Rheum - Immune

INTERNAL MEDICINE GRAND ROUNDS

AUTOANTIBODIES AND ASSOCIATED DISEASES

Peter Stastny, M.D.

The University of Texas Health Science Center at Dallas, Southwestern Medical School, Dallas, Texas

February 16, 1984

PREFACE

This is the third of a series of lectures devoted to autoimmunity and disease susceptibility associated with the MHC. The first dealt with human diseases associated with HLA-B27. In the second one, the topic was disease susceptibility related to HLA-D. The present lecture will attempt to perform the more difficult task of providing a perspective. Ostensibly, the focus is on certain interesting autoantibodies, however, much of the time will be devoted to an examination of how the immune system works and how it can go wrong. The medical literature is now available by telephone delivered to our home computers. Consequently, reviews are not valued for the number of references supplied. Rather, the reviewer's task, as always, is to provide a synthesis. If this is accomplished in some measure, I shall be grateful.

CONTENTS

1. AUTOIMMUNITY AND SELF-RECOGNITION

The ability to discriminate between self and nonself is fundamental to the existence of living organisms. Primitive metazoa like sponges have cell-surface recognition systems capable of identifying and destroying nonself (1,2). Thus, when two genetically identical sponges come in contact, the individuals fuse to form a single organism. Whereas, when two genetically dissimilar sponges are joined there is a reaction that leads to tissue destruction at the boundary between the two individuals (1,2). The surface structures recognized as nonself and the effector mechanisms that lead to destruction of the foreign tissue are still largely unknown. However, the resemblance to self/nonself recognition systems occurring in mammals is quite striking. It has been pointed out (3) that in both cases there are: a) cell-surface recognition structures, b) effector mechanisms that lead to destruction of nonself, and c) a high degree of polymorphism. There are more than 900 genetically distinct sponges that have the capacity to reject one another after apposition (1).

The self/nonself recognition systems of mammals are encoded by a chromosomal region called the major histocompatibility complex (MHC). In contrast to sponges, grafting of foreign tissues does not normally occur in mammals (with the exception of pregnancy). When grafting of tumors or skin was performed experimentally, however, it was found that rejection was due to the recognition

of cell surface structures, which were highly polymorphic and were capable of activating effector mechanisms which led to destruction of the foreign tissue (4). Whatever the meaning of the evolutionary history of this system might be, it is now clear that it regulates various aspects of the mammalian immune response. Beyond the requirement of discriminating between self and nonself (the allograft response) the ability of T cells to recognize all sorts of foreign antigens depends on the presence of self-MHC determinants on the surface of cells with which T cells interact. This will be discussed further in Section 7. It provides a new perspective to the concept of autoimmunity. Recognition of self by the cells of the immune system is now recognized as a normal activity and the term "autoimmunity", in this context, represents a physiologic state.

Table 1.

Self/Nonself Discrimination

Characteristics	Sponges	Mammals
Surface structures	?	MHC antigens
Polymorphism	High	High
Effects	Tissue destruction	Allograft rejection
Mechanism	?	Immune response

2. AUTOIMMUNE DISEASE

In 1900, Ehrlich and Morgenroth (5) injected goats with blood from other goats and looked for the development of hemolysins. These were regularly found and reacted with blood of many other

goats but never with that of the recipients. From these experiments Ehrlich recognized that "the organism has contrivances by means of which the immunity reaction, is prevented from reacting against the organism's own elements, and so give rise to autotoxins..." Although the nature of the internal regulating contrivances was not further understood, the term "horror autotoxicus", coined by Ehrlich, described the distinction between health and autoimmune disease for many years.

An explanation of how autoimmune reactions might be prevented was provided 50 years later by Burnet (6). He postulated that contact of antibody-forming cells with their respective antigens during fetal life leads to destruction or inactivation, with consequent elimination, of the corresponding clones. In this

Table 2.

Evolution of Ideas About Autoimmune Disease

Older Concepts	Present Concepts
Horror autotoxicus	Self-reactivity is normal
Forbidden clones, a qualitative abnormality	Immunoregulation and quantity of abnormal response may determine disease
Search for one main cause	Multifactorial etiology multiple genetic and environmental factors

Modified from (7)

manner self-tolerance was established and self-reactivity was avoided unless "forbidden clones" arose, either because their elimination was incomplete or by somatic mutation of lymphocytes. The progeny of such forbidden clones might be able to produce self-reacting antibodies.

The clonal selection theory, as will be discussed below, provides a widely accepted framework for understanding the working of the immune system, but the forbidden clone idea has been found difficult to fit with all the facts, as more was learned about normal and abnormal immune responses. It was found, for example, that B cells from normal persons, when stimulated by polyclonal activators made a variety of autoantibodies. This would indicate that indeed, B cells capable of making autoantibodies had not been eliminated. Furthermore, it is now known that the immune system is quantitatively regulated by a network of cells and humoral factors. Disordered regulation of the immune system and quantitatively excessive immune reactions are now believed to be associated with certain forms of autoimmune disease (7).

In many of the diseases we now consider to be the result of autoimmune reactions there is evidence that susceptibility is determined by multiple factors. Multiple genes appear to predispose to particular abnormalities, and environmental factors are required for triggering the development of disease manifestations.

3. ORGAN SPECIFIC AND NON-ORGAN SPECIFIC ANTIBODIES

The variety of self antigens (Tables 3 and 4) is enormous. It should be stressed though, that in many instances it is not at

Table 3.

Organ Specific Autoantibodies

Self Antigen	Disease
Cell membrane receptors	
Thyroid receptor	Graves' disease, thyroiditis
Acetylcholine receptor	Myasthenia gravis
Insulin receptor	Diabetes
Hormones	
Insulin	Diabetes
Thyroid hormones	Thyroiditis
Glucagon	Diabetes
Intrinsic factor	Pernicious anemia
Cell membranes	
RBC	Hemolytic anemia
Lymphocytes	SLE
Neutrophils	Neutropenia
Platelets	ITP
Extracellular	
Basement membranes	Goodpasture's, bullous pemphigoid
Intercellular substance	Pemphigus

all clear whether the autoantibody is a primary cause of disease, or merely a consequence of damage produced by another mechanism.

A convenient classification is to consider separately antibodies that react with antigens restricted to a single organ or tissue, the organ specific antibodies. I have included in this category

Table 4.

Non-Organic Specific Autoantibodies

Self Antigen	Disease
Intracellular	
Nucleolus	SLE, Sjogren's, Scleroderma
DNA	SLE
RNP	MCTD
Sm	SLE
Histones	SLE
Ribosomes	SLE
Mitochondria	Primary billary cirrhosis
Lysosomes	SLE
Microsomes	Thyroiditis
Melanosomes	Vitiligo
Filaments/tubules	SLE
Plasma proteins	
Immunoglobulin	RA
Complement components	SLE
Clotting factors	SLE

also antibodies reacting with certain hormones. A second group of autoantibodies is comprised of those that are not organ or tissue specific. One interesting feature of autoimmune reactions is that patients who tend to make organ-specific antibodies may develop them to more than one organ. Also, family members often can be found to make autoantibodies of the same or similar type. Moreover, patients with SLE, RA, MCTD, scleroderma, etc., tend to develop multiple autoantibodies against antigens that are not specific for a tissue or organ. Thus the classification into organ-specific and not organ-specific fits not only the antibodies, but to a certain extent divides patients with autoimmune disease into two broad categories of somewhat related conditions.

