Rheumatoid Arthritis—A Molecular Understanding

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The application of molecular immunology techniques in the study of rheumatoid arthritis has resulted in an explosion of knowledge on the risk factors for the disease, predictors of disease severity, the molecular mechanisms of inflammatory responses, and mechanisms of tissue destruction. We know, for example, that inheriting certain genes in the major histocompatibility complex partly dictates susceptibility and severity of rheumatoid arthritis. These genes and others in the major histocompatibility complex are critical for the occurrence of immune responses both constructive (prevention of infection, surveillance for malignant cells) and destructive (development of autoimmune diseases). We also now understand mechanisms of cell communication, regulation of immune responses, how the cells that mediate immune responses and tissue injury accumulate in tissues, and how the injury occurs. The knowledge itself is satisfying, but more important, based on this knowledge, effective and reasonably safe treatments that address basic mechanisms of the disease process have been developed and are now widely used. In fact, the newer treatments represent the "tip of the iceberg," and as our basic knowledge increases, so too will the armamentarium with which we can fight rheumatoid arthritis and other similar autoimmune diseases.

Ann Intern Med. 2002;136:908-922. www.annals.org

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For definitions of terms, see Glossary at end of text.

The etiology of rheumatoid arthritis remains elusive, although it appears that genetic, infectious, environmental, and hormonal factors are all involved in complex, interrelated ways (1–3) (**Table 1**). The various clinical manifestations of rheumatoid arthritis were well characterized before the advent of modern molecular technology. However, in recent years, application of that technology has provided much knowledge about inherited risk factors, the underlying immunopathogenesis, and the heterogeneity of the disease with respect to localized versus systemic manifestations. In this review, we explore some of the answers that these molecular techniques have provided to a series of clinical questions: 1) Who gets rheumatoid arthritis, and what determines its severity? More specifically, what role do the major histocompatibility complex (MHC)–encoded genes play in the nature and vigor of the immune response, and how does this relate to disease susceptibility? 2) How do inflammatory cells accumulate in affected tissues? 3) What do T cells do in the synovium? 4) How do the cells in the synovium effect tissue destruction?

Understanding rheumatoid arthritis at a molecular level is important for several reasons. First, this knowledge provides satisfying new models that help elucidate aspects of the disease. Moreover, molecular-level understanding may help clinicians in explaining the symptoms and the disease course to patients and their families. Perhaps most important, such knowledge should help make disease management more effective by identifying specific targets for the development of potential therapies for rheumatoid arthritis.

What Are the Biological Correlates of the Clinical Disease?

The classic clinical picture of rheumatoid arthritis usually emerges within a few months of disease onset. This clinical picture includes bilaterally symmetric painful swelling of the wrists and of the metacarpophalangeal and proximal interphalangeal joints of the hands.

The natural history of the disease in most patients involves chronic low-grade inflammation, eventually involving many joints, with periodic flares in the intensity of inflammation. Failure to treat aggressively leads to deformity and disability. Some patients with rheumatoid arthritis have an explosive onset of disease followed by an extended period of clinical remission, while others develop extra-articular disease, such as subcutaneous inflammatory nodules, vasculitis, or lung involvement. The risk for rheumatoid arthritis and its various clinical forms is most likely related to genetically influenced responses to environmental factors.

The clinical manifestations of rheumatoid arthritis are initiated by lymphocytes that localize to synovial tissue where, when activated, they cause pain and swelling. These lymphocytes produce protein mediators (cytokines) that initiate inflammation, attract other immune cells to the site, activate resident cells, and cause excess synovial fluid production. The T cells arrive through a complex process that mediates passage through the vascular endothelium and into the synovial tissue. In this process, T cells attach to the vessel lumen via surface molecules that recognize adhesion molecules

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Table 1. **Milestones in Understanding the Immunopathogenesis of Rheumatoid Arthritis***

Event	Evidence	Study, Year (Reference)
Genetic predisposition	HLA-DR4	Stastny, 1978 (4)
	DRB1*0401 disease-linked polymorphism	Nepom et al., 1987 (5)
	Homozygosity for DRB1*0401	Nelson et al., 1991 (6)
		Weyand et al., 1992 (7)
Environmental trigger	Infectious agents or toxins hypothesized	Firestein, 1991 (2)
		Wordsworth and Bell, 1992 (3)
Autoimmune events	Synoviocytes are antigen-presenting cells	Poulter et al., 1982 (8)
	T cells are found in inflamed synovium	Klareskog et al., 1981 (9)
		Burmester et al., 1983 (10)
		Weyand et al., 1992 (11)
Chronic inflammation	Proinflammatory cytokines in synovial fluid	Smith et al., 1989 (12)
	Interleukin-1	Xu et al., 1989 (13)
	Granulocyte-macrophage colony-stimulating factor	Brennan et al., 1990 (14)
	Interleukin-8	Buchan et al., 1988 (15)
	Tumor necrosis factor-α	Chu et al., 1991 (16)
Cell trafficking	Chemokines	Suzuki et al., 1999 (17)
	Adhesion molecule expression	Borzi et al., 1999 (18)
		Mack et al., 1999 (19)
Treatment	Monoclonal antibodies to T cells	Breedveld et al., 1999 (20)
	Cytokine and anticytokine therapy	Panayi, 1999 (21)
	Monoclonal antibodies to T-cell subsets	Schulze-Koops et al., 1998 (22)
	Monoclonal antibodies to adhesion molecules	Bresnihan et al., 1998 (23)
	T-cell–receptor peptide immunization and blocking	Moreland et al., 1999 (24)
	Antigen-presenting cell inhibition	Harriman et al., 1999 (25)
		Kavanaugh et al., 1994 (26)
		Kavanaugh et al., 1997 (27)

* HLA = human leukocyte antigen.

expressed on endothelial cells. Several interactions involving distinct pairs of adhesion molecules ultimately result in the localization of T cells to synovial tissue. After the T cells have arrived in the synovium, they can interact with resident macrophage-like type-A synoviocytes that may have acquired antigens, as yet unidentified, on their surface. As a consequence of this interaction, the T cells are activated and various cytokines are produced (**Figure 1** and **Table 2**). It should be noted that this process is not specific to rheumatoid arthritis or synovial tissue. Migration of immune cells into areas of tissue inflammation or injury is a nonspecific phenomenon, with specific activation occurring at the site (28).

These immunologic disturbances also lead to production of rheumatoid factor. Rheumatoid factor is usually a polyclonal immunoglobulin (Ig) M autoantibody response directed against the Fc portion of IgG, forming immune complexes. These generally appear in the serum early in the disease course and can be demonstrated in approximately 85% to 90% of patients within a year. How rheumatoid factor is involved in the disease process is unclear, but high levels of rheumatoid factor are associated with more severe disease (29, 30). This increased severity may result from enhanced inflammation from immune complex deposition and activation of complement or may simply reflect a generally more vigorous autoimmune response.

