

THE MOLECULAR BIOLOGY OF AUTOIMMUNITY

Daniel Muller, MD, PhD

“ . . . one might be justified in speaking of a horror
autotoxicus of the organism.”

EHRLICH AND MORGENROTH (1900)²⁸

The concept that the immune response is unable to react against “self” held firmly for about 50 years, despite mounting evidence to the contrary. It was not until the 1950s that experimental models were developed to satisfy “Witebsky’s postulates.”⁹⁰ These were postulates, modeled after Koch’s postulates, that required the passive transfer of an experimental disease using antibodies or immune cells to prove that the disease was autoimmune.

Innate and acquired immune responses have evolved to protect us against external and internal pathologic processes. Ideally, the immune system would not react to “self” and would react only to “non-self.” Additionally, whether it reacts to an invading microbe, neoplastic tissue, or a splinter of wood, the immune response optimally would react but not overreact. The ideal is seldom realized, however, and often it is the over-reaction that leads to disease. For example, the immune response to *Mycobacterium tuberculosis* in most cases leads to the containment of the bacteria by creating a wall of fibrous tissue. This results in minor

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From the Division of Rheumatology and Departments of Medicine, Medical Microbiology, and Immunology, School of Medicine, University of Wisconsin–Madison, Madison, Wisconsin

tissue damage. If the organism is not contained relatively quickly, however, the lung tissue involved may be extensive. If the area of tissue is too large, it may undergo central liquefaction necrosis and erode into an airway creating a cavity.¹¹⁹ Although many microbes may damage tissues directly, often the "double-edged sword" of the immune response both fights the organism and causes immunopathologic damage.

The IgE immune response likely evolved as protection from parasitic infestation.⁶⁴ In the absence of these parasites, however, we are left with an immune response that does not seem to have a protective role, and instead plays a detrimental role. Perhaps allergies may be a small price to pay for this protection in evolutionary terms, especially in areas of endemic infection. These immune responses, however, may range from the annoying to the life-threatening and become important diseases economically. Current investigations point to a person's genetic background as being the most important determinant for the immune response to internal and external antigens. These genes also are linked epidemiologically to the development of an autoimmune disease. A variety of environmental and, perhaps, stochastic factors, however, may interact with this genetic predisposition and result in the phenotypic expression of an autoimmune disease.

There are a myriad of internal and external factors related to autoimmune diseases. Figure 1 shows a simple model of factors contributing to the development of an autoimmune disease. A delicately balanced immune response may tip over into an autoimmune disease when an inflammatory immune response becomes chronic. On occasion, an infectious trigger can be identified. For example, diarrhea caused by an "arthritogenic" strain of an enteropathogenic organism such as *Yersinia*, *Shigella*, *Salmonella*, or *Campylobacter*, or a urogenital infection with *Chlamydia*, may trigger reactive arthritis. But, the risk of chronic reactive arthritis is increased 35-fold in persons who are genetically susceptible through having the major histocompatibility complex (MHC) gene HLA-

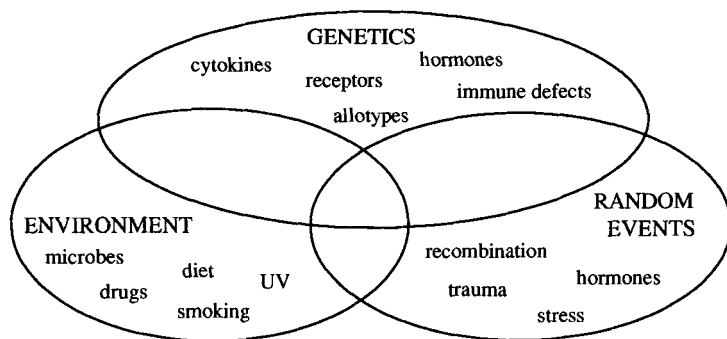


Figure 1. Basic model of factors interacting to contribute to the development of autoimmune disease.

B27.⁵ Similarly, the genetic predisposition to another chronic autoimmune arthropathy, rheumatoid arthritis (RA), is linked to the class II MHC gene DR4.^{42, 84} Although a microbe is suspected to be involved in RA, no organism has been linked satisfactorily to this disease.^{52, 91} In this article, we explore the variety of internal, external, and stochastic factors that contribute to the development of autoimmune disease. We focus on molecular mechanisms whereby internal genetic factors, such as MHC molecules, interact with external factors, notably microbes, along with stochastic factors that lead to autoimmune disease. We set the stage by examining how tolerance evolved to regulate autoimmune responses to a level we call normal autoimmunity.

TOLERANCE, NORMAL AUTOIMMUNITY, AND AUTOIMMUNE DISEASE

Tolerance

Tolerance protects us from autoimmune diseases. Traditionally we consider that "central tolerance" occurs when T or B lymphocytes are selected primarily in the thymus or bone marrow, respectively, to avoid reacting to self antigens.^{15, 39} In the case of T cells, positive selection occurs so that T cells recognize MHC molecules, and then negative selection eliminates T cells with high affinity for MHC with self peptides. For both B and T cells, negative selection may eliminate any lymphocyte clone with too high an affinity for "self." Secondary controls exist in the periphery, so called "peripheral tolerance," to suppress cells that have escaped this primary selection. Recently, however, the concepts that tolerance is maintained by central and peripheral controls that discriminate between "self" and "non-self" have been challenged.^{15, 39, 71, 126} Several observations argue against a simple "self/non-self" discrimination. We have easily detectable "normal autoimmunity," antibodies and immune cells that react to self-antigens, without pathologic consequences. Additionally, there are antigens that are sequestered in the periphery and are not used in primary selection for central tolerance. Further, we can demonstrate that the immune response often does not react to many things that are "non-self." Hence, the immune system may have evolved to react to "danger."⁷¹

