Autoimmune Diseases: The Failure of Self Tolerance

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The ability to discriminate between self and nonself antigens is vital to the functioning of the immune system as a specific defense against invading microorganisms. Failure of the immune system to "tolerate" self tissues can result in pathological autoimmune states leading to debilitating illness and sometimes death. The induction of autoimmunity involves genetic and environmental factors that have focused the attention of researchers on the trimolecular complex formed by major histocompatibility complex molecules, antigen, and T cell receptors. Detailed molecular characterization of these components points to potential strategies for disease intervention.

THE PHENOMENON OF AUTOIMMUNITY—CLINICALLY CHARacterized by such seemingly unrelated diseases as insulin-dependent diabetes mellitus (IDDM), multiple sclerosis (MS), rheumatoid arthritis (RA), myasthenia gravis (MG), and psoriasis—appears to be due to the failure of normal mechanisms of self tolerance, frequently under the stress of environmental stimuli, so that Ehrlich's dictum of *horror autotoxicus* (*1*) is contravened. Autoimmune diseases, as a group, affect 5 to 7% of the population, often with severe disability, and are thus a major cause of chronic illness. Whereas many autoimmune diseases involve an immune response against self molecules that are expressed in anatomically privileged extrathymic sites, others appear to be due to immune responses to ubiquitous nuclear and cytoplasmic antigens. Our growing knowledge of the normal mechanisms for induction of self tolerance during development of the T cell repertoire in the thymus (*2*) still leaves us ignorant of the mechanisms by which tolerance is established for extrathymic self molecules. For many self molecules that are the target autoimmune responses, it is not yet known whether the normal state of self tolerance depends on the absence of self-reactive T cells or on active suppression by self antigen–specific suppressor T cells.

Human autoimmune diseases can be classified in several ways. Of the more than 40 diseases known or thought to be autoimmune in nature, susceptibility to almost all is strongly influenced by genes encoded within the major histocompatibility complex (MHC), particularly the class I (HLA-A, B, C) and class II (Ia, HLA-D) MHC molecules (*3*).

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Within the MHC class II–associated diseases, there is a subdivision between organ-specific autoimmune disease and multisystem autoimmune disease. The organ-specific autoimmune diseases are characterized by autoantibody patterns that are primarily directed at a single organ or closely related organs (for example, β cells in the islets of Langerhans in IDDM). On the other hand, systemic autoimmune diseases are characterized by a variety of autoantibodies specific for nuclear and cytoplasmic molecules involved in DNA replication, DNA transcription, and messenger RNA (mRNA) translation. Some of the manifestations of systemic autoimmunity are due to direct effects of these autoantibodies, whereas others are due to antigen-antibody complex deposition.

Autoimmune disease can also be classified in terms of final effector mechanism. Among organ-specific autoimmune diseases, IDDM and MS appear to be due to the action of T cells (primarily $CD4^+$) (*4*), whereas hyperthyroidism (Grave's disease) and myasthenia gravis are the result of antireceptor antibodies specific for the thyrotrophic hormone (TSH) receptor and the acetylcholine receptor (AChR), respectively (*5*). Similarly in systemic autoimmunity, many of the manifestations of RA appear to be due to the effect of T cells (*6*), whereas much of the pathology in systemic lupus erythematosus (SLE) and polyarteritis nodosa is due to deposition of antigen-antibody complexes (*7*).

MHC class I–associated autoimmune diseases are a much smaller group and fall into two main categories: (i) the HLA-B27–related spondyloarthropathies, including ankylosing spondylitis, Reiter's syndrome, and reactive arthropathy, and (ii) psoriasis vulgaris, which is associated with HLA-B13, B16, and B17.

There are features common to both MHC class II– and class I–associated autoimmune diseases. Susceptibility in both cases is clearly multifactorial. Study of monozygotic twin pairs shows that concordance for disease is much less than 100%, and varies from less than 5% for MS to approximately 30% for IDDM (*8*). Thus, even in individuals with a proven susceptible genotype, not all individuals will develop autoimmunity. The implication of this finding is that environmental factors also play a major role. The association of many autoimmune diseases with preceding infection (*9–13*) raises the question of whether the initial autoimmune response might be triggered by an antigenically similar, cross-reacting environmental pathogen, a phenomenon often referred to as molecular mimicry.

A second important characteristic of autoimmunity is that susceptibility is polygenic. Because susceptibility is both multifactorial and polygenic, most autoimmune diseases are not inherited in a simple Mendelian segregation. The comparison of concordance rates for monozygotic twins with either HLA-identical dizygotic twins or HLA-identical siblings clearly indicates that MHC genes are an important but not a sufficient genetic factor in determining suscepti-

bility to autoimmunity. Quantitative estimates in IDDM and its murine counterpart, the nonobese diabetic (NOD) mouse, suggest that from three to six independently segregating genes may determine susceptibility to autoimmune destruction of the β cells in the islets of Langerhans (*14*).

Aside from relatively weak effects of immunoglobulin loci on susceptibility to hyperthyroidism and possibly MS (*15*), the other genes determining susceptibility have not yet been identified. The value of identifying these genes in order to predict disease susceptibility is obvious, and of great importance in diseases such as IDDM, for which islet cell destruction is largely asymptomatic until it is nearly complete.

Many autoimmune diseases have a peak incidence at or shortly after puberty, often with a second peak of incidence in the forties and fifties. As a general rule, MHC class II–associated diseases show female preponderance, most marked in SLE and hyperthyroidism, while MHC class I–associated diseases show male predominance, most marked in ankylosing spondylitis and Reiter's syndrome (*16*).

