDM can be summarized thus:

1. The spontaneous development of insulitis is accompanied by autoimmune T cells and autoantibodies to hsp65.[37] These autoimmune phenomena precede overt diabetes by several months. NOD mice that fail to develop IDDM do not manifest hsp65 autoimmunity.
2. T cell clones ($CD4^+$, $CD8^-$) responsive to a 24-amino-acid peptide in the human hsp65 sequence can transfer insulitis and hyperglycemia to prediabetic NOD mice. The T clones also can cause diabetes in an MHC-compatible strain of mice that does not otherwise develop diabetes, the NON-H-2^{NOD} strain. Active immunization of nondiabetic NOD or NON-H-2^{NOD} mice induces diabetes.[38]
3. The autoimmune destruction of beta cells and the development of IDDM can be aborted by specifically reducing the autoimmune response to the hsp65 peptide.[38] (How we can control reactivity to hsp65 will be explained later.)

We now have cloned and sequenced the hsp65 gene from a mouse insulinoma cell and have found that the mouse and human hsp65 molecules are 97% identical; they differ by only one amino acid in the key epitope.[39] Thus, the response to human hsp65 probably reflects autoimmunity to mouse hsp65. In other words, hsp65 autoimmunity is both necessary and sufficient for the development of IDDM in NOD mice. Inducing the immunity induces the disease and preventing the immunity prevents the disease.

Although the immunologic results are crystal clear, their relationship to the pathophysiology of beta cell destruction is perplexing. An organ-specific autoimmune disease is expected to have an organ-specific target antigen. The hsp65 molecule is inducible in all cells; why should anti-hsp65 T cells target to the islets? It is conceivable that hsp65 has a physiologic role in the assembly or secretion of insulin, which is unique to beta cells. If so, the T cells must have a way of detecting this unique form of hsp65 expression. It also is possible that hsp65 autoimmunity is a critical cofactor to an additional immune response more specifically focused on beta cells.

Note that the 64-kd antigen, another autoantigen related to IDDM, has

been identified as the enzyme glutamic acid decarboxylase.[40] Similar to hsp65, the expression of glutamic acid decarboxylase is not unique to beta cells. Thus, there is much to learn about the association between reactivity to particular autoantigens and the expression of autoimmune disease. Be that as it may, the importance of hsp65 autoimmunity is not limited to NOD mice. A preliminary study of T cells from newly diagnosed IDDM patients showed strong reactivity to the very same 24-amino-acid peptide discovered in the NOD model.[41]

Paradoxes

The results of the investigations of arthritis in rats and IDDM in mice indicate that epitopes of hsp65, bacterial as well as self, can be involved in autoimmune disease. Is it not dangerous for hsp65 to be a dominant antigen in microbial disease and a natural self-antigen in health? Clearly, the immunology of hsp65 cannot be explained by the reflex paradigm or by the mechanisms of deletion or anergy as the guardians of self-tolerance.[2, 6] Whether or not an autoimmune disease develops is not determined only by whether or not self-epitopes are recognized; hsp65 is recognized universally. The determination of health or disease is made distal to the act of recognition. It depends on how the immune system behaves subsequent to recognition. Recognition is only the first step; it merely defines the raw material upon which the immune system then acts. The nature of the immune response to hsp65 can dominate the responses to other antigens, it can be physiologically useful to the host in the host-parasite relationship, or it can be perniciously destructive in an autoimmune disease. How are these diverse programs implemented? Who decides which program to load? The hardware of the system is ready—the T cells and B cells have receptors for hsp65 epitopes; the antigen-presenting cells can process the epitopes; and the phagocytes, inflammatory cells, and complement are available. The outcome of hsp65 recognition depends on the software—the particular organizational program for implementation.

An Immunologic Brain

To return to the nervous system metaphor, what we are learning about immunity to hsp65 is not compatible with a view of the immune response as a stereotyped undiversified reflex. The diverse outcome of hsp65 immunity and its complexity supports a view of the immune system as a brain. The first step in the transition from reflex to brain is the interposition of an interneuron between sensory input and motor output, between recognition and response. The interneuron is the seed from which sprout the neural networks whose complexities create the integration of information within the nervous system leading to the behavioral repertoire, learning, memory, and adaptation. It should be clear to anyone who observes immune behavior that the immune system too can express a wide range of actions,

learn, remember, and adapt. To attribute all this to variations of primary recognition is to seriously oversimplify the system.

