Autoimmune Disease

Internal Medicine Grand Rounds
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"I now see why people chop wood -
it is so easy to understand the
results"

Albert Einstein

Introduction

Understanding the basis of self non-self discrimination has been one of the major thrusts of research in immunology. Autoimmunity is one of the major clinical consequences of the failure of the organism to discriminate between self and non-self.

Paul Ehrlich was the first to consider the importance to the survival of an animal for the immune system to discriminate between self and non-self. Before 1900, it had become clear then animals could react to a wide variety of foreign materials by producing antibodies with damaging potential. Bordet had shown, for example, that serum of a guinea pig immunized with rabbit erythrocytes was highly toxic to the rabbit. Ehrlich realized that essentially the same conditions that were necessary to elicit an antibody response to foreign materials also occurred when self-antigens were exposed to the immune system, such as after subcutaneous hemorrhage or after other tissue damage. This suggested to Ehrlich that an animal might have the means to refuse to form antibodies that would recognize antigens on his own tissues, since such antibodies could potentially be harmful.

In 1900, Ehrlich reported the first evidence that animals could discriminate self from non-self. He and Morgenroth had studied the response of goats to intraperitoneal administration of blood. If goats were injected with blood from another species, they produced antibodies that could lyse the foreign red cells. Similarly, goats injected with goat blood developed antibodies that could lyse red cells of the donor goat and many other goats but, failed to lyse the recipient goat's own erythrocytes. These results prompted Ehrlich and Morgenroth to coin the term "horror autotoxicus" or an organism's fear of developing immune responses to self-antigens that could potentially be injurious to his own tissues. Ehrlich and Morgenroth further postulated that "the organism has contrivances by means of which the immunity reaction, so easily produced by all kinds of cells is prevented from reacting against the organism's own elements and so give rise to autotoxins... only when the internal regulating contrivances are no longer intact can great dangers arise." Understanding the nature of those contrivances and the mechanisms involved in self non-self discrimination became one of the major concerns of research in immunology over the next 80 years.

Two general inferences were drawn from the concept of "horror autotoxicus," although neither was actually stated by authors. First, it was inferred that an absolute proscription existed against the development of all autoimmune phenomena and secondly, all autoimmune phenomena were thought necessarily to be harmful. Thus, autoimmunity was viewed only as an abnormal event with considerable pathologic potential. Over the years thinking about autoimmunity has evolved as knowledge of the immune system has expanded. The present review will examine our current understanding of autoimmunity with special reference to the role of autoimmune processes in the development of human disease.

Autoimmunity in Normal Animals

Since the original experiments of Ehrlich and Morgenroth, the paradigm of autoimmunity has included two elements. First, the generation of all autoimmune responses was believed to be precluded in normal

animals and, therefore, all manifestations of autoimmunity were, by definition, abnormal. Secondly, autoimmunity was considered not only to be abnormal but also harmful. A number of observations have indicated that these inferences are incorrect. Autoimmunity is neither rare, nor necessarily harmful. The serum of most normal animals contains naturally-occurring auto-antibodies that appear to cause no harm whatsoever. This was first observed in 1929, by Friedenreich who found that normal human serum agglutinated bacterial enzyme-digested human erythrocytes. The antibody nature of these serum factors could not be documented, however, for many years until the structure and function of the various immunoglobulin molecules had been elucidated. Since then it has become clear that a variety of different naturally-occurring autoantibodies exist in the serum of most, if not all, normal humans and a number of laboratory animals as noted in Table I. In most of these situations, the autoantibody is of the IgM class. Most of these reactivities are

Table I
Naturally-Occurring Autoantibodies

Factor	Species	Immunoglobulin class	Tissue specificity
Kidd-Friedewald	Rabbit,rat,dog	IgM	Tissue lysates
Anti-cell	Man, various animals	IgM	Neuraminidase-treated RBC, lymphocytes, sperm
Anti-immunoglobulins (anti-Fab)	Man, primates, rabbit	IgG, IgM	Fab fragments of IgG after papain
"pepsin agglutinators"		IgM	or pepsin digestion
Anti-erythrocyte	Man, mouse	IgM	Aged or bromelain-digested erythocytes
Anti-myelin	Man	IgG	Myelin

directed at determinants that are normally concealed in vivo, reguiring enzymatic digestion or cellular damage to become revealed. Nonetheless, they constitute evidence of naturally-occurring autoantibodies. Their physiologic role, in general, remains speculative, but they are not felt to be harmful, presumably because they are directed at antigens that are normally hidden.

A number of suggestions have been made about the function of naturally-occurring autoantibodies. Grabar suggested that these reactants played an important role in tissue catabolism serving as carriers or transporters that facilitated uptake by phagocytic scavenger cells of damaged materials generated at sites of inflammation.

Kay has suggested a role for naturally-occurring autoantibodies in the removal of senescent erythrocytes. As red cells age in vivo, they shed sialic acid. Such desialated erythocytes are recognized by naturally-occurring autoantibodies. The resultant opsonization facilitates the uptake of these aged erythrocytes by phagocytic cells in the liver and spleen. On the other hand, Najjar showed that erythocytes coated with autoantibody were more resistant to shear forces than uncoated red

cells. Thus, naturally-occurring autoantibodies could function to protect aged erythrocytes and promote their survival.

It has been suggested that anti-Fab autoantibodies (pepsin agglutinators) have a unique function as auxiliary immune reactants. The enzymes of phagocytic cells and those released at inflammatory sites can digest IgG to its Fab fragments. These fragments continue to be able to bind antigen, but are no longer able to fix complement or function as opsonins because of the loss of the Fc portion of the IgG molecule. Waller has suggested that anti-Fab autoantibodies are able to react with autoantigenic determinants exposed on such Fab fragments at inflammatory sites and thus convey the Fc-mediated functions of complement activation and opsonization.

These observations indicate that evidence of autoimmunity is not unusual, but can be found routinely in all animals. Recent studies have indicated, in fact, that an element of self-recognition is intimately involved in the generation and regulation of all normal immune responses to exogenous antigens. One example of this comes from studies examining the control of T cell activation. To be stimulated, T cells must interact not only with antigen but also with a macrophage, whose role is to present antigen to the T cell is an appropriate manner. Moreover, T cell activation has been shown to require that the T cell recognize not only macrophage-bound antigen but also cell surface antigens encoded by the major histocompatibility locus of the macrophage. This indicates that stimulation of T cells by all exogenous antigens involves a necessary step of self-recognition.

The second example of the involvement of self-recognition in normal immunoregulation is the immunological network as envisioned by Jerne and confirmed by many investigators. Jerne postulated that the body contained a network of self-reactive antibodies that tightly regulated immune responsiveness. The basic unit of this network of interactions consisted of idiotype-anti-idiotype interactions. In this system an idiotype refers to the region of the antibody molecule that binds antigen, while an anti-idiotype is an antibody directed against that region. The immune system is thus seen as consisting of a series of interactions between antibodies and mirror images or anti-antibodies. Exogenous antigen elicits the production of an antibody, bearing a particular idiotypic determinant. This idiotype is then able to induce synthesis of immunoregulatory autoanti-idiotype antibodies which themselves elicit the synthesis of additional anti-(anti-idiotype) antibodies. The immune system is thus seen as a tightly regulated series of complementary autoantibodies, with exogenous antigen serving only as the initial perturbing influence. A considerable amount of evidence has been developed to support this hypothesis. The existence of Jerne's immunological network is further evidence supporting the conclusion that self-reactivity is not proscribed but rather seems to be absolutely essential for normal homeostasis.

Mechanisms preventing autoimmunity in normal animals

The existence of naturally-occurring autoantibodies indicates that a level of autoimmunity normally exists in all healthy animals and that such autoantibodies are not harmful, but rather play a physiological role in the homeostasis of the organism. Although the evidence indicates that animals normally exhibit some degree of autoreactivity,

these autoantibodies usually occur in very low titers. Moreover, naturally-occurring autoreactivity appears to be restricted such that autoantibodies to only a very few self-antigens are detected. Thus, of the millions of autoantigens that exist in the body, naturally-occurring autoantibodies are directed predominantly at immunoglobulin determinants or at antigens that are normally concealed in vivo. These results support the conclusion that mechanisms must exist to prevent the development of autoimmunity to most self-antigens that are normally exposed to the immune system.