Almost all autoimmune diseases have a pronounced tendency for spontaneous exacerbations and remissions—a characteristic that suggests a fluctuating balance between positive and negative regulatory factors (such as helper versus suppressor T cells). Finally, most autoimmune diseases have a characteristic pattern of autoantibody production that is frequently a predictor, and in some instances a cause, of particular clinical manifestations. Thus, identification of the initial stimulus for production of certain autoantibodies is an important problem in understanding the pathogenesis of such diseases as neonatal lupus erythematosus, characterized by a typical skin rash and complete heart block and apparently due to the effects of maternal autoantibody to ribonucleoprotein complexes (*17*).

The key factors in the initiation of normal and autoimmune responses are shown in Fig. 1. To induce a $CD4^+$ T cell response, the T cell receptor must recognize a self or foreign peptide within the peptide-binding groove of a self, class II MHC molecule (*18*). Clearly, one major goal in understanding autoimmunity is to characterize the self or cross-reacting environmental antigens that contribute critical peptide epitopes; the MHC alleles that are most effective in presenting these self peptides; and the complexity of the T cell receptor repertoire, which is utilized for recognition of this self peptide–self MHC molecular complex. A detailed characterization of all three components in the development of an autoimmune response would contribute greatly to our understanding of the pathogenesis of the process, and to the development of methods for preventing it.

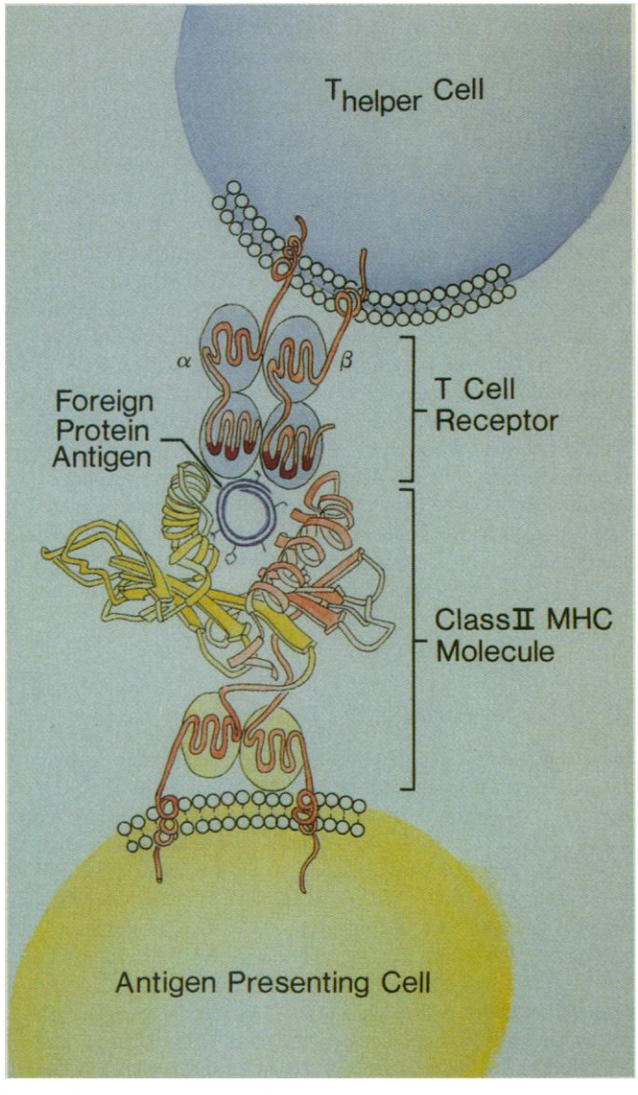

Fig. 1. The ternary complex. Schematic representation of the trimolecular interaction between an MHC molecule, peptide, and TCR involved in the induction of a normal or autoimmune response.

The Major Histocompatibility Complex in Autoimmunity

A large part of our understanding of the genetic basis of autoimmunity stems from the observation that susceptibility to many human diseases is associated with a particular MHC class I or class II allele (*3*). The first significant associations reported were with human class I allelic specificities. Subsequently, with the development of methods for typing HLA class II alleles, most diseases have been shown to be more strongly associated with class II MHC alleles. The initial class I associations reflect the known strong linkage disequilibrium within the MHC. Genetic analysis has also shown a role for the MHC in several murine models of autoimmunity (*19*). These findings, combined with the central role of class I and II molecules in the immune response, have focused attention on MHC function in the induction of autoimmunity.

The human class II region spans approximately 1.1 megabases and is located centromeric to the class I and class III (which includes complement components) regions on chromosome 6. The class II region (HLA-D) is complex. The DR, DQ, and DP encode cell surface heterodimers composed of a 34-kD α chain noncovalently associated with a 29-kD β chain (*20*). Each chain has two extracellular domains of approximately 90 amino acids, a transmembrane region, and a short cytoplasmic tail. On the basis of x-ray crystallographic analysis of class I MHC molecules, the outermost domains of each class II chain (α_1 and β_1) are predicted to fold together to form a groove, or cleft (*21, 22*), that appears to function in the binding of peptide fragments of protein antigens for the presentation to antigen-specific, class II–restricted T cells in the initiation of an immune response. A β-pleated sheet comprised of eight antiparallel strands forms the floor of the cleft; two α helices form the sides.