Central and peripheral tolerance, including clonal deletion, can be shown to be important mechanisms of tolerance. This more recent paradigm, where the immune response reacts to danger, however, portrays antigen-presenting cells as central to the regulation of immune responses in the periphery. For example, a circulating lymphocyte is "turned-off" if it encounters an antigen without an antigen-presenting cell. The antigen-presenting cell usually provides a costimulation signal.⁷⁸ In the absence of costimulation the lymphocyte may become unreactive or may self-destruct by programmed cell death.¹⁰⁷ Therefore, an inflammatory environment is required where the body senses destruction and only then

lymphocytes will be fully activated for effector functions. This dependence on antigen-presenting cells helps to localize immune responses, preventing systemic activation. This concept also helps to explain why we usually do not react to self antigens in sequestered peripheral sites, such as myelin basic protein around neurons or crystallin protein of the lens. This concept also may help to explain how an autoimmune response to sequestered antigen might arise. An inflammatory event, such as a viral or bacterial infection, exposes the self antigen. Subsequently, antigen-presenting cells could process the previously sequestered antigen and present these antigens to lymphocytes. The immune response would then be directed toward antigens that were not previously accessible, and an inflammatory autoimmune response would ensue.

Normal Autoimmunity

Normal autoimmunity can be defined as any part of the normal immune response reactive to self-antigens. For example, anti-DNA antibodies or anti-heat shock protein T-cell responses may play important roles in early responses to pathogens or neoplasia. These autoimmune responses may help activate other innate immune responses prior to the activation of acquired immune responses. Further, this normal autoimmunity could play a role in the "clean-up" at inflammatory sites near the end of immune responses. Autoantibodies such as rheumatoid factors are found in 4% to 14% of normal persons, and antinuclear antibodies can be found in 4% of normal individuals.⁴¹ These autoantibodies increase with age.¹¹⁵

Many of these normal autoimmune responses originate from subclasses of B and T cells that may have arisen earlier in the evolution of the immune response.⁵⁴ B cells expressing the CD5 marker produce antinuclear antibodies and rheumatoid factors usually of the IgM subclass.⁴⁶ Certain $\gamma\delta$ T cells react to heat shock proteins of human or bacterial origin.⁴³ These B and T cells are similar in that (1) they appear earlier in ontogeny and also may be seen earlier in phylogeny; (2) they proliferate in peripheral sites, likely without conventional central tolerance; and (3) the antibodies or T-cell receptors (TCR) on these lymphocytes may exhibit less random recombination events, that is, these receptors are more likely to be derived from germline sequences.

It is controversial whether these self-reactive antibodies and cells play pathologic roles in autoimmune disease. Do pathologic antibodies arise from these CD5 B cells expressing germline sequences or arise from more conventional B cells in which antibody genes undergo somatic recombination? The observation that pathologic antibodies undergo affinity maturation argues for somatic recombination in reaction to specific antigen; this is more likely to occur in conventional B cells.²⁴ Do $\gamma\delta$ T cells contribute to the etiology of autoimmune disease or do we find them in areas of chronic inflammation simply because they are trying to

clean up debris? Recent studies suggest that γ - δ T cells could play both normal and pathologic roles.^{26, 50, 123}

Autoimmune Disease

Autoimmune disease is estimated to be present in 5% to 7% of the population. It can be largely organ specific, such as in autoimmune thyroiditis or diabetes mellitus, or involve multiple organs systemically, such as in RA or systemic lupus erythematosus. We discuss internal and external factors as contributing either to the etiology, to the maintenance, or to direct damage in autoimmune diseases. *Etiology* refers to what factors need to be present in order to initiate inflammatory events directed toward self. *Maintenance* refers to what factors may contribute to keeping the disease active. *Direct damage* refers to what effector molecules or mechanisms participate in the immediate cause of damage. In practice, many factors such as T cells, cytokines, or macrophages may play all three roles.

MULTIPLE HIT HYPOTHESIS FOR ETIOLOGY, MAINTENANCE, AND DIRECT DAMAGE IN AUTOIMMUNE DISEASES

Estimates for the number of genes contributing to the etiology of an autoimmune disease range from 3 to 10.⁸⁹ For example, in a mouse model of lupus glomerulonephritis, gene mapping was used to identify four gene locations, where each gene location independently accounted for a part of susceptibility to glomerulonephritis.⁷⁴ In this study only one of the four genes was identified, the MHC locus; the other three genes could not be identified using this technique.

Two studies of animal models of autoimmune diabetes mellitus may serve to illustrate the multiple hit hypothesis. In the first study, three separate transgenic mice were developed to show the roles of MHC, TCR, and cytokines in autoimmune disease.⁴⁷ The first transgenic mouse had an allogeneic, or foreign, class I MHC molecule expressed only on the insulin-producing β cells of the pancreas. The second transgenic mouse had a TCR specific for this allogeneic MHC molecule. The third transgenic mouse expressed the cytokine interleukin-2 (IL-2) in the β cells of the pancreas. None of these mice developed autoimmune diabetes mellitus. The first two mice were bred together, to make a mouse with an allogeneic MHC molecule expressed on the β cells of the pancreas and a TCR specific for that allogeneic MHC molecule. T cells expressing the highest levels of the anti-MHC transgenic TCR were deleted in the thymus. In these mice with two transgenes, however, T cells having lower levels of the anti-MHC TCR were detected in the peripheral circulation. Surprisingly, these mice did not develop an anti- β cell immune response and did not get diabetes. When the double-

transgenic mouse strain, however, was bred with the third transgenic mouse strain, which expressed the cytokine IL-2 in the β cells of the pancreas, the animals developed diabetes. Hence, an autoimmune destruction of the β cells of the pancreas did not occur until three genes were present: (1) an MHC molecule expressed in the target organ, but not present in the thymus; (2) a TCR specific for that MHC molecule; and (3) an increase in locally produced cytokines that tipped the balanced immune response from tolerance to islet cell inflammation and damage.

In a second study, a transgenic viral gene was expressed in the β cells of the pancreas without causing disease.¹¹³ Another transgenic mouse had a costimulatory molecule, B7, expressed in the β cells of the pancreas without causing disease. Breeding these two together resulted in immune-destruction of the β cells and diabetes. If the viral transgene was expressed in the thymus as well as the pancreas, then the mice deleted most of the autoreactive T cells and no diabetes was seen. If these mice, however, were then infected with the virus, which expressed the same gene that was expressed in the pancreas, then the animals developed diabetes. Again, two to three factors contributed to the development of autoimmune disease: (1) a target molecule in the target organ, (2) a costimulation gene in the target organ, and (3) a viral infection that increased inflammatory cytokines at the site of immune destruction.

