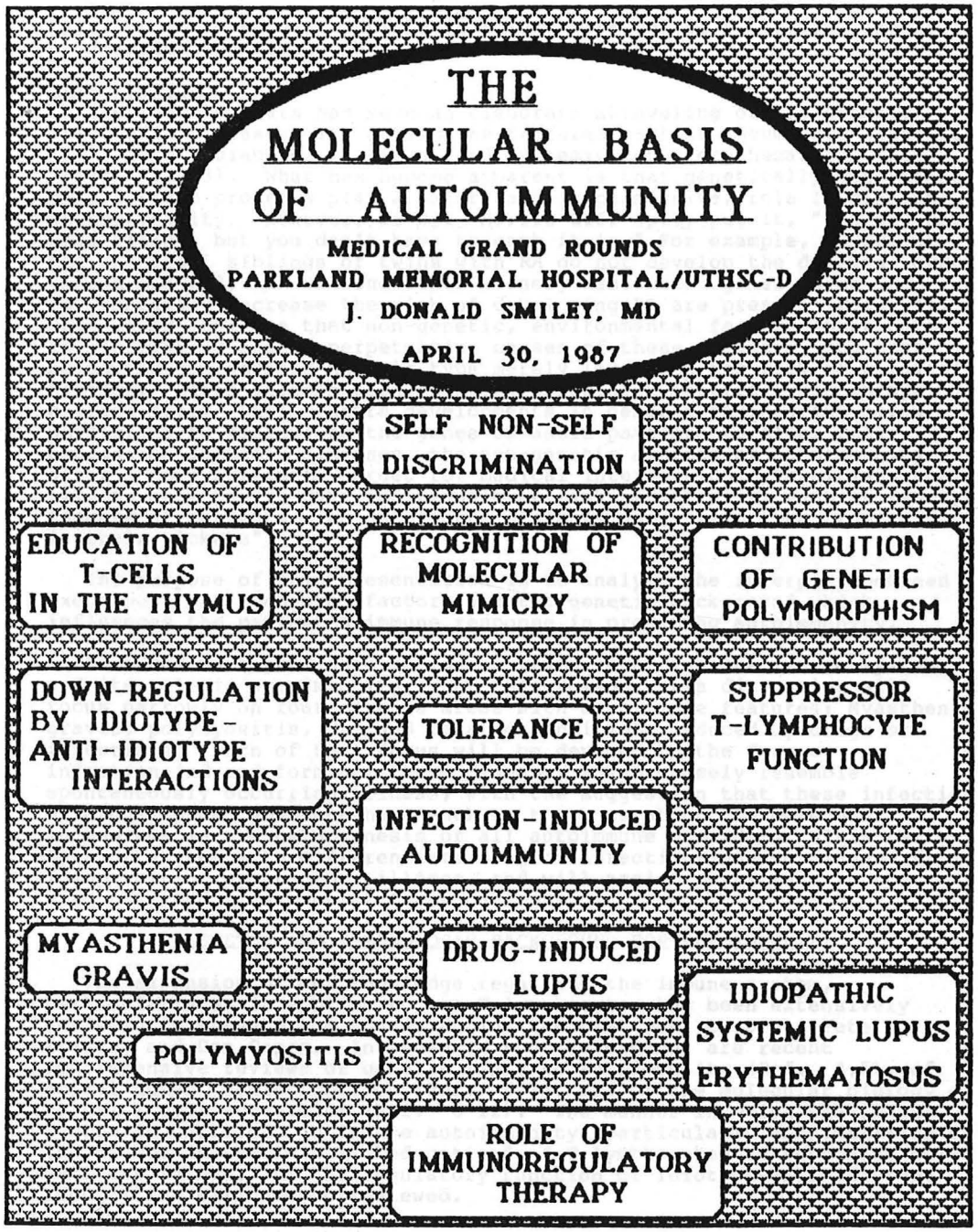

THE
MOLECULAR BASIS
OF AUTOIMMUNITY
MEDICAL GRAND ROUNDS
PARKLAND MEMORIAL HOSPITAL/UTHSC-D
J. DONALD SMILEY, MD
APRIL 30, 1987
SELF NON-SELF DISCRIMINATION
EDUCATION OF T-CELLS IN THE THYMUS
RECOGNITION OF MOLECULAR MIMICRY
CONTRIBUTION OF GENETIC POLYMORPHISM
DOWN-REGULATION BY IDIOTYPE-ANTI-IDIOTYPE INTERACTIONS
TOLERANCE
SUPPRESSOR T-LYMPHOCYTE FUNCTION
INFECTION-INDUCED AUTOIMMUNITY
MYASTHENIA GRAVIS
DRUG-INDUCED LUPUS
IDIOPATHIC SYSTEMIC LUPUS ERYTHEMATOSUS
POLYMYOSITIS
ROLE OF IMMUNOREGULATORY THERAPY

THE MOLECULAR BASIS OF AUTOIMMUNITY

"Mirrors would be well-advised to think twice before reflecting images."
Jean Cocteau (quoted by NK Jerne in Ref. 16)

INTRODUCTION

The last 10 years has seen an elaborate unraveling of the genetic factors in diseases with autoimmune features such as rheumatoid arthritis (RA), type 1 diabetes mellitus, and systemic lupus erythematosus (SLE) (Refs. 1,2,3). What has become apparent is that genetically-determined cell surface proteins play a major, albeit permissive, role in disease susceptibility. However, as Dr. Morris Ziff aptly put it, "You may have your ticket, but you don't have to cash it in." For example, 50-60% of the identical siblings of twins with RA do not develop the disease, even though HLA-Dw4, HLA-DR4 and other closely-associated genes which substantially increase the risk of developing RA are present (Ref. 1). This would indicate that non-genetic, environmental factors contribute the initiating and/or perpetuating causes of these diseases, and that being the appropriate genetic type merely increases the risk of clinical disease. Often persons with the risk-associated genetic types escape, disease-free. Until future developments in genetic engineering allow physicians to manipulate the genes of adult patients to alter their expression in these diseases, the non-genetic aspects of autoimmunity offer a more realistic target for medical intervention. Physicians would like to know how to take individuals at risk genetically, such as family members of patients with autoimmune disease, and, "keep them from cashing in their tickets".

The purpose of this presentation is to analyze the interplay between exogenous, environmental factors and the genetic background which influences the patient's immune response in producing autoimmunity.

Much has been published in recent years regarding the immune system and its role in autoimmunity. For this reason, this discussion will focus narrowly on four disease areas with autoimmune features: Myasthenia gravis, polymyositis, SLE and related conditions induced by drugs or infections. Much of this focus will be devoted to the drug or infection-induced forms of these diseases which closely resemble spontaneously occurring illness, with the suggestion that these infection or drug-induced versions have much to teach us about the molecular mechanisms in the pathogenesis of all autoimmune disorders. This new data often helps to differentiate drug or infection-induced disease from idiopathic or spontaneous illness, and will assist physicians in the diagnosis and treatment of these patients.

GENETIC AND IMMUNOLOGIC BACKGROUND FOR AUTOIMMUNITY

The expansion of our knowledge regarding the immune system, particularly as it applies to the T lymphocyte, has been extensively reviewed in these Grand Rounds during the past year by Drs. Peter Stastny and Don Capra. Included in your references are recent comprehensive reviews of genetic links to autoimmunity (Ref. 4,5), of normal human thymic function (Ref. 6,7), and of the molecular biology of normal T cell activation (Ref. 8-12). The manner in which the immune system is perturbed to induce autoimmunity, particularly abnormalities of the thymus in the initial "education" of T lymphocytes to recognize self antigens in man, and the regulatory function of idiotype-anti-idiotype recognition will now be reviewed.

EDUCATION OF T CELLS IN THE THYMUS TO RECOGNIZE SELF ANTIGENS

A critical aspect of thymic processing of T cells is the generation of lymphocytes which can detect foreign antigens, yet are not responsive to the self-antigens present in body tissues. As will be discussed later, thymic abnormalities such as thymoma or thymic dysplasia/hyperplasia are associated with autoimmune diseases including myasthenia gravis, aplastic states, SLE and polymyositis, suggesting that interruption of normal processing fails to impart the appropriate self-recognition.

During fetal life and childhood, the human thymus is a relatively large, lobulated organ with a well-defined external capsule surrounding a peripheral cortical region and a central medulla. Septate indentations divide each lobe and provide access for blood vessels which bring incoming stem cells into the subcapsular space. At any given time, only about 0.01% of thymic cells represent these immature stem cells. The remaining myriads of T cells are in various stages of processing into the mature T lymphocytes which will populate the lymph nodes, spleen, and bone marrow. Eventually, these peripheral T cells recirculate throughout the body, forming a vast repetoire capable of recognizing up to 750,000,000 different antigenic determinants. In normal persons, by puberty, this goal of peripheral dissemination has been reached, and the thymus involutes dramatically, so that in postmortem examinations on adults it may be difficult to locate any thymic tissue.

The use of frozen sections of human thymus and highly specific mouse monoclonal antibody reagents for immunohistological identification of T cells at different stages of maturation has greatly increased our understanding of thymic function during fetal and neonatal life (Ref. 6,7).