The most striking feature of MHC genes is their extensive polymorphism. In class II, the majority of variable residues are concentrated in the α_1 and β_1 domains. Moreover, polymorphic residues are clustered into three to four discrete hypervariable regions (HVR) (*23*). On the predicted model of class II structure, HVR sequences are located almost exclusively within the antigen-binding groove, supporting the hypothesis that these residues

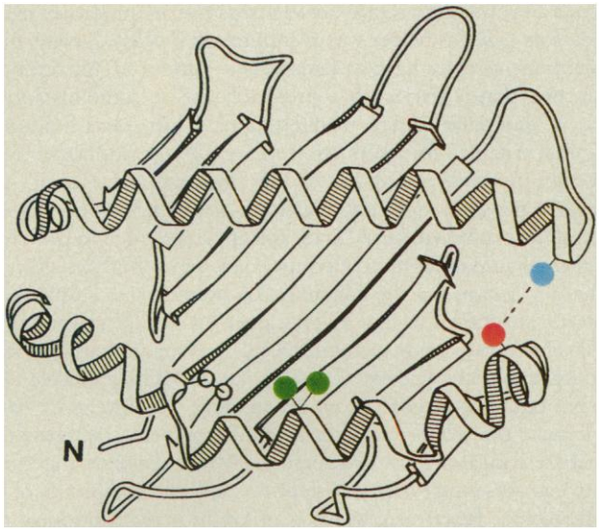

Fig. 2. Ribbon diagram of predicted model of class II MHC peptide-binding cleft as viewed from above [adapted from (*21*) with permission, ©1988 Macmillan Journals Ltd.]. Resistance to diabetes correlates with Asp^{57} (red dot) of the DQβ molecule (*26*). Susceptibility correlates with Val, Ser, and Ala. The susceptibility allele found in DRw6 PV patients has Asp^{57} at DQβ (*28*). Asp^{57} could form a salt bridge with a conserved Arg^{79} of the α chain (blue dot). Class II alleles with positively charged amino acids (for example, Lys and Arg) at positions 70 and 71, respectively, of DRβ1 (green dots) are overrepresented in patients with RA; negatively charged amino acids (for example, Glu and Asp) are found in DR4 PV patients (*91*).

contact the peptide or the T cell receptor, or both (*21, 22*). In this way, polymorphism in allelic HVR regions can alter the nature of the interaction among MHC molecules, antigen, and T cell receptors and thus control the specificity of the immune response to foreign and, presumably, self antigens.

To better understand the role of class II polymorphism in autoimmunity, several groups have undertaken detailed molecular analysis of class II alleles found in diseased individuals. The emergence of polymerase chain reaction (PCR) technology (*24*) has allowed direct nucleotide sequence analysis of class II genes from a large number of patients with various autoimmune conditions. One of the major conclusions of this work is that disease is not the result of mutant MHC alleles that are found exclusively in patients with autoimmune disease. In fact, the same sequences that are found in patients are also found in healthy individuals, although a particular allele may be represented at different frequencies in diseased as compared to healthy populations. However, for some diseases, it has been possible to identify short stretches of sequence or critical residues that play a major role in susceptibility and resistance to disease (*25*).

In each case, the critical residues correlated with disease are located within the class II binding site (Fig. 2). Clearly, nonconservative changes at any of these positions could alter the structure of the peptide-binding groove and affect class II function. Aspartic acid at residue 57 of the DQβ molecule (correlated with resistance to IDDM) could conceivably form a salt bridge with a conserved Arg^{79} of the α chain, in contrast to Val, Ser, or Ala found at residue 57 of DQβ in susceptibility alleles (*26*). The identification of particular epitopes within class II alleles important for disease development helps to explain the observation that certain autoimmune diseases are associated with more than one HLA haplotype. Allelic HVR are often shared among distinct class II alleles. In large part, polymorphism appears to have been generated as the result of the shuffling of a limited number of HVR between alleles, perhaps by gene conversion-like events (*27*). Although a particular class II epitope may be the single most important MHC-associated factor controlling the development of disease, other sequences (within the α- or β-chain gene, or both) are also likely to influence the conformation of the complete heterodimer.

Despite considerable efforts, it has not been possible to identify critical epitopes or residues for all MHC-associated diseases. In some cases, different epitopes could confer risk for the same disease. For example, negatively charged residues in the third HVR of DRβ are necessary for the development of pemphigus vulgaris (PV) in DR4 individuals, whereas Asp^{57} of a rare DQβ allele controls susceptibility in DRw6 patients (*28*). In addition, a particular clinical autoimmune condition may be heterogeneous in etiology or pathogenesis. Rather than being associated with a single locus, some diseases seem to be associated most strongly with a particular chromosomal combination of MHC alleles that, because of linkage disequilibrium, are inherited together as an extended haplotype. Individuals that inherit the A1, B8, and DR3 HLA specificities together on one chromosome have an increased risk of developing IDDM, MG, SLE, and celiac disease (*29*). This may indicate that more than one MHC molecule contributes to disease. Alternatively, MHC associations may actually reflect linkage to the genes for tumor necrosis factor and several other genes of unknown function that have recently been mapped to the MHC (*30, 31*). The observed HLA associations with SLE may be in part related to a partial deficiency of complement caused by the presence of C2 null or C4 null alleles in linkage disequilibrium with HLA antigens (*32*).

Although linkage disequilibrium has made it difficult to investigate MHC-disease associations, several lines of evidence indicate that class II molecules themselves are important in at least some diseases. (i) Specific class II allelic associations are often maintained in different ethnic backgrounds (*33*). (ii) Monoclonal antibodies directed against class II molecules can block the induction of autoimmune disease in several animal models (*34*). (iii) By nucleotide sequence analysis of class II alleles that are increased in frequency in diseased as compared to healthy populations, it has been possible to identify the class II locus (and pinpoint critical residues within that locus) that are strongly disease-correlated.

Most individuals who carry disease-susceptibility class II epitopes, however, do not develop disease. DR3 and DR4, susceptibility haplotypes for IDDM, are present in 40 to 50% of the general Caucasian population. This observation underscores the importance of other genetic and environmental factors in autoimmunity. Certain class II sequences appear to be necessary but not sufficient for disease development.