A number of early observations suggested that something unique about the fetal environment precluded the development of immune responses to foreign antigens and perhaps, also, to autoantigens. For example, in 1945 Owens demonstrated persistent erythrocyte mosaicism in adult dizygotic twin cattle resulting from antenatal exchange of stem cells through placental vascular anastomoses. Such evidence of "tolerance of immature tissues to foreign material" caused Burnet and Fenner to suggest that the immune system recognized "self-marked" materials at certain stages of its development and as a result became unresponsive to them. This hypothesis predicted that adult animals would be specifically unresponsive (tolerant) to a foreign antigen if they had been exposed to that antigen before complete maturation of the immune system. This prediction was confirmed by Billingham et al in 1953, who showed that adult animals were tolerant to allografts as a result of exposure to the donor animal's cells in fetal life. Similar results were subsequently made with a number of soluble protein antigens. These experiments showed that an animal could be made unresponsive to foreign antigens by exposure to them during fetal life.

The relevance of these findings to understanding the prevention of autoimmunity required the demonstration that unresponsiveness to self was not a genetically-determined property but rather was acquired during embryonic life. It remained possible that each animal carried genetic information that uniquely permitted it to recognize its own self-antigens, independent of fetal exposure to such determinants. Triplett carried out experiments to examine this possibility. He found that adult frogs that had been hypophysectomized as embryos rejected their own pituitaries when these organs were re-engrafted, whereas animals that had had only a partial hypophysectomy did not. These experiments indicated that the prevention of reactivity to self was acquired. Additional experiments by Catty in the rabbit supported this conclusion. Thus, rabbits could produce autoantibodies against determinants found on their own immunoglobulin molecules (allotypes), if production of that particular determinant was prevented by the neonatal administration of appropriate anti-allotype antibody. Upon subsequent immunization, such rabbits made antibody to the allotype whose production was suppressed, although control animals were unresponsive. In both the experiments with the frog and the rabbit, the experimental animals contained the genetic material to make the self-antigen, but did not become normally unresponsive to that antigen when it was removed surgically or its production was phenotypically suppressed. These experiments indicate that unresponsiveness to self-antigens is not genetically controlled in normal animals but rather is an acquired characteristic.

A mechanism for neonatal tolerance was subsequently suggested by Burnet as part of the clonal selection theory. The clonal selection theory postulated that there existed within an animal a large number of precursors of antibody forming cells, each of which could give rise to cells producing antibodies capable of reacting with a specific antigenic determinant. Precursors of a given clone could respond to contact with the corresponding antigen, but not

with unrelated antigens, by proliferation and differentiation into plasma cells with the production of their unique antibody molecule. The clonal selection theory has been tested in a number of ways and is generally accepted as fact.

The clonal selection theory, as hypothesized by Burnet and modified by Nossal, explained the absence of autoimmunity by postulating that clones of self-reactive cells were eliminated as a result of encounter with self-antigens during embryonic development. The central dogma of this theory was that during maturation of immunocompetent cells, the cells passed through a stage in differentiation during which contact with specific antigen would result in inactivation or deletion of the cell. Animals, therefore, would not produce antibodies or show other immune reactions against their own tissue antigens because clones of potentially self-reactive cells would have been deleted during embryonic development. Autoimmunity could only develop when this normal clonal-deletion mechanism was circumvented.

Burnet gave two possible explanations for the development of autoimmunity. First, he suggested that auto-reactive clones would escape deletion if the particular antigen was hidden from the corresponding clones of lymphocytes during the period of ontogeny when self-reactive lymphocytes were normally eliminated. Exposure later in life would therefore lead to a normal immune response and the production of autoantibodies. Secondly, Burnet suggested that some forms of autoimmune disease could be the result of somatic mutation of an immunologically competent cell committed to an exogenous antigen, leading to the development of a cell with the capacity to react to the host's own antigens. Proliferation of such a cell would lead to the emergence of a "forbidden clone" of self-reactive cells.

It should be emphasized that the clonal selection theory was formulated at a time when little was known about the organization of the immune system. For example, the distinction between T and B lymphocytes was unknown. Indeed, it was not clear at this time that small lymphocytes were even part of the immune system.

The clonal deletion theory of autoimmunity permits a number of predictions that can be experimentally tested. In general, the predictions have not been supported by the experimental results. For example, the basic tenet upon which the theory rests is that immature B lymphocytes are sensitive to tolerization whereas mature B cells are not. This conclusion is supported by the finding that fetal cells are extremely sensitive to tolerance induction, with abrogation of subsequent responsiveness resulting from exposure to extremely small concentrations of multivalent antigens that are immunogenic for mature B cells. However, Dresser and Mitchison subsequently demonstrated that specific unresponsiveness to antigens was not unique to immature animals but could also be induced in adult animals by challenging them with native, undenatured antigen. Moreover, challenging fetal animals with foreign antigens was found not to result uniformly in tolerance. On the contrary, in a number of situations, fetal animals respond with the development of an immune response. Burnet himself found this when he attempted to prove his own hypothesis. Thus, chickens, that had been injected with human erythrocytes as embryos were found to develop natural hemagglutinins to human erythrocytes before untreated controls. Finally, recent evidence indicates that sensitivity to tolerization of immature cells does not reflect heightened sensitivity of fetal B cells to inactivation

but rather a lack of mature, functionally-active helper T cells. When immature B cells are exposed to antigen in the presence of helper T cells, a positive immune response occurs. These results all indicate that the difference between adult and fetal lymphocytes is not absolute and, moreover, may be more quantitative than qualitative.

The clonal deletion theory of autoimmunity further predicts that precursors of self-reactive cells will not be found in normal adult animals. A number of experimental finding have refuted this prediction (Table II). For example, autoimmunity can be induced routinely by

Table II

Evidence that Self-Reactive
Lymphocytes Persist in Normal Adults

I.	Laboratory Animals
	A. Induction of autoimmunity by immunization
	B. Induction of autoimmunity by administration of polyclonal B cell activators
	C. Induction of autoantibody formation in vitro by polyclonal B cell activators
II.	Man
	A. Autoantibody formation during infection, inflammation, drug reactions
	B. Presence of circulating autoantigen-binding B cells
	C. Induction of autoantibody formation in vitro by polyclonal B cell activators

immunizing normal animals with autologous tissues with various adjuvants. Secondly, autoimmunity can be induced in experimental animals by the in vivo administration of polyclonal B cell activators, such as bacterial endotoxin, that directly stimulate a large number of B cell clones, or by in vitro stimulation with these materials.

In man, autoantibody formation is frequently observed during a number of infectious processes, as a result of tissue damage or following the administration of a number of pharmaceutical agents (Table III). B cell precursors of autoantibody producing cells can be detected in normal individuals by their capacity to bind the specific autoantigen by means of their surface membrane immunoglobulin receptors. Both thyroglobulin binding-B cells and DNA-binding B cells have been found in normal humans. Finally, autoantibody production can be stimulated in vitro by culturing normal human lymphocytes with various polyclonal B cell activators. Thus, stimulation of human lymphocytes with either pokeweed mitogen or Epstein-Barr virus has been observed to result in production of anti-immunoglobulin autoantibodies (rheumatoid factor) as part of the polyclonal response. All of these experimental results indicate that precursors of self-reactive B cells reside within normal animals.

The clonal deletion theory would predict that self-reactive lymphocytes could exist within adults, but only if the relevant self-antigen was

Table III
Exogenous Factors Causing Autoimmunity in Normal Subjects

Clinical Condition	Autoantibody
Infection	
1. Bacteria	
a. Subacute bacterial endocarditis	RF
b. Syphilis	RF, anti-cardiolipin, anti-RBC (P)
c. β-hemolytic streptococci (A)	anti-myocardium
d. Leprosy	RF, ANA
e. Tuberculosis	RF, ANA
2. Mycoplasma	
a. M. pneumoniae	cold agglutinins (anti-I)
3. Parasites	
a. Schistosomiasis	RF, ANA, anti-collagen
b. Trypanosomiasis	RF
c. Leishmaniasis	RF
d. Malaria	RF, anti-cardiolipin
4. Viruses	
a. Epstein-Barr virus	RF, ANA, anti-lymphocyte, anti-smooth muscle, anti-I, anti-i anti-cardiolipin, anti-thyroglobulin anti-P
b. Cytomegalovirus	RF, ANA, anti-lymphocyte, anti-I anti-smooth muscle
c. Hepatitis B	RF, ANA, anti-lymphocyte
d. Herpes zoster	RF
e. Influenza A	RF
f. Rubella	RF
Drugs	
Procainamide	ANA, anti-lymphocyte
Hydralazine	ANA, anti-lymphocyte
Phenytoin	ANA, anti-lymphocyte
D-penicillamine	ANA, anti-acetylcholine receptor, anti-intercellular substance of epidermis
Tissue Damage	
Myocardial infarction	anti-myocardium
Inflammatory arthritis	anti-collagen

RF, rheumatoid factor; ANA, anti-nuclear antibody

sequestered away from the immune system. While many autoantigens, such as the lens, brain and spermatozoa, appear to be sequestered from the immune system, the observation that a number of autoantigens that were the target of autoimmune reactions, such as thyroglobulin, could be found in the circulating blood of normal neonates and adults refuted this idea.