TABLE I. STAGES OF T CELL DIFFERENTIATION IN THE HUMAN THYMUS

	Anatomical Location	Associated Non-T Cells	CD-Antigens on T Cell Surface	Histological Features	Per Cent of Thymic T Cells
Stage I	subcapsular space	subcapsular epithelium thymosin+ thymopoietin+ MHC-I+	None	stem cells TdT+	0.01
			CD2,CD7	large thymic blasts TdT+	0.5-5.00
Stage II	cortex	1.cortical epithelium MHC-I&II++ 2.nurse epithelium MHC-I&II++ 3.macrophage MHC-I+	CD2,CD7 CD1,CD4,CD8	common cortical thymocytes TdT+	60-80.00
Stage III	cortical-medullary junction	1.dendritic MHC-I&II+++	CD2,CD7,CD6 CD4,CD3 (70%) MHC-I+	mature medullary thymocytes	15-20.00
	medulla	2.medullary epithelium thymosin+ thymopoietin+ MHC-I+	CD2,CD7,CD6 CD8,CD3 (30%) MHC-I+		

MHC-I = Class I HLA-A/B/C
MHC-II = Class II HLA-DR/DQ/DP
CD = Cluster Designation-surface antigen
TdT = Terminal Deoxynucleotidyl Transferase nuclear enzyme

Stem cells arriving in the thymus from the bone marrow first appear in the space beneath the dense thymic capsule. This space is lined by the subcapsular epithelium which produces thymosin, thymopoietin, and perhaps other unrecognized hormones. Like most other cells in the body, thymic stem cells have germline DNA in those regions of their seventh and fourteenth chromosomes which ultimately will code for the antigen recognition system on the mature T cell surface. The hormones from subcapsular epithelial cells stimulate a random process of gene rearrangements of the germline T cell genes to code for a different antigen receptor on each T cell.

These rearranging T cells now enter the thymic cortex where they make up 60-80% of all thymic lymphocytes. These cortical T cells soon acquire surface membrane proteins which include CD1, CD2, CD7, CD4 and CD8, (the latter two thought capable of reacting with Class I=HLA-A/B/C, and Class II=HLA-DR/DQ/DP histocompatibility antigens, respectively), in addition to the rearranged gene for the beta chain of the T cell antigen receptor.

These cortical T cells have also undergone other differentiation which has altered their purine metabolism, greatly reducing the intracellular level of three enzymes which break down deoxypurines: purine nucleoside phosphorylase, 5'-nucleotidase, and adenosine deaminase (Ref. 6). The reduction in the intracellular level of these enzymes allows the rapid accumulation of deoxynucleotides, particularly dATP and dGTP. These changes favor rapid DNA synthesis, but also lead to toxic levels of deoxynucleotides which literally threaten thymic cortical T cells with biochemical suicide.

FIGURE 1a. METABOLISM OF DEOXYNUCLEOTIDES IN T CELLS
(Modified from Janossy, Curr Top Pathol 1986)

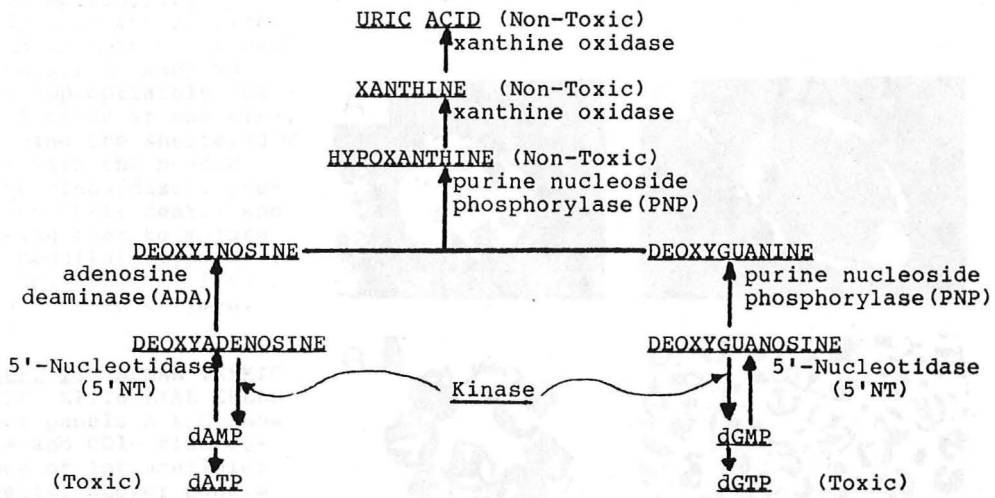
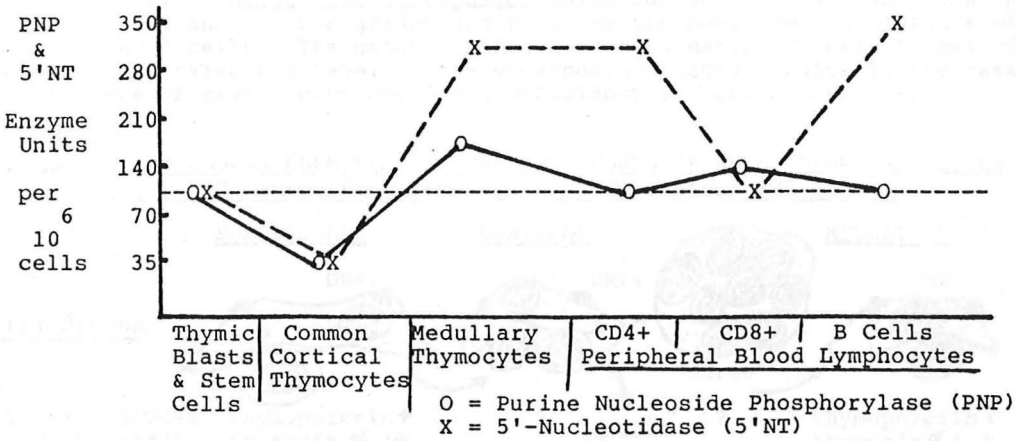


FIGURE 1b. PURINE METABOLISM IN THYMOCYTES AND T CELLS
(Modified from Hoffbrand, Clin Haematol 1982, Ref. 13)



However, the thymic cortex is uniquely prepared to deal with this situation with three types of non-T cells. The first of these is a cortical epithelial cell which has a large amount of both Class I=HLA-A/B/C, and Class II=HLA-DR/DQ/DP histocompatibility antigens on its surface available for reaction with the surface receptors on cortical T cells. If self-recognition occurs, the selected T cell is then transferred to a second type of non-T cell, a nurse epithelial cell (See Fig. 2), which, unlike the subcapsular epithelial cell, does not make thymosin or thymopoietin, but does have both Class I and Class II histocompatibility antigens on its surface. The nurse epithelial cell can engulf as many as seven appropriately reactive T cells at one time, providing the sheltered T cells with the needed deoxynucleosidases, preventing their death, and allowing them to mature into medullary-type cells containing higher levels of the needed enzymes.

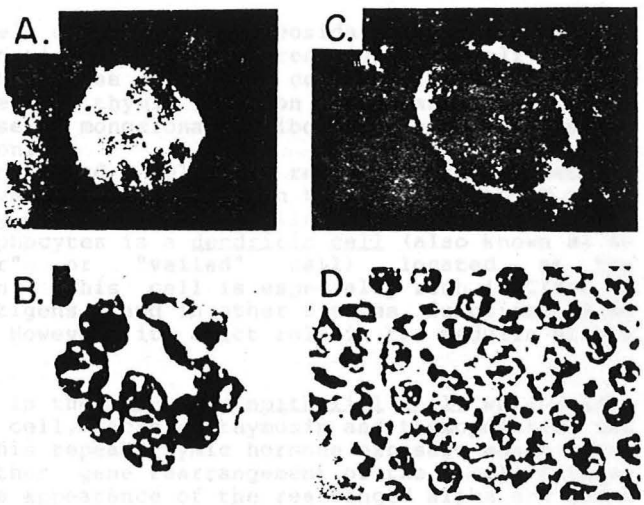
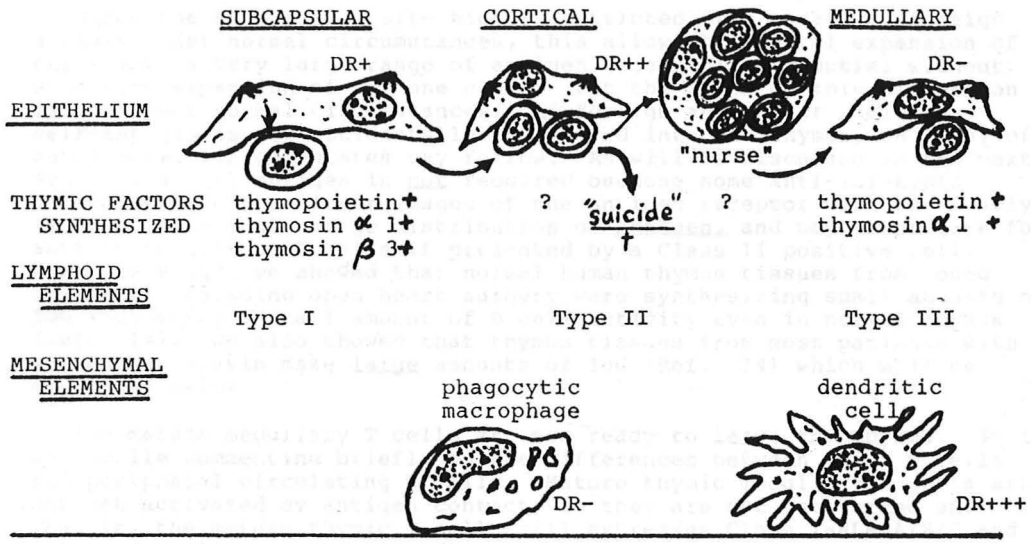


FIGURE 2. HUMAN THYMIC NURSE EPITHELIAL CELLS Upper panels A & C show CD1+ and CD1- fluorescence of intracellular T cells. Lower panels B & D show same nurse cells by phase contrast. (Ritter, 1981, Ref. 7).