These experimental models demonstrate the validity of the multiple hit hypothesis. This hypothesis is somewhat similar to what has been hypothesized for the development of neoplasia.³¹ Persons may require a certain number of genetic and environmental "hits" for full expression of an autoimmune disease—for example, (1) a particular MHC molecule, such as HLA-B27 is hit number one; (2) an infection with a mucosal pathogen, such as *Chlamydia* is hit number two; and (3) perhaps a cytokine response that is higher than normal in certain tissues, such as the synovium, would be hit number three. The end result in this hypothetical case is reactive arthritis.

ETIOLOGIC FACTORS

Table 1 outlines one classification scheme for the major contributors to the development of autoimmunity. Any known part of the normal immune response can be linked to either the etiology, maintenance, or a destructive process in some autoimmune disease.⁹⁷ All of these factors are linked to autoimmune disease using epidemiologic methods. We cannot transfer experimentally most autoimmune diseases from humans to animals. The major exception is myasthenia gravis, where anti-acetylcholine receptor antibody can transfer disease from humans to animals.⁶⁶ Therefore, very few of these factors can be proved to cause autoimmune disease in humans. Hence, we are unable to fulfill Witebsky's postulates for most of the factors in Table 1.⁹⁰

Table 1. MULTIFACTORIAL CONTRIBUTING FACTORS TO AUTOIMMUNE DISEASE**Genetics (Internal Factors)**

Cell surface receptors and ligands: MHC molecules, T-cell receptors, adhesion molecules, costimulation molecules, apoptosis molecules
 Antibody: Gm allotypes, idiotypes, antigen processing in B cells
 Immunodeficiency: Complement, IgA
 Cytokines: interleukins, interferons, TNF, TGF- β
 Innate Cells: macrophages and dendritic cells (antigen processing), neutrophils, mast cells, eosinophils
 Hormones: estrogens, androgens, corticosteroids
 Neuronal: substance P, vasoactive intestinal peptide (VIP), others
 Other: peptide transporters, aging of the immune system

Environment (External Factors)

Microbes: bacteria and viruses (includes superantigens), parasites (?), fungi (?)
 Iatrogenic: drugs, exposure of sequestered antigen (infection, trauma, or surgery)
 Physical: ultraviolet irradiation, smoking
 Nutritional: caloric intake, fatty acids, vitamins, minerals (zinc)

Stochastic (Random Factors)

Underproduction of suppressive cytokines or overproduction of inflammatory cytokines
 Exposure of sequestered antigen by trauma or infection
 Somatic recombination of immunoglobulin variable regions (affinity maturation)
 Recombination of T-cell receptor genes (variable gene repertoire)
 Hormonal fluctuation
 Stress

MHC: Structure and Function, Molecular Mechanisms, Diagnosis and Prognosis

Considering all the internal factors related to etiology listed in Table 1, it is the MHC genes that confer the highest increased risk for developing an autoimmune disease. Further, our growing knowledge of the function of these genes may hold some of the strongest clues for proving molecular mechanisms and interfering with those mechanisms to specifically shut down autoimmune reactivity. Several of the epidemiologic associations with specific MHC molecules and autoimmune diseases are listed in Table 2.¹⁰⁵ We outline the structure and function of MHC molecules, current theories of molecular mechanisms, and how MHC typing may play a role in predicting prognosis in the future.

MHC Structure, Function, and Epidemiology

HLA molecules are cell surface proteins encoded in the human MHC locus on chromosome 6. Figure 2 illustrates the gene and protein structure in schematic form. T cells recognize self and foreign linear peptides 8 to 20 amino acids long bound within the MHC peptide binding groove. Self peptides occupy the groove if a foreign peptide is not present, as peptides stabilize the MHC structure. This is the so-called "trimolecular complex" of TCR, MHC molecule, and peptide.

Table 2. HLA AND AUTOIMMUNITY: EPIDEMIOLOGIC LINKS BETWEEN MHC ANTIGENS AND DISEASE

Antigen	Disease
A3	Hemachromatosis
B27	Spondyloarthropathies (ankylosing spondylitis, Reiter's, reactive arthritis)
B5	Ulcerative colitis
Cw6	Psoriasis
DR3	Systemic lupus erythematosus
	Graves' disease
DR4	Insulin-dependent diabetes mellitus
	Rheumatoid arthritis

CD4+ T helper cells usually bind to class II MHC with peptide, whereas CD8+ T cytotoxic cells bind to class I MHC with peptide.¹⁸ Despite differences in structure, class I and class II MHC molecules bind peptides in a similar manner.⁶⁸ HLA molecules are members of the immunoglobulin superfamily. This is a family of cell surface receptors used for cell-cell communication and includes antibody molecules and TCR.

Certain of these HLA molecules confer an increased relative risk for autoimmune disease as shown using epidemiologic methods (Table 2). For example, the relative risk for reactive arthritis in an HLA-B27 positive person is about 35-fold greater than for HLA-B27 negative persons. Approximately 8% of the Caucasian population has HLA-B27, but persons with reactive arthritis are about 75% HLA-B27 positive.⁵ Hence, relative risk = [# cases B27 pos / # cases B27 neg] × [# controls B27 neg / # controls B27 pos] = [75%/25%] × [92%/8%] = 34.5. The highest relative risk documented, about 100-fold, is for HLA-B27 increasing the risk of ankylosing spondylitis. In comparison, the class II molecule HLA-DR4 may confer an increased relative risk for RA of about 4- to 6-fold.⁸⁴

Evidence that it is the HLA-B27 molecule itself that increases the risk of ankylosing spondylitis or reactive arthritis, and not a gene that is simply closely linked within the MHC region, is as follows^{58, 59, 92, 106}:

- Most patients are HLA-B27 positive.
- Components of arthritogenic bacteria cross-react with HLA-B27 cells.
- Components of arthritogenic bacteria can be found in synovial fluid.
- Peptides from arthritogenic bacteria share sequences with HLA-B27.
- Transgenic mice or rats with HLA-B27 get "reactive" arthritis.
- Transgenic rats with HLA-B27 are "cured" in a germ-free environment.