Finally, the clonal deletion theory postulated that autoreactive cells might arise in the adult as a result of somatic mutation of immunocompetent cells with the production of a "forbidden clone" of autoreactive cells. One prediction of this somatic mutation theory is that the autoantibody produced would all be derived from cells that had arisen from one mutant cell in the adult and therefore should be monoclonal. In fact, the anti-erythrocyte (anti-I) antibody that causes the idiopathic cold agglutinin syndrome has been shown to be monoclonal. However, in a number of autoimmune diseases in man and experimental animals, autoantibodies have been shown to be heterogeneous both with regard to the determinant (epitope) on the autoantigen recognized and the immunoglobulin class (isotype) and the antigen combining region (idiotype) of the autoantibody. This indicates that autoimmune responses tend to be polyclonal in nature. A second prediction of the "forbidden clone" theory is that the autoantibodies found in different individuals are unlikely to have the same idiotype if the clones of auto-reactive cells arose by random somatic mutation. This prediction is based upon the fact that there is a tremendous degree of potential heterogeneity in the variable regions of the heavy and light chains of the immunoglobulin molecule that can combine with different antigenic determinants present on a complex antigen. Therefore, idiotypic cross reactions between antibodies directed at the same antigen, but obtained from different members of the same outbred species, such man, are rare. Indeed, there is considerable diversity in the anti-thyroglobulin response in patients with Hashimoto's thyroiditis where no cross-reacting idiotypes between individual patients have been demonstrated. On the other hand, a relatively high incidence of shared idiotypes has been observed among patients with other autoimmune diseases. Thus cross-reacting idiotypes have been found on human cold-agglutinins, rheumatoid factors and anti-acetylcholine receptor antibodies. These results cast doubt that somatic mutation with the emergence of unique "forbidden clones" explains the development of autoimmunity.

Alternatives to Clonal Deletion

These observations have cast doubt on the overall validity of the clonal deletion hypothesis as the only explanation for self non-self discrimination. Despite this, it seems reasonable to conclude that some form of clonal inactivation does play a role in the prevention of autoimmunity. Evidence for this includes the finding that the number of autoantibodies found is much smaller than the potential number of autoantigens implying that some deletion of reactivities has occurred. Even in situations when autoantibodies are formed to self-antigens, the number of determinants recognized appears to be quite restricted. For example, human autoantibodies to thyroglobulin recognize a much smaller number of determinants on the thyroglobulin molecule than antibodies found in the serum of rabbits immunized with human thyroglobulin. Thus, human autoantibodies react with only two or three epitopes on thyroglobulin, whereas rabbits immunized with human thyroglobulin make antibodies to more than forty determinants. This finding implies that the autoimmune response is greatly restricted compared to the response of a heterologous species reacting to the same molecule as a foreign antigen, and suggests that functional deletion of part of the self-reactive repertoire has occurred. Despite such restrictions on autoresponsiveness, it remains clear that precursors of self-reactive clones persist in normal animals.

A number of additional mechanisms may explain the finding that some self-reactive B cell precursors escape inactivation. The concentration of the autoantigen may be too low to cause B cell deletion. Alternatively, the avidity of the receptor on the lymphocyte for the autoantigen may be the critical determinant of the fate of immature autoreactive cells. Support for this suggestion comes from the findings that immature B cells are more easily tolerized by multivalent than by mono- or paucivalent antigens and high avidity clones of immature B cells are more effectively tolerized than low avidity clones. This may explain why the majority of autoantibodies exhibit low avidity binding to their specific autoantigens. Despite the deletion of high avidity clones of autoreactive cells, additional mechanisms appear to be essential to prevent the development of activation of the autoreactive lymphocytes that persist in normal adults.

Insight into the mechanisms that might prevent activation of self-reactive lymphocytes developed as additional knowledge became available about the organization of the immune system. It became apparent from the work of Claman, Good, Mitchinson and Miller that the immune system can be broadly divided into two classes of lymphocytes, B lymphocytes whose progeny secrete antibody and T lymphocytes that are responsible for the varied manifestations of delayed-type hypersensitivity. Moreover, subpopulations of T lymphocytes were found to play a necessary helper role in the induction of antibody responses to a number of antigens. The observation by Weigle that T lymphocytes could be more effectively tolerized than B cells and that T cell tolerance was more long-lived and required low doses of antigen for its induction suggested that the lack of activation of self-reactive B cells could result from an inadequacy of T cell help. A number of findings support this point of view. First, antibody production to a number of autoantigens such as thyroglobulin, erythrocyte antigens, acetylcholine receptors, water soluble antigen extracted from liver, and perhaps others requires helper T cell activity. Secondly, autoimmunity can be regularly induced by immunizing animals with self-antigens that had been modified so as to stimulate helper T cells that could then cooperate with autoreactive B cells.

A number of recent experimental protocols have suggested that some antigen specific helper T cells may persist in normal adults. These have been documented for both thyroglobulin and collagen. However, further delineation of the organization of the immune system disclosed additional mechanisms that could prevent the development of autoimmunity. Thus, the demonstration by Gershon of the immunomodulatory role of suppressor T lymphocytes and the aforementioned finding of the immunoregulatory role of the idiotype-anti-idiotype network suggested that these might act to prevent the development of autoimmunity. As a result, prevention of autoimmune responses need not involve total elimination of all self-reactive clones of lymphocytes, as long as immunoregulatory mechanisms exist to suppress potential self-reactivity.

The Development of Autoimmunity

Three general processes are thought to be involved in limiting the activity of the potentially autoreactive cells that persist in adults. These include: 1) sequestration of self-antigens such that they are excluded from the immune system, 2) unresponsiveness or inadequacy of helper T cell activity, and 3) limitation of potential reactivity by

suppressive mechanisms. Derangements of these normal processes may predispose to the development of autoimmunity in various circumstances. Thus, autoreactivity could result from 1) alterations in self-antigens, 2) stimulation of the immune system leading to the generation of augmented T cell help or to direct activation of B cells, or 3) an inadequacy of normal immunoregulatory influences. One or more than one of these mechanisms may be important in the generation of autoimmunity in any one situation.

1) Alterations in self-antigens

a. Altered availability of self-antigens

Although a number of antigens such as thyroglobulin that formerly had been considered to be sequestered from the immune system have been found to circulate, there does appear to be effective segregation of some antigens from the immune system. For example, sperm and the lens and ciliary body of the eye contain antigens that can stimulate an immune response when local inflammation or injury allows them to enter the general circulation. Similarly, myelin basic protein of the central nervous system is a protein that develops late in ontogeny and is segregated from the immune system. Immunization with this material leads to the development of encephalitis.

b. Exposure to cross-reacting exogenous antigens

A second mechanism for the development of autoimmunity involves exposure of the organism to exogenous antigens that stimulate the production of antibody or antigen-primed lymphocytes which cross-react with self-antigens. A number of examples of organ specific autoimmune disease, such as the encephalomyelitis that develops after immunization with Pasteur-type rabies vaccine appear to involve such a mechanism. Similarly, immunization with thyroglobulin obtained from a closely related species induces autoimmunity to autologous thyroglobulin.

c. Altered immunogenicity of self-antigens

Alteration of self-antigens may also predispose to the development of autoimmune responses. Drug interactions, viral infections or partial digestion or denaturation resulting from inflammation or senescence have been implicated as possible mechanisms by which self-antigens could be altered and thus rendered immunogenic. It has been suggested that generation of autoimmunity in this situation may not be pathologic but rather the expression of a normal mechanism designed to facilitate the disposal of damaged body constituents. Notwithstanding this potentially important physiologic process, it is clear that exposure to partially digested autologous tissue can lead to the development of autoimmune disease under certain circumstances. A number of examples of experimental autoimmune disease elicited by immunizing normal animals with altered self-antigens have been documented. Thus, thyroiditis can be induced by immunizing animals with papain-digested thyroglobulin, leukocyte-enzyme-digested thyroglobulin or haptenated thyroglobulin. Similarly, hapten-coupled erythrocytes stimulate the production of Coombs-positivity and acetylated renin induces anti-renin antibodies. Although the mechanism involved has not been clearly elucidated in each of these situations, stimulation of helper T cells by the altered self-antigen that can then cooperate with autoreactive B cells or effector T cell precursors appears to be a

likely mechanism. The relevance of these models is called into question, however, by the clinical finding that radioiodine destruction of the thyroid in man does not induce autoimmunity.