Some typical examples of autoantibodies and their associated diseases will be given below in Sections 9 and 10.

4. THE IMMUNE SYSTEM

Key features of the highly developed and complex immune systems of mammals are the ability to discriminate between self and nonself, the capacity to react in a specific manner to all sorts of foreign substances, the regulation of the response, quantitatively, through an elaborate interplay of enhancing and inhibiting factors, and the development of a wide variety of effector mechanisms (8).

The clinician comes into contact with the end results of various immune responses and therefore the operation of effector mechanisms is easy to understand. Hemolysis in an acute transfusion reaction results from the activation of the complement sequence and produces the lysis of red blood cells. In a case of cellulitis we recognize the hallmarks of acute inflammation, activated by a specific immune response against the products of an invading microorganism. The inflammatory reaction itself, however, is not specific. The clinical picture in a case of acute gout is so similar, that an acute infection is often suspected. However, no immune response is involved in the reaction to urate crystals. It is just the inflammatory response that is similar. When we skin test with PPD we elicit delayed-type hypersensitivity. A whole series of cell mediated immune mechanisms were developed by the immune system to cope with intracellular parasites. They are very effective to eliminate or control many viruses and bacteria. However, we also know that such responses may be harmful. A good example is when the inflammatory reaction of cell mediated immunity takes place in the central nervous system. Such an immune response can be more harmful than the virus. Patients with hay fever, urticaria, or asthma have immune responses that activate the effector mechanisms of allergy. Therapeutic interventions can sometimes be devised that deal with these effector mechanisms and do not interfere with the initial immune response. The anti-inflammatory drugs can inhibit inflammation from any cause. Anti-histamine drugs counteract the pharmacologic effects of histamine without regard to the initial trigger.

5. ANTIBODY DIVERSITY

Kabat and Mayer wrote in 1948 (9), "When an animal receives one or more parenteral injections of certain foreign materials - proteins, red blood cells or tisue extracts from another species, or of bacteria or bacterial products - there generally appear in the serum, within a few days, substances which possess the unique property of reacting with the material injected. These substances are termed **antibodies** and the materials which stimulated their production are called **antigens.**" Immunologists have pondered the remarkable ability of the immune system to manufacture specific antibodies to so many diverse antigens.

A major triumph was the recognition of the chemical nature of the elements involved. I quote from the same source: "Antibodies are now (1948) generally accepted as proteins and belong to the class of serum globulins. Doubts of earlier investigators as to the protein nature of antibodies were finally resolved by the accumulation of an overwhelming mass of evidence culminating in the preparation of analytically pure antibodies and the determination of their molecular weights and other physico-chemical properties. As yet little or nothing is known of the specific modifications of chemical structure required for the formation of an antibody molecule instead of a molecule of normal serum globulin. In most instances, these changes must be very subtle since no method of distinguishing between certain normal

globulins and antibodies has been found" (9).

Further discussion of the origin of antibody specificity and diversity is avoided as the book was essentially a manual of laboratory methods. Antibodies are useful chemical reagents, was the main message, before going into a detailed description of virtually all the methods of quantitative immunochemistry known at the time.

About three years later (1951) a symposium was held at the New York Academy of Medicine. The proceedings, published in 1953, start with an article by Felix Haurowitz entitled "Theories of antibody formation." Haurowitz and his colleagues were impressed by the fact that antibodies could be produced against all types of products of the chemical laboratory such as arsanilic or tartranilic acid. The proposals of Ehrlich (1900) that cells had receptors and that these receptors were replicated when the corresponding antigen was presented, could not be accepted. Haurowitz wrote: "It is unimaginable that the organism of an animal contains preformed receptors against such synthetic chemicals."

Instead it was postulated that antibody function originates through complementary adaptation of the shape of the antibody molecule to the shape of the determinant group of the antigen. Haurowitz believed this occurred during protein synthesis in the presence of antigen. All of this was, of course, wrong and has been for the most part forgotten. Newer textbooks don't even

mention these ideas. I quote (11):

"... the majority of the heterogeneity (of antibodies) lies in the amino acid sequence of the part of the molecule which combines with antigen. It is accepted that for an amino acid sequence in a polypeptide chain there is a corresponding sequence of nucleotides in the DNA of the cell which made the polypeptide chains. Efforts made to show that this is not the case for antibody molecules have so far failed, and there is no reason to believe that the actual process of synthesis of antibodies is in any way different from other proteins."

We now recognize that Ehrlich (5) was essentially correct when he postulated the existence on the outer surface of cells of preformed receptors that had the capacity to interact specifically with foreign substances. He was the first to postulate that antigen recognition structures on cell surfaces were identical in binding specificity to humoral antibodies.

Burnet (6) considered lymphocytes to be heterogeneous in the sense that each cell was genetically predetermined in its capacity to synthesize only one type of antibody molecule, specific for only the corresponding antigenic determinant. Such a determinant would not have any effect on the majority of lymphoid cells but would select those few already equipped with the ability to synthesize the antibody with the correct complementary structure. As a result of interaction of that

lymphocyte with antigen, the cell would be stimulated to proliferate and differentiate to produce a progeny of cells which, being derived from a single ancestral cell, would represent a clone of antibody-producing cells.

It is now clear that what seemed unlikely to the early immunochemists is indeed true: genetic mechanisms have evolved that are capable of generating the whole spectrum of antibody diversity. The antibodies created contain not only those that are specific to current antigens, but also those that appear useless, but might be reactive to antigens to be encountered in the future (12).

Table 5.

Understanding Antibody Diversity

1901	Ehrlich	Preformed receptors
1951	Haurowitz	Antigen serves as template
1959	Burnet	Clonal selection theory
1970	Hood & Talmage	Germ line basis for antibody diversity
1970	Weigert et al	Somatic diversification basis for antibody diversity
1979-84	Data from several labs	Molecular genetics of Ig: multiple germline genes which undergo somatic rearrangement and hypermutation

How is this accomplished? Two hypothesis have been proposed:

- the **germ line theory** (13) has it that all the information required for antibody genes is stored in the zygote or germ line;

- the **somatic theory** (14) proposes that somatic mutation or recombination generates functional variants of antibody genes. Thus antibody diversity arises in large part anew in each individual during its development.

Protein and gene sequencing experiments have provided the definitive information on how antibody diversity is generated (15). Both of the above are true. The germ line provides considerable diversity, probably as many as 100 to 1000 genes. In addition, diversity is generated by random and flexible recombination between the DNA segments that comprise the variable regions. There are two segments of DNA in the V region of light chains (V,J) and three in the V_H regions (V,D,J). It has been calculated that with all the possible joinings of these DNA segments it is possible to generate 5×10^7 combinations (12). In addition, evidence of true somatic mutations has also been obtained (15).

Given the enormous flexibility in the immunoglobulin gene system it becomes critical to have a mechanism for selection at the cellular level to construct a useful defense system for the organism.