Currently, the major missing piece to this scenario is the arthritogenic peptide. This peptide has been termed the "holy grail" of reactive arthritis.²⁰ Delineation of this peptide, or peptides, may establish the

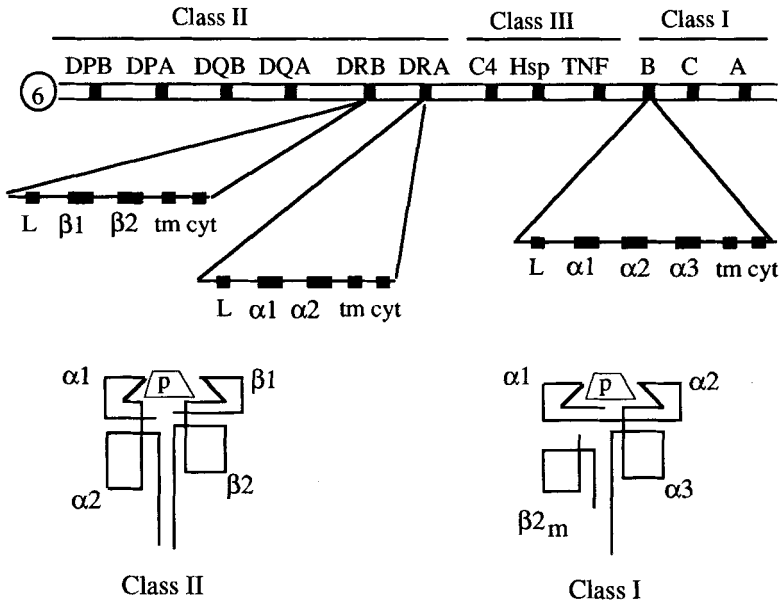


Figure 2. Schematic of HLA, the human MHC locus, on the short arm of chromosome 6. Shown are the structures of introns and exons and resulting proteins of the class I and class II MHC loci. L = leader exon; $\alpha 1$, $\alpha 2$, $\alpha 3$, $\beta 1$, $\beta 2$ are exons coding for the external protein domains of class I and II MHC molecules; tm = transmembrane exons; cyt = cytoplasmic exons. $\beta 2m$ = $\beta 2$ -microglobulin, encoded on chromosome 15, the light chain noncovalently associated with class I MHC molecules; p = peptide bound in the peptide binding groove of class I or class II MHC molecules. The binding groove of class I MHC is formed by the outermost two domains of the single heavy chain, $\alpha 1$ and $\alpha 2$. Class II MHC forms a similar binding groove from the outermost domain of each separate chain, $\alpha 1$ and $\beta 1$. Other immunologically active molecules are encoded in the MHC locus; C4 = component 4 of the complement cascade; Hsp = heat shock protein; TNF = tumor necrosis factors α and β .

molecular mechanism linking MHC and bacterial infection with reactive arthritis, and may lead to specific treatment.

Models of Molecular Mechanisms Linking MHC and Autoimmune Diseases

Models of possible molecular mechanisms are illustrated in Figure 3. The first three models show how an invading microbe might interact with an MHC molecule to contribute to the cause of autoimmune disease. The last two models show how MHC molecules alone may increase the risk of an autoimmune disease. One simple model postulates that the MHC molecule is a receptor for a microbe (Fig. 3A). The microbe binds to the MHC molecule allowing entry of that microbe into a cell or organ. For example, arthritogenic bacteria bind to HLA-B27 possibly

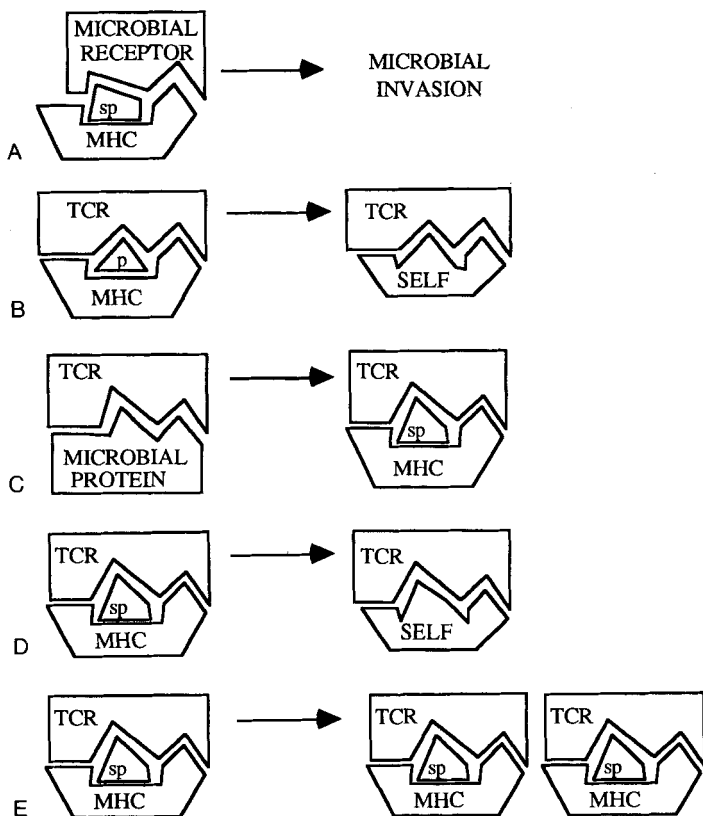


Figure 3. Models of molecular mechanisms postulated to explain the epidemiologic link between HLA genes and autoimmune diseases. *A*, MHC is a receptor for an invading microbe. *B*, MHC with microbial peptide resembles a self-protein: breaks tolerance. *C*, A microbial protein resembles MHC with self-peptide: breaks tolerance. *D*, MHC influence on TCR repertoire: failure to delete self-reactive TCR. *E*, Inappropriate display of MHC: normally negative or lower level MHC. See text for details of each model. sp = self peptide; p = pathogenic microbial peptide; MHC = major histocompatibility complex molecule; TCR = T cell receptor.