The cortical T cells which fail to recognize self MHC-Class I&II antigens die of biochemical suicide, and are taken up by a third type of non-T cell, a phagocytic macrophage, which has only Class I antigens on its surface, and which degrades and recycles the DNA, RNA and proteins of the dying T cells. The genetic failure of more mature T cells to switch back on and raise the level of the deoxynucleotidases results in the rare occurrence of severe combined immunodeficiency syndromes (Ref. 6).

FIGURE 3. CELLULAR ELEMENTS OF THE THREE ZONES OF HUMAN NEONATAL THYMUS (Modified from Janossy, Curr Top Pathol 1986)



The reduction in the levels of the deoxynucleosidases acts to restrict the future survival of cortical T cells not reactive with self surface proteins. The action of the nurse epithelial cell to protect the "good guys" is a fascinating aspect of thymic function which has only recently been clarified by the use of monoclonal antibody reagents to identify each stage of cell maturation.

From the cortex, self-selected T cells are released into the medulla and make up 15-20% of thymic lymphocytes. In the medulla, the T cells now encounter two totally different non-T cells. The first non-T cell reacting with medullary lymphocytes is a dendritic cell (also known as an "interdigitating reticular" or "veiled" cell) located at the medullary-cortical junction. This cell is especially rich in Class II (HLA-DR/DQ/DP) surface antigens, and in other tissues, functions as an antigen presenting cell. However, its exact role in the medulla of the thymus is unclear.

The second non-T cell is the medullary epithelial cell, which, like the subcapsular epithelial cell, secretes thymosin and thymopoietin, and is Class I (HLA-A/B/C). This repeat thymic hormone exposure again turns on the stimulus for further gene rearrangement of the T cell antigen receptor resulting in the appearence of the rearranged alpha and gamma chain genes, loss of CD1, and especially acquisition of CD3 and CD6 surface antigens.

With the CD3 complex, and CD4 or CD8 in place, the maturing T cell has for the first time acquired the necessary machinery to divide when stimulated by specific antigen (Ref. 8,9). Medullary T cells now begin to separate into two discrete subpopulations with 70% losing their CD8 surface antigen becoming T helper/inducer type cells, and 30% losing their CD4 surface antigen becoming T cytotoxic/suppressor type cells. This makes mature medullary T cells similar to peripheral T cells in terms of the ratio of CD4/CD8 positive cells.

Since the thymus is a site highly restricted from access to foreign antigen under normal circumstances, this allows horizontal expansion of cells with a very large range of antigen recognition potential without selective expansion of any one clone. But the focus of this discussion is not about normal circumstances. If foreign antigen or modified self-antigen is now accidentally introduced into the thymus, an array of autoimmune clinical states may follow. As will be discussed in the next section, actual antigen is not required because some anti-idiotypic antibodies are the mirror-images of the antigen receptor with the nearly identical chemical charge distribution of antigen, and may substitute for antigen to turn on T cells if presented by a Class II positive cell. Many years ago, we showed that normal human thymus tissues from young children undergoing open heart surgery were synthesizing small amounts of IgG indicating a small amount of B cell activity even in normal thymus (Ref. 14). We also showed that thymus tissues from most patients with myasthenia gravis make large amounts of IgG (Ref. 14) which will be discussed below.

The mature medullary T cells are now ready to leave the thymus. It is worthwhile commenting briefly on the differences between these T cells and peripheral circulating T cells. Mature thymic medullary T cells are not yet activated by antigen contact, so they are not yet turned on. That is, the mature thymic T cell still expresses Class I=HLA-A/B/C and only a few Class II=HLA DR/DQ/DP surface antigens, and has only low levels of interleukin-2 (IL-2) receptors. After antigen exposure, greatly increased amounts of Class II=HLA-DR/DQ/DP and IL-2 receptors will be expressed.

CD4-positive T cells (70%) not only augment the immune response of B cells (Helper subset), but have a separate sub-population for activating cytotoxic and/or suppressor T cells (Inducer subset) (Ref. 9,12). The CD8-positive T cells (30%) are even more heterogeneous, but can be divided into two major types: A cytotoxic subset which has an identical T cell antigen receptor to the CD4-helper T cell, made up of an alpha and a beta chain, but differs from it by not expressing CD4 or Class II=HLA-DR/DQ/DP proteins on its surface.

CD8-positive cells also include the suppressor subset which has a different T cell antigen receptor which does not express either the alpha or the beta chain, but possibly contains the gamma chain (Ref. 8). However, the CD8-suppressor subset does express Class II=HLA-DR/DQ/DP antigens on its surface when functionally activated by CD4-inducer T cells. A number of new monoclonal antibodies have been developed which have allowed the identification of the four types of peripheral T cells (See Table II, next page).

TABLE II. MONOCLONAL ANTIBODIES WHICH IDENTIFY ACTIVATED T CELL SUBSETS
(Modified from Romain & Schlossman, Adv Intern Med 1986)

m-Antibody Designation	T-Helper	T-Inducer	T-Cytotoxic	T-Suppressor
CD4	+	+	-	-
CD8	-	-	+	+
Class II-DR/DP/DQ (not monoclonal)	+	+	-	+
4B4	+	-	-	-
Leu8	-	+	±	±
2H4	-	+	±	±
JRA	-	+	±	±
CD2,CD3,CD6,CD7	+	+	+	+
Tac (a-IL2-R)	+	+	+	+

IDIOTYPIC NETWORKS AND AUTOIMMUNITY

The concept of idiotype-antiidiotype was first introduced by Henry Kunkel and his coworkers, Mart Mannik and Ralph Williams, at the Rockefeller Institute in 1963 (Ref. 15). They observed that a unique antibody response in rabbits following immunization with purified human antibodies to blood group A, dextran or levan caused a substantial portion of the rabbit antibodies formed to be directed against that part of the gamma globulin molecule later shown to represent the antigen-combining site. Later, the antigen-combining site, unique for each different myeloma, or for the antibody from any one clone of B cells, was called the idiotype, and antibodies directed against different parts of the idiotype, called anti-idiotypic antibodies. At first this finding was seen as fascinating only in the sense of emphasizing the unique diversity of antibodies derived from different clones of B cells, but later the broader significance of anti-idiotypic antibodies was understood (Ref. 14).

Within a given individual, anti-idiotypic antibodies against a new idiotype appear when the level of the idiotype reaches a critical level (somewhere between 0.1 and 1.0% of the total gamma globulin level in the animal), and inhibit further synthesis of that particular monoclonal antibody. Levels of new antibody below a critical level do not appear to generate an anti-idiotypic response. This type of control mechanism has enormous advantage since as long as antigen is abundant, and antibody is being used up, synthesis accelerates, but when antibody excess appears, the idiotype synthesis is turned off with enough for protection, but not with multiple myeloma.

Experimental infusion of different levels of purified anti-idiotypic antibodies have shown high concentrations to be potent inhibitors of the expression of antibodies of the corresponding idiotype, while low concentrations may enhance new antibody synthesis, probably because some anti-idiotypic antibodies (as internal mirror images of the antigen receptor) share the chemical charge distribution of the original antigen (Ref. 16).

What does all of this have to do with autoimmunity? Like the idiotype of antibodies, a similar clonally-unique antigen receptor is found on the surface of T cells. Although less well-studied, feedback regulation by excess T cell idiotype is also believed to occur (Ref. 17,18). How do responses to self-antigens fit into the T and B cell systems? Most immunologists agree that normal B cells in vitro have an almost unlimited range of responses, including the formation of antibodies to most auto-antigens. More recently, the same autoreactivity has been shown for T cells (Ref. 19,20). Small amounts of auto-antibodies and small numbers of auto-reactive T cells are present in healthy people (Ref. 16,18). How then can the normal immune system keep these autoimmune responses to a minimum?

Generation of T cell antigen receptor diversity in the thymus and of antibody idiotypic diversity in the bone marrow each requires random rearrangements of the germline genes which code for antigen recognition, and evolutionary selection of which germline genes survive almost certainly has reflected the antigen pressures to which the organism has been subjected. At first glance, the threat of microorganisms would seem the most likely selective force, but Jerne and others (Ref. 16,17,18) have stressed that self-antigens are even greater selectors of these germline genes. Self-antigens are present in enormous concentrations in the host at all times, including the period of early education of T cells in the thymus. "Forbidden clone abortion" in thymic development sounds good, but it turns out that autoreactive T and B cells are alive and well in normal people. For this reason, a completely different theory for control of excess autoimmunity seems necessary.