2) Stimulation of the immune system

A number of models have explored the capacity of stimulation of the immune system to predispose to the development of autoimmunity. One example of the role of such immunostimulation is provided by the effect of adjuvants on the production of autoimmunity. Autoantigens become much more immunogenic when administered with complete Freund's adjuvant or bacterial endotoxin. Moreover, autoantibody formation is frequently seen as a result of the adjuvant-like effect of a variety of infectious processes.

a. Augmented T cell help

One mechanism precluding the development of autoimmunity in normal individuals appears to be the absence of sufficient helper T cell activity to support such responses. It is possible that non-specifically activated T cells could by-pass the need for specific autoreactive helper T cells with the resultant generation of autoimmune responses. One experimental design used to test this hypothesis has been the graft versus host reaction. In this situation, allogeneic lymphocytes are injected into a recipient animal. When appropriate combinations of animals are used, the T lymphocytes of the donor respond to the histocompatibility antigens of the host. One result of this stimulation is that B lymphocytes of the recipient are induced to proliferate as a result of signals from the activated donor T cells. It is known that this non-specific T cell stimulation can lead to the production of antibodies that normally require specific T cell help. During graft versus host reactions, a number of auto-antibodies are formed, including anti-nuclear, anti-erythrocyte (Coombs positive) and anti-lymphocyte antibodies. The antibodies produced have been shown to be true autoantibodies in that they are produced by recipient cells and react against recipient tissues. In addition to the induction of autoantibodies, true autoimmune diseases develop in animals undergoing chronic graft versus host reactions. These include autoimmune hemolytic anemia and immune complex-mediated glomerulonephritis. The complexes eluted from the kidneys of such animals contain IgG anti-lymphocyte and anti-erythrocyte antibodies and fragments of red cell and lymphocyte surface antigens. In the related model of homologous disease of the rat, Stastny, Stembridge and Ziff pointed out the development of lesions in the skin, joints, and heart that had similarities to those found in systemic lupus erythematosus and scleroderma. Lesions resembling those of scleroderma and Sjogren's syndrome have recently been described in human bone marrow allograft recipients experiencing chronic graft versus host disease.

b. Direct B cell stimulation

Recent evidence indicates that a variety of materials can directly stimulate B cells to differentiate into antibody secreting cells. These agents stimulate multiple clones of antibody forming cell precursors irrespective of their genetic commitment to secrete specific antibodies and, hence, are referred to as polyclonal B cell activators. A variety of reagents function as polyclonal B cell activators, including bacterial lipopolysaccharide, staphylococcal aureus protein A, Epstein-Barr virus, pokeweed mitogen and a host of other bacterial products, enzymes and miscellaneous reagents. Since autoreactive B cell precursors

exist in normal man, stimulation with polyclonal B cell activators could well result in the development of autoimmunity. It has been reported that administration of bacterial lipopolysaccharide or other polyclonal B cell activators to normal mice leads to the production of anti-DNA antibodies, IgM rheumatoid factor, anti-thymocyte antibodies, and antibodies to autologous erythrocytes and autologous serum albumin. Similarly, mice infected with Trypanosoma brucei develop autoimmune responses to DNA, autologous erythrocytes and thymocytes. In man, in vitro stimulation with pokeweed mitogen, a T cell dependent polyclonal B cell activator, and Epstein-Barr virus, a T cell independent polyclonal B cell activator, has been shown to lead to the production of IgM rheumatoid factor by peripheral blood lymphocytes obtained from normals and from patients with rheumatoid arthritis. Similarly, in vitro stimulation with pokeweed mitogen has been shown to lead to the production of antibodies directed against acetylcholine receptors.

3) Deficiency of normal immunoregulatory influences

a. Suppressor T cell defects

It has become apparent that besides their role as helper cells and effector cells in delayed-type hypersensitivity reactions, T cells also contain a subpopulation of cells that function as active suppressors of immune responses. The possible role of these cells in preventing autoimmune reactions has stimulated an enormous amount of investigation and it now seems reasonably clear that abnormalities in suppressor T cell function can predispose to the development of autoimmunity in certain situations.

A number of experimental models have clearly suggested that suppressor cells play a central role in preventing the development of autoimmunity. For example, suppressor T cells triggered by a specific epitope on the myelin basic protein molecule can prevent the development of experimental allergic encephalomyelitis stimulated by a second epitope on the same molecule. Similarly, antigen specific suppressor T cells can be generated by appropriate administration of soluble thyroid extracts that prevent the development of experimental thyroiditis in mice or spontaneous thyroditis in Buffalo rats or obese chickens.

Additional supporting evidence for the role of suppressor cells in preventing autoimmunity comes from two sorts of experiments. First, neonatal thymectomy, treatment with anti-lymphocyte serum or low dose irradiation have been shown to accelerate a number of spontaneously-occurring autoimmune diseases in laboratory animals including the systemic lupus erythematosus-like syndrome of New Zealand mice and autoimmune thyroiditis of obese chickens and Buffalo rats. Neonatal thymectomy may, in addition precipitate autoimmunity in otherwise normal strains of experimental animals. These results are said to relate to selective removal of suppressor T cells, although other explanations such as non-specific adjuvant-like effects of the various treatments have not be rigorously ruled out. Secondly, a number of spontaneous autoimmune diseases of laboratory animals have been shown to be retarded by administration of normal T lymphocytes.

In man, the investigation of the role of suppressor cell abnormalities in autoimmune disease has involved an examination of non-specific suppressor T cell function with attempts to correlate abnormalities

with manifestations of autoimmunity or autoimmune disease activity. Abnormalities have been detected in a number of diseases including systemic lupus erythematosus. This work is somewhat unsatisfying, however, because of the inability to establish a clear link between the suppressor cell defect and the development of autoimmunity. It is not known, for example, whether the suppressor cell activity measured actually regulates autoantibody production. Moreover, it is not clear whether the defects measured are the cause of the self-reactivity, the result of the autoimmune reaction or a concomitant but not essential feature of the process.

Two aspects of the suppressor cell defects that characterize systemic lupus erythematosus deserve mention in this regard. First, suppressor cell abnormalities, in general tend to vary with disease activity, with diminished suppressor cell function and number found during active disease and a reversion toward normal during periods when the disease is quiescent. This finding suggests that generalized suppressor cell dysfunction is not a primary event in the autoimmune process. Secondly, evidence has been presented that anti-lymphocyte antibody, which is characteristically produced by lupus patients, may cause suppressor T cell dysfunction. This again supports the conclusion that suppressor cell abnormalities may not be the primary abnormality in systemic lupus erythematosus.

Regardless of the lack of conclusive evidence in man, suppressor T cells clearly play an important role in maintaining unresponsiveness to self-antigens in a number of animal models and therefore abnormalities in their function could be expected to predispose to the development of autoimmunity.

b. Defective idiotype control

As mentioned above, the antigen-combining region of an antibody molecule (idiotype) can act as an autoantigen eliciting the production of autoanti-idiotype antibodies, which are thought to function as immunoregulatory molecules. One possible mechanism to explain the unrestricted production of autoantibodies, therefore, could involve defective production of specific autoanti-idiotype antibodies. Abdou and colleagues have recently presented evidence that patients with active systemic lupus erythematosus lack autoanti-idiotype antibodies direct against anti-DNA antibodies. Such autoanti-idiotypes are routinely detected in the serum of patients with inactive lupus and of normals who have had close contact with lupus patients. These investigators, furthermore, have found that this autoanti-idiotype antibody is capable of mediating complement-dependent lysis of DNA-binding B cells. These results suggest the possibility that defects in idiotype control may predispose to the development of autoimmunity. This hypothesis is especially intriguing because it relates the excessive production of harmful autoantibodies to a deficiency in the elaboration of normal immunoregulatory autoantibodies.

One or a combination of these mechanisms may be involved in the development of different human autoimmune diseases. Table IV indicates diseases in which these various precipitating factors may pertain. It is clear however that the etiologic mechanism in most autoimmune disease is

unknown.