allowing access from the intestinal or urogenital mucosal surfaces to the lymphatics or to the blood stream. Alternatively, binding to the MHC receptor allows a microbe to enter a joint space. A recent study documents the specific interaction of arthritogenic bacteria with HLA-B27.⁵⁷ The bacterium likely does not multiply significantly in the joint, as there is usually no evidence for a septic joint. The immune response directed toward long-lived bacterial cell wall material, however, could result in chronic inflammation within the joint.⁴⁰

There are documented instances of shared sequences between microbial proteins and MHC molecules.^{10, 85, 91} Two models of "molecular

mimicry" between MHC and microbial proteins or peptides are presented. In the first model (Fig. 3B) MHC with an arthritogenic microbial peptide "looks like" a self protein. Therefore, the combination of hydrophobic, hydrophilic, and weaker molecular interactions forms a surface that is similar enough to allow for binding of the TCR to either the MHC with bound arthritogenic peptide or a self protein. For example, HLA-B27 with a *Shigella* peptide looks like human connective tissue and breaks tolerance to a ligament protein or to a ligament peptide bound to MHC. T cells then can be activated by ligament tissue. Acute inflammation could be followed by "maintenance" factors, which propagate a cycle of chronic inflammation that results in an autoimmune disease we diagnose as reactive arthritis. In the second model (Fig. 3C) a microbial protein looks like MHC with self peptide. For example, a protein from *Shigella* looks like HLA-B27 with collagen peptide, and breaks tolerance to self (HLA-B27 with a collagen peptide). T cells activated by "B27 + peptide" activate immune cascades, leading to acute inflammation and then chronic inflammation. In a similar manner, B cells could be induced to make antibody to ligament proteins, leading to the loss of tolerance and chronic inflammation.

MHC molecules influence the T-cell repertoire. Conventional T cells recognize self and foreign peptides in the "context" of MHC. T cells are "trained" in the thymus by a succession of steps.¹⁵ Positive selection allows TCR to bind to, and thereby "recognize," MHC with and without bound self peptide. Negative selection acts to eliminate T cells that would bind to self MHC with a high affinity and, thereby, become activated. If a certain MHC molecule fails to delete a self-reactive T cell then that T cell may escape from the thymus into the peripheral circulation. Subsequently, secondary peripheral tolerance controls could fail. For example (Fig. 3D), persons with HLA-DR4 could fail to delete a small number of self-reactive T cells that recognize a collagen sequence present in the synovial membrane, or a collagen peptide bound to HLA-DR4. These T cells escape secondary suppression and lead to the slow onset of chronic inflammation of the joints that we clinically diagnose as RA. Additionally, an HLA-DR4 molecule could fail to positively select competent suppressive cells that would act as the secondary layer of protection against self-reactive T cells. This same argument could be used to explain how a class I or II MHC molecule could be protective; that is, epidemiologically negatively linked to an autoimmune disease.¹⁰⁹ The MHC molecule would eliminate potentially reactive T cells or select for peripherally suppressive T cells.

In the section on tolerance we postulated that the immune response has evolved to respond to any destructive process, not necessarily to recognize self and non-self. In this model, antigen-presenting cells are important for providing a second costimulation signal for T cells to become activated.⁷⁸ Any factors that result in an increase in antigen-presenting cells would predispose to the recognition of self antigen. In this last model, MHC molecules could be linked to autoimmune diseases through abnormal expression on tissues (Fig. 3E). Class I MHC mole-

cules are expressed on nearly all nucleated cells, whereas class II MHC molecules usually are expressed only on tissues of hematopoietic origin, particularly those cells that can act as antigen-presenting cells (i.e., macrophages, dendritic, and B cells). Class II MHC, however, can increase on many tissues and can be expressed on normally negative tissues in the presence of pro-inflammatory cytokines such as interferon- γ .⁶² For example, in autoimmune diabetes mellitus, normally negative β cells in pancreatic islets are induced to express class II MHC due to local inflammation.³³ This class II MHC could allow the recognition of sequestered islet proteins not normally bound to class II MHC, breaking tolerance, and leading to chronic inflammation. There are some tissues that have particularly low or no class I MHC, notably neurons.⁷⁰ Neural cells, and cells in other organs, also can increase the amount of class I MHC on the cell surface after exposure to interferon- γ . Therefore, the increased expression of class I MHC in target tissues could lead to inflammatory autoimmune disease.

MHC and the Molecular Biology of Diagnosis and Prognosis

The diagnosis of autoimmune diseases is largely clinical, often using published criteria.⁹⁵ We use the laboratory for evaluation of nonspecific inflammation (sedimentation rate, C reactive protein), and somewhat more specific assays of rheumatoid factor and antinuclear antibodies. Certain autoantibodies are highly specific for diagnosis, notably anti-Smith and anti-double-stranded DNA in systemic lupus. Biopsy is usually required for the diagnosis of vasculitis.⁶⁵ Currently, there is no proven clinical use for HLA typing. Recent studies, however, may point to a change in this in the future.