Antibody-producing cells mature in two steps (3), antigen-independent and antigen-dependent differentiation (Fig. 1). The antigen-independent stage includes proliferation and differentiation to produce individual lymphocytes with antibody receptor molecules on their surface, identical in specificity to

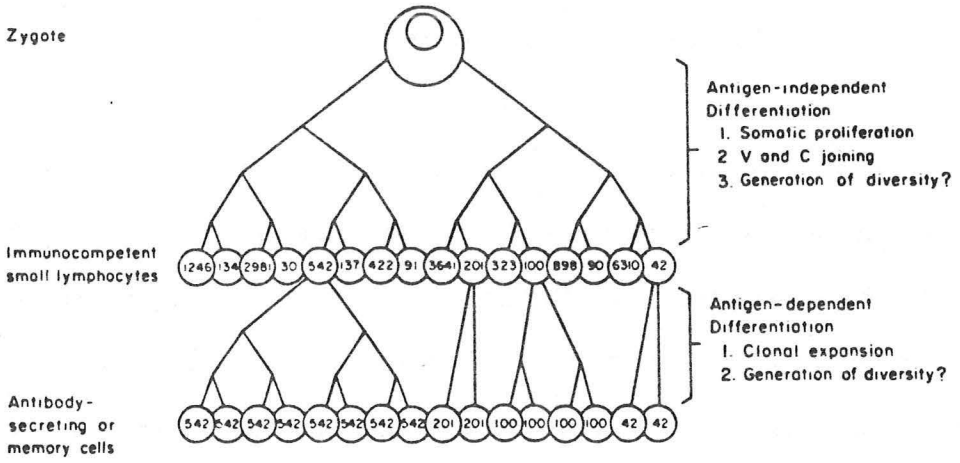

Figure 1. A model of somatic differentiation of antibody-producing cells. This model suggests that there are two stages of development—one antigen-independent that leads to unit differentiation and the other antigen-dependent that leads to clonal selection and amplification. The different numbers signify the commitment of distinct lymphocytes to a different molecular species of antibody.

From L. Hood

FIGURE 1. Antibody genes and other multigene families.

the antibody these cells will eventually secrete. The second stage is the antigen-driven process of clonal selection, differention and expansion. It yields expanded clones of specific antibody-producing cells as well as specific memory cells that can be triggered to differentiate into specific antibody-producing cells by a second exposure to the same antigens.

6. T CELLS

In order to simplify the subject an important element was left out of the discussion of B cell activation: the fact that the majority of B cells cannot be triggered to respond by antigen alone, but that they require in addition, certain factors provided by helper T cells (16). This would not be all that complicated if one could assume that T cells are very much like B cells. That they have specific immunoglobulin receptors on their surface and that they are triggered to proliferate and differentiate by contact with specific antigen in accordance with the clonal selection process already described. Unfortunately this also, is not quite so simple.

T cells are not able to be activated by antigen alone. In fact, there are many different T cell subsets with differing activation requirements. Most T cells, particularly those with cytotoxic or helper function, manifest a dual specificity for both nominal antigen and for a product of the MHC. Thus activation takes place only when the T cell is presented with nominal antigen in the context of the appropriate MHC product (17).

The T cell receptor continues to be a mystery. Even the question of whether the dual specificity of T cells is due to two separate receptors, or a single receptor with dual specificity, is not entirely resolved (18). Undoubtedly, the antigen receptors of T cells are in some way related to immunoglobulin on the basis of

shared idiotypy. However, since not much more is known about the molecular structure of the antigen receptors of T cells, further discussion of this topic would not be productive in the present context.

7. THE ROLE OF THE MHC

The discovery that T cells respond to antigen only in an MHC-restricted fashion has completely changed our notion of T cell antigen recognition. Cytotoxic cells attack only targets that express allogeneic MHC antigens or targets that combine a nominal antigen with self class I MHC products (19). Helper T cells respond to antigen only when it is presented associated with products of the HLA-D region of the MHC (20). This function requires the participation of antigen-presenting cells which

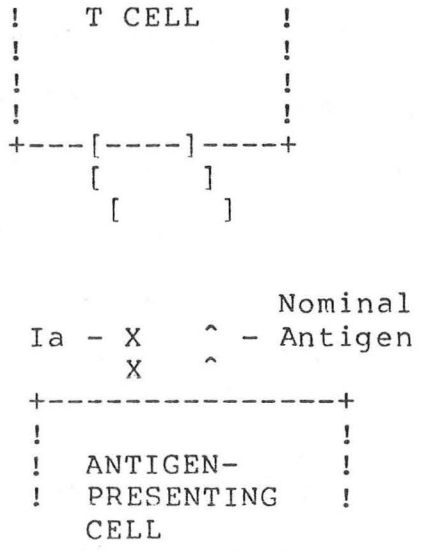

FIGURE 2. HLA-restricted antigen presentation.

carry Ia-like antigens on their surface (Figure 2). The key feature is that triggering of T cells requires the presence on the surface of the antigen-presenting cells of both nominal antigen and a class II MHC product. The T cell receptor(s) determines the specificity. It selects the epitope of nominal antigen and the determinant of the MHC structure that will trigger a response.

The value of such restriction of the T cell activation process is quite obvious. It is a mechanism that focuses the triggering of T cell activity in close proximity to the surface of a target or an activating cell. While antibodies constitute effective defense against organisms or foreign substances located in the extracellular space, they are relatively inefficient against invaders that take residence within host cells. The T cell system was developed largely to cope with the problem of intracellular parasites. Virus-infected cells are killed by cytotoxic T cells which can function effectively only if they are triggered in close proximity to their target. The need for viral and self membrane-associated antigens accomplishes that strategy. The focusing of helper-cell activity to the surface of the antigen-presenting cells, where the machinery for antibody production can be assembled, achieves a similar effect.

In inbred mice it was relatively easy to show that some animals made strong immune responses to certain antigens, of limited heterogeneity, while other strains of mice made very low or no responses (22). Control of such immune responses was found to be

linked to the MHC and mapped to the I (immune response) region (23), which is equivalent to the HLA-D region of man. It is now clear that the I-region products, the class II molecules, are the products of the Ir genes. Low responses, or failure to respond, may be due to either a lack of T cells with the appropriate receptors, or to the effect of immunoregulatory influences. Immunoregulation will be discussed in the next section.

While human antigen-specific HLA restricted T cell clones can be easily produced and studied in the laboratory, evidence for haplotype-associated defects in the T cell repertoire have not yet been clearly observed in man. In mice, it has been postulated (24) that "holes" in the T cell repertoire might arise when a response to self-plus-x, might trigger a harmful autoimmune reaction. In other words, such T cell clones would be eliminated or inactivated to prevent disaster.

8. IMMUNOREGULATION

The clonal selection theory held that the intensity of the immune response to a given antigen depends on the numbers of immunologic cells that bear receptors that bind the antigen. Unresponsiveness or tolerance reflects an absence of cells that bear appropriate receptors. However, by the early 1970's there were indications that reality was more complex and that the original clonal selection theory would require major revisions.

There were observations that infusion of T cells from donors unresponsive to a particular antigen into normal mice, rendered the recipient mice specifically unresponsive (25). The experiments indicated that absence of immunologic activity could reflect an active process. Also, analysis of graft-versus-host responses showed that the intensity of the reaction was determined not simply by the number of responding T cells, but by interactions among different types of lymphocytes (26). These observations, which established the existence of suppressor cells, led to efforts to make an analysis of the cellular interactions between different types of T cells that regulate immunologic reactivity (27).