This theory (Ref. 17) suggests that much of the germline gene repetoire is related to self-antigen recognition. The resulting selection of self-recognizing clones of both B and T cells is then followed in the restricted environments of early development by anti-idiotypic clones against the specific receptors for self antigens on B and T cells which down-regulate the self-responders. These idiotype/anti-idiotype partners for auto-antigens differ in a very important way from the idiotype/anti-idiotype antibodies for exogenous antigens. Anti-idiotypic responses against autoantibodies are clonally restricted (Ref. 19,20,21). That is to say, anti-idiotypic antibodies against an autoantibody show a remarkable degree of oligoclonal restriction (Ref. 22,23,24) while the anti-idiotypic antibodies against a myeloma protein generated in a rabbit, or against a clone of antibodies directed against an exogenous antigen are polyclonal, with at least 30-100 different anti-idiotypic antibodies formed for each idiotype against most foreign antigens. This might be explained by more germline DNA recognition of most autoantigens while highly rearranged DNA genes code for recognition of foreign antigens (Ref. 17).

The theory also suggests that a substantial proportion of the long-lived T and B memory cells are anti-idiotypic autoregulators. It is estimated that at any given time about half of the T and B cell populations are in the long-lived category, and that they are replaced at an annual rate of about 5% (Ref. 16). As the individual grows older, there is a gradual attrition of the anti-idiotypic autoregulators, perhaps accounting for the increases in autoimmunity in older persons (Ref. 25).

Bacteria or viruses that can mimic self-antigens, or that are able to survive in a protected intracellular environment in spite of a hyperimmunized host are then seen as "hard to deal with" because they mimic the "self" situation and cause anti-idiotypic antibodies or T cells to arise which down-regulate the antibodies directed against them (Ref. 16). A later section addresses the link between the mimicry generated by infection or chemical exposure and autoimmunity.

IMMUNOLOGIC FEATURES OF IDIOPATHIC MYASTHENIA GRAVIS

Myasthenia gravis (MG) is a relatively rare disorder associated with HLA-DR3/B8, and characterized by muscle weakness which worsens with use. It is now known to be a prototypic anti-receptor autoimmune disease with autoantibodies against the nicotinic acetylcholine receptor (AChR) (Ref. 26). These anti-AChR antibodies are under the direct control of autoreactive T cells which arise in the thymus as a result of abnormalities which impair normal self-recognition. Although very small amounts (<1.0 unit/liter of serum) of anti-AChR antibody may be found in normal subjects, 91% of patients with active MG severe enough to interfere with daily activities show anti-AChR above 1.0 unit/liter. Over 70% of asymptomatic or minimally active patients with MG also show anti-AChR levels above 1.0 unit (Ref. 27).

In patients with MG, unlike in normal adults, the thymus is usually enlarged, either as a result of lymphoid dysplasia/hyperplasia characterized by lymphoid follicles in the medulla containing B lymphocytes, plasma cells and immune complexes; or as a result of the development of a malignant thymoma of epithelial cell elements (Ref. 6,14). Most patients with thymic dysplasia show intrathymic synthesis of anti-AChR antibodies in a concentration (when compared to total IgG synthesis) which is ten times greater than peripheral blood lymphocytes, lymph nodes or bone marrow (Ref. 28). Those with thymomas appear to synthesize their AChR-antibody exclusively in the peripheral lymphoid tissues (Ref. 6). This is thought to explain the therapeutic benefit of thymectomy only in MG patients with dysplasia/hyperplasia, but not in those with thymomas (Ref. 6).

Autoreactive AChR-specific helper T cell lines can be isolated from blood mononuclear cells of patients with MG (Ref. 29,30), and can augment AChR-antibody production in vitro in the presence of AChR, supporting the role of these T cells in autoimmune regulation in MG. CD8-positive suppressor T cells which recognize the AChR are also present in the peripheral lymphoid tissues of patients with MG, and exert substantial, although incomplete, down-regulation of AChR-antibody production (Ref. 31). In spite of clinical improvement of idiopathic MG in most patients with thymic dysplasia/hyperplasia after thymectomy, changes in the abnormal CD4+/CD8+ T cell ratio (which is consistently elevated above normal in the blood of patients with active disease) does not occur, even one year after thymectomy (Ref. 32).

INDUCTION OF MYASTHENIA GRAVIS BY D-PENICILLAMINE

D-Penicillamine (D-Pen) was first used medically to lower the free copper levels in body fluids of children with Wilson's disease (absence of ceruloplasmin, cirrhosis of the liver, Parkinsonism and Kayser-Fleischer rings of copper deposited on the periphery of the iris of the eye). D-Pen is a four-carbon compound with an amino group on one end and a thiol (sulfhydryl) group on the other, representing only a small fragment of the penicillin-G molecule. It has been shown to have low toxicity in animal studies, and forms mercaptides with lead, mercury, gold, and copper which are rapidly cleared by the kidney. D-Pen is also used to treat cystinuria. D-Pen forms a soluble, mixed disulfide with cysteine and prevents the formation of highly-insoluble cystine renal stones.

Fortuitous observation of a patient with Wilson's disease who also had rheumatoid arthritis led Jaffe and others to use D-Pen in adult RA patients where it was found to be tolerated by about 65% of patients with beneficial improvement in arthritis activity in approximately 50% of patients. Because D-Pen also blocks an enzyme responsible for cross-linking of collagen in growing tissues causing an increase in the "soluble" collagen fraction, it has also been used to treat patients with scleroderma (progressive systemic sclerosis) with equivocal results (Ref. 33).

The mechanism of action of D-Pen in RA is now generally believed to be modulation of T lymphocyte function with immunosuppression of the disease (Ref. 34,35). However, D-Pen also significantly reduces the level of IgM, including IgM-rheumatoid factor (RF), believed to be T cell independent (Ref. 36). The RF-reduction may be secondary to D-Pen's effect on T cells which regulate the synthesis of IgG causing a fall in IgG-containing immune complexes, which are the stimuli for IgM-RF synthesis (See below).

Unfortunately, both in Wilson's disease and in RA, about 35% of patients develop toxicity to the drug. Some of these toxic reactions have features of autoimmune diseases, including membranous glomerulonephritis, MG, SLE, polymyositis, pemphigus, pemphigoid, Goodpasture's disease, Sjogren's syndrome and progressive systemic sclerosis (Ref. 37). Since most D-Pen is given to treat rheumatoid arthritis, that is the disease in which the autoimmune complications have often been observed. In particular, 0.4-1.0% of RA patients treated with D-Pen for several months develop MG (Refs. 38,39,40,41). It was Masters, et al,(Ref. 42) in 1977 who first determined that patients with D-Pen-induced MG had antibodies to the AChR. They also showed many of the same patients to have antibodies to striated muscle (Ref. 42). D-Pen-induced MG differs in several ways from the idiopathic form of the disease (See Table III).

Much effort has been made to identify genetic markers which will predict which patients with RA taking gold or D-Pen will be at risk for toxicity. Of significant interest is the finding of two independent groups that patients who are HLA DR3/B8 are 32 times more likely than other genetic types to develop <u>proteinuria</u> (?membranous glomerulonephritis) to either drug (Ref. 46,47), and while HLA-DR3/B8 is the largest risk group for idiopathic MG, this type is <u>not</u> at increased risk to develop D-Pen-induced MG (See Table III, Ref. 44,45).

TABLE III. SEROLOGICAL AND GENETIC DIFFERENCES BETWEEN IDIOPATHIC AND D-PENICILLAMINE-INDUCED MYASTHENIA GRAVIS

	Idiopathic MG	D-Pen MG	(Ref.)
Anti-AChR Antibody			
Titer-Units/l serum	56-72	6.5	(43)
Avidity	0.61	0.38	(43)
K (x10 to minus 12)	24-57	73.7	(43)
D			
% Kappa Light Chain	66-79	58.0	(43)
% IgG	10-16	18.0	(43)
3			
HLA-Types	DR3/B8	DR1/Bw35	(44,45)

D-Pen use in scleroderma (progressive systemic sclerosis) (Ref. 33,48,49), and primary biliary cirrhosis (Ref. 50,51) has generated isolated case reports of D-Pen-induced MG, and of interest, in the two patients in which lymphocyte typing was done, they were not HLA-DR3/B8 as would be expected in 60-80% of patients with idiopathic MG (Ref. 26), but rather typed for Bw35 (Ref. 33) and DR1 (Ref. 50), again characteristic of the genetic types at risk for the drug-induced form of the disease. In contrast to this finding, Steen, et al (Ref. 48) reported four patients with scleroderma who developed MG after being treated with D-Pen, and all four were HLA-DR5.