The diagnosis of a spondyloarthropathy (ankylosing spondylitis, Reiter's syndrome, and reactive arthritis) usually is made without typing for HLA-B27. Certain treatments may be more appropriate in HLA-B27-related disease, however. If an occult chlamydial infection is suspected, such as epididymitis or prostatitis, then antibiotic therapy may be indicated.⁶¹ An atypical presentation of B27-related disease may best be treated with a high dose of indomethacin or even phenylbutazone if a nonsteroidal anti-inflammatory agent with fewer side effects is not sufficient. Further, sulfasalazine or a cytotoxic drug may be indicated for resistant disease.⁴ The following clinical presentations may arouse suspicion of an atypical spondyloarthropathy and the consideration for HLA typing for B27⁵: in adults, incomplete Reiter's or chronic dactylitis ("sausage" fingers or toes in reactive arthritis), pustular psoriasis (keratoderma blennorrhagica), circinate balanitis, chronic heel pain (plantar or Achilles enthesitis), acute anterior uveitis, complete heart block or aortic regurgitation, and in children, pauci- or poly-articular arthritis, cervical spine arthritis, or chronic plantar fasciitis may warrant testing for HLA-B27. In children, a positive test also may be helpful to prompt regular screening for asymptomatic complications such as anterior uve-

itis and cardiac disease. Note that the sensitivity and specificity of diagnosis using HLA typing varies according to ethnic group.⁵

MHC typing currently is not standard testing for RA. Early studies showed an increased relative risk for RA from having HLA-DR4, as identified using serologic typing. Newer methods use oligonucleotide typing to examine defined sequenced subregions within class II molecules. These subregions have been dubbed the "shared epitope," where DR4, DR1, and DRw14 share amino acid sequences that increase the relative risk for severe RA.⁴² Several amino acids of this subregion face into the peptide binding pocket of the class II molecule.¹⁰² Therefore, the contribution to increased relative risk may occur through binding of pathogenic peptides and possibly molecular mimicry. The DR shared epitope, however, also could act as a receptor for microbes, in shaping the T-cell repertoire, and in inappropriate expression of class II MHC (Fig. 3). Similar to HLA-B27, these molecular mechanisms are postulated to be important, but none have been proved.

There is an increased risk of severe RA in persons homozygous for DR4. This is not true for HLA-B27, where a single allele confers maximum risk for reactive arthritis. Further, there seems to be a "dose-response" relationship in the severity of RA with different DR alleles.¹¹⁸ In comparison, there is no such relationship documented with HLA-B27. This dose-response relationship in RA has led to the speculation that we could use newer methods of DR genotyping to predict the phenotype of RA. That is, if the person had a diagnosis of RA and had a genotype consistent with developing severe erosive RA, we would consider using cytotoxic, and perhaps combined therapy, early in the disease course. A recent study, however, evaluated genotyping to predict disease severity and found it not strongly associated with outcome in RA after 2- to 5-years' duration.²⁷

Multifactorial Etiology—Additional Factors

Genetic studies indicate that 3 to 10 genes could contribute to the phenotypic expression of an autoimmune disease.⁸⁹ HLA molecules and infectious agents only partially explain our understanding of the etiology; other genes involved in the etiology of autoimmunity are listed in Table 1. The TCR would be a likely candidate for conferring increased susceptibility to autoimmune diseases, as it is the cell surface receptor that binds to MHC with peptide. The wide diversity of TCR sequences, however, has made this analysis difficult.^{13, 104} Immunoglobulin allotypes, variable regions, and deficiencies all have been associated with the etiology of autoimmune disease.^{6, 93} Inherited deficiencies in complement are highly associated with increased relative risk for systemic lupus and lupus-like disease.^{6, 77} Genes associated with peptide transport to MHC molecules during antigen processing, and also encoded within the MHC, may play a role in susceptibility to autoimmunity.⁹ Variations in apoptosis genes leading to a failure to remove self-reactive T and B

cells have been postulated to contribute to etiology.¹⁰⁷ Genetic defects in the Fas and Fas ligand genes of the tumor necrosis factor (TNF) family of cell surface receptors lead to lupus-like disease in the *lpr* and *gld* mouse models, respectively.⁸¹

Cytokines have been implicated in susceptibility to autoimmune disease.⁷⁶ A current paradigm considers the balance between type 1 (Th1) cytokines, interferon- γ and IL-2, versus the type 2 (Th2) cytokines, IL-4 and IL-10, secreted by CD4+ T helper cells as important in the regulation of immune responses. In this model, Th1 cytokines predispose to certain autoimmune diseases such as RA, and by pushing the immune response toward Th2 cytokines, might ameliorate the disease. Conversely, Th2 cytokines may predispose toward other autoimmune diseases such as systemic lupus.⁴⁴ IL-12 also fits into the Th1 category, predisposing to similar autoimmune diseases, but is secreted by macrophages.¹¹⁰ IL-6 does not clearly fit into the Th1/Th2 paradigm but has been implicated in the polyclonal stimulation of a wide variety of immune cells in its contribution to autoimmune disease.⁴⁹ TNF- α and - β map to the MHC region (see Fig. 2) and the TNF- α locus is polymorphic with two alleles.⁷² One allele predisposes to increased levels of TNF- α , and more severe disease, during malaria infection, but may be protective against systemic lupus.⁵³ Transforming growth factor- β may play a role in suppressing autoimmune responses, but also may contribute to the underlying immune suppression to infectious agents in autoimmunity.¹¹⁴

Hormones play an important role in the regulation of metabolism, and this holds true for immune cells as well.¹²⁰ In the sexual dimorphism of autoimmune diseases—in systemic lupus erythematosus the female to male ratio is 10:1—sex steroids appear to directly affect the immune response.⁵¹ Further, estrogen has been shown in experimental models to affect directly immune cell function.⁷⁹ Activated neutrophils may play an important role in the transformation of drugs to forms more likely to induce lupus.⁵⁵ Cells and molecules not usually thought to be a part of the immune system may play significant roles. Endothelial cells may present antigens through MHC molecules. Close connections between the neuronal, endocrine, and immune systems play central roles in the general regulation of immune responses, and contribute to the susceptibility to autoimmune disease.^{67, 120}

External Factors

Environmental factors have been shown to be linked epidemiologically to autoimmune diseases. We have highlighted the role of microbial peptides from infectious agents binding to MHC molecules for presentation to T cells (see Fig. 3). These are the best models in our current understanding of possible molecular mechanisms. There are other bacterial products that interact with immune cells in unique ways. Certain microbial proteins stimulate nearly all T cells that carry a defined subclass of TCR. These microbial products are called *superantigens*.⁶⁹ They

bind directly to both TCR molecules and MHC molecules, linking the two in a unique manner by binding outside of the normal peptide binding site of MHC. This polyclonal activation of T-cell subsets has been postulated to contribute to autoimmune disease.⁸⁶ Bacterial DNA itself can stimulate the release of interferon- γ from T cells. Recent studies show that the bacterial DNA stimulates macrophages to release IL-12 and TNF- α , which then act together to stimulate interferon- γ from T cells.⁴⁵