T Cell Receptors

It is now well established that T cells recognize antigen as denatured peptide fragments bound by MHC class I or class II molecules (*18*). Recognition by T cells is carried out by a heterodimeric T cell receptor (TCR) that is generated, in a manner analogous to that of immunoglobulins, by the somatic recombination of noncontiguous germline variable (V), diversity (D), and joining (J) genes to form a continuous variable region gene (*35*). It is estimated that this strategy generates 10^{15} distinct TCRs, a sufficient number to account for the different antigens the immune system will encounter (*36*). However, not all individuals within a species are equivalent with regard to their genomic potential for TCR diversity. In different mouse strains, the TCR repertoire has been contracted by extended deletions within the TCR V_β locus that result in the loss of particular subsets of V_β loci from the genome (*37, 38*).

As discussed in detail elsewhere in this issue, the random somatic generation of TCRs also permits the generation of receptors capable of recognizing self peptides. In the mouse, immature T cells, reactive with either the self I-E molecule or particular alleles of minor lymphocyte stimulating (Mls) antigens in the context of specific class II alleles, are eliminated during intrathymic T cell maturation (*2*). Since not all mouse strains express I-E (due to the absence of a functional I-E α or β chain) or possess the relevant Mls allele–class II combinations, the mature TCR repertoire will vary between strains according to the presence of at least these two unrelated genes (*39*). Similar events may also occur in humans, but have not yet been directly demonstrated.

Studies of genetic linkage in different autoimmune animal populations implicate the variable region genes of the TCR in the etiology of autoimmune disease. In murine collagen-induced arthritis, it has been shown that despite the presence of a permissive MHC haplotype, mouse strains that have a genomic deletion resulting in the loss of 50% of the V region genes of the TCR β-chain loci are resistant to arthritis induction (*38*).

Although genomic deletions in the human TCR V_α and V_β region genes have not been identified, these loci do exhibit limited sequence polymorphism (*40*). This permits the pattern of inheritance of different alleles and chromosomal combinations of alleles (haplotypes) to be examined in families with several affected members or in populations of patients with autoimmune disease. Restriction fragment length polymorphism (RFLP) analysis of the germline V region genes of the TCR α and β loci has suggested that, although the association is not absolute, distinct TCR alleles may segregate with disease (for example, type I diabetes and MS) (*41*).

Immunocytochemical studies of tissues undergoing autoimmune destruction suggest a primary role for distinct T cell subsets in several organ-specific autoimmune diseases. Infiltrating T cells have been found in the pancreas of a newly diagnosed IDDM patient (*42*), the thyroid gland of Grave's patients (*43*) and freshly demyelinated plaques in the central nervous system of MS patients (*44*). However, studies in the animal models of autoimmune diseases have been the most informative. Transfer of splenic T cells from diabetic, arthritic, and encephalitic animals results in transfer of disease to genetically compatible, healthy animals (*45, 46*). Fractionation of splenic T cells into subsets of $CD4^+$ (helper/inducer) and $CD8^+$ (cytotoxic/suppressor) cells demonstrates the necessity of the $CD4^+$ T cell subset in most instances (*46, 47, 48*). Inhibition of disease by monoclonal antibody to CD4 confirms the $CD4^+$ T cell requirement (*49*). Since CD4 is found predominantly on class II–restricted T cells, this is consistent with the MHC class II association with autoimmune disease.

Antigen-specific T cell lines and clones have been established from animals immunized with mycobacteria, which transfer an autoimmune arthritic condition (*50*); and from animals immunized with myelin basic protein (MBP), which transfer the murine equivalent of MS (*48, 51*). Recently $CD4^+$ and $CD8^+$ T cell clones have been established from the spontaneously diabetic NOD mouse by culturing T cells infiltrating the NOD pancreas with isolated pancreatic islet cells (to serve as antigen) and the T cell growth factor, interleukin-2 (IL-2). Unlike the results in the models of RA and MS, both the $CD4^+$ and $CD8^+$ T cell clones are required to transfer diabetes in NOD mice (*52, 53*).

Sequence analysis of the murine experimental allergic encephalomyelitis (EAE)–inducing $CD4^+$ T cell clones specific for a peptide derived from amino acid residues 1 to 11 of MBP and restricted to $I\text{-}A^u$ revealed extensive homology in both chains of the TCR (*54, 55*). All clones utilized a single V_α and seven of the eight, a single V_β region gene. Furthermore, extensive identity was seen in J_α and J_β, as well as with template-independent, junctional sequences. The MBP-specific TCR in the rat model of EAE also demonstrated very limited heterogenity and used a V_β gene homologous to that used by the murine EAE-inducing clones (*56*). The prominent role of these T cells in EAE was shown by the inhibition of disease induction, as well as the moderation of ongoing disease by a monoclonal antibody specific for the common V_β (*54, 57*).

In contrast to these results, EAE-inducing T cell clones specific for MBP 89 to 101 or 89 to 100 and restricted to $I\text{-}A^s$ display significantly greater TCR V region diversity. Although the V_α region genes have not yet been analyzed, a minimum of three V_β region genes are used. The predominant V_β region gene occurs at a frequency of 50% (*58*).

The results implicating a role for particular subsets of TCR-bearing T cells in the induction and maintenance of autoimmune conditions in the animal models are provocative. However, these models, with the exception of the spontaneous occurrence of diabetes in the NOD mouse, require immunization with a specific antigen, often in the presence of a strong adjuvant, to elicit autoimmunity. In human studies, T cell clones derived from the cerebrospinal fluid, but not the peripheral blood, of MS patients showed restricted heterogeneity in the TCR V_β region genes expressed (*59*). The extent of heterogeneity in cloned T cells derived from the synovial fluid of RA patients is controversial (*60*). Thus, it is unclear whether the findings of limited heterogeneity of self-reactive T cells moderating autoimmune disease seen in animal systems will be mirrored in human disease.