Who Gets Rheumatoid Arthritis, and What Determines Its Severity?

Twin and family studies originally suggested that the cause of rheumatoid arthritis was genetic, infectious, or both (3). Diligent searches for infectious agents that trigger the disease process have been uniformly unrewarding. Moreover, concordance studies in monozygotic twins indicate that up to one third of the unaffected twins of patients with rheumatoid arthritis can expect to become affected. In addition, the slightly increased disease prevalence in dizygotic twins and in offspring of affected parents suggests that susceptibility may involve a polymorphic genetic component (an allele or gene variant expressed $<$100% of the time).

What Is the Role of the Major Histocompatibility Complex Genes and Their Products?

The most polymorphic genetic locus in mammals is the MHC. Susceptibility to rheumatoid arthritis and the

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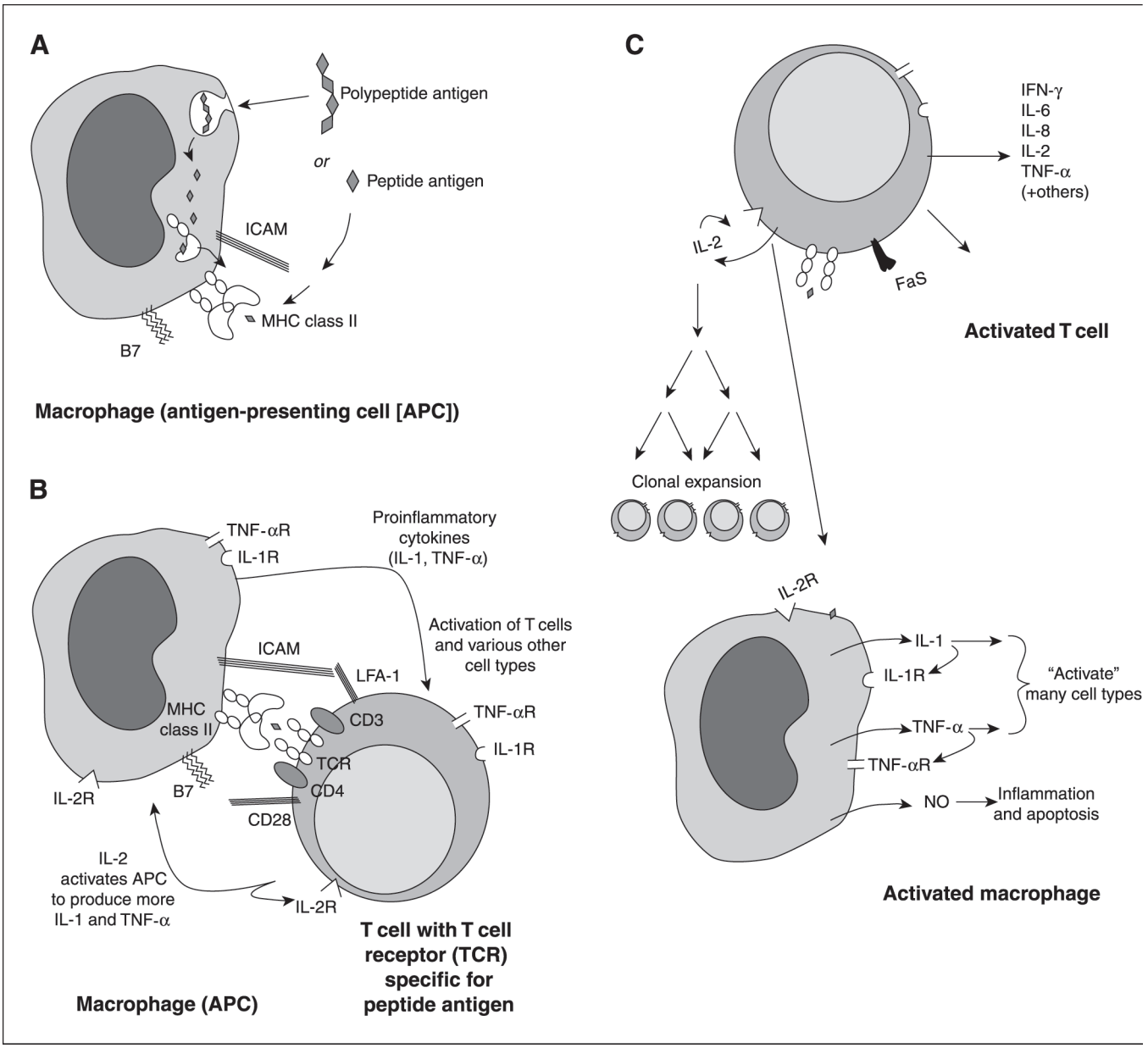
Figure 1. **Interactions between T cells and antigen-presenting cells.**



A. Polypeptide antigen undergoes pinocytosis by an antigen-presenting cell and is enzymatically digested; small peptides are then associated with a class II–related molecule in the cell and subsequently inserted with human leukocyte antigen class II α and β chains on the cell surface. Small peptide antigens may bind directly to surface class II molecules. **B.** The antigen-presenting cells interact with a passing T cell that bears the appropriate T-cell–antigen receptor—that is, one that "fits" the T-cell receptor. Antigen-presenting cells and T cells are held together by cell interaction molecules LFA-1/ICAM-1 and costimulatory molecules B7/CD28. T cells and antigen-presenting cells become activated to produce cytokines. **C.** Activated T cells and antigen-presenting cells continue to produce cytokines and other mediators of inflammation. CD = cluster of differentiation; FAS = fatty-acid synthesis; LFA = lymphocyte function antigen; ICAM = intercellular adhesion molecule; IFN = interferon; IL = interleukin; MHC = major histocompatibility complex; NO = nitric oxide; TNF = tumor necrosis factor.

severity of its manifestations are dictated by inheritance of a particular gene expressed in the MHC locus. Inheritance of this gene, often referred to as the disease-associated allele, from both parents increases risks for development and greater severity of rheumatoid arthritis, including extra-articular manifestations.