The discovery by Cantor and co-workers (28), of surface markers that identified different lymphocyte subsets, allowed the isolation of cellular components of the immune system and an analysis of their respective roles in the events that govern the intensity and type of the immunologic response. It was observed that the immune response is controlled in a highly precise manner by messages passed among at least three types of T cells: inducer cells, regulatory cells, and effector cells (29). The majority of immunologic cells are not effector cells but regulatory cells that respond to signals or messages generated from within the immune system itself.

The inducer cells can activate a variety of effector cells, such as B cells, which make antibody, or monocytes and inflammatory cells that participate in delayed-type hypersensitivity. In

addition, they activate other T cells which emit inhibitory signals. The intensity of inhibition depends on the genetic background of the host, the nature of the antigenic stimulus, and the intensity and quality of the inducer signals. The observed

Table 6

Surface Markers of Lymphocyte Subsets in Man

Marker	Detection Method	Percent of T Cells with Marker	Proposed Function
		(%)	
Receptor for sheep erythrocytes (E)	Rosetting	100	Total T cell function
Fc receptor for IgM	Rosetting	60-70	helper/inducer cytotoxic
Fc receptor for IgG	Rosetting	10-20	suppressor
Histamine receptor	adherence	10-20	suppressor
TH_1	immunofluorescence	40-60	helper/inducer
TH_2	immunofluorescence	20-30	suppressor, cytotoxic
OKT11	immunofluorescence	100	Total T cell function
OKT3	immunofluorescence	85-95	Total T cell function
OKT4 (Leu 3)	immunofluorescence	50-60	helper/inducer
OKT8 (Leu 2)	immunofluorescence	20-30	suppressor, cytotoxic

immune response depends on the relative potency and timing of the inhibitory signals relative to the potency and timing of the inductive signals that are passed to the effector cells, such as B cells (30). It is suggested that elimination of regulatory

cells, which participate in feedback inhibition, can result in the development of autoimmune disease.

Similar markers of T cell subsets can also be identified in man (31,32) (Table 6). An extensive literature has developed based on the use of commercially-available antibodies to quantitate T cell subsets in every conceivable disease or condition. Unfortunately, often the users of such reagents have assumed a simplistic interpretation of the subsets of lymphocytes identified by the available markers. Much of the published work in this field lacks sophistication. For example the T4 subset contains not only helper cells, but also inducer and suppressor cells (33), as well as cytotoxic cells against class II MHC antigens (34). The T8 subset contains at least suppressor and killer cells. Thus, when a subset defined by these broad markers, is altered in number in a group of patients it is usually not clear which functional type of T cell is really affected. The problems are compounded even further when the results of such investigations are expressed as T4/T8 ratios. When results are expressed as ratios it is impossible to tell which of the two quantitations was abnormal. Furthermore, when patients are studied during the active stages of a disease, the alterations observed may be secondary to the disease process. It is unlikely that they reflect a primary alteration that bears a causal relationship to the development of the disease. A good case in point are studies performed in patients with SLE. Profound changes in T cell subsets are reported during active disease while similar patients studied when their disease is in

remission show essentially normal values.

Smolen and coworkers recently published an excellent study of T cell subsets in 32 patients with SLE (35). The helper/inducer subset determined with antibody Leu 3a was normal in 9, decreased in 16 and increased in 7 of the patients. Only 10 patients had abnormal percentages of the suppressor/cytotoxic subset determinant with Leu 2a. An attempt was made to correlate the Leu 3a/Leu 2a ratio with clinical features of SLE. All 8 patients with low ratios had significant renal involvement. These patients also had a tendency to onset of SLE before age 20, presence of thrombocytopenia and reduction of Islet peripheral T cells. In contrast, patients with high helper/suppressor ratios tended to have development of SLE after 20 years of age, and presence of multisystem disease, with lymphadenopathy, but were less likely to have involvement of the kidney. The largest group of patients (16/32) had normal helper/suppressor T cell ratios. They often had widespread SLE disease with CNS involvement as well as renal disease. No conclusions were drawn about the pathogenetic significance of the changes observed.

One frequently reads speculations about failure of immune regulation a primary cause of autoimmune disease. However, diffuse nonspecific failure in the regulation of the immune response, although possible, is not likely to be a common event. Autoimmune disease is usually quite specific. Patients get thyroid disease, or thrombocytopenia, or myasthenia gravis, or

immune complex disease due to antibodies against native DNA. If failure of immunoregulation does play a role, as is very likely, it must be a specific failure of the regulation of the immune response to a given antigen, in a selective manner.

At this point we reach the edge of knowledge in this field. It is becoming clear that in order to understand the pathogenesis of autoimmune disease, the specific lymphocyte subsets involved must be known in each case. The immunologic events may include a failure of immunoregulation leading to the development of an immune response that causes a specific lesion. In most cases, this will involve the participation of specific T cells, even when the eventual outcome is the production of auto antibodies as in the examples that will be discussed in the next two sections. The inducer T cells, and also some of the specific regulatory T cells, are almost certainly restricted by MHC determinants. Most of the diseases of this type appear to be associated with certain MHC haplotypes. Just how having a certain haplotype predisposes an individual to the development of a given disease, is one of the most interesting problems facing immunologists today (17). We think that perhaps susceptibility is conditioned by the T cell repertoire which is to some extent at least, MHC-haplotype dependent.

9. AUTOANTIBODIES TO ENDOCRINE ORGANS

A. Thyroid

Autoimmune disease of the thyroid is the most typical and well

studied of the idiopathic autoallergic disorders (36). Both humoral and cell-mediated mechanisms are thought to be involved in the pathogenesis and genetically-determined factors, possibly controlling the immune response, appear to play a role.

Clinically the autoimmune diseases of the thyroid include both goitrous and atrophic thyroiditis as well as primary thyrotoxicosis. Autoimmune thyroiditis is characterized by invasion of the gland by lymphocytes and presence of high titers of circulating antibodies against thyroid antigens. Several recent studies indicate that antibodies are largely produced by cells concentrated in the diseased organ (37,38). Cultures of thyroid lymphocytes were found to synthesize large amounts of microsomal and/or thyroglobulin antibody.

In atrophic thyroiditis the gland is destroyed with resulting mixedema. In Hashimoto's thyroiditis with goiter, the gland appears to compensate for the destructive effect by formation of new thyroid tissue.

Formation of four distinct auto-antibodies against normal constituents of thyroid tissue has been associated with thyroiditis:

a) thyroglobulin antibodies
b) antibodies against microsomal antigens
c) Antibodies against second colloid component
d) Antibodies to thyroid cell-surface antigens

Table 7.

Autoantibodies to Thyroid Antigens

Antigen	Properties	Function
Thyroglobulin	Mainly IgG, also IgA, IgM, not C fixing	No clear function
Microsomal	IgG, complement fixing	Cytotoxic, also ADCC
Second colloid (CA2)	IgG, some IgA, does not fix C	None
Thyroid cell surface	IgG and IgM	ADCC
Thyroxine	IgG	Binds and prevents hormone action
TSH receptor	IgG	May stimulate or inhibit
Thyroid cell surface	IgG	Growth promoting

Modified from (36).

Graves' disease is associated with the production of thyroid stimulating antibodies. These are IgG antibodies that bind to the TSH receptor and produce activation of the thyroid with resulting chronic oversecretion by the gland.