Table IV
Suggested Mechanisms for the Development
of Human Autoimmunity

I. Alterations in self-antigens
   A. Altered availability
      1. Sympathetic ophthalmia
      2. Anti-sperm antibody formation following vasectomy
   B. Exposure to cross reacting exogenous antigens
      1. Post-rabies vaccine encephalitis
      2. Acute rheumatic fever
   C. Altered immunogenicity
      1. Drugs
         - α-methyldopa-induced Coombs positivity
      2. Virus infections
         - ? Insulin-dependent diabetes mellitus
      3. Inflammation
         - anti-collagen antibodies

II. Stimulation of the immune system
   A. T cell activation
      1.
   B. B cell activation
      1. Epstein Barr virus infection
      2. African trypanosomiasis

III. Defective immunoregulation
   A. Depressed suppressor T cell activity
      1. Systemic lupus erythematosus
      2. Autoimmune hemolytic anemia
   B. Defective idiotype control
      1. Systemic lupus erythematosus

Immunopathogenic mechanisms in autoimmune diseases

A variety of mechanisms may cause tissue injury in autoimmune disease. These have largely been documented in experimental animals, but comparable examples have been found in man. It should be emphasized that nearly all the examples of human autoimmune disease appear to be caused by autoantibodies. Although experimental allergic encephalomyelitis in guinea pigs and rats appears to be mediated by myelin basic protein responsive T lymphocytes, and not by autoantibody, no autoimmune disease in man has been convincingly demonstrated to be caused by autoreactive T cells. There is some evidence to suggest that polymyositis may be mediated by T cells reacting against autoantigens on muscle cells, but this requires additional verification. The lack of autoimmune disease caused by self-reactive T cells may relate to the greater difficulty of

proving that a pathologic lesion is caused by delayed-type hypersensitivity as opposed to antibody. On the other hand, the evidence that T cell but not B cell reactivity to many self-antigens is deficient and that autoimmunity is easily induced by techniques that by-pass the need for specific self-reactive T cells predicts that T cell-mediated autoimmune diseases should be rare. The T cell basis for experimental allergic encephalomyelitis may be explained by the sequestered nature of the antigen.

A number of antibody-mediated mechanisms may cause autoimmune disease. (Table V)

Table V
Mechanisms of Antibody-Mediated Tissue Damage in Autoimmune Disease

Mechanism	Example: Autoantigen	Example: Disease
Receptor Binding		
1. Receptor loss	Acetylcholine receptor	Myasthenia gravis
2. Receptor stimulation	TSH receptor	Graves' disease
3. Receptor blockade	Insulin receptor	Kahn type B syndrome
Blocking or Neutralization	Intrinsic factor	Pernicious anemia
	C3 convertase (C3bBb)	Hypocomplementemic nephritis
Stimulation	Intercellular substance of epidermis	Pemphigus vulgaris
Complement activation	Glomerular basement membrane	Goodpasture's syndrome
	Intercellular substance of epidermis	Pemphigus vulgaris
	Erythrocyte (anti-I,anti-i)	Cold agglutin-mediated hemolytic anemia
Opsonization	Erythrocyte	Autoimmune hemolytic anemia
	Platelet	Autoimmune thrombocytopenia
Immune complex deposition	DNA, RNP, IgG nucleoprotein, lymphocyte membrane	Systemic lupus erythematosus

a. Receptor Binding

Three distinct clinical entities have been shown to be related to autoantibodies directed at cellular receptors. These are myasthenia gravis, Graves' disease and the Kahn type B syndrome of insulin resistance and acanthosis nigricans. A possible role for autoantibodies directed at β_2 adrenergic receptors in allergic rhinitis and asthma has also recently been reported.

In myasthenia gravis, autoantibodies are directed against the nicotinic acetylcholine receptor of skeletal muscle. The unique specificity of these autoantibodies is indicated by the finding that they do not recognize the muscarinic acetylcholine receptors of cardiac or smooth muscle. Most of these antibodies do not actually recognize the acetylcholine binding site and do not alter receptor function. Rather, they bind to adjacent sites on the receptor molecule and lead to an actual loss of receptors. The post-synaptic membrane of myasthenics contains about one-third the normal number of receptors and many of the remaining receptors have antibody bound to them. The loss of receptors is mediated by two separate mechanisms. The first involves complement-mediated destruction of the receptors. The second mechanism appears to be independent of complement and results from antibody-mediated cross-linking and subsequent ingestion of the receptor by the muscle cells with digestion of the receptor within secondary lysosomes. Since the rate of de novo synthesis of new acetylcholine receptors is not increased, the net result is a diminution in the number of receptors available on the post-synaptic membrane.

In Graves' disease, serum antibodies to the receptor for thyroid stimulating hormone (TSH) are found. In contrast to the anti-acetylcholine receptor antibodies found in patients with myasthenia gravis, the autoantibodies found in patients with Graves' disease competitively inhibit the binding of TSH and, in addition, activate the receptor. The result is clinical hyperthyroidism.

Patients with the Kahn type B syndrome of insulin resistance and acanthosis nigricans have antibodies to insulin receptors. These antibodies appear to bind at or near the site on the receptor for insulin binding and thus inhibit insulin binding. In some cases these antibodies act as partial agonists.

More recently, antibodies to β_2-adrenergic receptors have been identified in one patient with allergic rhinitis and in two patients with asthma. These antibodies appear to be directed at a determinant in or near the ligand binding site on β_2 receptors since they inhibit agonist binding. This autoantibody is specific for β_2-adrenergic receptors since it does not inhibit ligand binding by heart β_1 adrenergic receptors.

The mechanisms for the development of these autoantibodies may include any of the above described possibilites. One additional possibility deserves mention. This is based upon the observation that anti-idiotype antibodies raised against antibodies directed at insulin or β-adrenergic receptors have the capacity to mimic the activity of the original ligand. Thus anti-idiotype antibodies against antibodies to insulin inhibit the binding of insulin to adipocytes and, in addition, stimulate metabolic activity of cells in a manner comparable to insulin. Similarly, anti-idiotype antibodies directed at antibodies to β agonists specifically inhibit binding of β agonists and stimulate adenylate cyclase activity. These results suggest the possibility that autoanti-receptor antibodies in some syndromes may reflect an abnormal production of an anti-idiotype antibody through as yet unexplained mechanisms. Since these diseases are often associated with the production of multiple autoantibodies, the possibility exists that cross reacting anti-idiotypes and not anti-idiotypes induced specifically by the anti-ligand antibody may be functioning as anti-receptors.

b. Blocking or neutralization

A second antibody mediated mechanism of autoimmune disease involves blocking of function or neutralization. A example of this is pernicious anemia in which an autoantibody against intrinsic factor may exert either of two activities. The antibody may bind to intrinsic factor at or near the site of vitamin B_{12} binding and inhibit the formation of B_{12}-intrinic factor complexes. Alternatively, antibody may bind to intrinsic factor at a site distant from the vitamin-binding site but block the absorportion of the vitamin B_{12}-intrinsic factor complex in the distal ileum. A second example of this immunopathogenic mechanism is that of C3 nephritogenic factor found in patients with hypocomplementemic nephritis, systemic lupus erythematosus and partial lipodystrophy. This autoantibody reacts with a C3 convertase (C3bBb) involved in the amplification loop of complement activation. Such interaction stabilizes the C3 convertase and prolongs it biological half-life significantly, thus causing increased activation of C3.

c. Stimulation of protease activity

Recently, an additional mechanism of antibody-mediated tissue damage has been reported by Lazarus and co-workers who were examining the antibody against the intercellular substance of the epidermis characteristic of patients with pemphigus. These antibodies have been shown to stimulate cultured epidermal cells to release a protease that can dissolve the intercellular substance resulting in acantholysis. This epidermal cell activation occurs in the absence of complement.

d. Other antibody-mediated mechanisms

Other antibody-mediated mechanisms of tissue damage have also been implicated in the pathogenesis of various autoimmune diseases. Complement-mediated damage to basement membrane appears to be the most likely cause of tissue injury in Goodpasture's syndrome, a disease caused by the production of antibodies to glomerular basement membranes. Similarly, some of the blister formation in pemphigus appears to be caused by autoantibody mediated complement activation, leading to a loss of cohesion of epidermal cells. Finally, hemolysis in cold-agglutinin-mediated hemolytic anemia appears to involve complement-mediated lysis of antibody coated red cells.

In a number of conditions such as autoimmune thrombocytopenic purpura or autoimmune hemolytic anemia, autoantibodies appear to function primarily as opsonsins, promoting the uptake of platelets or erythrocytes by macrophages in liver and spleen.

Many of the clinical features of systemic lupus erythematosus appear to be caused by the deposition of immune complexes. This has been documented for the glomerulonephritis that is a well-recognized feature of this disease. Of importance, the immune complexes deposited in the kidney of these patients contain autoantibodies and autoantigens. Autoantibodies against native-DNA, single-stranded DNA, ribonucleoproteins, nucleoproteins, immunoglobulin G and lymphocyte membrane determinants have been demonstrated in the immune complexes eluted from the kidneys of patients with lupus nephritis.

Other antibody-mediated processes such as antibody-dependent cellular cytotoxicity have been suggested as contributing to the pathogenesis of

Hashimoto's thyroiditis and autoimmune hemolytic anemia, but the evidence remains circumstantial.