Lymphocyte Networks and T Cell Vaccination

If the immune system acts like an integrating brain rather than like a monotonic reflex, where are its interneurons, its lymphocyte networks? Experimental investigation of immune networks is in an early stage. Nevertheless, lymphocyte networks have been described by several groups, and the work of Coutinho[42] and Rajewsky[43] and their colleagues may be consulted. My appreciation of the power of T cell networks has come about through my work and that of my colleagues aimed at controlling autoimmune diseases by T cell vaccination. The term "T cell vaccination" was coined by us to describe the prevention and therapy of an autoimmune disease by the administration of attenuated autoimmune effector T lymphocytes.[44] Our success in isolating and growing the virulent T cells responsible for an autoimmune disease, experimental autoimmune encephalomyelitis,[45] led us immediately to try to prevent this disease by vaccinating rats with the same T cells, attenuated by irradiation.[46]

Since then, T cell vaccination has been applied successfully to prevent and even to treat a variety of experimental autoimmune diseases such as thyroiditis,[47] adjuvant arthritis,[48] and IDDM.[38] The vaccination effect can be induced by synthetic peptides comprising parts of the sequence of the T cell receptor,[49] indicating that anti-idiotypic immunity to the autoimmune T cell receptors is involved in controlling the autoimmune process. Indeed, anti-idiotypic T cells of both the CD4+ and CD8+ types have been isolated[50] and an anti-idiotypic CD8+ T clone was shown to prevent experimental autoimmune encephalomyelitis in vivo.[51]

The general success of T cell vaccination in the animal models has justified preliminary phase I trials in multiple sclerosis and rheumatoid arthritis.[6] Distinct from the potential value of medicinal T cell vaccination as a therapeutic maneuver in clincal autoimmunity, study of the antiautoimmune mechanisms aroused by T cell vaccination has provided evidence for the existence of natural T cell networks. It seems that animals may respond to T cell vaccination by activating or amplifying T cell networks that already exist before vaccination. In other words, T cell vaccination exploits natural regulatory networks whose physiologic function is to control the expression of autoimmunity.

The evidence for natural networks has been discussed elsewhere.[2, 44, 52, 53] What we know specifically about hsp65 networks can be stated briefly. Both adjuvant arthritis in rats[48] and IDDM in mice[38] can be cured by administering T cells responsive to the epitopes of hsp65 involved in the particular disease. Freedom from disease can be attributed to anti-idiotypic networks of T cells (unpublished data, 1991). Recognition of hsp65 itself is a given in both health and disease; disease or health depends on the behavior of the network of anti-idiotypic T cells organized around hsp65 and the T cells that recognize it.

It has been reported that administration of the 180 through 188 peptide of mycobacterial hsp65 can ameliorate adjuvant arthritis.[54] Moreover, we have found that IDDM too can be treated by vaccinating NOD mice with the 24-amino-acid peptide specific for IDDM.[38] Interestingly, we find that peptide vaccination induces the same immune regulation of the response to human hsp65 as does T cell vaccination with anti-hsp65 T cells.[38] Indeed, peptide vaccination seems to activate anti-idiotypic T cells responsive to the anti-hsp65 T cells that cause IDDM (unpublished observation, 1991). This is what would be expected of an immune network.[53]

Immunologic networks are not only anti-idiotypic. There is evidence that T cells can regulate other T cells through recognition of their state of activation.[55] Moreover, it is becoming clear that networks of lymphokines and cytokines have a great influence on the quantity and quality of the effector cells and molecules deployed by the immune system.[56] These elements integrate to determine the biologic meaning of the response (Fig 2).