Microbes are not the only external factors that could initiate an autoimmune disease. Prescribed and recreational drugs, ultraviolet light, and smoking all may contribute to the etiology or maintenance of autoimmune disease.^{25, 48, 100, 124} Many drugs can cause hypersensitivity vasculitis and drug-induced lupus.^{25, 100} Ultraviolet light is an important factor in the exacerbation of systemic lupus; all patients need to wear sunscreen and avoid sun exposure.¹²⁴ Most external factors are likely to be those that a majority of persons would encounter at some time in their lives. Only in the right setting, with the particular genetic background and perhaps other random factors in place does an acute inflammatory event progress to chronic damage. For some autoimmune diseases an external factor may not be needed at all. Experiments with mice prone to autoimmune diabetes show that a germ-free environment may increase disease.⁶³ One mechanism may be that normal B cells specific for antigens from normal bacterial flora help to exclude pathogenic self-reactive B cells from lymph nodes.²² Therefore, in some cases of a genetic predisposition to autoimmune disease, the immune response may require a certain level of antigenic stimulation; otherwise it turns in upon itself.

Stochastic Factors

The concordance of monozygotic twins for RA and systemic lupus is only 10% to 30%.^{16, 121} Hence, other factors aside from genetics or even environmental exposure may play important roles (see Table 1). One example of a stochastic, or random, event would be the somatic recombination of antibody genes during affinity maturation leading to a pathogenic autoantibody.^{24, 29} This could occur in one twin but not the other, leading to discordance in the expression of the autoimmune disease. Another example would be the release of sequestered antigens after infection or trauma, allowing presentation of these antigens on the surface of antigen-presenting cells and leading to the activation of T and B cells.

Stress has been linked to the onset of autoimmune disease.^{8, 11, 73, 88} Animal models of behavioral conditioning show that the CNS can influence directly the immune response.² Other behavioral manipulations, such as experimental stress, can increase experimental arthritis.³ Recent investigations clearly document the close connections between the nervous and the endocrine and immune systems.¹²⁰ Levels of endogenous corticosteroids change with stress and can have significant effects on

immune responses. The hypothalamic-pituitary-adrenal axis mediates changes in systemic corticosteroids through the influence of corticotropin-releasing factor synthesized in the hypothalamus. Cytokines, such as IL-1, IL-6, and TNF can affect directly brain tissue, particularly the hypothalamus. Hence, there is direct two-way communication between the CNS and the immune system. Stress alone does not cause autoimmune diseases. It is clear that when coping mechanisms fail, however, significant changes in the immune response may interact with genetic and environmental factors to complete the multiple hits needed for the expression of autoimmune disease.^{11, 88}

MAINTENANCE

We are starting to define the molecular mechanisms contributing to the etiology of autoimmune diseases. What factors are important in the transition from a single inflammatory event to chronic inflammation, however, are least well understood. Autoimmune diseases can present with a relentless downhill course. Most commonly, however, there is a fluctuation in the disease activity with complete or partial remissions followed by exacerbations. Hormonal fluctuation can influence the course of autoimmune disease. Remissions in RA during pregnancy are common, followed by subsequent post-partum exacerbations.⁸³ Exacerbations, as well as the onset, of an autoimmune disease often can be linked to a stressful event. Likely, many of the same factors listed in Table 1 will be linked to the maintenance of autoimmune diseases. This is a largely unexplored area of research, however, and many questions remain. Are there multiple episodes, perhaps subclinical, that must occur? Is there a threshold of tissue damage that, once crossed, leads to a failure to resolve the inflammation? Once this threshold is reached, is there a nonspecific immune response that is more polyclonal and, therefore, would be less suitable to downregulation by specific therapy? These questions will need to be addressed in experimental models and in careful clinical studies.

MECHANISMS OF DAMAGE

We know the most about the direct events that cause tissue damage. Antibodies can cause damage to cells and tissues through direct lysis, opsonization, stimulating and blocking activity, and immune complex deposition. In hemolytic anemia or thrombocytopenia, antibodies can cause cell damage either through direct lysis, by fixing complement, or through opsonization, by the removal of antibody coated cells in the reticuloendothelial system.¹¹⁶ Graves' disease is characterized by hyperthyroidism mediated by the "long-acting thyroid-stimulating" antibody that directly acts on the TSH receptor. In myasthenia gravis anti-acetylcholine receptor autoantibodies can block the receptor. In chronic

disease, however, the receptors are removed through direct damage to the neuromuscular junction.⁶⁶ Serum sickness is the prototype immune complex disease, and can present with arthritis, renal disease, and vasculitis. The antigen can be a drug, a vaccine, or an infectious agent. These antibody and antigen complexes deposit in tissues and can fix complement, activate neutrophils and platelets, and attract mononuclear cells.²⁵

Innate cells, such as neutrophils and macrophages, may transform drugs and antigens for the induction of autoimmune disease.^{18, 55} These cells also can release myriad important inflammatory factors and, therefore, play central roles in direct damage to tissues.⁸² A recently discovered immune mediator, nitric oxide, also may play a role in the synovitis seen in RA.¹¹² T cells can directly lyse cells through the pore-forming protein perforin, which works in concert with secreted serine esterases.¹⁴ T cells also can induce programmed cell death, apoptosis, using cell surface receptors that are members of the tumor necrosis family.^{81, 107} Although CD8+ T cells are considered the major mediators of cytotoxic T-cell activity, CD4+ T cells also can acquire the ability to lyse target cells.⁸⁰ T cells are thought to play roles in the pathogenesis of RA, systemic lupus, spondyloarthropathies, diabetes mellitus, and multiple sclerosis. Animal models clearly show the roles of T cells in the etiology of autoimmune disease. The role of T cells in mediating direct damage to tissues during autoimmune disease in humans, however, remains to be proven.