Autoimmunity versus autoimmune disease

The presence or absence of pathological consequences resulting from self-reactivity determines whether autoimmunity leads to the development of an autoimmune disease. The essential feature of an autoimmune disease is the demonstration that tissue injury is caused by the immunological reaction of the organism with its own tissues. Autoimmunity on the other hand refers merely to the presence of antibody or lymphocytes that reacts with self-antigens and does not necessarily imply that the development of self-reactivity has pathogenic significance.

Autoimmunity is far more common than the development of an autoimmune disease. In a survey of 3492 adult subjects living in a rural Australian town, autoantibodies of one form or another were found in 21.6% with a prevalence in females of 27.5% and in males of 13.7%. The prevalence increased with the age of the subjects varying from 10.7% in 21 to 30 year olds to 33.5% in individuals 71 to 75 years of age.

Autoimmunity may be seen as an isolated event or in the setting of specific clinical syndromes. For example, autoreactivity may develop during various infectious or inflammatory conditions. The expression of autoimmunity may be self-limited as occurs following a number of infectious processes or persistent as is seen in old age. In both circumstances there is a tendency to develop autoreactivity directed against a variety of different tissue or organs. The presence of autoimmunity however, does not necessarily mean that the observed self-reactivity is the cause of tissue damage. Even in the presence of organ pathology, it can be difficult to determine whether the presence of autoimmunity bespeaks an autoimmune disease.

Although autoimmune mechanisms have been implicated in a wide range of disorders, identification of the specific roles played by immune processes in the pathogenesis has been difficult. The characteristics of an autoimmune response that determine whether it will lead to an autoimmune disease have not been rigorously investigated. A number of points can be made however. The first involves the persistence of the autoimmune response. Autoimmune disease appears to require a sustained response. Brief bursts of autoimmunity such as occur after various infections or after the administration of a polyclonal B cell activator such as bacterial endotoxin to animals rarely results in the development of an autoimmune disease. However, many individuals have lifelong evidence of autoimmunity but never develop an autoimmune disease.

a. Characteristics of the autoantibody

Characteristics of the autoantibody such as avidity, capacity to cross-link receptors, complement fixing capability and isotype appear to play a role in determining whether autoimmune disease will result.

When autoimmunity generates immune complexes, the critical feature that determines their pathogenicity is size. Large complexes (> 19s) are rapidly cleared from the circulation and appear to cause

little tissue pathology. Smaller complexes persist in the circulation for a longer period of time and have the capacity to be deposited in vessel walls, activate complement and trigger inflammation. Gallo and co-workers have recently shown that the charge of the circulating complex may also be a factor in determining its tissue localization. Thus, cationic but not anionic or neutrally-charged immune complexes have been shown to be localized preferentially in the subendothelial or subepithelial region of the glomerular basement membrane. The nature of the antigen in the immune complex may also contribute to its potential pathogenicity. For example, DNA binds directly to the collagen of glomerular basement membrane. This may provide a means to target DNA-containing complexes to the kidney. In addition, free DNA may directly bind to the glomerular basement membrane and set up a nidus for the development of in situ immune complexes with the capacity to activate complement and initiate inflammation.

A critical determinant of whether autoimmunity will lead to an autoimmune disease appears to be the nature of the epitope recognized by the autoantibody. Complex autoantigens may have multiple determinants (epitopes), each of which may stimulate the production of autoantibodies. This has been shown for a number of autoantigens including myelin basic protein, acetylcholine receptors, insulin receptors and thyroglobulin. The tests currently used clinically examine for autoantibodies only by measuring reactivity directed at crude preparations of intact autoantigen. The presence of unique antibodies directed at individual epitopes which may be of pathogenic significance therefore cannot be determined. Indeed, since identical epitopes may exist on a number of apparently unrelated autoantigens, the use of such crude techniques may give the impression that multiple autoantibodies exist when only a single reactivity is present. A good example of this is demonstrated by the recent work of Stollar and co-workers. These investigators produced hybridomas that secreted monoclonal anti-DNA antibodies. These were formed by fusing plasmacytoma cells with spleens cells of MRL/1 mice, a strain that spontaneously develops systemic lupus erythematosus. It was found that some of these monoclonal anti-DNA antibodies actually reacted with the diester-linked phosphate groups in the phosphate-sugar backbone of the nucleic acid. These antibodies were also found to bind to similar structures on a number of naturally-occurring phospholipids. As a result of this latter activity, the antibody reacted with cardiolipin and prolonged the activated partial thromboplastin time presumably by binding to the phospholipid (Thrombofax) used in the test system to substitute for the in vivo role of platelets. Thus, a single monoclonal autoantibody can produce a number of the serological features of systemic lupus erythematosus, including reactions with native DNA and other polynucleotides, reactions with cardiolipin (biologic false positive serologic test for syphilis) and anticoagulant activity characteristic of a lupus anticoagulant.

Athough animals may generate autoreactivity against a number of epitopes on an autoantigen, there is evidence from a number of experimental models and human systems to suggest that reactivity against only one or a few of these epitopes may be capable of mediating autoimmune disease. The other antibodies may not be pathogenic and in fact may protect the animal from the pathogenic antibody. Since the tests currently used clinically are, in general, unable to distinguish between these various antibodies,

it is not possible to identify patients with autoimmunity at risk to develop autoimmune disease.

In experimental animals, evidence for the role of specific anti-epitopes in the generation of autoimmune disease comes from the study of experimental allergic encephalomyelitis, a disease caused by immunization with myelin basic protein. This small molecule (molecular weight of 18,000 daltons) contains at least eight distinct determinants that induce delayed-type hypersensitivity and three that stimulate antibody production. Only a small segment of the molecule is responsible for inducing encephalitis; the encephalitogenic fragment is distinct from the other segments that induce delayed-type hypersensitivity and humoral immune responses. An additional segment of the protein can induce unresponsiveness and prevent the development of encephalitis when given to the animal before immunization with the intact molecule. Prevention of encephalitis results from the stimulation of specific suppressor T cells. Therefore, the lack of responsiveness to this "sequestered antigen" in normal adults may actually represent active stimulation of specific suppressor cells resulting from slow release of this tolerogenic determinant from the central nervous system. These results obtained with a reasonably simple autoantigen emphasize the complexities inherent in analyzing the mechanisms involved in the development of autoimmunity. Moreover, they indicate the importance of analyzing the response to individual epitopes in understanding the relationship of autoimmunity to autoimmune disease.

In man, evidence of the importance of epitope analysis in understanding the pathogenesis of autoimmune disease comes from the study of patients with myasthenia gravis. Although 90% of the patients have autoantibodies to acetylcholine receptors, the total concentration of antibody in the blood does not predict the severity of muscle weakness. There is, however, a clear relationship between muscle strength and autoantibody titers within individual patients. Moreover, there is a better correlation of strength with anti-acetylcholine receptor antibody titers between individuals when the ability of the autoantibody to accelerate acetylcholine receptor degradation in muscle culture is taken into account. These results support the idea that a spectrum of autoanti-acetylcholine receptor antibodies is produced in patients with myasthenia gravis, only some of which are important in inducing muscle weakness.

b. Genetics of the host

It has become clear from the work of a number of investigators that the capacity of animals to respond to a variety of antigens is controlled by immune response genes encoded within the major histocompatibility complex of the animal. Recent work in laboratory animals has indicated that responses to a variety of autoantigens including insulin, collagen, thyroglobulin, myelin basic protein and acetylcholine receptors is also controlled by these immune response genes.

This has stimulated considerable interest in the possibility that autoimmune disease in man is associated with the expression of certain

histocompatibility types. The existence of such genetic associations is further emphasized by the finding of an increased familial incidence of many diseases with autoimmune features including systemic lupus erythematosus, Graves' diseases, Hashimoto's thyroiditis and pernicious anemia. The familial incidence is even more striking when evidence of autoimmunity rather that overt autoimmune disease is examined.

A detailed discussion of the human major histocompatibility region (HLA-region) is beyond the scope of this discussion. The point to be made is that the HLA region of man consists of a number of major loci, of which the HLA-D and HLA-DR regions appear to be homologous to those containing immune response genes in laboratory animals. An association has been found between particular alleles of the HLA-D and DR antigens and various diseases with autoimmune features (Table VI).