Even when D-Pen is stopped when patients are found to have developed MG, the disease may persist for many weeks because of the presence of circulating anti-AChR antibodies. Although usually a mild disease, D-Pen-induced MG may resemble idiopathic MG, and be severe, life-threatening, and poorly responsive to anticholinesterase medications. Successful treatment with plasmaphresis of two such refractory D-Pen-induced MG patients has been reported (Ref. 52) with both patients quickly regaining normal muscle power. In addition, clinical response of the underlying RA to D-Pen may precede the appearence of MG, and tempt the physician to give the patient gold therapy in the place of the D-Pen. Unfortunately, this may reactivate the MG, suggesting that both gold (which forms a mercaptide with thiol groups on cell surface proteins) and D-Pen (which forms a mixed disulfide with the same thiol groups) can share the induction of the autoimmune response of MG (Ref. 53).

IMMUNOLOGIC ABNORMALITIES IN IDIOPATHIC POLYMYOSITIS

Polymyositis is a clinical syndrome characterized by muscle weakness, a peculiar vasculitis of the skin (around the eyes, in light exposed areas of the face and upper neck, and over the extensor surfaces of the finger joints, knees and elbows), and occasionally myocarditis. It has multiple initiating causes. The cause is unknown in 60% of patients while the remaining 40% of polymyosytis patients also have SLE, MCTD, scleroderma, cancer including thymoma, toxoplasmosis, or are receiving treatment with D-Pen.

These initiating causes share, to a variable degree, a final common pathway of T-lymphocyte-mediated autoimmunity against rapid contracting, striated muscle fibers present predominantly in the proximal limb girdles, neck and thoracic areas, and myocardium. The classical study by Johnson, Fink and Ziff in 1972 (Ref. 54) showed that the lymphocytes infiltrating polymyositis muscle biopsies would secrete lymphocytotoxin (now also known as Tumor Necrosis Factor-Beta), a lymphokine subsequently shown to be released after specific muscle antigen stimulation by CD4+ cytotoxic T lymphocytes (Ref. 55). This T-cell mediated muscle lysis explains why high dosages of prednisone can be life-saving in most polymyositis patients since prednisone promptly shuts off lymphocytotoxin (TNF-beta) release in over 60% of patients.

POLYMYOSITIS AND OTHER AUTOIMMUNE DISORDERS INDUCED BY D-PEN

Although only 0.2 to 0.4% of caucasian patients with RA who receive D-Pen develop polymyositis (Ref. 37,56,57,58), 1.2% of Japanese patients with RA treated with D-Pen develop the disease (Ref. 72). The two caucasian patients who were genetically typed (Ref. 58), and 50% of the Japanese patients (Ref. 59) who developed polymyositis while taking D-Pen were HLA-DR1, making genetic susceptibility to develop polymyositis similar to D-Pen-induced MG (See Table III).

D-Pen-induced polymyositis may be severe with fatal outcome in spite of treatment (Ref. 56). The clinical appearence of both MG and polymyositis after D-Pen is delayed, usually occurring after more than one year of treatment with D-Pen (Ref. 57). When D-Pen is stopped, the polymyositis slowly remits, but may recur if D-Pen is restarted (Ref. 58). Antinuclear antibodies were present in the serum of 12 of 15 patients (Ref. 59), and may persist after D-Pen is stopped, emphasizing that other autoimmune manifestations may accompany the muscle involvement. Anti-JO-1, an autoantibody present in 30% of patients with idiopathic polymyositis, has also been observed with D-Pen-induced polymyositis (Ref. 60).

As mentioned above, D-Pen therapy can induce other autoimmune states, including a variety of nephropathies. Ntoso, et al, (Ref. 61) describe two patients with scleroderma who developed diffuse, proliferative glomerulonephritis with serological changes of SLE which was reversible when treated with high dose pulse methylprednisolone after stopping D-Pen. They also review other forms of renal disease seen after D-Pen use, including membranous glomerulonephritis with heavy proteinuria (Ref 61). Yung and Hambrick (Ref. 62) describe a patient who developed pemphigus on D-Pen, and reviewed all reported cases through 1982.

GENETIC/IMMUNOLOGIC ASPECTS OF IDIOPATHIC SLE

The combination of HLA-DR3/B8 can be thought of as the "German Gene", since its frequency is greater in Germanic peoples than in other racial groups. To put it mildly, those Germans really got around! It also carries the dubious honor of being the "Autoimmune Gene", since it has been associated with an increased risk of developing SLE, myasthenia gravis, Type 1 diabetes mellitus, chronic active hepatitis, Sjogren's syndrome, Graves' disease, Addison's disease, pernicious anemia, celiac disease, and dermatitis herpetiformis (63).

McCombs and her coworkers (Ref. 63) found 7 of 11 B8-positive normal persons to have the DR3 antigen while none of 12 B8-negative normal persons had the DR3 antigen, suggesting a linkage disequilibrium in the HLA-DR3/B8 combination. They also found B8 positive donors to have greater numbers of both B and T lymphocytes with more CD4+ T cells and fewer CD8+ T cells, giving an overall CD4/CD8 ratio of 2.49 compared to 1.69 for B8-neg donors. B8+ donors also had more IgM+ B cells, higher gamma globulin levels and greater Ig synthetic responses after poke weed mitogen stimulation. The B8+ donors also showed formation of less suppressor factors when their T cells were stimulated by concanavalin A. These findings would suggest that persons with the HLA-DR3/B8 genetic type develop alterations in lymphocyte subsets and lymphocyte functions which explain their predilection to develop autoimmunity (Ref. 63).

Just how this genetic variation could cause autoimmunity has captured the imagination of geneticists, immunologists, and physicians involved in the care of patients with autoimmune diseases, such as SLE. One area would be the role of these MHC-proteins in the T-cell surface receptors which were discussed earlier. An interesting Black Jamaican family has been described by Stohl, Crow and Kunkel (Ref. 64) at the Rockefeller Institute. Homozygous members of this family lack the CD4-epitope on their helper/inducer T cells, and three family members had SLE associated with generalized lymphadenopathy. Involved patients share the HLA-DR5/Bw35 combination, and are homozygous for absence of the CD4-epitope on their T cells. Remember that the AIDS virus uses the CD4-epitope as its specific receptor, and selectively depletes CD4+ T cells. Also recall that many AIDS patients, like this unusual subset of SLE patients, have significant generalized lymphoadenopathy.

Another fascinating link between genetic aberrations and SLE was simultaneously discovered by Kotzin, et al, (Ref. 65) and Noonan, et al, (Ref. 66) in NZW mice. Remember that these white mice are clinically well, but when crossed with NZB mice the NZB/NZW-F1 hybrid develops florid lupus kidney disease, and a full range of other autoimmune phenomena resembling human SLE. The inbred NZB mice have mild hemolytic anemia, but usually do not have the severe glomerulonephritis characteristic of the hybrid NZB/NZW animals. These investigators (Ref. 65,66) have shown that NZW mice have a deletion of a significant segment of their germline DNA, Cβ1-Dβ2-Jβ2 elements, which codes for the beta chain of the T cell antigen receptor.

It would be reasonable to assume that this genetic deletion in combination with some other genetic defect in the NZB mouse strain, prevents the normal thymic "education" of T cells in the hybrid NZB/NZW mouse. This impairs the usual suppression of selected autoimmune responses. Singer, et al, (Ref. 67) studied MRL-lpr/lpr mice, which develop SLE-like disease, and showed selective abnormalities in the expression of the Vβ8 gene of the beta chain of the T cell antigen receptor; and Hashimoto, et al, (Ref. 68) studied C3H/HeJ gld/gld mice, also prone to develop SLE, and found unusual increased rearrangements of the beta chain gene when compared to the normal C3H/HeJ mouse controls. They (Ref. 68) speculated that premature emigration of T cell clones from the thymus to peripheral lymphoid tissues, before the clones had been "educated" for appropriate self recognition, resulted from the influence of the abnormal gld-gene on thymic function. They pointed out that gld/gld mice will not express lupus if thymectomized (Ref. 68).

These various genetic defects involve different parts of the beta chain gene of the T-cell antigen receptor. They impact on thymic processing of T cells in different mouse strains which develop SLE-like disease, indicating a spectrum of genetic changes in SLE in the mouse. Almost certainly, a similar variation in genetic defects are present in human patients with the disease.

Taking a somewhat different approach, human idiopathic SLE can be broken down into several clearly identifiable serological and clinical variants. For example, by examining the in vitro effects of anti-T cell antibodies from SLE patients on T cell subsets, and T cell functions, Morimoto, et al (Ref. 69), were able to divide SLE patients into four immunologically and clinically different groups (See Table IV). Remember that 75-90% of patients with active SLE have IgM-cold-reactive anti-T cell antibodies, and that the level of these anti-T-cell autoantibodies correlate with lymphopenia in active SLE. The levels of these anti-T cell autoantibodies parallel the titers of anti-n-DNA, rising and falling during exacerbations and remissions of disease (Ref. 70,71).