The nervous system processes information about the self using a series of neural networks that encode in the brain a functional representation of the body. Recall the neurologic homunculus pictured in neurology textbooks. It now seems that the immune system too processes information about self-molecules such as hsp65 using lymphocyte networks that encode a representation of the self-antigen, a type of immunologic homunculus.[2, 15, 52] This encoding of hsp65 within the networks of the immunologic homunculus may be the cause of the immunologic dominance of this antigen.

Recent research in immunology has advanced greatly our knowledge of the chemistry of the processing, presentation, and recognition of antigen. It was hoped that an understanding of the molecular basis of recognition would suffice to explain the behavior of the immune system. It should not be surprising that this hope will go unfulfilled. The behavior of the nervous system, the other system we use to process information, also cannot be predicted merely by knowing the chemistry of receptors and synapses.

Recognition is only the beginning of the story. The immune system has been selected during evolution, not to recognize the difference between self and nonself, but rather to adapt the individual to the environment in a way that promotes survival. The test of the appropriateness of an immune response is whether the response is beneficial, irrespective of whether the molecule recognized is self or foreign. For example, if a well-regulated response to a self-hsp65 epitope will help rid the person of a microbial infection or a tumor cell, then the autoimmune response is beneficial. In contrast, an immune response to a harmless foreign antigen can trigger an allergy that may damage health and handicap survival. The immune system decides how best to respond to what it recognizes on the basis of what it has learned from past experience. The immunologic networks that encode experience and weigh decisions constitute the immune system's brain.

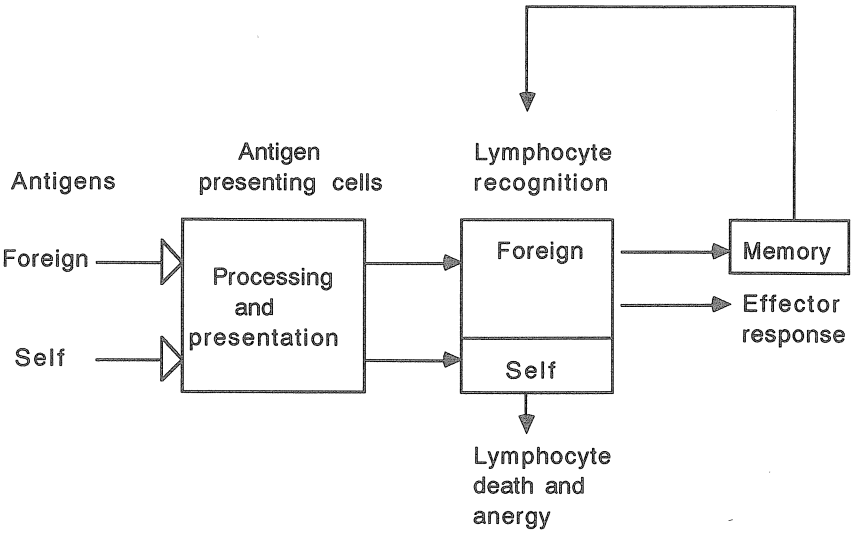

FIG 2.
Brain paradigm of the immune system. This paradigm differs from the reflex paradigm in Figure 1 in that lymphocyte recognition of processed antigens is merely the gateway into the system and there is no absolute difference between the lymphocytes that recognize foreign or self-antigens. Lymphocyte recognition of antigen accomplishes two ends: it forms cell networks by cell activation, death and anergy; and it activates these networks to produce an immune response. The networks include lymphocytes (idiotypic and ergotypic), factors (lymphokines and antibodies), and other cell types (endothelial and tissue cells that communicate with lymphocytes by way of cytokines and adhesion molecules). The output of the networks is the integration of information that directs the responses to the antigens. There may be no response to some antigens. Responses to other antigens may include memory (adaptations of the existing networks, the formation of new networks, and changes in the numbers of lymphocytes with receptors for the antigens). Integration of the various networks modifies the parameters of the response, including effector and regulatory T and B cells, effector and regulatory antibody and lymphokine molecules, and the kinetics of the response and traffic of the cells to various sites. The immune response also can feed back to influence the processing and presentation of antigens. According to the brain paradigm, the pressures of natural selection have molded an immune system whose primary job is not to distinguish between self and non-self, but to aid survival of the organism in a changing environment.

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