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Table 2. **Important Cytokines and Their Activities in Rheumatoid Arthritis***

Cytokine	Activity Related to Rheumatoid Arthritis
Interleukin-1	Activates T cells; activates metalloproteinases that destroy cartilage
Interleukin-2	Stimulates proliferation of activated T cells and production of many other cytokines
Interferon-γ	Enhances expression of class II human leukocyte antigen on antigen-presenting cells; enhances intercellular adhesion molecule expression and favors TH1 cell growth
Tumor necrosis factor-α	Enhances class II expression on antigen-presenting cells; enhances expression of intercellular adhesion molecules
Interleukin-4	Anti-inflammatory, downregulates TH1 cytokine production; stimulates B-cell differentiation and antibody production
Interleukin-6	Causes B-cell differentiation and enhances antibody production; stimulates production of acute-phase reactants by hepatocytes
Interleukin-8	Neutrophil chemotactic factor; attracts neutrophils to the synovial compartment
Interleukin-10	Anti-inflammatory; in concert with interleukin-4, inhibits interferon-induced expression of class II human leukocyte antigen
Transforming growth factor-β	Profibrotic; enhances production of tissue inhibitor of metalloproteinase, causing decreased production of collagenase and other destructive enzymes; possibly also responsible for late-stage fibrosis and ankylosis; suppresses expression of class II major histocompatibility complex antigens induced by interferon-γ Suppresses production of tumor necrosis factor-α, interleukin-1, interleukin-6, and neutrophil chemoattractant
Interleukin-12	Activates and causes proliferation of natural killer cells; favors activation and growth of TH1 cells

* TH = T helper.

In humans, the MHC locus encodes the cell-surface proteins known as the human leukocyte antigens (HLAs). These antigens define the tissue type of an individual—in effect, they express each person's molecular identity, much as the red blood cell antigens define blood type. The HLA molecules are divided into two major types, class I and II, based on the structure of the cell-surface proteins they specify. There are three types of class I antigens, designated as HLA-A, HLA-B, and HLA-C. Similarly, the three class II antigen types are designated HLA-DR, HLA-DP, and HLA-DQ. One gene from each locus is inherited from each parent, and the array of HLAs inherited from one parent is called a *haplotype*. At each locus, one of several alleles can be inherited (HLA-A1, HLA-A2, HLA-A3, and so forth).

Stastny (4) identified a gene associated with rheumatoid arthritis in this polymorphic group of MHC genes. He found that approximately 70% of white patients with classic rheumatoid arthritis, compared with 28% of patients with nonrheumatoid arthritis, expressed HLA-DR4. This association with HLA-DR4 has been demonstrated in most populations studied. In the white population, the presence of HLA-DR4 confers a relative risk of approximately 3.5 for rheumatoid arthritis (that is, individuals who have the HLA-DR4 genotype are about 3.5 times more likely to develop rheumatoid arthritis than white persons with other DR types). Some nonwhite populations exhibit a different association. For instance, rheumatoid arthritis in the Native American population is associated with HLA-DR9.

Through use of DNA-based tissue-typing techniques, such as site-specific probes and the polymerase chain reaction (PCR), specific rheumatoid arthritis–associated alleles have been identified. These alleles contain a particular sequence in the DRB1 gene that encodes a specific amino acid motif called the *shared epitope* (**Figure 2**). The shared epitope occurs in the β1 chain of HLA-DR4 and also on β1 chains encoded by some other DR genes, namely, DR1, DR6, and DR10. The epitope common to these HLA-DR molecules is found from amino acids 70 to 74 (31).

In nonwhite persons with rheumatoid arthritis, the disease-associated alleles differ only minimally from each other in amino acid sequence. (As noted, inheriting this allele from both parents not only increases the risk for rheumatoid arthritis, it is associated with more severe disease [32, 33]). This phenomenon has been documented best in white patients, but it also occurs in patients from Asian and some populations in India. African-American patients with rheumatoid arthritis do not exhibit a strong HLA-DR association, and those who have the disease-associated allele do not appear to develop more severe disease. Thus, in these patients, currently unknown risk factors for severity of disease are probably expressed.

What Is the Role of the Disease-Associated Allele in the Rheumatoid Arthritis Disease Process?

The disease-associated allele appears to be involved in both disease susceptibility and disease severity. As the

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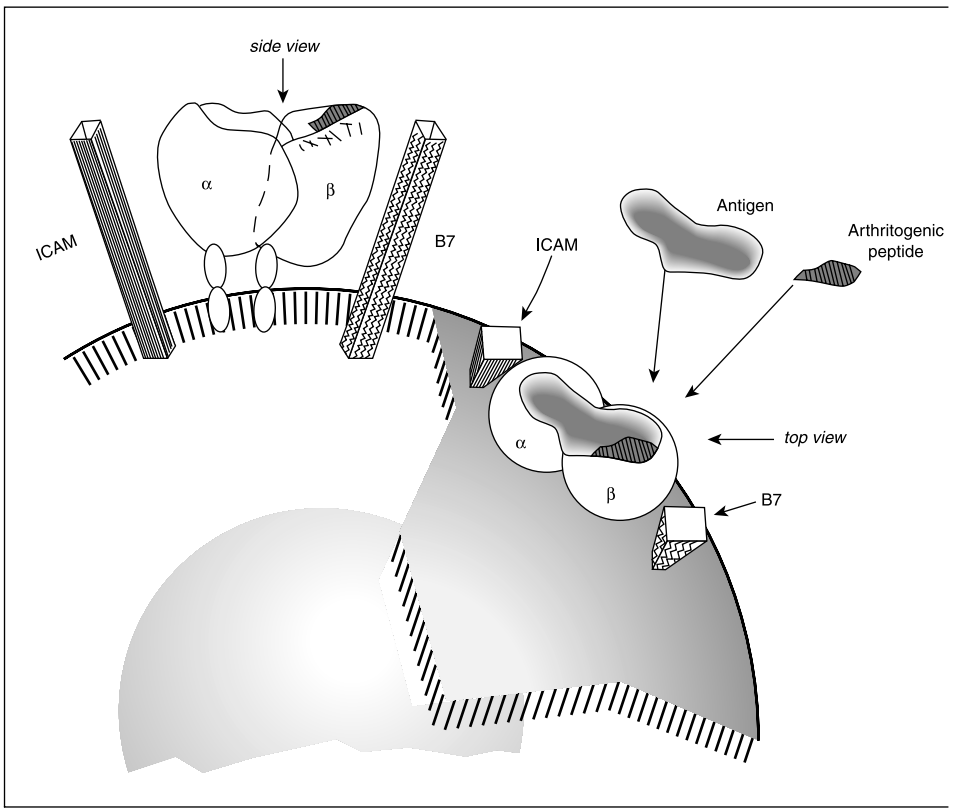
Figure 2. **Class II molecules of the major histocompatibility complex (top and side views).**



The antigen-binding cleft formed by the intertwining of the α and β chains of the molecule shows the peptide sequence known as the "shared epitope" in the β chain (shown as the cross-hatched area). The shared epitope is specified by the disease-associated allele in genes associated with rheumatoid arthritis (for example, human leukocyte antigen DR4). The shared epitope may serve as the binding site for an arthritogenic peptide or may itself be the autoantigen that activates T cells. ICAM = intercellular adhesion molecule.

susceptibility factor, the molecules encoded by this allele could bind an environmental factor (toxin or infectious agent) if this were the root cause of rheumatoid arthritis. However, the existence of a common extrinsic factor seems unlikely because no such factors have been identified through several investigations.