In addition to the autoantibodies that stimulate thyroid secretion, antibodies that promote thyroid growth have been recently described (39) and their significance has been reviewed (40).

The following immune effector mechanisms are thought to be operative in patients with autoimmune thyroid diseases:

1. Cytotoxic T lymphocytes reactive to thyroid cell surface antigen;
2. Antibodies against cell membrane antigens activate antibody dependent cellular cytotoxicity (ADCC);
3. Complement-fixing antibodies may produce cytolysis by antivation of complement;
4. TSH receptor antibody blocks receptor, causing hypothyroidism;
5. TSH receptor antibody stimulates thyroid secretion;
6. TSH receptor antibody promotes growth of the gland, producing goiter.

B. Diabetes

The autoimmune nature of the process that leads to island cell destruction in Type I diabetes is not so well established. However, the evidence in favor of this view is becoming more and more convincing (41). Some of the main features will be briefly summarized here.

The histology of the islet cells, at the onset of disease, shows mononuclear cell infiltration with selective destruction of beta cells. The picture resembles other immunologically-mediated lesions, such as those of thyroiditis.

Association of type I diabetes with other autoimmune diseases, especially other organ-specific autoimmune endocrinopathies such as idiopathic Addison's disease, Graves' disease, Hashimoto's thyroiditis and pernicious anemia will be discussed further below.

Association of type I diabetes with HLA-DR3 and HLA-DR4 is well established. It suggests that "immune response genes" are somehow related to susceptibility for development of Type I diabetes.

Table 8

Autoimmune Nature of Type I Diabetes

Feature	Findings
Immunopathology	Lymphoid infiltration
Association with autoimmune disease	Addison's, thyroiditis, organ-specific antibodies, polyglandular failure
Association with MHC	HLA-DR3 and DR4
Antibodies to islet cells	Cytoplasmic and cell surface
Cell-mediated immunity	Some evidence in BB rat model
Alteration in lymphocytes	Ia-bearing T cells

Autoantibodies to islet cells of two main types have been described. Most of the early studies were performed by immunofluorescence on tissue sections and detected **cytoplasmic antigens** (42). Such antibodies are found in 60-80% of type I

diabetes at the time of diagnosis and they decrease with time so that only 20% or less of the patients remain positive after 2-5 years. A recent study of nondiabetic monozygotic twins of diabetic patients showed that this antibody may appear many years in advance of eventual hyperglycemia in the second twin (43).

Antibodies against **islet cell surface antigens** have also been detected in recent studies which made use of suspensions of live islet cells (44). Because they react with the cell surface, such antibodies are thought to be more likely to have a role in beta cell destruction. Indeed such antibodies have been noted to have cytotoxic activity, to be able to induce radioactive chromium release and to inhibit glucose-induced insulin secretion from suspension of living islet cells (45).

Evidence is mounting that type I diabetes is a chronic disease of insidious onset, somewhat similar to other autoimmune disease states. The abrupt onset of hyperglycemia apparently does not occur until 90% of the islet cells have been destroyed. Given the available time, if methods can be perfected, the possibility of identifying individuals at risk and perhaps of preventing progression, by appropriate therapy, may be possible in the future (41).

C. Polyglandular failure

In some diseases such as myasthenia gravis or Hashimoto's thyroiditis the autoimmune response is predominantly organ-

specific. In other diseases, such as systemic lupus erythematosus, the autoantibodies produced react with components of most tissues (such as DNA or RNP). Finally, there is a group of conditions where the autoimmune response, though organ specific, is broad, involving several different organs. An example of this last group is polyendocrinopathy or polyglandular failure. For example, in patients with polyendocrine diseases, autoantibodies have been found that react with the pancreas, thyroid, pituitary, and gastric mucosa. Clinically, these patients tend to have combinations of diseases involving multiple endocrine organs, as well as certain other features such as vitiligo or sometimes candidiasis. A typical constellation might be Graves' disease, pernicious anemia, and myasthenia gravis.

Many of the individual illnesses of the polyglandular failure syndrome such as myasthenia gravis, type I diabetes, or Graves' disease have an increased frequency of certain HLA antigens. Eisenbarth and coworkers (46) evaluated eleven patients with the polyglandular failure syndrome. Seven had hypothyroidism, 3 had hypogonadism, Graves' disease existed in one, Addison's disease was present in 7, 6 had pernicious anemia, one myasthenia gravis, one hypoparathyroidism and 4 had diabetes (Table 9). The gene frequencies of HLA-B8 and the A1,B8 haplotype were increased in this group of patients as compared with a control population. Of interest also was that 11 of 42 relatives were similary affected by a polyglandular failure illness.

Table 9

Polyglandular Failure Syndrome*

Features	Patients										
	1	2	3	4	5	6	7	8	9	10	11
Hypothyroid	+	+	+	+	+	-	+	-	-	-	+
Hypogonad	+	-	-	-	-	-	+	-	+	-	-
Graves' disease	-	-	-	-	-	-	-	+	-	-	-
Addison's disease	-	-	+	+	-	+	+	-	+	+	+
Pernicious anemia	+	+	-	+	+	-	+	-	-	-	+
Myasthenia gravis	-	-	-	+	-	-	-	-	-	-	-
Hypoparathyroid	-	-	-	-	-	-	-	-	-	+	-
Type I diabetes	-	-	-	-	-	+	-	+	+	-	-
HLA-A1	-	-	-	+	-	+	+	+	+	-	-
HLA-B8	-	-	-	+	-	+	+	+	+	-	-

* Modified from (46).

Valenta and coworkers (47) recently reported a family in which 44 members, in four generations, had various combinations of Addison's disease, hypothyroidism, diabetes, and ovarian failure. Two cases of scleroderma also existed in this family. All of the diseased individuals had inherited the HLA-B8 antigen. None of the B8-negative family members developed autoimmune disease in spite of the fact that some of them had circulating autoantibodies in their serum.

Recently it has become possible to produce monoclonal antibodies by somatic cell hybridization of human B cells from patients with autoimmune disease. Satoh and coworkers (48) reported that they had produced autoantibody-producing hybridomas from the peripheral lymphocytes from patients with diabetes and other associated autoimmune abnormalities. Nine human hybridomas synthesized autoantibodies and 7 of the 9, were reactive with multiple organs. They reacted with anterior pituitary, thyroid follicles, gastric mucosa, and pancreatic islet cells. In addition some of them also reacted with cytoskeletal elements of human embryo fibroblasts and HeLa cells.

The high proportion of autoantibody-producing hybridomas that react with multiple organs suggests that multiple-organ-reactive autoantibodies are common. The fact that they react with some of the same organs that serum from patients with polyendocrine disease reacts with, is of interest. Such antibodies should make it possible to identify the autoantigens involved in patients with polyglandular failure.

10. AUTOANTIBODIES TO NUCLEAR ANTIGENS

I have chosen to end this discussion with an examination of autoantibodies to nuclear antigens that develop in patients with systemic lupus erythematosus and related diseases. The astounding variety of autoantibodies produced in this group of patients (49) requires perhaps more extensive treatment than can

be devoted today. However, there are a number of good reasons why these antibodies should be included. In systemic lupus erythematosus the autoantibodies against native-DNA constitute useful markers of disease activity and of propensity toward the development of renal disease. Autoantibodies to Sm antigen appear to identify a subset of SLE patients. Antibodies to nuclear ribonucleoprotein (nRNP) are associated with a clinically distinct group of patients with mixed connective tissue disease (MCTD). Antibodies against two other small ribonucleoprotein have turned out to be extremely interesting. Most noteworthy is the antibody called Ro (or SS-A) that reacts with a small ribonucleoprotein that is predominantly cytoplasmic. Antibodies against Ro have been found to be associated with the development of neonatal lupus erythematosus and with congenital heart block. Additional antibodies are of interest because of their predominant association with scleroderma (scl-70), the CREST syndrome (anticentromere), or with polymyositis (PM-1).