Target Antigens of the Autoimmune Process

Why certain self antigens are selected as the targets of autoimmunity is not known. In fact, for many diseases, the target antigens have not been identified. Thus far, the major approach to this issue has been to isolate and determine the specificity of lymphocytes active in autoimmune responses. In this manner, several B cell and some T cell self-epitopes have been defined.

There is a fundamental difference in the manner by which B cells and T cells recognize antigens, foreign or self. The immunoglobulin molecules (receptors) of B cells are capable of binding native antigen alone in solution. Since B cells recognize primarily intact protein, antibody-antigen interactions are often dependent on three-dimensional conformation. T cells are blind to intact native antigen molecules; antigen must be processed (cleaved into peptide fragments) and the peptide fragments are then presented on the surface of specialized antigen-presenting cells in association with class II or class I MHC molecules, which serve as the target for $CD4^+$ and $CD8^+$ T cells, respectively (*18*).

B cells are capable of binding self components, and low levels of nonpathologic "natural" autoantibodies are found in the normal state (*61*). Although autoantibodies are a predominant feature of many diseases, the factors that lead to their production and their role in pathogenesis remain largely unresolved. In many cases, autoantibodies may simply be epiphenomena, appearing as a result of, rather than being responsible for, the primary autoimmune process. For example, type I diabetes seems to be primarily mediated by T cells, even though a large majority of patients develop antibodies to pancreatic β cells. Autoantibodies may be involved in effecting or perpetuating tissue damage rather than initiating it.

However, in some diseases a restricted autoantibody specificity is directly linked with pathogenesis. Antibodies from PV patients immunoprecipitate a 210-kD heterodimer from the surface of epidermal cells that (as predicted by in vitro studies) may cause the release of plasminogen activator and lead to the loss of cell adhesion, to acantholysis, and to skin blisters (*62*). Antibodies that bind the

TSH receptor or the AChR lead to the clinical manifestations of Grave's disease and MG (*5*). In contrast, in SLE there appears to be a generalized disregulation of the immune system such that autoantibodies are generated to several nuclear constituents including: single- and double-stranded DNA, Z-DNA, histones, DNA-histone complexes, and ribonucleoproteins. These antibodies may participate in the immune complex formation that is related to many of the clinical manifestations of SLE (*7*). Secreted immunoglobulins can themselves be targets of an immune response. Immunoglobulin M antibody to immunoglobulin G rheumatoid factor (RF) is found in the serum and synovial fluid of RA patients and in MRL/l (lymphoproliferative) mice (*63*).

Autoantibodies can be valuable in the study of disease in several respects. The principal importance of some anti-self responses may be in their predictive value. Antibodies to insulin and to a 64-kD islet β-cell antigen can precede the development of IDDM by several years (*64*). The level of autoantibody production sometimes correlates with disease severity; lupus nephritis is associated with a high titer of antibodies to DNA (*7*). Analysis of target antigens recognized by autoantibodies may identify T cell epitopes critical for disease induction (perhaps located on the same target molecules) or point to environmental triggers of autoimmunity.

Although autoantibodies can clearly be involved in the effector phase of autoimmunity, the induction of disease most likely involves T cells. Because B cells require T cell help, most immune responses begin with the activation of $CD4^+$ T helper cells in a ternary complex with MHC and peptide. Thus, the control of self-nonself discrimination is largely the responsibility of the T cell compartment.

Difficulties in experimental manipulation have hampered the characterization of T cell epitopes in human disease. However, antigenic targets of autoreactive T cells have been studied in animals and disease-inducing peptides of self proteins have been defined in some cases. In EAE, amino acids 1 to 9 and 89 to 101 of MBP are the major disease-inducing determinants of the $I\text{-}A^u$ and $I\text{-}A^s$ alleles, respectively (*54*).

The characterization of T cell epitopes in several other diseases is also under way (*55*). A number of distinct peptides derived from the α subunit of the AChR have been shown to stimulate T cells from MG patients and Lewis rats (the best characterized model for MG). T cells specific for thyroglobulin and P2 protein (amino acids 66 to 78) or peripheral myelin can induce experimental autoimmune thyroiditis in mice (an animal model possibly relevant to Hashimoto's thyroditis) and experimental autoimmune neuritis, respectively. T cells reactive to retinal S-antigen cause experimental autoimmune uveitis in several species. The target antigen of T cell clones isolated from mice susceptible to collagen-induced arthritis (restricted to $I\text{-}A^q$) is type II collagen.

Rat adjuvant arthritis is another experimental animal model for human RA. The disease can be induced by intradermal injection of killed *Mycobacterium tuberculosis* in complete Freund's adjuvant. T cell lines from arthritic rats that transfer a transient form of the disease in irradiated, nonarthritic hosts are specific for hsp60, a 65-kD mycobacterial heat shock protein (*65*). Recently, a T cell clone isolated from human rheumatoid synovium was also shown to be hsp60-reactive (*66*). These findings and the remarkably conserved nature of stress proteins, suggest that hsp60 may be the autoantigen in rheumatoid arthritis. The human clone was phenotypically $CD4^-CD8^-$ and expressed the γδ (rather than the more common αβ) TCR. The ligands for γδ T cells are not yet well characterized, and the role of these cells in self-nonself immune reactions is unclear.

Thus far, T cell epitopes have been characterized only in experimentally induced autoimmunity in which animals are exogenously administered self or self-related antigens. The target antigens for spontaneous models of disease in animals [such as IDDM in NOD mice or SLE in (NZB×NZW) F_1 mice] and for most human autoimmune conditions are not known. The availability of clonal populations of T cells that cause disease, along with recent molecular advances that have improved our understanding of T cell recognition, should facilitate the isolation of T cell target antigens in many of these diseases. Autoimmune T cell clones might then be used to screen tissue-specific expression libraries to identify self-epitopes.