Other possible explanations have been suggested. For example, according to a hypothesized scenario called "molecular mimicry," the allele-encoded epitope might bind a self-peptide (auto-antigen) that mimics an environmental antigen, resulting in activation and proliferation of T cells. Finally, the disease-associated amino acid sequence might itself be the auto-antigen in rheumatoid arthritis. This possibility arises from the fact that HLA-DR molecules are intimately involved in T-cell ontogeny. It is possible that the disease-associated epitope causes a positive selection rather than a deletion of autoreactive clones as T cells interact with class II molecules during their development in the thymus.

How Do T Cells Get into the Synovium?

The synovial lining is a well-vascularized tissue that seals the joint, maintaining negative pressure and ensuring the presence of lubricating fluids in the joint space. The outer layers of the joint capsule consist of a dense collection of collagen fibers. Toward the joint space, some fat cells are seen, and finally, lining the joint space, a 2- to 4-cell–thick synovial membrane exists. This membrane consists of type A and type B synoviocytes. Type A synoviocytes perform phagocytic functions and can interact with T cells as antigen-presenting cells (34). Type B cells are fibroblastoid in appearance. Type A synoviocytes and type B synoviocytes can synthesize hy-

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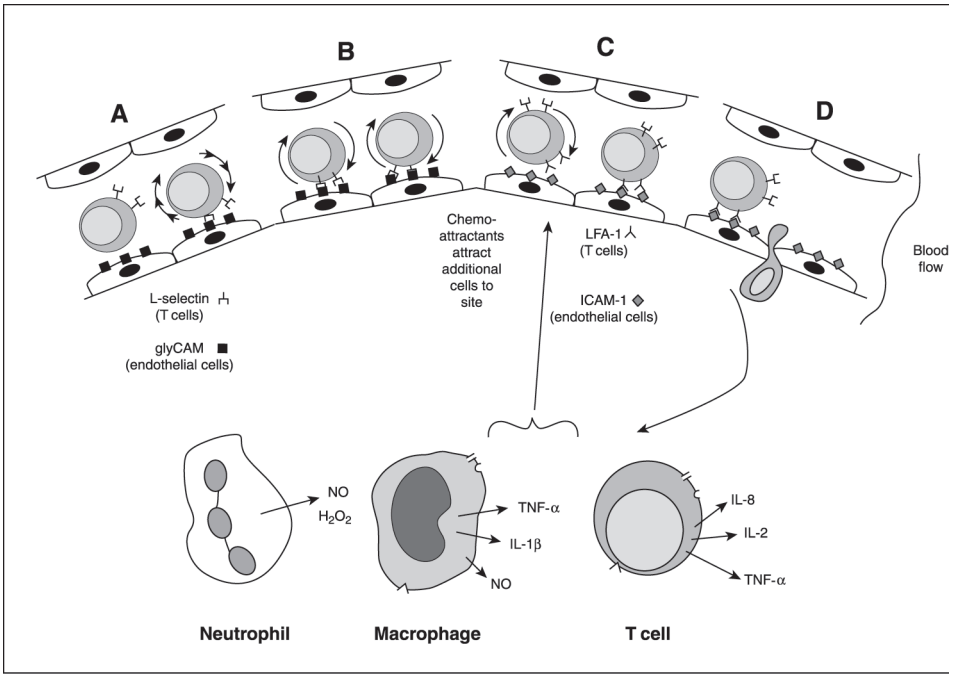
Figure 3. **Lymphocyte adhesion and migration into tissues.**



A. and **B.** At or near sites of inflammation or tissue injury, L-selectin–positive lymphocytes interact with glyCAM-1 on vascular endothelial cells. This interaction causes lymphocytes to adhere loosely to the endothelium, roll along, and slow down. **C.** As the lymphocytes progress along the endothelium, they become attached in a more shear-resistant fashion through interaction between lymphocyte function antigen-1 (*LFA-1*) on lymphocytes and intercellular adhesion molecule-1 (*ICAM-1*) on endothelial cells. **D.** Lymphocytes leave the vessel and enter tissues through gap junctions between endothelial cells. **Bottom.** As shown in **Figure 1**, T cells are activated at sites of tissue inflammation through interaction with resident antigen-presenting cells. The resultant cytokine secretion attracts other cell types to the area and mediates tissue damage (in the case of rheumatoid arthritis and in other similar autoimmune diseases), clean-up functions (in the case of infections), or tissue repair (after injury). GlyCAM = glycosylation-dependent cell adhesion molecule; H_2O_2 = hydrogen peroxide; IL = interleukin; NO = nitric oxide; TNF = tumor necrosis factor.

aluronic acid, the major component of normal synovial fluid. This unique physiology and the lack of a true basement membrane around synovial vessels allow fluid to accumulate in the joint and contribute to localization of the inflammatory cells in the rheumatoid synovium.

Leukocytes enter tissues through a multistep process of 1) adhesion to the walls of blood vessels, 2) migration through the wall, and 3) extravasation into the tissues (**Figure 3**). Pairs of adhesion molecules mediate the binding and migration steps (**Table 3**). These events normally occur at specialized portions of vessels called *postcapillary high endothelial venules* (35). In the course of physiologic, noninflammatory leukocyte migration, the initial binding step occurs when lymphocytes that express one of a particular pair of adhesion molecules on their surface (L-selectin) attach to the other one of the pair on high endothelial venules, glycosylation-dependent cell adhesion molecule-1 (GlyCAM-1). This attachment has low affinity, and the cells roll along the vessel inner surface much more slowly than the passing unattached blood elements. Rolling makes it possible for these leukocytes to more firmly attach to the vessel wall, through the expression of more shear-resistant adhesion molecules. The latter include lymphocyte function antigen (LFA)-1 on the surface of the leukocyte and intercellular adhesion molecule (ICAM)-1 on the surface of the vascular endothelial cells. Once bound in a shear-resistant fashion, the leukocyte flattens and migrates to endothelial cell junctions, where other ligand pairs mediate diapedesis into tissues.