Antibodies to DNA

Since the discovery of the LE phenomenon, and the development of the immunofluoresence technique for the determination of antinuclear antibodies, autoantibodies against nuclear constituents have been closely associated with our understanding of SLE (50). Antibodies against DNA generally fall into two major classes. One which recognizes determinants on double stranded DNA (dsDNA) and single stranded DNA (ssDNA). The other, which recognizes antigenic determinants exposed on ssDNA only.

Antibodies reactive with ds/ssDNA are usually referred to as antibodies to native-DNA. They occur in high titer only in SLE and are an important cause of lupus nephritis. Complexes of DNA-anti-DNA have been observed in serum and deposited in the kidneys of such patients. The presence and titer of antibodies to native DNA appears to correlate clinically with SLE activity. Together with the measurement of levels of circulating complement (CH50) and complement components (C3, C4), determination of antibodies against dsDNA are used to follow the course of SLE patients and as a guide for treatment.

Antibodies to determinants of ssDNA only, can be found in SLE as well as in other diseases. Of interest also are antibodies against nuclear histones. They have been observed in about 30% of patients with SLE and in virtually all patients with SLE induced by drugs such as hydralazine or procainamide.

Autoantibodies to Sm and to nuclear ribonucleoprotein (nRNP)

Extracts of nuclei contain several distinct antigens against which autoantibodies can be produced. The generic name anti-ENA describes a group of antibodies which were found to hemagglutinate red cells sensitized with extractable nuclear antigen (ENA). Eventually it became clear that two main antigens could be detected by this technique: the ribonuclease-sensitive, nRNP antigen, and a ribonuclease-resistant antigen, Sm (51). These antibodies are also commonly studied by immunoprecipitation in double diffusion.

The Sm antibody originally described by Tan and Kunkel (52), was found to be unique to SLE, where it is found in 25-20% of the patients.

Antibody to nRNP can be found in SLE in approximately 30-40% of the patients, but it is present in 95-100% of patients with mixed connective tissue disease (MCTD). Thus high titer anti-nRNP is a marker of MCTD where it is present without any of the other antibodies commonly found in SLE (such as anti-dsDNA, anti-Sm, etc.) (49). It can be found in low titers in some patients with other conditions such as scleroderma or Sjogren's syndrome. Clinically, patients with MCTD selected on the basis of high titer anti-nRNP in the absence of other nuclear autoantibodies, have been found to have polyarthralgia or arthritis, Raynaud's phenomenon, hypergammaglobulinemia, problems with esophageal mobility and myositis (51). In other words, an overlap of certain features of lupus scleroderma and polymyositis. Another feature of this group of patients is that they generally obtain a beneficial response after treatment with corticosteroids.

Antibodies to Ro (SS-A) and La (SS-B)

There are still other antigens, present in large enough amounts in tissue or cell extracts, to precipitate with the corresponding autoantibodies from sera of certain lupus patients. Ro has been shown to be immunologically identical to SS-A and La to SS-B (53). The SS designations refer to the fact that these

Table 10

Antibodies to Nuclear Antigens*

Antigen	Clinical Association
ds DNA	SLE, rare
ds/ss DNA	Active SLE, 60-70%
ss DNA	SLE and other rheumatic diseases
Histone	Drug LE (95-100%), RA (15-20%), SLE (30%)
Sm	SLE (30-40%)
nRNP	MCTD (95-100%), low titer in some SLE, scleroderma
Ro (SS-A)	Sjogren's, SCLE, neonatal lupus, congenital heart block
Scl-70	Scleroderma (15-20%)
Centromere	CREST (70-90%)
PM-1	Polymyositis

* Modified from (49).

antibodies were observed with high frequency in serum of patients with Sjogren's syndrome. Like Sm and nRNP, Ro and La are antigens on small particles comprised of protein and RNA (54). Anti-Ro antibodies react with determinants of small ribonucleoproteins which are predominantly cytoplasmic. Anti-La reacts with ribonucleoprotein particles which contain numerous small cellular RNA's. In addition, anti-La antibodies

immunoprecipitate RNP particles containing Epstein-Barr virus encoded RNA from EBV infected cells, and adenovirus encoded RNA from adenovirus infected cells.

Ro particles bear both the Ro and the La determinants, thus Ro is a subset of La. Small cytoplasmic RNP's have recently been assigned roles related to protein synthesis in mammalian cells. It is thought that similarly, Ro RNA's might play a role in translation or transport of proteins specified by certain nRNA's.

Antibodies to Ro has become of special interest recently because they have been detected in sera of mothers and infants with neonatal lupus.

The prevalence of anti-Ro in SLE ranges between 25 and 30%. Clinically, except for the presence of an increased frequency of photosensitivity and of rheumatoid factor, patients with Ro antibodies did not differ from other SLE patients (49).

It has been suggested that complexes of Ro and anti-Ro antibodies may play a role in the pathogenesis of SLE nephritis in some patients (55). This was based on a study of two patients who developed diffuse SLE renal disease in the absence of antibodies against native DNA. Maddison and Reichlin (55) obtained acid eluates of kidney tissue and demonstrated the presence of anti-Ro, present in much higher concentration in the kidney than in the serum.

Provost and coworkers (56) have described a group of SLE patients in whom the ANA test, performed in the usual manner is negative, and who had a high frequency (62%) of anti-Ro antibodies. Clinically this group of patients tended to have photosensitive skin rash.

Subacute cutaneous lupus erythematosus (SCLE) was described by Sontheimer and coworkers (57-59) using a well defined skin rash to characterize a distinct subset of lupus patients. This group of patients had a remarkable, usually photosensitive, subacute, non-scarring, lupus skin rash. They also had a high frequency (63%) of anti-Ro antibodies (59). HLA-DR3 was found in 20 of 26 patients (77%) compared to 22% in controls (58).

Neonatal lupus erythematosus is a rare, transient disease characterized by annular skin lesions (56, 60, 61). The lesions may be present at birth or develop shortly thereafter. They generally heal and disappear by the age of six months, although residual hypo- or hyperpigmentation may persist. Histologically the skin lesions show characteristic features of lupus erythematosus. The syndrome may also be associated with cardiac conduction defects (62). The conduction defects may occur in the absence of cutaneous disease and are considered secondary to carditis and fibrosis of the atrial septum in the region of the cardiac conduction pathway. Mothers of these infants may be asymptomatic, but they usually have anti-Ro antibodies which are passsed across the placenta to the fetus.

Lee and coworkers (63) recently reported on seven infants with neonatal lupus in six families which were studied by HLA typing. All seven infants had transient cutaneous lesions, congenital heart block, or both. Five of six mothers were asymptomatic and one had Sjogren's syndrome. Six of seven infants and all six mothers had antibodies to Ro in their sera. The infants became seronegative by age 8 months. Five mothers were positive for HLA-DR3, no HLA associations were seen in the infants. It was concluded that HLA-D region genes may be associated with autoantibody production but not with the other events in tissue injury.