TABLE IV. ANTI-T CELL ANTIBODIES IN SLE. IMPACT ON T CELL FUNCTIONS
(Modified from Morimoto, et al, JCI 1984)

Combinations of Anti-T Cell Antibodies	CD8/CD4 Killing Ratio	Inhibition of Suppressor Function	Clinical Features (% of SLE Patients)
Group A -Both Anti-CD4 Inducer Anti-CD8 Suppressor	1.2	0.8	Severe renal disease, Thrombocytopenia (43%)
Group B -Only Anti-CD8 Suppressor	3.3	3.5	(21%)
Group C -Only Anti-CD4 Inducer	1.2	1.0	Severe renal disease, Thrombocytopenia (21%)
Group D -To Other T Cell Antigens	1.8	1.2	(15%)

The exact mechanism by which anti-T cell autoantibodies in SLE cause lymphopenia is still not certain. Winfield, et al (Ref. 71), were able to show that an unidentified surface antigen (not CD3, CD4, CD8, CD11, DR-framework, beta-2-microglobulin, or IL-2-receptor=Tac) was stripped off normal peripheral blood T cells by the IgM-cold reactive anti-T cell antibodies, and after washing, would be reexpressed on the T cell surfaces in a few hours. These same anti-T cell antibodies are enriched in serum cryoprecipitates from patients with active SLE, presumably representing immune complexes formed with the unidentified T cell surface antigen (Ref. 71). Winfield, et al, speculated that impaired T cell suppressor functions could be explained by the impact of these anti-T cell autoantibodies (Ref. 71).

On the other hand, Sano, et al (Ref. 72), showed that anti-T cell autoantibodies in the serum from 6 of 15 Japanese patients with SLE showed reactivity with Tac-antigen (believed to be an epitope of the IL-2 receptor) on T cell lines. Others (Refs. 73-78) have shown that IL-2 production in vitro by CD4+ and CD8+ T cells in SLE is deficient after PHA plus PMA stimulation, but is not deficient after Con A plus PMA stimulation (Ref. 79).

The IL-2 secretion defect in SLE T cells disappeared after removal of spontaneously activated suppressor cells (Ref. 74), or after resting the SLE T cells in culture for 2-3 days before stimulating them (Ref. 75), suggesting that a rapidly reversible defect occurs on circulating T cells in vivo in SLE.

ANTI-n-DNA ANTIBODIES, T-CELLS AND RENAL DISEASE IN SLE

Anti-n-DNA antibodies are found in a majority of patients with SLE, and correlate with the presence of diffuse proliferative glomerulonephritis, central nervous system disease and low C3 and C4-complement levels in the serum. Recently it has been shown that these autoantibodies to DNA are oligoclonal, and share idiotypic cross-reactivity with the anti-DNA antibodies of unrelated patients with SLE. The idiotype of one monoclonal anti-DNA antibody obtained from a cell line from a patient with active SLE has been termed 16/6 (Ref. 80). This 16/6 idiotype was shared in the serum of 67% of SLE patients with anti-DNA antibodies, and it was also found in the serum of 50% of their asymptomatic first-degree relatives, but in only 3% of normal control sera.

The presence of the 16/6 anti-DNA idiotype in the serum correlated highly with a defect in T suppressor cell function in both the patients with SLE and in some of the asymptomatic first-degree relatives who had also shown the 16/6 idiotype in their serum (Ref. 80). This was interpreted as showing the expression of anti-n-DNA antibodies could be linked to a genetically transmitted defect in T cell suppressor function allowing autoantibody expression.

Mouse monoclonal anti-idiotypic antibodies against anti-DNA offer reagents which allow quantitation of similarities in these antibodies shared from one SLE patient to another. Livneh, et al (Ref. 81) have generated such a mouse monoclonal reagent (8.12) which recognizes a unique negatively-charged (cationic) anti-n-DNA antibody containing a lambda-light chain which is found in the serum of 50% of SLE patients. The authors (Ref. 81) believe this is a subset of antibodies which are preferentially deposited in the kidney, and that this mouse monoclonal reagent (8.12) will have predictive and diagnostic value in deciding which SLE patients are destined for severe renal disease.

D'Agati, et al (Ref. 82), used monoclonal reagents to stain the infiltrating T lymphocytes in cryostat sections of kidney biopsies from 26 patients with SLE. There was a substantial increase in the CD8+ T cells ($47.3 \pm 11.0\%$) compared to CD4+ T cells ($32.5 \pm 11.3\%$) in the interstitial space in the kidney. Monocytes made up 7.9%, B cells only 3.9%, and the remaining mononuclear cells were "null". The CD4/CD8 ratio was less than 1.0 in 22 of the 26 biopsies. There was no correlation with the CD4+ and CD8+ T cell distribution in the peripheral blood of each patient at the time of the kidney biopsy.

Discordant results showing more CD4+ than CD8+ T cells in the renal lymphocytic infiltrate had been reported earlier by Caligaris-Cappio, et al (Ref. 83) in renal biopsies from 11 SLE patients. In view of the small numbers of renal biopsies involved in both studies, and the immunologically opposite functions which CD4+ helper T cells may have compared to the CD4+ inducers (of CD8+ suppressor T cells), the differences between these studies are understandable, and both may be correct. The overall conclusion would be that T cells may be playing a greater role in producing immunologic injury of the kidney in SLE than has been thought in the past.

Finally, a group of atypical SLE patients have been described in which the antinuclear antibody (ANA) is negative, and which have been shown to have unusual clinical and serological features of autoimmunity (See Table V, Ref. 84).

TABLE V. FEATURES OF ANTINUCLEAR ANTIBODY-NEGATIVE LUPUS
(Modified from Hughes & Asherson, Adv.Nephrol. 1985)

Clinical	Serologic
33% Systemic Features:	
Nephritis and CNS Disease	67% Pos. Anti-Ro + La
Photosensitive dermatitis	Positive RA Latex
Mouth ulcers	Anti-ss-DNA
Non-scarring alopecia	
Arthralgias	25% Hematologic Abnormalities
Fever	Thrombocytopenia
Malaise, fatigue	Leukopenia
Sjogren's syndrome	+Hemolytic Anemia
20% Pleurisy and Pericarditis	

DRUG-INDUCED LUPUS (DIL)

Perhaps no other area of exogenously induced-autoimmunity has been better studied than drug-induced lupus (DIL). This was the subject of a recent Grand Rounds by Dr. Eliot Goldings which was subsequently published with Dr. John Cush (Ref. 85). They have joined other authors (Ref. 86,87) in suggesting that the diagnostic criteria for DIL include: 1) Absence of history suggestive of idiopathic SLE prior to drug ingestion; 2) Development of antinuclear antibodies, and at least one feature of lupus during sustained drug therapy; and 3) Rapid improvement of clinical features and gradual disappearance of autoantibodies after stopping the drug. They (Ref. 85,86,87) also emphasize the relative rarity of renal or central nervous system involvement, and the rarity of anti-double stranded DNA antibodies or low serum complement levels. This is not to say that patients with DIL never have renal disease. Virtually all forms of nephritis have been reported in 0-13% of DIL patients, depending on the drug involved, with hydralazine (Apresoline) being the most frequent cause of renal involvement (Ref. 85). Since hydralazine would have been given for hypertension, it is tempting to speculate that clinically silent renal disease causing high blood pressure preceded the use of the drug, and that idiopathic SLE was really present prior to hydralazine use.

In an attempt to explain the development of DIL in molecular terms, attention has recently been focused on the action of hydralazine and chemically-related drugs on the complement system. Hydralazine, and probably other hydrazine derivatives, bind covalently to the C4-component of complement (Ref 88). This may interfere with a variety of complement-mediated functions, including the solubilization of immune complexes (Ref. 89), allowing the accumulation of immune complexes and the development of lupus-like illness. In addition to hydralazine, Tartrazine (Food, Drug and Cosmetic Yellow No. 5) which is metabolized to sulfophenylhydrazine, has been shown, when taken in large amounts, to cause DIL (Ref. 90).

DIL produced by hydralazine has also been associated with pulmonary hypertension in a patient in whom there was a C4-null allele (Ref. 91). That is, the patient was heterozygous with only one of the two normal C4 genes, giving only 50% of the usual C4 level, and allowing easier depletion of the remaining C4 by interaction with hydralazine. This patient was also found to be HLA-DR4 (Ref. 91), a genetic type noted by others to be present in 73% of DIL patients and found in only 25% of patients with idiopathic SLE (Ref. 86,92). As in idiopathic SLE, DIL occurs much more frequently in women with a female:male ratio of 4:1 (Ref. 92).

For a number of years, physicians have known that procainamide (Pronestyl, Procan-SR) leads to the appearance of a positive ANA in 40% of patients, and mild to moderate DIL in 1-5% of patients who receive over 500 grams of the drug (Ref. 93,94). However, the assumption has been made that symptomatic patients could merely be switched to quinidine, since prior to 1985, only 11 patients taking quinidine had been reported as developing DIL. In the last two years, 10 more patients have been reported with DIL related to quinidine (Ref. 95,96,97,98,99). This should alert physicians to the possibility that a patient with DIL induced by procainamide, after being changed to quinidine, may continue to have symptoms of DIL, now due to the quinidine.