Although mechanically similar, inflammation-induced localization of leukocytes in tissues involves ligand pairs that differ from those discussed above. Synovial macrophages also use some of the same ligand-receptor pairs as those involved in lymphocyte extravasation to gain access to the synovial membrane. Tak (36) and Koch (37) and their colleagues have observed the expression of specific adhesion molecules by synovial cells, E-selectin, on synovial

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Table 3. **Adhesion Molecule Pairs and Functions in Rheumatoid Synovium***

Adhesion Molecule Pairs (Endothelium/Leukocyte)	Expression Pattern	Function
LFA-3/CD2	Constitutive	Activation events
VCAM-1/VLA-4	Inductive	Migration events
ICAM-1,2/LFA-1	Constitutive and inductive	Activation and migration events

* CD = cluster of differentiation; ICAM = intercellular adhesion molecule; LFA = lymphocyte function antigen; VCAM = vascular cell adhesion molecule; VLA = very late antigen.

endothelium, and vascular cell adhesion molecule (VCAM)-1 and ICAM-1 on endothelial cells and macrophages). These authors have postulated that all three ligands and their respective binding partners are important for leukocyte trafficking into inflamed synovium. Control of the inflammatory localization of leukocytes in tissues involves the regulated expression of both chemo-attractants and adhesion molecules. Cytokines contribute to lymphocyte extravasation by effecting the expression of adhesion molecules or through chemotaxis; numerous cytokines regulate cellular adhesion, including interleukin (IL)-1β and tumor necrosis factor (TNF)-α—two cytokines abundant in inflamed rheumatoid arthritis synovium.

How Are T Cells Identified, and What Do They Do?

Cell-surface markers called cluster-differentiation (CD) antigens identify subtypes of lymphocytes. These markers are called antigens not because they cause immune responses in the host's body but because specific antibodies can be raised against them in laboratory animals. B lymphocytes are defined by the presence of CD19 and CD20; T lymphocytes are defined by the presence of CD3 and CD4 (T-helper cells) or CD8 (cytotoxic cells). Cells belonging to the $CD8^+$ subpopulation have also been associated with suppressor functions, whereas $CD4^+$ helper T cells are also known to mediate delayed-type hypersensitivity reactions. The T cells that accumulate in synovium are predominantly $CD4^+$ and express the phenotype of so-called "memory cells" ($CD45RO^+$, $CD29^{bright}$). Memory T cells accumulate in synovial tissue because they express adhesion molecules, allowing them to bind to vascular endothelium and to subsequently move into the synovium (38). Such T cells may be involved in the pathogenesis of rheumatoid arthritis by mediating delayed-type hypersensitivity or by providing help for B-cell differentiation and antibody production (including rheumatoid factor), or both (39).

As previously noted, one feature of T-cell activation is cytokine production. These effector molecules can be considered microenvironmental hormones because they usually affect their target cells in a very small radius around the cells producing them. $CD4^+$ helper cells are categorized into two functional sets on the basis of the cytokines they produce (**Table 4**) (40). So-called TH1 cells—T-helper 1 cells—characteristically produce a series of proinflammatory molecules (such as IL-2, interferon [IFN]-γ, TNF-α, granulocyte-macrophage colony-stimulating factor) that mediate delayed-type hypersensitivity reactions. In contrast, TH2 cells produce a group of cytokines (IL-4, IL-5, IL-6, and IL-10), all of which affect B-cell differentiation and activation. The complex interactive control of the immune system is well illustrated by the observation that some of the cytokines produced by TH2 cells (IL-4, IL-5, IL-10, and transforming growth factor-β [TGF-β]) also have potent anti-inflammatory activity (41–45) that can downregulate TH1 immune responses (46).

The immunohistopathology of the synovium early in the course of rheumatoid arthritis represents a classic response of delayed-type hypersensitivity involving proinflammatory TH1 cytokine production (1). It is thought that later in the disease course, synovial type A or macrophage-derived cytokines—specifically IL-1β, TNF-α, and IL-8—mediate the ongoing destruction of cartilage, subchondral bone, and other joint-related tissues (2, 47, 48). **Table 2** provides details of these cytokines. Interleukin-8 is probably responsible for attracting the neutrophils that compose the main cell type found in rheumatoid arthritis joint effusions. Production of IL-6 by activated T lymphocytes (and other cell types, including macrophages and synoviocytes) also contributes substantially to the disease process. Interleu-

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kin-6 prompts B cells to differentiate and mature into antibody-secreting cells. In fact, increased IL-6 levels correlate with increased levels of rheumatoid factor. Moreover, IL-6 enhances bone resorption and may play a role in the periarticular osteoporosis characteristic of early rheumatoid arthritis. It also stimulates the production of acute-phase reactants by hepatocytes; the level of IL-6 in serum is highly correlated with the level of C-reactive protein, an indicator of disease activity, in rheumatoid arthritis (49). The cytokines produced by helper T cells are generally more central elements in immune regulation, and the balance between the various cytokines is likely to be important in most microenvironments.

Schulze-Koops and colleagues (50) have reported increased levels of TH1 and TH2 cytokine mRNA in peripheral blood cell extracts of patients with active rheumatoid arthritis. The increased levels have been seen with IL-2, interferon-γ, and IL-4, suggesting more TH1 immune activity. Interleukin-1β, which is produced primarily by cells of the macrophage–monocyte series, is also commonly found in rheumatoid arthritis synovial fluid (12, 51) and in synovial macrophage-like cells (52). The effects of IL-1β on nonlymphoid cells include activation of metalloproteinases that are involved in cartilage destruction (53). Other cytokines found in synovium include TGF-β (14, 16), granulocyte-macrophage colony-stimulating factor (13), and IL-8 (54). On the anti-inflammatory side, Katsikis and coworkers (55) have found IL-10 mRNA in monocytic cells of synovial tissue in patients with rheumatoid arthritis and also in patients with osteoarthritis. They also demonstrated that these monocytic cells produced IL-10 and that anti–IL-10 treatment of cultured synoviocytes resulted in increased production of the inflammatory cytokines, TNF-α and IL-1, consistent with a downregulatory role for IL-10 at the local level.

Transforming growth factor-β is also present in large amounts in rheumatoid arthritis synovial fluid. Because TGF-β is fibrogenic (56), it may be involved in the fibrosis in rheumatoid arthritis synovium and the eventual ankylosis of the joints. In persons with rheumatoid arthritis, IL-6 and TGF-β have been shown to downregulate metalloproteinase synthesis and secretion, probably by stimulating production of tissue inhibitors of metalloproteinases (45).

The normal synovial microenvironment probably represents a state of homeostasis in which occasional TH1 responses are balanced by TH2 activation. Why this goes awry in patients who develop rheumatoid arthritis and how the cytokines work at the tissue level are unclear. At the macro level, the balance between TH1 and TH2 immune activation is important in most specialized microenvironments. An example is the normal first-trimester placenta, where both TH1 and TH2 cytokines are produced in abundance but where successful completion of pregnancy requires a skew toward a TH2 microenvironment (57). Because some cytokines, IL-6 and TGF-β in particular, exhibit activities that could be beneficial or detrimental to the immunopathogenesis of rheumatoid arthritis, their functions at the cellular level, both alone and in concert with other cytokines, beg further study.