The relation between congenital heart block and maternal connective tissue disease was further investigated in a study by Scott and colleagues (62), who obtained serum specimens from 21 patients and 41 mothers. Anti-Ro was found in 34/41 mothers tested and in 7 of 8 serum specimans collected from affected children when they were less than three months old. It was concluded that anti-Ro serves as a marker for the risk of isolated congenital complete heart block. The evidence is strong that this antibody is the cause of the lesion but it is also possible that a closely related antibody, as yet unidentified, could be responsible.

11. CONCLUSION

The immune system carries out its function of protecting the

individual through a complex evolutionary mechanism which is practised over and over again in each member of the species.

Discrimination between self and nonself is accomplished through the recognition structures of the MHC which exist on the surface of cells. Antibody diversity is generated through both evolutionary (germ line) and somatic means. Something similar must apply also to the receptors of T cells, but the details remain to be worked out. A complex network of amplifying and inhibitory signals regulates the quantitative expression of immunologic activity and determines the end result at the effector level.

Derangements in this complex system can result in disease. The abnormality can consist of inappropriate, excessive, effector activity, resulting in tissue damage, or of insufficient responsiveness, resulting in immune failure. Autoimmune diseases involve the development of a specific immune response directed against self antigens which results in tissue lesions or impairment of function.

The patterns of autoimmune disease involving the thyroid, the autoimmune component of type I diabetes and the syndrome of polyglandular failure were discussed as examples of organ-specific autoimmunity. A variety of autoantibodies are produced against nuclear antigens. Of particular interest are antibodies against small ribonucleoprotein particles (anti-Ro) which are

markers for subacute cutaneous lupus, neonatal lupus, and congenital heart block. The immune response to this autoantigen, but not the development of tissue lesions, appears to be associated with immune response genes of the main histocompatibility complex.

REFERENCES

1. Hildemann, WH, Johnson, IS, and Jokiel, PL, Immunocompetence in the lowest metazoan phylum: transplantation immunity in sponges. Science **204:** 420-422, 1979.
2. Hildemann, WH, Jokiel, PL, Bigger, CH, and Johnston, IS, Allogeneic polymorphism and alloimmune memory in the coral, montipora verrucosa. Transplantation **30:** 297-301, 1980.
3. Hood, L, Antibody genes and other multigene families. Federation Proc **35:** 2158-2167, 1976.
4. Klein, J, Biology of the mouse histocompatibility - 2 complex. Principles of immunogenetics applied to a single system. Springer-Verlag, New York, 1975.
5. Ehrlich, P, On immunity with special reference to cell life, Proc Roy Soc **B66:** 424, 1900.
6. Burnet, FM, The clonal selection theory of acquired immunity. University Press, Cambridge, England, 1959.
7. Smith, HR, and Steinberg, AD, Autoimmunity - a perspective. Ann Rev Immunol **1:** 175-210, 1983.
8. Gell, PGH, Coombs, RRA, and Lachman, PJ, Clinical aspects of immunology. Blackwell, Oxford 1975.
9. Kabat, EA and Mayer, M, Experimental immunochemistry. Charles Thomas, Springfield, Ill., 1948.
10. Haurowitz, F, Theories of antibody formation. **In:** The nature and significance of the antibody response. AM Pappenheimer, editor, Columbia University Press, New York, 1953, pp. 3-12.

11. Munro, AJ, The genes for antibodies and the origin of diversity, In: Clinical aspects of immunology. PGH Gell, RRA Coombs, and PJ Lachmann, editors, Blackwell, Oxford, 1975.

12. Honjo, T, Immunoglobulin genes. Ann Rev Immunol **1:** 499-528, 1983.

13. Hood, L, and Talmage, DW, Mechanism of antibody diversity, germ line basis for variability. Science **168:** 325-334, 1970.

14. Weigert, MG, Cesari, IM, Youkovich, SJ, and Cohn, M, Variability in the lambda light chain sequences of mouse antibody. Nature **228:** 1045-1047, 1970.

15. Gearhart, PJ, Generation of immunoglobulin variable gene diversity. Immunology Today **3:** 107-112, 1982.

16. Reinherz, EL, Morimoto, C, Penta, AC, and Schlossman, SF, Regulation of B cell immunoglobulin secretion by functional subsets of T lymphocytes in man. Eur J Immunol **10:** 570-572, 1980.

17. Stastny, P, Ball, EJ, Dry, PJ, and Nunez, G, The human immune response region (HLA-D) and disease susceptibility. Immunological Rev **70:** 113-153, 1983.

18. Goodman, JW, and Sercarz, EE, The complexity of structures involved in T-cell activation. Ann Rev Immunol **1:** 465-498, 1983.

19. McMichael, AJ, Ting, A, Zweering, HJ, and Askonas, BA, HLA restriction of cell mediated lysis of influenza virus infected human cells. Nature **270:** 524-526, 1977.

20. Rodey, G, Luehrman, L, and Thomas, D, In vitro primary immunization of human peripheral blood lymphocytes to KLH: evidence for HLA-D region restriction. J Immunol **123:** 2240-2254, 1979.

21. Nunez, G, Ball, EJ, and Stastny, P, Accessory cell function of human endothelial cells. I. A subpopulation of Ia positive cells is required for antigen presentation. J Immunol **131:** 666-673, 1983.

22. Benacerraf, B and McDevitt, HO, The histocompatibility-linked immune response genes. Science **175:** 273-279, 1972.

23. McDevitt, HO, Deak, BD, Shreffler, DC, Klein, J, Stimpfling, JH, and Snell, GD, Genetic control of the immune response. Mapping of the Ir.1 locus. J Exp Med **135:** 1259-1278, 1972.

24. Nagy, ZA, Baxevanis, CN, Ishii, N, and Klein, J, Ia antigens as restriction molecules in Ir-gene controlled T-cell proliferation. Immunological Rev **60:** 59-83, 1981.

25. Gershon, RK and Kondo, K, Infectious immunological tolerance. Immunology **21:** 903-914, 1971.

26. Cantor, H and Asofsky, R, Synergy among lymphoid cells mediating graft-versus-host response. J Exp Med **131:** 235-246, 1970.

27. Gershon, RK, "Clonal selection and after", and after. New Engl J Med **300:** 1105-1107, 1979.

28. Cantor, H and Boyse, EA, Lymphocytes as models for the study of mammalian cellular differentiation. Immunological Rev **33:** 105-124, 1977.

29. Cantor, H and Gershon, RK, Immunological circuits: cellular composition. Federation Proc **38:** 2058-2064, 1979.

30. Green, DR, Flood, PM, and Gershon, RK, Immunoregulatory T-cell pathways. Ann Rev Immunol **1:** 439-463, 1983.

31. Reinherz, EL, Meuer, SC, and Schlossman, SF, The human T-cell receptor: analysis with cytotoxic T cell clones. Immunological Rev **74:** 83-112, 1983.

32. Lanier, LL, Engleman, EG, Gatenby, P, Babcock, GF, Warner, NL, and Herzenberg, LA, Correlation of functional properties of human lymphoid cell subsets and surface marker phenotypes using multiparameter analysis and flow cytometry. Immunological Rev **74:** 143-160, 1983.