Epitopes for both class II–restricted $CD4^+$ and class I–restricted $CD8^+$ T cells may be involved in the pathogenesis of some diseases. In this respect, the differential intracellular trafficking pathways of antigen processing and presentation for class I versus class II MHC–restricted T cells (*67*) should be kept in mind. Once antigenic targets of autoreactive T cells are identified, their role within the autoimmune cascade must still be assigned, and the targets of effector T cells distinguished from that of T cells involved in the initiation of autoimmunity.

Environmental Agents Implicated in Autoimmunity

Numerous bacteria and viruses have been implicated in the etiology of autoimmune disease. Usually the association is indicated by antibody titers, or a recent history of viral infection, concurrent with the initial presentation of disease. Attempts have been made to substantiate the initial correlation by identification and isolation of the infectious organisms with equivocal results. There may be several reasons for the absence of consistent identification: (i) specific autoimmune diseases may represent the cumulative effect of several different disease-inducing events, only some of which are associated with a particular infectious agent; (ii) host antibody or drug treatment may interfere with detection; (iii) the isolation procedures may not be sufficiently sensitive; (iv) infection may have occurred either much earlier than presentation of overt symptoms or at a different location than that sustaining immune-mediated damage; (v) several different infectious organisms may indirectly trigger autoimmunity as a consequence of local inflammation.

Infectious agents may activate self-reactive and inflammatory cells of the immune system by establishing a condition of low level, chronic latency (*68*). By enabling researchers to detect nucleic acid sequences representing 1 in 10^{12}, the PCR has increased the sensitivity to the level needed for detection of endogenous viral sequences in mammalian tissues. By probing PCR-amplified DNA, one to five copies of the Epstein-Barr virus genome per 10^5 cell equivalents were detected, and a positive correlation was found between the presence of the Epstein-Barr viral sequences in salivary gland tissue and primary Sjögren's syndrome. Although a low percentage of normal salivary glands were also positive (*69*), this is not surprising. The mechanism of initiating autoimmunity may require that the infectious agent be present in the general population at a sufficient frequency to infect those individuals carrying the particular combination of susceptible HLA and non-HLA genes required for autoimmunity to develop.

In addition to the infectious agents discussed later, several drugs and toxins have been shown to precipitate autoimmune disease (for example, procainamide and SLE) (*70*).

Cytokines

As products of activated cells of the immune system, cytokines function as an interactive communication network to coordinate the immune response in the development of inflammation, specific

immunity, and hematopoeisis. The effect of individual cytokines are pleiotropic and depend on the cell type and activation state. In addition, not only do different cytokines possess overlapping repertoires, but when acting in concert on the same cell, a combination of cytokines may function in an additive, synergistic, or antagonistic manner. To further complicate the analysis, cytokines are often produced only transiently and at picomolar concentrations in order that the response be limited to a discrete, local radius. Because of their importance in coordinating the immune response and the interwoven nature of their effector function, it is clear that an abnormality in regulation of their production or the reception of their signal could contribute to the development of autoimmunity (*71*).

The T cells infiltrating autoimmune lesions have been shown in several cases to secrete IL-2 and to bear the IL-2 receptor (*72*). Il-2 is known to be produced shortly after nonspecific or antigen-specific activation of T cells and to enhance the activation state of many different cells of the immune system (*73*). The examination of the synovial fluid of inflamed joints of patients with RA and the demyelinated plaques of MS patients has shown the presence of several cytokines, especially granulocyte-macrophage colony stimulating factor (GM-CSF), gamma interferon (IFN-γ), and tumor necrosis factor–α (TNF-α) (*74*). GM-CSF and IFN-γ enhance expression of class II determinants; the effect of TNF-α is complicated by its differential effect on distinct target cells and the presence of other cytokines (*71*). Unlike other known cytokines, the genes of TNF-α and the functionally related TNF-β (or lymphotoxin) map within the MHC complex, near the H-2D locus in mice and the HLA-B in humans (*30, 75*). Therefore, it is possible that a disease susceptibility allele of these genes exists in linkage disequilibrium with a class I or II disease susceptibility allele.

Mechanisms

Having identified some of the genetic, environmental, and immunologic components of autoimmunity, how do these factors function to cause disease? The precise mechanisms leading to the breakdown of tolerance are not known for any disease. Several critical events are likely to be involved (Table 1).

First, an MHC susceptibility allele must be capable of binding and presenting the self (or foreign) antigens that initiate the autoimmune process. MHC molecules may also select epitopes for the induction of antigen-specific T suppressor cells. In this way, polymorphisms within MHC alleles control the activation of T cell subsets involved in normal and autoimmune responses.

A second requirement for autoimmunity is the existence of T cells with anti-self reactivity. Whereas anti-self specificities may be encoded in the germline, the rearrangement of TCR genes is a somatic event, which may in part explain disease discordance in identical twins. The expression of certain MHC molecules in conjunction with polymorphic self antigens in the thymus [Blackman *et al.* (*76*), this issue] causes clonal deletion of entire sets of T cells expressing particular TCR V_β elements—an event referred to as negative selection (*2*). The ability of DQβ alleles having Asp at position 57 to delete potentially autoreactive T cells may be one possible explanation for the dominant resistance seen in IDDM (selection for T suppressor cells may be another). However, potentially autoreactive T cells may be included in the repertoire that is selected (positively) by critical residues encoded by an MHC susceptibility allele. The mechanisms that allow anti-self T cells to escape tolerance induction must await a better understanding of nonresponsiveness to extrathymic self antigens [see Goodnow *et al.* (*77*) and Burkly *et al.* (*78*), this issue]. The existence of antigen-specific T suppressor cells that decrease the number of T cells with anti-self reactivity and the functional inactivation of anti-self T cells (clonal anergy) have been postulated as potential mechanisms by which peripheral tolerance is maintained (*79*).