T cells that accumulate in synovium may be involved in the pathogenesis of rheumatoid arthritis by mediating delayed-type hypersensitivity or by helping B-cell differentiation and Ig production (39). Major histocompatibility complex class II determinants or class II–antigen combinations on antigen-presenting cells in synovial tissue may select and stimulate the growth of arthritogenic T-cell clones. The expansion of T cells that share the same receptor suggests that these T cells are responding to the same antigen or to a group of highly

Table 4. **Functions of CD4$^+$ (Helper) T-Cell Subsets***

T-Cell Subsets	Functions
TH1	Transfers and mediates the delayed-type hypersensitivity reaction Produces IL-2, interferon-γ, tumor necrosis factor-α, LT, IL-3, and granulocyte-macrophage colony-stimulating factor
TH2	Produces IL-4, IL-5, IL-10, IL-3, and granulocyte-macrophage colony-stimulating factor Cooperates with B cells to generate vigorous IgM, IgG1, IgA, and IgE responses
TH0	Mixed phenotype: predominates in peripheral blood of nondiseased humans; may be driven toward TH1 or TH2

* Ig = immunoglobulin; IL = interleukin; LT = lymphotoxin; TH = T helper.

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related antigens. Several (11, 58–62) but not all (63–67) studies have shown clonal expansion of T cells in the synovium of patients with rheumatoid arthritis. Thus, the responses vary considerably, and the evidence does not suggest a single (or even a few) restricted disease-causing T-cell clones. This is not surprising considering the numerous events that determine the T-cell repertoire and the heterogeneity of the disease, even in an individual.

What Is the Mechanism of Tissue Destruction in Rheumatoid Arthritis?

The mechanisms that ward off infections and protect against malignant transformation of cells are the same as those that destroy tissue in rheumatoid arthritis. In patients with rheumatoid disease, these mechanisms of tissue destruction target normal tissue rather than invading microorganisms or malignant cells. The several ongoing destructive processes in rheumatoid arthritis are mainly orchestrated by T cells (**Figure 4**). These destructive processes include production of proinflammatory cytokines and activation of cytotoxic T cells, macrophages, and other cells that then produce metalloproteinases capable of digesting cartilage.

Neutrophils, the predominant cells in the synovial fluid of patients with rheumatoid arthritis, kill pathogens primarily through oxygen-free radicals. Enzymes in neutrophils permit addition of an electron to oxygen, resulting in production of superoxide radicals; removal of electrons by superoxide dismutase forms hydrogen peroxide and the hydroxyl radical (OH). All of these oxygen products are highly toxic. Increased levels of reactive oxygen species have been found in rheumatoid arthritis synovial fluid, but their exact role in damaging tissue is unknown. Nitric oxide (NO) in tissue maintains vascular tone and is a neurotransmitter. It is produced constitutively in various cells as a result of activity of the enzyme NO synthase. An inducible form of this enzyme is present in macrophages, polymorphonuclear leukocytes, natural killer cells, and endothelial cells. Nitric oxide resulting from activity of the inducible enzyme mediates the killing of some intracellular pathogens and tumor cells. In addition, NO causes chondrocyte apoptosis (68), affects the ratio of TH1 to TH2 cells (69), and plays a role in various other inflammatory and destructive processes (70, 71). Increased NO levels have been seen in the serum and in synovial macrophages of patients with rheumatoid arthritis (72). In murine models, the blocking of NO production in tissues prevents and treats autoimmune disease (73).

T cells and macrophages in the synovium of patients with rheumatoid arthritis also produce various cytokines that damage tissue directly or via "downstream" effects, such as induction of enzyme synthesis. TH1- and TH2-associated cytokines are known to mediate inflammation in the synovium and damage joint tissues. Production of IL-2 by activated $CD4^+$ TH1 lymphocytes causes myriad downstream effects, including stimulation of macrophages to produce IL-1β. Activated TH1 cells also produce TNF-α, as do autonomously functioning macrophages. Both IL-1 and TNF-α are involved directly in tissue destruction—IL-1β via activation of metalloproteinases, particularly collagenase and stromelysin, and TNF-α via its direct cytotoxic role.

Apoptosis is known to increase in rheumatoid arthritis synovial tissue (74). As noted, destruction of chondrocytes by apoptosis is one mechanism of tissue damage in rheumatoid arthritis. However, inhibition of apoptosis may also play a role in the immune response in rheumatoid arthritis synovial tissue. During an immune response, apoptosis primarily eliminates "activated" cells—that is, those cells that have been programmed to perform a specific function, such as enzyme or cytokine synthesis. Apoptosis prevents unregulated creation of cell products. As shown in **Figure 3**, activated T cells express increased amounts of a surface molecule called Fas. Fas-ligand, a substance that binds to the Fas molecule, is normally present in the blood, and the interaction between Fas and Fas-ligand initiates apoptosis. Interleukin-1β and TNF-α can also initiate cell death by this mechanism when they occupy their respective cell-surface receptors (75–77). Intracellular proteins in the Bcl-2 family can inhibit or enhance apoptosis. Increased expression of Bcl-2 in $CD4^+$ $CD28^-$ (memory) T cells that results in resistance to apoptosis may allow clonal growth of these autoreactive cells in synovial tissue (78).

The Effect of New Knowledge on Therapy

Until we know the exact cause of rheumatoid arthritis and can therefore direct therapy at the inciting cause or at the earliest steps in the pathophysiologic sequence,