Table VI

HLA-D or HLA-DR
Associations with Diseases Having
Autoimmune Features

I.	Diseases associated with HLA-D3 or DR3	
	a. Systemic lupus erythematosus	(6.5)*
	b. Primary Sjögrens syndrome	(19)
	c. Myasthenia gravis	(2.3)
	d. Graves' disease	(4.4)
	e. Hashimoto's thyroiditis	(3.5)
	f. Idiopathic Addison's disease	(8.8)
	g. Insulin-dependent diabetes mellitus	(3.8)
	h. Chronic active hepatitis	(2.2)
II.	Diseases associated with HLA-D4 or DR4	
	a. Seropositive rheumatoid arthritis	(5.0)
	b. Pemphigus	(3.2)
	c. Insulin-dependent diabetes mellitus	(6.4)
III.	Diseases associated with HLA-D2 or DR2	
	a. Systemic lupus erythematosus	(3.9)
	b. Autoimmune thrombocytopenia	(10)

*Estimated relative risk of an individual with the particular HLA-D or DR antigen having the disease.

The mechanisms leading to the HLA-D-associated susceptibility to autoimmune diseases are unknown at this time. The complex nature of the genetic control of autoimmune diseases is illustrated by animal models of autoimmune disease. One such model is the spontaneous autoimmune thyroiditis that develops in the obese strain of white leghorn chickens. The development of this disease has been shown to result from the coincidence in these animals of at least three distinct genetically controlled abnormalities. The first is a predisposition to make a vigorous response to thyroglobulin and is controlled by genes of the

major histocompatibility complex. The second abnormality involves disordered thymic maturation predisposing to precocious peripheralization of effector T cells. Finally, these animals have an abnormality of their thyroid glands which precedes the development of thyroiditis. This abnormality is characterized by an increased uptake of iodine in the face of large concentrations of thyroxine and an alteration in the nature of the iodinated products synthesized. These latter abnormalities are controlled by genes that are not associated with the major histocompatibility complex. The genetic control of the expression of autoimmune thyroiditis has been further examined in the murine model. In this model, thyroiditis is induced in normal mice by immunization with autologous thyroglobulin and the adjuvant, bacterial endotoxin. The development of experimental thyroditis in the mouse appears to be controlled by at least three separate genes in the major histocompatibility complex, with two controlling the magnitude of the immune response and a third modifying the severity of the disease in high responder mice. In addition, genes outside the major histocompatibility complex also appear to play a modifying role in the disease. These relatively simple experimental models of organ specific autoimmune disease emphasize the difficulty of analyzing the role of genetic factors in human autoimmune disease. In experimental models of systemic autoimmune disease such as the New Zealand mouse model of systemic lupus erythematosus, recent studies have indicated that the coincidence of at least six different genes, none of which is a generalized autoimmunity gene, is required to account for the varied manifestations of autoimmunity in this strain.

A number of other genetic characteristics appear to predispose to the development of autoimmunity in various situations. For example, Berman and Patrick found that susceptibility to paralysis of mice immunized with acetylcholine receptors was linked both to genes of the major histocompatibility locus and genes on an additional chromosome that control structural determinants on the constant region of the immunoglobulin heavy chains (allotypes). This latter genetic locus is closely linked to the region that contains structural genes for the variable region of immunoglobulin heavy chains. Of interest, the incidence of paralysis in murine experimental myasthenia gravis could not be predicted by the concentration of murine-specific anti-acetylcholine antibodies or the presence of antibodies that could increase the rate of acetylcholine receptor degradation but only by the histocompatibility type and allotype of the mouse. Recently, Nakao and co-workers have found an association between immunoglobulin allotypes and myasthenia gravis in Japanese.

An additional genetic marker that may predispose to the development of autoimmunity is acetylator phenotype. The capacity to metabolize drugs rapidly by means of n-acetyl-transferase is inherited as a dominant trait. Slow acetylators are more likely to develop either the procainamide or hydralazine-induced lupus syndrome. A recent report by Reidenberg and co-workers has suggested that the slow acetylator phenotype may also predispose to the development of idiopathic systemic lupus erythematosus.

Another genetic factor that appears to play a role in the development of autoimmune disease is sex with postpubescent females being more

likely to develop a number autoimmune diseases including for example, systemic lupus erythematosus, myasthenia gravis in young adults, Graves' disease and Hashimoto's thyroiditis. The interrelationship between sex, HLA type and autoimmune disease is indicated by the finding that myasthenia gravis is associated with the HLA-B8, D3 haplotype in young women but not when the disease presents in older men. The role of sex in autoimmune disease has been most thoroughly studied in systemic lupus erythematosus and the effect appears to be predominantly mediated by hormones. In SLE, there is a female preponderance when the disease presents after puberty and before menopause. There is an increased incidence of SLE in patients with the Klinefelter syndrome (XXY). Patients with lupus, both male and female, have abnormal estradiol metabolism as evidenced by an increased rate of 16 α hydroxylation of estradiol. Finally, castrastion and androgen treatment of female New Zealand mice prolongs life while castration of male mice accelerates the illness. Of interest, castration and androgen treatment of older female mice with established disease prolongs survival but does not affect autoantibody titers, suggesting that one effect of androgen is to inhibit to disease-inducing potential of the autoantibodies. Similar results have been seen when these mice are treated with prostaglandin E_1.

Autoimmunity verus autoimmune diseases

Manifestations of autoimmunity are found in association with a number of pathological conditions. However, the presence of the autoimmune phenomena does not necessarily imply that the morbid process is an autoimmune one. The presence of self-reactivity may be either the cause or the consequence of the pathological process, or merely an associated, but not essential feature of the disease state. An autoimmune disease is a pathological condition resulting from the activity of antibody or lymphocytes reacting with self-antigens. The mere presence of circulating autoantibodies, lymphocytic tissue infiltrates or circulating cells that can be stimulated by self-antigens is not sufficient for the diagnosis of an autoimmune disease. A number of attempts have been made to establish formal criteria for the diagnosis of autoimmune diseases. The first such attempt was made by Milgrom and Witebsky in 1962. Based upon Koch's postulates, they suggested five criteria for the diagnosis of an autoimmune disease.

Criteria for Definition of an Autoimmune Disease

1. Antibody or cellular immune reaction in patients with disease.
2. Specific antigen in tissue or organ involved in disease.
3. Production of antibody in experimental animals by immunization with the antigen.
4. Reproduction of the disease in an immunized experimental animal.
5. Passive transfer of the disease with serum or immunologically competent cells.

Although a number of autoimmune disease, such as Graves' disease, myasthenia gravis and Goodpasture's syndrome meet these criteria, they are unnecessarily restrictive in that they cannot be applied to systemic autoimmune diseases and they do not consider the possible pathogenic role of immune complexes. In addition, there is a great deal of reliance on animal models. The following is an attempt to formulate

new criteria for the diagnosis of autoimmune diseases. It should be viewed as tentative and to be used merely as a guide for thinking about the problem.

Table VII
Human Autoimmune Disease: Presumptive
Evidence For an Immunologic Pathogenesis

1. Absence of an exogenous etiology
2. Presence of autoantibody or evidence of cellular reactivity to self
3. Documentation of relevant autoantibody or lymphocytic infiltrate in the pathologic lesion
4. Demonstration that self-reactivity can cause the disease
   a. Passive transfer
   b. Relevant animal model
   c. Relevant effects demonstrated in vitro

Using these liberalized criteria, the list of diseases that can be documented to be autoimmune remains quite small as seen in Table VIII.

Table VIII
Autoimmune Diseases

I. Organ Specific
   a. Sympathetic ophthalmia
   b. Goodpasture's syndrome
   c. Graves' disease
   d. Myasthenia gravis
   e. Autoimmune thrombocytopenia
   f. Autoimmune hemolytic anemia
   g. Pemphigus vulgaris

II. Systemic
   a. Systemic lupus erythematosus

Most of the diseases included are organ specific autoimmune diseases in which the pathologic process involves only a single organ or tissue. One interesting feature of these diseases is the tendency for there to be overlap such that an individual with one specific autoimmune disease tends to develop multiple other manifestations of autoimmunity without necessarily the development of associated organ pathology. Thus, for example, patients with myasthenia gravis may develop anti-nuclear antibodies, anti-thyroid antibodies, rheumatoid factor, anti-lymphocyte antibodies and polyclonal hypergammaglobulinemia. This may relate to the association between the occurrence of the HLA-B8/DR3 haplotype and a number of these conditions, including myasthenia

gravis and Grave's disease.

A number of organ specific diseases with associated evidence of autoimmunity have been excluded from this list. Hashimoto's thyroiditis was excluded because of the lack of evidence that the autoantibodies present actually cause the pathologic lesions. Thus, it has not been possible to transfer the disease to monkeys with serum. Moreover, there is no neonatal thyroid damage in the offspring of affected mothers, although placental transfer of anti-thyroid antibodies has been demonstrated. Finally, serum from Hashimoto's patients has not been convincingly shown to be toxic for unaltered cultured thyroid cells. Similarly, pernicious anemia has been excluded because of the lack of evidence that the autoantibodies actually cause atrophic gastritis. This is emphasized by the occurrence of the disease in young adults with agammaglobulinemia.