Table 1. Genetic, immunologic, and environmental factors involved in the breakdown of self tolerance.

1.	Presence of an MHC susceptibility allele that is able (i) To bind and present target self antigen (ii) To select for anti-self T cells
2.	Existence of self-reactive T cells based on (i) Germline V, D, J, and constant (C) region elements (ii) Somatic rearrangement (iii) Positive and negative selection in thymus (iv) Escape from tolerance induction in the periphery
3.	Exposure of self antigens to immune system based on (i) Release of antigens from sequestered sites (ii) Ectopic expression of class II molecules on nonlymphoid cells
4.	Lymphokines and other constimulatory signals necessary to activate self-reactive T cells (absence may lead to peripheral tolerance by clonal anergy)
5.	Non-MHC loci (mostly unidentified, although TCR and immunoglobulin genes have been linked to disease in some cases)
6.	Environmental trigger (microbial or toxin) due to (i) Inflammation leading to lymphokine release and ectopic class II expression (ii) Molecular mimicry

Third, target antigens must be available for presentation by an MHC susceptibility allele to anti-self reactive T cells to complete the ternary complex. The initiating antigens of autoimmunity may be derived from a pool of cell surface and intracellular molecules (perhaps from anatomically isolated peripheral tissues) that are not normally exposed to the immune system. Bottazzo and colleagues proposed that ectopic expression of class II molecules would allow the presentation of sequestered self antigens to an immune system that was previously blind to their existence, and thus, nonresponsive (*80*). Recent work in transgenic mice suggests that local lymphokine production may also be necessary for the induction of anti-self T cells (*81*).

Thus far, this discussion has focused on the immunologic events that lead to the breakdown of tolerance and the development of autoimmunity. But what gets it all started? What is (or are) the triggering event (or events)? In many diseases, it appears that autoimmunity is preceded by some environmental insult. Tissue damage, as a result of viral or bacterial pathogens, or toxins, may be the key event that allows for (i) release of sequestered antigens from immunologically privileged sites or (ii) local inflammation resulting in lymphokine release and subsequent ectopic expression of class II MHC molecules, or both (i) and (ii).

Microbial determinants that are sufficiently similar to cross-react with host determinants, but sufficiently different to break immunological tolerance may also provoke autoimmunity (*82, 83*). Thus, an immune reaction initially directed against a pathogen could result in an anti-self response. Although a direct causal relation is yet to be established in most cases, there are a number of known or postulated examples of such molecular mimicry (Table 2). The functional importance of these sequence similarities is supported in two ways. First, the implicated pathogen can sometimes be demonstrated in, or recovered from, patients (for example, *Shigella flexneri* in mini-epidemics of Reiter's syndrome after *Shigella* dysentery outbreaks) (*84*). Second, autoantibodies specific for a self antigen often also have specificity for viral or bacterial determinants (*9, 10, 85*). A special instance of molecular mimicry has been proposed for ankylosing spondylitis and Reiter's syndrome to explain the high incidence of the class I HLA-B27 allele in patients with these syndromes

Table 2. Molecular mimicry.

Disease	Proteins bearing homologous sequences	Infectious organism	Reference
Rheumatic heart disease	Cardiac myosin/cell wall M protein	Group A *Streptococci*	(*9, 10*)
Anklyosing spondylitis, Reiter's syndrome	HLA-B27/nitrogenase	*Klebsiella pneumoniae* Yersinia pseudo tuberculosis	(*11, 12*)
Multiple sclerosis	Myelin basic protein/DNA polymerase	Epstein-Barr virus, hepatitus B virus	(*92*)
Rheumatoid arthritis	Core protein of cartilage/proteoglycan wall component	*Mycobacterium tuberculosis*	(*65*)
Systemic sclerosis	DNA topoisomerase I/p30 gag protein	Retrovirus	(*93*)
Myasthenia gravis	AChR/capsid protein VP2	Poliovirus	(*94*)
Celiac disease	A-gliadin of wheat gluten/early region E1b protein	Adenovirus type 12	(*95*)
Acute proliferative glomerulonephritis	Vimentin/cell wall M protein	*Streptococcus pyogenes* type 1	(*12, 96*)

*First protein is the self antigen, protein after the solidus is the microbial antigen.

(greater than 90% for ankylosing spondylitis, 80% for Reiter's syndrome). The amino acid sequence of HLA-B27 has been shown to share a region of five or six amino acids with a *Klebsiella pneumoniae* nitrogenase protein.

Once the bacteria or virus provokes cross-reactive tissue destruction, its presence is no longer necessary, since continued tissue injury generates more self antigen. In the case of group A *Streptococci*, which infect the skin or nasopharyngeal mucosa (*10*), and rheumatic heart disease, the site of infection may be distant from the site of the final autoimmune pathology (*11, 12, 86*). These factors complicate the identification of the initiating agent.

In summary, autoimmunity (i) develops in genetically susceptible individuals, (ii) may be triggered by environmental agents operating by nonspecific inflammation or molecular mimicry, or both, (iii) is the result of the sum of genetic and environmental factors that overrides normal mechanisms of self tolerance, and (iv) is most often mediated by T cells or is characterized by an underlying defect or deregulation in the T cell compartment.

An important goal of future studies in autoimmunity will be to arrange contributing factors in a temporal sequence. What are the initiating events versus subsequent events? The causes versus the consequences? Most likely, there will be no single pathway, but rather a complex network of interactions (no doubt different for each disease) leading to autoimmunity.