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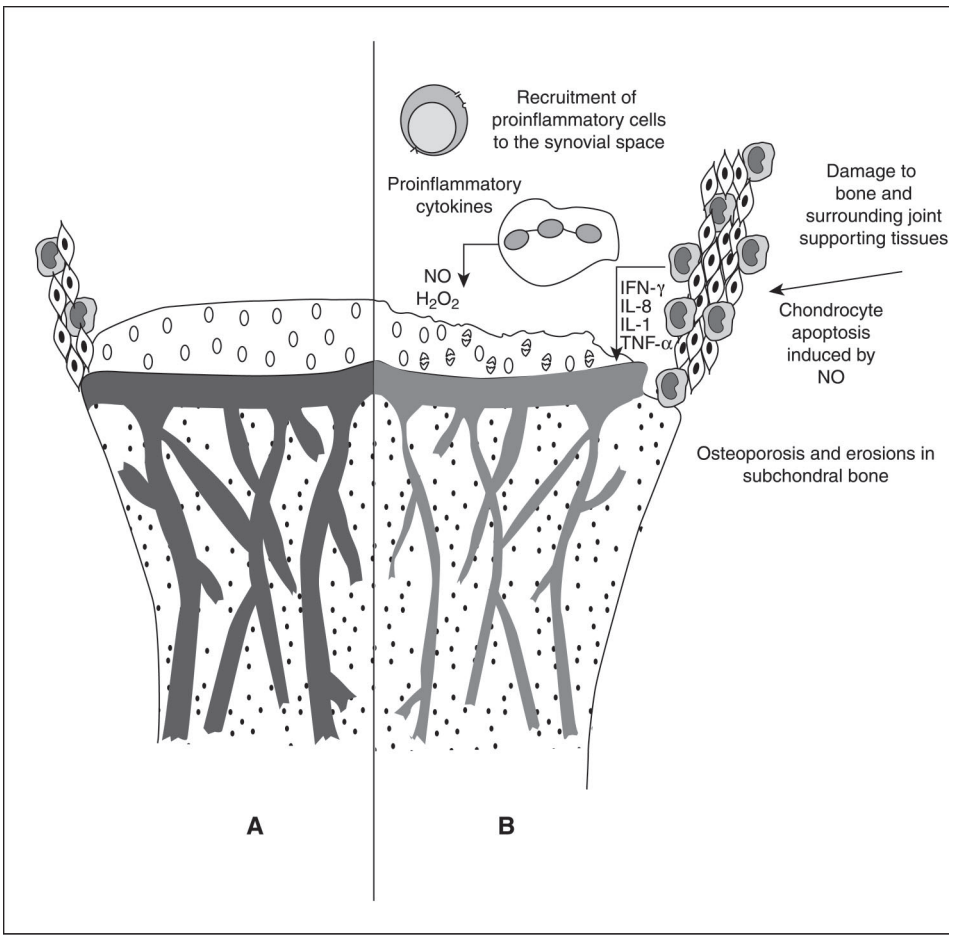
Figure 4. **Synovial cell proliferation, damage of cartilage, subchondral bone, and periarticular tissues in rheumatoid arthritis.**



A. Normal joint. **B.** Joint affected by rheumatoid arthritis. The various aspects of immune activation and lymphocyte and neutrophil attraction to sites of inflammation (as discussed in the text and in **Figures 1–3**) act in concert to produce the clinical signs of joint inflammation and damage. H_2O_2 = hydrogen peroxide; IFN = interferon; IL = interleukin; NO = nitric oxide; TNF = tumor necrosis factor.

the molecules mediating joint damage are the logical targets of anti–rheumatoid arthritis therapy. Our understanding of the immunopathology of rheumatoid arthritis has already generated several effective therapies. For example, understanding of rheumatoid arthritis as an immune-mediated disease led to the use of "nontargeted" immunosuppressive drugs, such as hydroxychloroquine, methotrexate, 6-mercaptopurine, cyclosporin, and leflunamide, all of which have proven fairly effective in ameliorating symptoms and improving quality of life for patients with rheumatoid disease. Other advancements in drug development have resulted from knowledge of the inflammatory–destructive process.

More recently, targeted therapies have been directed at the molecules shown to cause tissue injury. These approaches, generally called cytokine or anticytokine therapies, are based on inhibition of the proinflammatory cytokines (79). The primary targets of these therapies have been TNF-α and IL-1β, although development of other targeted treatment is under way (**Table 5**). Anticytokine treatment of rheumatoid arthritis is effective, readily available, and increasingly used. Inhibition of TNF-α by either a recombinant soluble TNF-α receptor (etanercept) (24) or a monoclonal antibody that reacts with and inhibits the function of TNF-α as a mediator of inflammation (infliximab) (25) results in objective and subjective improvement of rheumatoid arthritis. A recombinant IL-1–receptor antagonist (IL-1ra,

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anakinra) that binds the IL-1 receptor on monocytes without activating them also appears to be effective, according to preliminary studies (23). Other studies suggest that treating rheumatoid arthritis with both IL-4 and IL-10 may benefit some patients (80, 81). As noted in **Table 2**, these TH2-related cytokines are potent inhibitors of the proinflammatory TH1 cytokines.

Monoclonal antibodies that react with the cell-surface molecules of cells involved in autoimmune responses may also be effective (21). Mostly, studies have focused on $CD4^+$ T cells (22, 82, 83); however, antibodies that inhibit leukocyte migration into synovial tissue have also shown potential benefit for clinical application (26, 27). Antibodies that react with CD4 are potently immunosuppressive because they deplete $CD4^+$ T cells and thus may precipitate the appearance of cancer or other autoimmune diseases. This has led to the development of a potentially safer alternative—a "nondepleting" anti-CD4 monoclonal antibody (84). Therapies based on the molecular interaction between T cells and antigen-presenting cells are being developed. Although the search for clonal T cells in the synovial tissue, fluid, and blood of patients with rheumatoid arthritis has yielded variable results, it is clear that the interaction between T cells and the disease-associated allele on HLA-DR molecules plays an important role in immunopathogenesis. Peptides designed to bind HLA-DRB1 molecules can prevent the development of collagen-induced arthritis in HLA-DRB1 transgenic mice (85). Similarly, synthetic molecules that bind to the peptide groove formed by the HLA-DR4 allele associated with rheumatoid arthritis have been designed (86) and provide the opportunity for vaccine development (87). Studies of cell culture have shown that similar synthetic peptides can inhibit proliferation of cultured T cells bearing that particular T-cell receptor (88). Vaccination against T-cell–receptor peptides also blocks the interaction between $HLA\text{-}DR^+$ antigen-presenting cells and $CD4^+$ T cells and would be expected to inhibit specific autoimmune responses. T-cell–receptor peptide vaccination has been performed in a phase I trial (89).

Another potential biotechnology-based treatment that has been shown to be feasible in a laboratory setting is the use of antisense oligonucleotides or gene therapies that would inhibit production of cytokines or other mediators (90). In a laboratory study (91), the in vitro transfer of genes that mediate apoptosis resulted in the death of cultured rheumatoid synoviocytes but not of cartilage. Similarly, treatment of rabbits with experimental arthritis by injecting Fas-ligand into the joints resulted in the death of synovial cells but not of chondrocytes (92).