33. Thomas, Y, Rogozinski, L, Irigoyen, O, Friedman, S, Kung, P, Goldstein, G, and Chess, L, Functional analysis of human T cell subsets defined by monoclonal antibodies. IV. Induction of suppressor cells within the OKT4+ population. J Exp Med **154:** 459-467, 1981.

34. Ball, EJ and Stastny, P, Cell-mediated cytotoxicity against HLA-D region products expressed in monocytes and B lymphocytes. IV. Characterization of effector cells using monoclonal antibodies against human T-cell subsets. Immunogenetics **16:** 157-169, 1982.

35. Smolen, JS, Chused, TM, Leiserson, WM, Reeves, JP, Alling, D, and Steinberg, AD, Heterogeneity of immunoregulatory T-cell subsets in systemic lupus erythematosus. Correlation with clinical features. Am J Med **72:** 783-790, 1982.

36. Doniach, D and Roitt, IM, Thyroid auto-allergic disease, **In:** Clinical aspects of immunology. PGH Gell, RRA Coombs, and PJ Lachmann, editors, Blackwell, Oxford, 1975, pp. 1355-1386.

37. Weetman, AP, McGregor, AM, Lazarus, JH, and Hall, R, Thyroid antibodies are produced by thyroid-derived lymphocytes. Clin Exp Immunol **48:** 196-200, 1982.

38. McLachlan, SM, Dickinson, AM, Malcolm, A, Farndon, JR, Young, E, Proctor, SJ, and Smith, BR, Thyroid autoantibody synthesis by cultures of thyroid and peripheral blood lymphocytes. I. Lymphocyte markers and response to pokeweed mitogen. Clin Exp Immunol **52:** 45-53, 1983.

39. Valente, WA, Vitti, P, Rotella, CM, Vaughan, MM, Aloj, SM, and Grollman, EF, Antibodies that promote thyroid growth. A distinct population of thyroid-stimulating autoantibodies. New Engl J Med **309:** 1028-1034, 1983.

40. Doniach, D, Chiovato, L. Hanafusa, T, and Bottazzo, CF, The implications of "thyroid-growth-immunoglobulins" (TGI) for the understanding of sporadic nontoxic nodular goitre. Springer Sem Immunopathol **5:** 433-446, 1982.

41. Naji, A, Silvers, WK, and Barker, CF, Autoimmunity and type I (insulin-dependent) diabetes mellitus. Transplantation **36:** 355-361, 1983.

42. Bottazzo, GF, Dean, BM, Gorsuch, AN, Cudworth, AG, and Doniach, D, Complement fixing islet-cell antibodies in type I diabetes: possible monitors of active beta-cell damage. Lancet **1:** 668, 1980.

43. Srikanta, S, Ganda, DP, Eisenbarth, GS, and Soeldner, JS, Islet cell antibodies and beta cell function in monozygotic triplets and twins initially discordant for type I diabetes mellitus. New Engl J Med **308:** 322, 1983.

44. Van de Winkel, M, Smets, G, Gepts, W, and Pipeleers, D, Islet cell surface antibodies from insulin-dependent diabetics bind specifically to pancreatic B cells. J Clin Invest **70:** 41-49, 1982.

45. Doberson, MJ, Scharff, JE, Ginsberg-Fellner, F, and Notkins, AL, Cytotoxic autoantibodies to beta cells in the serum of patients with insulin-dependent diabetes mellitus. New Engl J Med **303:** 1493, 1980.

46. Eisenbarth, GS, Wilson, PW, Ward, F, Buckley, C, and Lebovitz, H, The polyglandular failure syndrome: disease inheritance, HLA type, and immune function. Ann Int Med **91:** 528-533, 1979.

47. Valenta, LJ, Bull, RW, Hackel, E, and Bottazzo, GF, Correlation of the HLA-A1,B8 haplotypes with circulating autoantibodies in a family with increased incidence of autoimmune disease. Acta Endocrinol **100:** 143-149, 1982.

48. Satoh, J, Prabhakar, BS, Haspel, MV, Ginsberg-Fellner, F, and Notkins, AL, Human monoclonal autoantibodies that react with multiple endocrine organs. New Engl J Med **309:** 217-220, 1983.

49. Tan, EM, Autoantibodies to nuclear antigens (ANA): their immunobiology and medicine. Adv Immunol **33:** 167-240, 1982.

50. Tan, EM, Special antibodies for the study of systemic lupus erythematosus. Arthritis Rheum **25:** 753-756, 1982.

51. Sharp, GC, Anti-nRNP and anti-Sm antibodies. Arthritis Rheum **25:** 757-760, 1982.

52. Tan, EM and Kunkel, HG, Characteristics of a soluble nuclear antigen precipitating with sera of patients with systemic lupus erythematosus. J Immunol **96:** 464-471, 1966.

53. Alspaugh, M, and Maddison, P, Resolution of the identity of certain antigen-antibody systems in systemic lupus erythematosus and Sjogren's syndrome: an interlaboratory collaboration. Arthritis Rheum **22:** 796-798 1979.

54. Lerner, EA, Lerner, MR, Hardin, JA, Janeway, CA, and Steitz, JA, Deciphering the mysteries of RNA-containing lupus antigens. Arthritis Rheum **25:** 761-766, 1982.

55. Maddison, PJ and Reichlin, M, Deposition of antibodies to a soluble cytoplasmic antigen in the kidneys of patients with systemic lupus erythematosus. Arthritis Rheum **22:** 858-863, 1979.

56. Alexander, EL and Provost, TT, Ro (SSA) and La (SSB) antibodies. Springer Sem Immunopathol **4:** 253-273, 181.

57. Sontheimer, RD, Thomas, JR, and Gilliam, JN, Subacute cutaneous lupus erythematosus. A Cutaneous marker for a distinct lupus erythematosus subset. Arch Dermatol **115:** 1409-1415, 1979.

58. Sontheimer, RD, Stastny, P, and Gilliam, JN, Human histocompatibility antigen association in subacute cutaneous lupus erythematosus. J Clin Invest **67:** 312-316, 1981.

59. Sontheimer, RD, Maddison, PJ, Reichlin, M. Jordon, RE, Stastny, P, and Gilliam, JN, Serologic and HLA associations in subacute cutaneous lupus erythematosus, a clinical subset of lupus erythematosus. Ann Int Med **97:** 664-671, 1982.

60. Kephart, DC, Hood, AF, and Provost, TT, Neonatal lupus erythematosus: new serologic findings. J Invest Derm **77:** 331-333, 1981.

61. Miyagawa, S, Kitamura, W, Yoshioka, J, and Sakamoto, K, Placental transfer of anticytoplasmic antibodies in annular erythema of newborns. Arch Derm **117:** 569-572, 1981.

62. Scott, JS, Maddison, PJ, Taylor, PV, Esscher, E, Scott, O, and Skinner, RP, Connective-tissue disease, antibodies to ribonucleoprotein, and congenital heart block. New Engl J Med **309:** 209-212, 1983.

63. Lee, LA, Bias, WB, Arnett, FC, Huff, JC, Norris, DA, Harmon, C, Provost, TT, and Weston, WL, Immunogenetics of the neonatal lupus syndrome. Ann Int Med **99:** 592-596, 1983.