Future Prospects

Although the exact pathways leading to the failure of normal mechanisms of self tolerance are unclear, knowledge of the factors that predispose individuals to autoimmune conditions allows intervention at two levels. Molecular probes that detect polymorphisms in MHC and other genes that are highly associated with disease would facilitate the clinical screening of individuals at risk of developing disease before the onset of clinical symptoms. For example, oligonucleotide probes specific for DQβ have been used in a family study to show that haplotypes carrying Asp^{57} are significantly increased in frequency among nondiabetic siblings, while non-Asp^{57} haplotypes are increased in diabetic siblings (*87*).

Second, specific immunosuppressive strategies may be designed to block the function of MHC and TCR molecules in disease. Monoclonal antibodies directed against MHC interfere with disease induction in several animal models (*34*). Where there is a limited heterogeneity of T cell receptors related to disease (for example, EAE), anti-TCR antibodies are effective. Cloned T cell lines capable of disease transfer and peptides with the sequence of the cloned TCR have also been used to vaccinate against the development of EAE (*88*). The mechanism of disease suppression here may involve immune network interactions. More recently, the possibility of blocking peptide therapy for autoimmune disease is being explored. It may be possible to produce peptides with very high affinity for the antigen-binding cleft of an MHC susceptibility allele that are able to compete with the peptide that is activating anti-self reactive T cells. Such high-affinity peptides are effective in preventing the induction of EAE (*89*). These experiments suggest that as the MHC and TCR sequences in human diseases are cataloged, new immunotherapies can be envisioned. To this end, mice made transgenic with human MHC susceptibility alleles and the SCID/hu mouse (*90*) offer experimental models for the detailed characterization of MHC and TCR molecules in human disease, and the design and testing of therapeutic protocols.

As target antigens of disease are identified, more refined manipulation of abnormal immune responses may become possible. It may be possible to specifically delete (physically or functionally) only those lymphocytes with anti-self reactivity, or activate antigen-specific suppression. Furthermore, the identification of non-MHC genes that contribute to genetic predisposition must be part of the next phase of studies in autoimmunity. Clearly, candidate genes such as those coding for TCR, immunoglobulin, lymphokines, and other loci regulating immune responsiveness should be and are being studied. Perhaps the biggest challenge in the future will be the search for the environmental events that trigger self-reactivity. Although antibodies to pathogens and sequence homologies between microbial and self antigens are abundant, direct evidence for how environmental factors act is lacking.

In the end, a clear vision of the contributing factors and events along the pathway to autoimmunity must await a better understanding of the basic mechanisms by which self tolerance to a wide variety of self components is established and maintained. However, as confirmed by several articles in this issue, recent progress foreshadows a promising future.

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The Influence of Allogeneic Cells on the Human T and B Cell Repertoire

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Clinical transplantation is often complicated by rejection episodes, in which the immune system of the recipient reacts to the foreign transplantation (HLA) antigens on the graft. This immune response includes humoral and cellular components. In the first, B lymphocytes form antibodies to the HLA alloantigens. In the second, $CD8^+$ T lymphocytes recognize and react to HLA class I antigens, and $CD4^+$ T cells react to HLA class II antigens. The frequency and severity of these rejection episodes can be diminished by immunosuppressive drugs, HLA matching between donor and recipient, and immune modulation by blood transfusion. Effective HLA matching between donor and recipient is not always possible and often not necessary. Insight into the factors that influence the T and B cell repertoire after blood transfusion might lead to new approaches to improve graft survival.

EVER SINCE BILLINGHAM, BRENT, AND MEDAWAR SHOWED that the injection of allogeneic (a different individual's) cells into a newborn mouse induces lifelong immunological tolerance for the donor's tissues and organs in a proportion of recipient animals (*1*), transplantation immunobiologists have attempted to achieve a similar effect in the adult animal and in humans. However, in adult mice and rats tolerance can only be induced with physical, pharmacological, and biological immune modulators such as azathioprine, prednisone, cyclosporine A (CsA), total body irradiation, total lymphoid irradiation, antilymphocyte globulins, monoclonal antibodies, or combinations thereof. The multiplicity of protocols used underlines that in contrast to the immune system of the newborn, heroic suppressive measures are needed before the immune system of the adult will accept allogeneic cells as "self." The mechanisms leading to tolerance of allogeneic cells and tissues are only partially understood. It is clear that it is not due solely to deletion of the alloreactive T and B lymphocytes. In many instances, the function of these alloreactive cells is actively suppressed by regulatory mechanisms involving both T cells and humoral factors. Recently, the work done in this field has been lucidly summarized (*2, 3*).

The present overview is confined primarily to studies on the induction of tolerance in humans (and other primates), which although clinically relevant have less detailed immunologic mechanisms than do studies in rodents, because well-defined congenic inbred strains are not available, and because, often, in vivo experiments cannot be performed. The effect of pretransplant blood transfusions (PTBTs) on humoral and cellular immunity and on the outcome of the organ transplant will be emphasized. In humans as in all other species, individuals vary widely, both qualitatively and quantitatively, with respect to the specificities recognized by the T and B cell allorepertoire. In extreme cases, certain individuals may lack cytotoxic T lymphocyte precursors to specific alloantigens of the major histocompatibility complex (MHC; in humans, HLA). We shall refer to this situation as a "hole" in the T cell repertoire. The influence of individual variability and especially of the natural holes in the repertoire should be taken into account in attempts to induce transplantation tolerance in humans.

We shall first briefly review the state of the art in organ transplantation and its current challenges, then describe the events that led to the identification of the holes in the T and B cell repertoire, speculate on the possible mechanism, and finally suggest how these findings could lead to new approaches to the biological management of clinical organ transplantation.

Historical Perspective

Fully 20 years after the report of Billingham *et al.* (*1*), it was realized that the infusion of allogeneic cells could down-regulate the homograft reaction although it did not induce tolerance in humans. Clinical renal transplantation, which started in 1955, played a central role in this achievement. It was first successfully done between monozygotic twins. Simultaneously it was shown that

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