The future holds great promise for the development of drugs that specifically inhibit the molecules that mediate inflammation and tissue destruction. Currently, these approaches appear not only effective but also reasonably safe. Some caution is advised, however, as it is easy to be seduced by the novelty and inventiveness of the technology. Aside from the concerns about unregulated and potentially harmful immunosuppression, infusion of monoclonal antibodies that are not "humanized" induces systemic effects, including fever, rashes, and hy-

Table 5. **Currently Used and Potential Biotechnology-Derived Treatments for Rheumatoid Arthritis***

Target	Drug or Agent
T cells	Recombinant T-cell–receptor peptide immunization
	Monoclonal antibody versus T-cell receptor
	Monoclonal antibody against CD4
	Immunization with activated T cells
T cells and B cells	Monoclonal antibody versus IL-2R
Intercellular adhesion molecules	Monoclonal antibodies versus ICAM-1 or LFA-1
Tumor necrosis factor-α	Recombinant soluble tumor necrosis factor–receptor protein
	Monoclonal antibody versus tumor necrosis factor-α
IL-1	IL-1–receptor antagonist
Proinflammatory (TH1) cytokines	Recombinant IL-10 and IL-4
	Targeted gene therapy
IL-6 receptor	Monoclonal anti–IL-6R
T-cell receptor	Synthetic peptides that bind specific T-cell receptors
Antigen-presenting cells	Synthetic peptide that binds to *0401 sequence on HLA-DR4

* HLA = human leukocyte antigen; ICAM = intercellular adhesion molecule; IL = interleukin; LFA = leukocyte function antigen; TH = T helper.

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potension, and re-exposure to foreign proteins poses the danger of severe allergy, including anaphylaxis. The application of molecular immunology techniques has enhanced our understanding of the pathogenesis of autoimmune diseases, such as rheumatoid arthritis. More important, treatments based on interruption of specific immune and cellular functions are already improving the quality of life for our patients and are clearly "the wave of the future" with respect to treatment of many autoimmune diseases.

GLOSSARY

Adhesion molecules: Cell-surface molecules that allow attachment of cells to one another. The two groups of molecules are known as integrins and selectins. Integrins on T cells, such as lymphocyte function antigen-1 and very late antigen-4, pair with selectins, such as intercellular adhesion molecule (ICAM)-1 and vascular cell adhesion molecule (VCAM)-1, on vascular endothelium during the process of movement of T cells from vessels to tissues. Pairing of certain integrins and selectins is also important in the interaction between T cells and antigen-presenting cells.

Allele: An alternative form of a gene occurring in some but not all individuals of a population. Also, intraspecies variation at a specific gene locus.

Antigen: A molecule that can bind to specific receptors on T cells or B cells. The specific receptors are the T-cell receptor on T cells and immunoglobulin molecules on B cells. T cells recognize antigenic molecules when they are attached to human leukocyte antigens on the surface of antigen-presenting cells.

Antigen-presenting cells: Cells in the myeloid lineage that are able to bind small peptides and "present" them to T cells. When this occurs in the presence of certain adhesion molecules, an immune response is initiated.

Antigen-processing cells: Cells in the myeloid lineage that are able to perform pinocytosis or engulf polypeptides and enzymatically cleave them into smaller antigenic units that are then moved to the cell surface along with human leukocyte antigen molecules where they are presented to T cells.

Apoptosis: Also known as "programmed cell death." Systematic enzymatic cleavage of a cell nucleus and surface membrane resulting in death of the cell. Apoptosis occurs in the thymus to eliminate autoreactive T-cell clones and to terminate immune responses. Apoptosis of cells does not initiate an inflammatory response, whereas necrosis of cells does.

Autoreactive T cells: T lymphocytes that are able to recognize and react against self molecules.

CD markers: CD stands for cluster of differentiation. Antibodies—initially polyclonal and later monoclonal—that were raised against lymphocytes developed the ability to divide lymphocytes into several subsets that could be defined by their function (for example, T-helper cells, T-suppressor cells, and natural killer cells). For simplicity, the members of the International Workshop on Human Leukocyte Differentiation Antigens have designated antigen groups (at present, >150 have been defined) on lymphocytes that are identified by similar antibodies as clusters. For example, the cluster of antigens that defines T cells in general is called CD3.

Chemokine: A hormone-like product of activated cells that attracts other cells to a particular site.

Clonal response: Proliferation of activated T or B cells having the same antigen receptor.

Cytokine: Intercellular messenger proteins. Hormone-like products of many different cell types that are usually active within a small radius of the cells producing them.

Delayed-type hypersensitivity: Classic type IV immune response. T cells, generally on second (memory) contact with an antigen, become activated and cause tissue inflammation via production of lymphokines and chemo-attractants.

Diapedesis: The process of a cell leaving the vascular space and migrating through gap junctions between endothelial cells into tissues. Literally means "leaping through."

Epitope: The antigenic portion of a molecule or the portion that actually combines with the T-cell receptor or an antibody molecule.

Extravasation: Movement of cells from the vascular to the extravascular space.

Human leukocyte antigen: The cell-surface molecules encoded by genes in the major histocompatibility complex on chromosome 6. These molecules are divided into two classes (class I and class II) on the basis of their structure. Class I and II molecules attach and present antigens to $CD8^+$ (cytotoxic and suppressor cells) T cells and $CD4^+$ (helper) T cells, respectively. Human leukocyte antigens were originally known as histocompatibility or transplant antigens because they were defined as the targets of transplant rejection. The human leukocyte antigens in class I are expressed on virtually all cells other than erythrocytes, and those in class II are found almost exclusively on antigen-presenting cells, B cells, and activated T cells.

Immune response: A series of events whereby T or B cells become activated when they encounter an antigen for which they have a specific receptor. Activated T cells produce lymphokines that foster proliferation of the activated cells (clonal expansion) and additional immune cell recruitment and activation. Activated B cells proliferate, also under the influence of lymphokines, and secrete the antibody specific for the antigen.

Interleukin: A hormone-like messenger protein produced by immune cells that acts on leukocytes.

Ligand: A molecule that binds or links to another molecule.

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Lymphokine: A hormone-like intercellular messenger protein produced by lymphocytes.

Major histocompatibility complex: The paired set of genes on human chromosome 6 that encode the cell-surface molecules known as the human leukocyte antigens. The set of genes inherited from one parent is known as a haplotype.

Polymorphism: The presence of alleles in a population at a frequency that is higher than expected and that cannot be explained by mutation, suggesting a positive selection mechanism.

T-cell receptor: The antigen-binding molecule on the surface of T cells. The general T-cell population has T-cell receptors specific for any or all antigens they would encounter during the individual's lifetime. When an antigen invades the body, a T cell recognizes it as having a "preformed" receptor that is specific for it.

T-cell repertoire: The array of possible T-cell immune responses.

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Acknowledgments: The authors thank Drs. John Abruzzo, Pam Norton, Andrew Koenig, and Stacy Fitch for their careful review and helpful suggestions in the preparation of this manuscript.

Grant Support: By the Mona Schneidman Foundation.

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