Finally, several drugs or chemicals have recently been added to the long list of medications unequivocally associated with DIL or the severe exacerbation of idiopathic SLE. These include griseofulvin (fatal exacerbation of SLE, Ref. 100), captopril (Ref. 101), and, of all things, alfalfa sprouts which contain L-canavanine (Ref. 102,103)! The molecular mechanism by which each drug induces DIL is usually unknown, but the modification of the complement system by hydralazine mentioned above (Ref. 88,89,90,91), or the modification of T-cell activation by D-penicillamine (Ref. 34,37), (?) captopril (Ref. 101), or L-canavanine (Ref.102,103) should now be considered possible mechanisms. Unlike idiopathic SLE patients, DIL patients show normal reticuloendothelial system Fc receptor function allowing them to clear immune complexes via the Fc-receptor on IgG more rapidly than patients with active idiopathic SLE. This could account for the much lower incidence of renal or central nervous system vasculitis in DIL than occurs in idiopathic SLE (Ref. 104).

Although not absolute, certain antinuclear antibodies are helpful in the immunotaxonomy of connective tissue diseases (See Table VI, Ref. 105, 106,107,108,109). For example, the differences in the ANA patterns between DIL and idiopathic SLE may be very useful to the clinician. Particularly, an ANA with a homogeneous (diffuse) staining pattern indicating autoantibodies against histones, deserves further study.

The homogeneous pattern is the ANA-type found in 95-100% of patients with DIL, and in 10-20% of patients with RA. Anti-histone antibodies are also found in 30-70% of sera from patients with idiopathic SLE, but in sufficient amounts to create a homogeneous staining pattern in only about one third of SLE patients (Ref. 106). Other ANA staining patterns are infrequent in DIL and RA. However, some ANAs have differential clinical value (See Table VI, next page).

TABLE VI. CANDIDATE ANTINUCLEAR ANTIBODIES IN THE IMMUNOTAXONOMY OF CONNECTIVE TISSUE DISORDERS

(Maini, Scand J Rheumatol 1985;Fritzler, Bull Rheum Dis 1985)

Disease (% pos.)	Autoantibody against	Disease (% pos.)	Autoantibody against
DIL (95-100%)	histone	CRST (27-90%)	centromere
SLE (47%)	ds-DNA	MCTD (95%)	U1-RNP
SLE (15-30%)	Sm	Scleroderma(15-30%)	Scl-70
SLE/Sjogren's(35/65)	SSA/Ro Ag	Polymyositis(87%)	PM-1
ANA-Neg Lupus(67%)	SSA/Ro Ag	Polymyositis (31%)	JO-1
Sicca syndrome (55%)	SSB/La Ag	PM/Scl Overlap(55%)	Ku
		Scleroderma(20%)	Nucleolar RNA

Therefore, all patients with features of lupus who have a positive ANA with a homogeneous staining pattern should be evaluated for possible DIL. The anti-histone antibodies seen in 10-20% of patients with RA have been shown to cross-react with a 10-amino-acid antigenic determinant on the Fc part of IgG which is shared with nuclear histone (Ref. 109). These dual specificity autoantibodies in RA, therefore, simultaneously qualify as "rheumatoid factors", and as "ANAs" (Ref. 109).

Unfortunately, only about one-third of patients with a positive ANA show specificity to a single nuclear antigen, 26% identified two, 11%, three, and 3%, four (Ref. 105). Another 28% of sera with a positive ANA reacted with "unidentified" nuclear antigens suggesting multiple autoantibodies in most patients with a positive ANA (Ref. 105).

In symptomatic patients with DIL secondary to procainamide, IgG anti-histone antibodies against the central histone peptides, of the H2A and H2B histone classes are predominantly present, while in asymptomatic patients, anti-histone antibodies are of the IgM class, and are directed against all histone classes (Ref. 110). Idiopathic SLE patients, on the other hand, have anti-histone antibodies against the N-terminal and C-terminal peptide regions of all native histone classes (111,112). By contrast, the anti-histone antibodies formed in DIL induced by hydralazine react with the trypsin-resistant (Not C-terminal or N-terminal) regions of H3 and H4 classes of histones (Ref. 112). These specific, but variable anti-histone responses among SLE and two different groups of DIL patients suggest genetic or other selective mechanisms for the kind of autoantibodies each group of patients will form, and makes DIL look more like a collection of different diseases with multiple mechanisms and causes.

Not all ANA's in DIL are directed against histones. Weisbart, et al, (Ref. 113) showed 15 of 18 patients with DIL secondary to procainamide to have anti-guanosine antibodies reactive with single stranded (denatured) DNA, while only 3 of 24 asymptomatic patients (also with positive ANA's while taking procainamide) showed anti-guanosine antibodies. Again, this suggests genetic or other selection of patients capable of a given autoimmune response which will produce illness in DIL.

Finally, the gold standard for autoantibodies against native or double stranded DNA (dsDNA) has been the Crithidia lucilia test. A kinetoplast at the base of the flagella of this small non-pathogenic protozoa normally contains no histone, RNA or nucleoprotein, and stains only with antibodies for native, non-denatured, double-stranded DNA. When positive, the C. lucilia test was thought to indicate idiopathic SLE, and to rule out DIL. However, Drs. Deng, Sontheimer, Lipscomb and Gilliam (Ref. 114) encountered 14 positive sera from patients with RA, and DIL who had no good clinical reason to have anti-dsDNA antibodies, and in whom homogeneous patterns of staining of the fluorescent ANA had been observed. They were able to show that the kinetoplast contains histone during the first 2 days of its cell cycle, and loses this as it becomes more mature, and that acid extraction to remove histone, but not dsDNA, converted the test to negative in these 14 sera. Alas, even gold standards sometimes contain lead!

INDUCTION OF AUTOIMMUNITY BY INFECTION OR EXPOSURE TO CHEMICALS

The presence of antigens on microorganisms or in chemicals in the environment which share determinants with autoantigens creates the opportunity for mimicry which may result in activation of autoimmunity. Here again, genetic variation may give some individuals tissue antigens which are more similar immunologically to a viral, bacterial or chemical determinant than another person's antigens are. The various forms of reactive arthritis, such as rheumatic fever, Reiter's disease or inflammatory bowel disease show such genetic selectivity.

SYPHILIS AND THE LUPUS ANTICOAGULANT

Little did Wasserman realize, when he used cardiolipin to detect antibodies against syphilis, that the time would come when his test would be used far more often to detect autoantibodies. Biological false positives for VDRL or RPR probably outnumber true positives ten to one. Only recently has the pathological significance of the BFP reaction been fully appreciated. The presence of antibodies in patients which show cross-reactivity between the phospholipid, cardiolipin, and phospholipids in platelet membranes, and other clotting factors has identified a subset of lupus patients (and others without clinical lupus) who have major thromboembolic complications, increased fetal loss and thrombocytopenia (Ref. 115,116). We now know that the mechanism of the often catastrophic events is related to the presence of the "lupus anticoagulant" (LAC) which which is an IgG autoantibody against phospholipids involved in the clotting cascade, as well as part of the platelet membrane. This LAC usually (Ref. 116,117), but not always (Ref. 118,119), correlates with the anti-cardiolipin reactivity in these patients, and often decreases substantially when the patients are treated with moderate doses of prednisone (20-40 mg/day) (Ref. 119).

The shared phosphodiester linkage in phospholipids and in DNA and RNA has led to the suggestion that a common loss of control for recognition of self could also explain the frequent coexistence of anti-DNA anti-RNA and LAC in patients with SLE. Because of this syndrome of thromboembolic phenomena in the presence of LAC in SLE, Bruneau, et al (Ref. 120). reviewed 16 non-lupus patients who had developed vascular complications while taking estrogen-containing oral contraceptives. None of these non-lupus patients had detectible levels of LAC, although 13 of the 16 had antibodies against ethinylestradiol (Ref. 120).

BACTERIAL AND VIRAL INFECTIONS AND MOLECULAR MIMICRY

The phenomenon of shared antigenic determinants between host and microbe probably occurs more fequently than has been reported (Ref. 121). Table VII (next page) shows a list of cross-reactions between microbes and mammalian tissues. It is not known whether the autoantibodies which result from these infections are a matter of "Self + X", where X = microbial antigen in close association with an autoantigen resulting in overlapping determinants; or occurs because the microbe, by sharing cross-reactivity with a certain tissue, leads to acute tissue injury. This then releases a cross-reactive antigen which then takes over and drives a more chronic autoimmune response. A considerable body of data support the second conclusion. For rheumatic heart disease, the antibody level to streptococcal polysaccharide A remains elevated for years in patients with chronic carditis, even though they are kept on penicillin prophylaxis, and have, therefore, no significant streptococcal antigen exposure (Ref.122).

TABLE VII. DISEASE ASSOCIATIONS WITH MICROBE/TISSUE CROSS-REACTIONS
(Modified from Zabriskie & Gibofsky, 1986)

Disease Association	Organism	Tissues Affected
Rheumatic Fever	Streptococcus pyogenes	Heart, Joints Brain, Skin, Thymus
Glomerulonephritis	Streptococcus pyogenes	Kidney
Ankylosing Spondylitis	Klebsiella, Shigella fl. Yersinia, Campylobacter, Salmonella, Chlamydia ?	Fibrocartilage, Eyes, Aorta, B27 Lymphocytes
Pneumococcal pneumonia	Streptococcus pneumoniae	Blood Group Substances
Gram Negative Bacterial Infections (50%)	Gram negative bacteria	
Ulcerative Colitis	Escherichia coli	Colon
Chagas' Disease	Trypanosoma cruzi	Neuronal tissues, Heart

Probably more significantly, rheumatic heart disease patients have high titers of anti-streptococcal polysaccharide A antibody, but this antibody has a low affinity for the strep antigen, yet cross-reacts strongly with the heart valve glycoprotein which is immunologically similar.