Systemic autoimmune diseases differ from organ specific diseases in that pathologic lesions are found in multiple, diverse organs and tissues. Systemic lupus erythematosus represents the prototype of these disorders because of its protean manifestations of autoimmune-induced tissue pathology.

A number of the autoantibodies found in SLE have clearly been implicated in some of the pathologic features of the disease. This is not meant to imply either that production of the autoantibody is necessarily unique to SLE or that the presence of the autoantibody is always associated with tissue damage. As pointed out above, the relationship of autoantibodies and tissue damage is not completely understood. Classically, SLE has been thought of as a disorder in which immune complexes are the major pathogenic entity. While immune complex deposition appears to be the major pathogenic mechanism in lupus renal disease, additional autoimmune processes can be implicated in the etiology of other features of the disease. Table IX lists some of the clinical features of SLE and the autoantibodies involved.

Table IX
Immunopathogenic Mechanisms in
Systemic Lupus Erythematosus

Mechanism	Autoantibody	Pathologic consequence
Blocking or neutralization	Anti-lymphocyte	Depressed T lymphocyte function, Inhibition of suppressor T cell function
	Anti-coagulants	Inhibition of clotting
	Anti-neuron	CNS dysfunction
	Anti-neutrophil	Depressed phagocytosis
Opsonization	Anti-erythrocyte	Hemolytic anemia
	Anti-platelet	Thrombocytopenia
	Anti-lymphocyte	Lymphopenia
	Anti-neutrophil	Neutropenia
Immune Complex Deposition	Anti-DNA, anti-lymphocyte, anti-nucleoprotein, Anti-RNP, anti-IgG	Renal disease

A number of other systemic diseases characterized by autoimmune features have not been included. Acute rheumatic fever has been excluded because it remains unclear whether the organ pathology is caused by lymphocytes or antibody stimulated by the Streptococcus but cross-reacting with host tissues or alternatively recurrent immune responses to widely disseminated non-degradable immunogenic products of the Streptococcus. In the other systemic diseases with autoimmune features, such as rheumatoid arthritis, polymyositis, scleroderma and chronic active hepatitis, the role of autoimmunity in tissue pathology remains unclear. The evidence of the role of the hepatitis B virus or other viruses in the etiology of polyarteritis nodosa makes it unlikely that this is an autoimmune disease. It would seem reasonable to think of these conditions as diseases with associated autoimmune features rather than true autoimmune diseases. Additional information could warrant their reclassification.

Conclusion

After 80 years of research, a tremendous amount has been learned about immunoregulation. From our current vantage point we can agree with Ehrlich that "horror autotoxicus" is a guiding principle of immunobiology. The immune system of an animal is remarkable not because it avoids the development of autoimmunity, but because of the efficiency with which it usually avoids mounting harmful immune responses directed at self.

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GLOSSARY

Adjuvant - A compound capable of potentiating an immune response

Allele - One of two genes controlling a particular characteristic present at a genetic locus

Allotype - Structure found on an immunoglobulin molecule of different members of the same species. Each allotype is encoded at one genetic locus and inherited as an allele.

Antibody - An immunoglobulin that is produced in response to introduction of an antigen and which has the ability to combine with the antigen that stimulated its production.

Antibody-dependent cellular cytotoxicity - A form of lymphocyte-mediated cytotoxicity in which an effector cells lyses an antibody-coated target cell, by recognition of the Fc region of the cell-bound antibody through an Fc receptor present on the effector cell.

Antigen - A substance that can induce a detectable immune response when introduced into an animal.

Antigen-combining site - The part of an immunoglobulin molecule that binds antigen.

Avidity - The strength of binding of antibody and antigen molecules.

B-lymphocyte (B cell) - Lymphocytes that are the precursors of antibody secreting cells.

Cardiolipin - A phospholipid derived from beef heart that is used as the substrate to detect reaginic antibody.

Clonal selection - The idea that lymphocytes are preprogrammed to react with a specific antigen and that the reaction with the antigen induces proliferation (clonal expansion) and differentiation into mature antibody secreting cells or effector of cell mediated immunity.

Clone - A group of cells, all of which are progeny of a single cell.

Cold agglutinin - Antibodies that agglutinate erythrocytes more efficiently at temperatures below 37°C.

Coombs test - A technique for detecting red-cell bound immunoglobulin. In the direct Coombs test, red blood cells taken directly from a sensitized individual are agglutinated by anti-immunoglobulin antibodies. In the indirect Coombs test, a patient's serum is incubated with test red blood cells and the sensitized cells are then aggluinated with an anti-immunoglobulin antibody.

Cross reaction - The reaction of an antibody with an antigen other than the one that induced its formation.

Effector cell - T lymphocyte stimulated to mediate cytotoxicity, suppression or help.

Endotoxins - Lipopolysaccharides that are derived from the cell walls of Gram-negative microorganisms and have toxic and pyrogenic effects when injected in vivo. They also function as adjuvants and polyclonal B cell activators.

Epitope - The simplest antigenic determinant present on a complex antigenic molecule. Alternatively, the precise region of an antigen recognized by an antibody.

Fab - An antigen-binding fragment containing one intact light chain and part of one heavy chain produced by enzymatic digestion of an IgG molecule with papain.

$F(ab')_2$ - A fragment obtained by pepsin digestion of immunoglobulin molecules containing parts of 2 heavy chains and 2 intact light chains linked by disulfide bonds. It contains antigen-binding activity. A $F(ab')_2$ fragment and an Fc fragment comprise an entire immunoglobulin molecule.

Fc - A crystallizable fragment obtained by papain digestion of IgG molecules that consists of the carboxyl terminal half of 2 heavy chains. It contains no antigen-binding capability but determines the biological characteristics of the intact immunoglobulin.

Freund's complete antigen - A oil-water emulsion that contains killed Mycobacteria. It acts as an adjuvant and enhances immune responses to antigens incorporated in the emulsion.

Graft versus host reaction (GVH) - The clinical and pathological result of reactions of immunocompetent cells contained in a graft against the cells of a histoincompatible and immunodeficient recipient.

Hapten - A substance that is not immunogenic but can react with an antibody of appropriate specificity.

Helper T cells - A sub-set of T cells that cooperate with B cells in antibody formation.

HLA (human leukocyte antigen) region - The major histocompatibility complex in man located on chromosome 6.

Hypervariable regions - Regions of the variable region of the immunoglobulin molecule that have great variability of amino acid sequence when compared to other immunoglobulin molecules and form the antigen binding sites.

Ia antigens - Cell surface antigens encoded by the I region of the major histocompatibility complex.

Idiotype - A unique antigenic determinant present on a homogeneous antibody or myeloma protein. The idiotype represents the antigenic determinant of the antigen-combining site of an antibody.

Immune response (IR) genes - Genes located within the major histocompatibility complex that determine the capacity of an animal to respond to a number of T cell dependent antigens.

Immununogen - A substance that stimulates an immune response when introduced into an animal.

Immunoglobulin - A glycoprotein composed of two heavy and two light chains that functions as an antibody.

Immunoglobulin class - A subdivision of immunoglobulin molecules based on unique antigenic determinants in the Fc region of the heavy chains. The classes of immunoglobulin are IgM, IgG, IgD, IgA and IgE.

I region - The portion of the major histocompatibility complex that contains genes which control immune responses.

Major histocompatibility complex (MHC) - A group of genes in close proximity that encodes the histocompatibility antigens of members of a species. In man, the MHC is located on the short arm of chromosome 6.

Polyclonal B cell activator - Substance that can stimulate multiple clones of B cells to proliferate and differentiate into antibody secreting cells irrespective of their antigenic specificity.

Suppressor T cells - A sub-set of T lymphocytes that suppresses antibody synthesis by B cells or inhibits other cellular immune reactions by effector T cells.

T lymphocyte (T cell) - Thymus-derived lymphocyte that participates is a variety of cell-mediated immune reactions.

Thymus - The central lymphoid organ that controls the ontogeny of T lymphocytes.

Thymus-dependent antigen - Antigen that depends on T cell interaction with B cells for antibody synthesis.

Thymus-independent antigen - Antigen that can induce antibody formation without the participation of T cells.

Tolerance - Acquired inability to respond to a specific antigen.

Variable region (V region) - The amino terminal portion of the heavy or light chain of an immunoglobulin molecule containing considerable heterogeneity in amino acid sequence compared to the constant region.