IMMUNOSUPPRESSIVE THERAPY

Most of our therapies are directed toward tissue damaging events or broadly to the overall immune response. Aspirin-type drugs reduce inflammatory mediators such as prostaglandins. Corticosteroids have wide anti-inflammatory activity for many cells.³² The molecular target for corticosteroids in lymphocytes may be the transcription nuclear factor- κ B (NF- κ B).^{7, 94} NF- κ B plays a central role in inducing cytokines and MHC and other cell-surface adhesion molecules. Recent evidence suggests that aspirin-type drugs at high doses also may inhibit NF- κ B.⁶⁰ The antimalarial drugs hydroxychloroquine and chloroquine may inhibit processing and presentation of antigen on class II MHC by raising the pH level within endosomes.³⁴ Cytotoxic agents such as methotrexate, azathioprine, and cyclophosphamide inhibit rapidly dividing cells by disrupting DNA synthesis.⁹⁷ Total lymphoid irradiation is immunosuppressive without the need for maintenance therapy, but may carry a higher risk for infectious complications as compared with cytotoxic drugs.¹²⁷ Cyclosporin A and FK506 are immunosuppressant drugs that have partial specificity for transcription factors in lymphocytes and can be used to treat autoimmune disease.^{56, 87, 108} Cytotoxic drugs, plasmapheresis, leukapheresis, and extracorporeal photochemotherapy may di-

rectly remove effector molecules or cells, but these methods also may have immunoregulatory effects.⁹⁷

The major problem in using these largely nonspecific inhibitors of the immune response is that the immune and inflammatory mechanisms targeted are the same mechanisms we use to fight microbial invaders and neoplastic disease. Hence, chronic use of the most powerful of these therapeutic agents leads to an increased risk for infectious, and sometimes neoplastic, disease.

IMMUNE-SPECIFIC TREATMENT IN ANIMAL MODELS AND CLINICAL TRIALS

Investigations into the molecular biology of the autoimmune diseases has led us to the brink of understanding the etiology of these diseases. We have used this information to design therapies that show great promise in well-defined animal models. We have been able to target "immune-specific" molecules to block experimental autoimmune disease. These molecules are involved in cell-cell communication and include soluble factors, such as cytokines, and cell surface ligands, such as specific TCR and MHC molecules. Experimental autoimmune disease has been blocked successfully using treatment directed at each element of the trimolecular complex of MHC molecule, bound peptide, and TCR. Table 3 lists successful immune-specific treatment strategies directed toward the etiologic molecular mechanisms.

One recent elegant study used peptide treatment in a well-defined model of experimental autoimmune encephalitis (EAE), a model of human multiple sclerosis.¹⁹ Disease is induced with an injection of cloned pathogenic T cells specific for a defined pathogenic peptide of myelin basic protein bound to class II MHC. An analogue of this pathogenic peptide injected into animals can reverse paralysis and regress inflammatory CNS infiltrates. This treatment appears to shift cytokines, increasing IL-4 (Th2), and driving out non-specific T cells from the CNS lesions. The T cells specific for myelin basic protein remain in the CNS but are "tolerized" and, therefore, inactive. This study illustrates the possibility of specific treatment in human multiple sclerosis even in the face of active disease.

The leap to treating the more complex and heterogeneous presentation of autoimmune diseases in humans has been more daunting. Experimental therapy using monoclonal antibody to immune molecules, such as cytokines, or to general T-cell markers, such as CD4, has shown early promise.^{30, 75, 103} These methods, however, suffer from a lack of specificity, leading to the possibility of generalized immune suppression and susceptibility to infectious and neoplastic disease. The possible use of oral tolerance to a specific antigen, such as collagen in RA or myelin basic protein in multiple sclerosis, is fascinating but will require further study.^{98, 111, 117}

Table 3. IMMUNE-SPECIFIC TREATMENT IN ANIMAL MODELS AND CLINICAL TRIALS**Animal Models**

- Arthritis (mouse models):
 - anti-T-cell receptor antibody³⁷
 - anti-class II MHC monoclonal antibody¹²²
 - oral type II collagen to induce tolerance¹²⁵
- Diabetes mellitus (non-obese diabetic mice):
 - anti-class II MHC¹¹⁷
 - peptide therapy²³
 - anti-T-cell receptor monoclonal antibody⁹⁶
 - cytokine therapy⁵²
- Experimental autoimmune encephalitis (multiple sclerosis model in mice and rats):
 - anti-class II MHC¹⁰¹
 - blocking peptides^{19, 36}
 - anti-T-cell receptor monoclonal antibody¹
 - T-cell vaccination³⁵
 - oral myelin basic protein²¹

Human Trials

- Multiple sclerosis:
 - interferon³⁸
 - oral myelin basic protein¹¹⁷
- Rheumatoid arthritis:
 - anti-lymphocyte subsets (anti-CD4) monoclonal antibody⁷⁵
 - anti-cytokine (anti-TNF) antibody³⁰
 - alloimmunization with mononuclear cells⁹⁹
 - oral collagen^{98, 111}

SUMMARY

The identification of those factors contributing to the etiology of, maintenance of, or direct damage in an autoimmune disease is the first step on the road to shutting down the cycle of injury to tissues. We are just beginning to understand the pathogenic factors—internal, external, and stochastic—that lead to autoimmune disease. Certain of these factors could be used to identify individuals at risk for autoimmune disease prior to the onset of clinical symptoms. Currently, we aim our therapy broadly at the immune response or at known end mechanisms of damage. We would like to target internal genetic factors that lead to the etiology or maintenance of the autoimmune disease. For example, we might use synthetic peptides to block pathogenic peptides from binding to MHC, thereby inactivating self-reactive T lymphocyte clones. Animal models show that this is possible. In the future we hope to be able to prescribe treatment to shut down specifically the immune response to self tissues while leaving intact the general immune response to invading pathogens.

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Address reprint requests to

Daniel Muller, MD, PhD
 Department of Medicine, Division of Rheumatology
 University of Wisconsin-Madison
 2605 Medical Sciences Center
 1300 University Avenue
 Madison, WI 53706-1532