This suggests that the patient with rheumatic carditis has switched his immunological allegience from the initial streptococcal antigen to his own heart autoantigen, and in so doing, has modified the antibody binding affinity (Ref. 122). It also means the rheumatic carditis patient is now independently driving an autoimmune process in the absence of additional initiating bacterial antigen, and that the mechanism for suppressing self-recognition of heart antigens has broken down.

Why are only 1-3% of patients at risk for the development of rheumatic fever after streptococcal pharyngitis, and the other 97-99%, normal? Not only beta hemolytic streptococci, but other infectious agents select genetically susceptible hosts. At least four different suggestions for microbial disease association with HLA-I&II surface molecules have been made (Ref. 121). These are outlined in Table VIII.

TABLE VIII. SUGGESTED MECHANISMS FOR HLA AND DISEASE ASSOCIATIONS
(Modified from Zabriskie & Gibofsky, 1986)

1)	HLA antigen might be structurally similar to a surface antigenic component of the microbe.
2)	HLA antigen may be part of a neoantigen, formed in combination with an infectious agent.
3)	HLA antigen may be a receptor for the infectious agent or for a secreted product or toxin.
4)	HLA antigen may be in linkage disequilibrium with an "immune response gene" in the D-region which identifies the microbe's surface as "self".

The potential to generate an animal model of RA in genetically selected rats by injection of Freund's adjuvant (mineral oil containing killed Mycobacterium tuberculosis) led two groups to explore the degree of genetic control in man of the immune response to M. tuberculosis and its relationship to the pathogenesis of RA. Ottenhoff, et al, (Ref. 123) looked at skin test responses of 86 Spanish leprosy patients who had been genetically typed using 4 different strains of mycobacteria. There was an enhanced skin test response to M. tuberculosis in DR4 patients compared to patients with other DR-types. On the other hand, DR4 patients did not show enhanced responses to the other three mycobacterial antigens. They speculated that the immune paralysis of most of these leprosy patients to the commonly shared mycobacterial antigens between M. leprae and M. tuberculosis may have enhanced their ability to demonstrate the function of the immune response gene linked to DR4 which responded to the strain specific antigens in M. tuberculosis (Ref. 123).

An independent group at the Weizmann Institute (Ref. 124) simultaneously showed that an acetone-precipitable fraction of M. tuberculosis cross-reacts with a component of human cartilage, and that patients with RA for less than 10 years show enhanced T cell proliferation in vitro to the acetone-precipitated M. tuberculosis antigen when compared to the responses of non-RA patients. Patients with RA for more than 10 years were similar to osteoarthritis controls (Ref. 124).

Table IX lists infectious agents of interest to this discussion.

TABLE IX. MHC (HLA-I&II) ASSOCIATIONS WITH INFECTIONS AND "AUTOIMMUNE" DISEASES

INFECTIOUS AGENT	MHC-ASSOC.	UNIQUE FEATURE	(REF.)
Mycobacterium tuberculosis E-B Virus, Bacterial cell wall proteoglycan	DR4	Rheumatoid Arthritis	(123,124) (125,126)
Mycobacterium leprae	DR3 DQw1	Tuberculoid Leprosy Lepromatous Leprosy	(121,123)
Borrelia burgdorferi	DR2	Chronic Lyme Disease	(121)
Shigella flexneri, Yersinia enterocolitica Salmonella species Campylobacter species	B27	Reiter's Disease ? Ankylosing Spondylitis	(121)
Hepatitis B Virus	DR3	Chronic Active Hepatitis	(127,128)
Mumps Virus	DR3/DR4	Type I Diabetes	(1,129)
Type C Virus	DR3,DR2 DQw1	Systemic Lupus Erythematosus	(121)
Measles Virus, Canine Distemper Virus	DR2	Multiple Sclerosis	(121)

THERAPEUTIC INTERVENTIONS IN AUTOIMMUNE DISEASES

Like chemotherapy for cancer, the suppression of serious autoimmune disease with high doses of corticosteroids or immunosuppressive medications means subjecting normal body tissues to potentially massive injury. The patient is either temporarily or permanently maimed by the therapy, and often is left with a shortened life expectancy because of subsequent lethal infections or malignancy. Long term steroids cause muscle wasting, and osteoporosis, and alkylating agents lead to lymphoproliferative neoplasms and sterility.

Therefore, the ideal therapeutic agent would be one focused more specifically on the problem causing the autoimmunity. Two successful immune interventions have encouraged future targeted therapy designed to inactivate a selected cell population causing tissue injury. Kirkman, et al, (Ref. 130), treated 19 patients undergoing acute rejection of renal allografts with monoclonal mouse antibody directed against a determinant of the CD6 (T12) surface protein of T cells. Each patient received about 1.5 mg of m-anti-CD6 daily for 10 days, enough to maintain detectible levels of free antibody in the serum during that time. Eleven of the patients were also receiving Cyclosporine and 8 were on azathioprin (Imuran). Seven had also received one or more high-dose steroid pulses, and one patient, horse anti-thymocyte globulin and plasmapheresis with evidence of continued renal rejection.

Although obviously not the cleanest experiment, 7 patients showed a good response, and 4 others had "equivocal" improvement with all 11 still having functional kidneys an average of 7 months after the m-anti-CD6 treatment (Ref. 130).

Reinerz, et al, (Ref. 131) using the same m-anti-CD6 (T12) plus complement, completely depleted a maternal bone marrow of T cells *in vitro* prior to its use to immunologically reconstitute a child with severe combined immunodeficiency (genetic absence of both B and T cells). The graft was HLA-mismatched, and therefore, it was possible to show that the full normal range of T and B cells which the child acquired were of maternal origin. The narrow specificity of the m-anti-CD6 for T cells meant that most body cells suffered no adverse effect in these two groups of patients. If human monoclonal antibodies of the right specificity become available in the future, selective immunosuppression will become the treatment of choice for a wide range of life-threatening autoimmune diseases.

Meanwhile, physicians must still rely on broader based immune suppression with drugs (Ref. 132,133). Karlsson-Parra, et al, (Ref. 134) have analyzed the changes in the various T cell subsets following different forms of therapy of previously untreated patients with active RA, with equivocal results. However, Fahey, et al (Ref. 132), and Miller and Steinberg (Ref. 133) have throughly reviewed immunoregulatory drugs, their pharmacology and the rationale for their use in connective tissue diseases with autoimmune features. Table X gives suggested guidelines for the use of these agents in rheumatologic diseases.

TABLE X.
GUIDELINES FOR USE OF IMMUNOREGULATORY AGENTS IN AUTOIMMUNE DISEASES
(From Miller & Steinberg, Immunology of Rheumatic Diseases, 1985)

Prerequisite	Agent(s)	Disease Condition
Life-threatening?	Alkylating agents	Lupus nephritis
Severely debilitating?	MTX AZA	Severe RA
Failure of conventional therapy?	Total body irradiation	Life-threatening RA
Reversible disease?	All	SLE nephritis, RA lung
Absence of infection?	Most	RA,SLE,PM,Wegner's
Defined disease parameter to follow?	All	All (must be something objective)
No contraindications?	MTX (alcoholic?) (pt. compliant?)	RA
Alternate treatment plan?	All are toxic	All
Potential drug interactions?	AZA/allopurinol MTX/NSAIDS	Psoriatic/gout RA
Proximity of patient for close follow-up?	All	All

Horse anti-thymocyte globulin has been successfully used to reverse gold-induced bone marrow aplasia (Ref. 135). This would suggest that a T cell mediated allergic reaction was preventing stem cell maturation, and that elimination of the autoreactive clone returned the bone marrow to normal function. The one-time-shot due to generation of future potential for fatal anaphylaxis makes this approach to a chronic autoimmune disease of very limited potential value.

The fungal metabolite, cyclosporine (CS) is the forerunner of a new generation of immunosuppressants. Its use in renal transplantation was reviewed in depth in these Grand Rounds by Dr. J. Harold Helderman two weeks ago. It has proven particularly valuable in organ transplants where it often completely blocks rejection. Its mechanism of action is believed to be on the antigen/IL1 activation of IL2 release by T cells, leaving the undifferentiated T cell in an early phase of its cell cycle (G-0 or early G-1 phase) (Ref. 136). CS has a significant effect on circulating autoantibody responses only when given in high doses and for long periods of time (Ref. 136). Its use in controlled studies of autoimmunity, so far, has primarily been limited to animal models where dramatic suppression of disease, such as SLE in mice, has occurred (Ref. 136). Human studies in patients with chronic uveitis who were treated with CS (Ref. 137) have shown abolition of skin tests for keyhole limpet hemocyanin (KLH) and tetanus toxoid, but no effect on antibody responses to these antigens, even though both antigens are T cell dependent. The renal toxicity, large expense, and equivocal results on the humoral immune response in man make the future usefulness of CS in autoimmune diseases uncertain.

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