Rheumatoid Arthritis: New Insights into an Old Adversary

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Internal Medicine Grand Rounds

October 16, 1997

When one does not see
What one does not see,
One does not even see
That he is blind...

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Rheumatoid arthritis is a chronic inflammatory condition that effects approximately 0.8 percent of the world population (1,2). Although there are frequent systemic manifestations of inflammation, the primary pathophysiologic events occur within the synovial tissue lining diarthrodial joints. Despite an intense amount of investigation, the cause of rheumatoid arthritis and the precise pathophysiologic events remain uncertain. Moreover, standard therapy remains largely empirical and, frequently not successful (3). As a result, more than 50% of persons with rheumatoid arthritis become work disabled within ten years of disease onset (4). Moreover, rheumatoid arthritis is associated with an increased mortality, with the standardized mortality ratio ranging from 1.3 to 3.0 (5,6).

In the past few years, concepts about the pathophysiology of rheumatoid arthritis have changed rapidly. These changes have resulted from intensive laboratory investigation coupled with focused clinical research. Part of the clinical investigation has involved therapeutic interventions, usually of biologic agents, that have been directed at known or suspected physiologic derangements that have been postulated to be important in disease pathogenesis. These interventions have been employed to test hypotheses about pathogenesis as well as to identify potential targets of therapeutic intervention in rheumatoid arthritis. The results of these interventions have shaped thinking about potential immunopathogenic mechanisms in rheumatoid arthritis. Moreover, new targets for therapy have emerged from these studies that may well provide the basis for substantial symptomatic benefit in rheumatoid arthritis, although the goal of a cure or prevention remains remote.

Immunopathogenesis of rheumatoid arthritis

In 1948, the discovery of the presence of the autoantibody, rheumatoid factor, in the serum of patients with rheumatoid arthritis was instrumental in designating this disease as an autoimmune condition (7-9). Before this, rheumatoid arthritis had been grouped with a series of conditions loosely categorized by the presence of fibrinoid necrosis at the site of inflammation and thought, therefore, to be primarily "collagen vascular" diseases (10). The documentation that rheumatoid factor was an autoantibody and its production was associated with rheumatoid arthritis implied that there was an autoimmune component to this disease. For the next 25 years, most research on the immunopathogenesis of rheumatoid arthritis focused on the role of rheumatoid factor and immune complexes in mediating disease pathology.

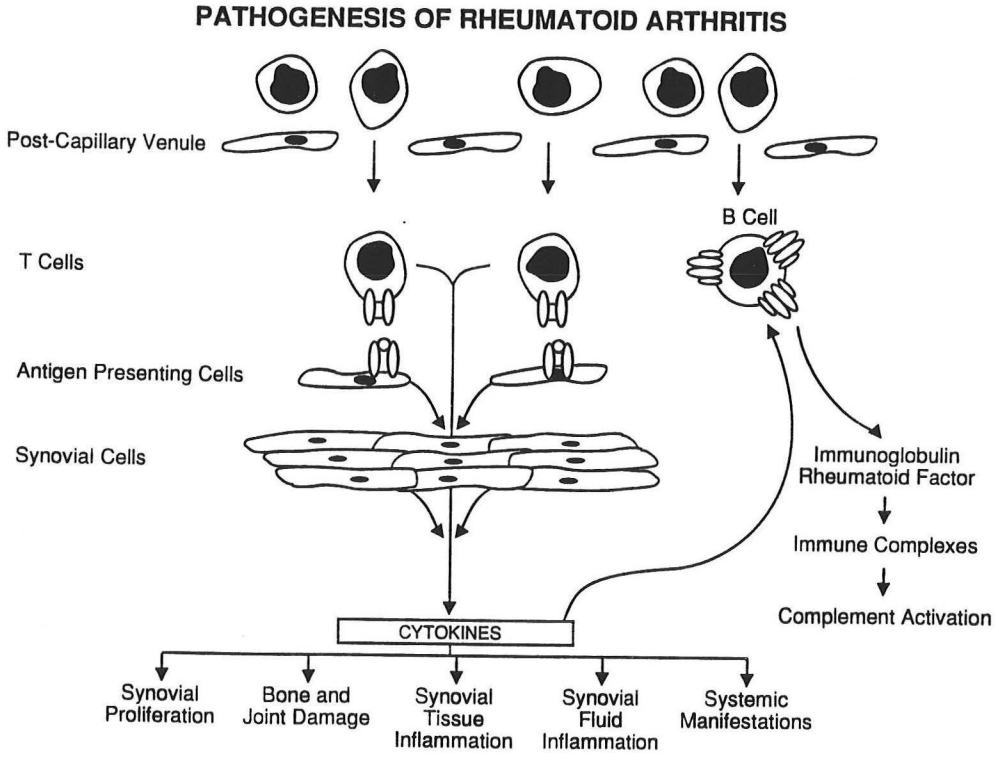
In 1975, van Boxel and associates were the first to note that the rheumatoid synovium was heavily infiltrated with T lymphocytes (11). This finding was rapidly confirmed by many other groups (12, 13), who demonstrated that synovial tissue contained aggregates of CD4+ T cells with an activated phenotype (12-14). The observation that rheumatoid synovial tissue was heavily infiltrated with T cells along with the finding of Stastny that rheumatoid arthritis was associated with particular alleles of the class II MHC complex (15), refocused thinking about the immunopathology of rheumatoid arthritis from the possibility that it was largely an immune complex mediated disease to the probability that the disease involved activation of CD4+ T cells, presumably responding to arthritogenic antigens. The data supporting a role for CD4+ T cells in the immunopathogenesis of rheumatoid arthritis are summarized in Table 1.

Table 1

Evidence that CD4+ T Cells Play a Central Role in Rheumatoid Inflammation

1. Rheumatoid synovium is infiltrated with CD4+ T cells
2. CD4+ T cells in the rheumatoid synovium exhibit an activated phenotype
3. Progressive rheumatoid arthritis is associated with specific HLA-DR alleles

The data available at that time supported the conclusion that recognition of an arthritogenic peptide presented in the context of a relevant HLA-DR molecules to clones of CD4+ T cells with appropriate T cell receptors (TCR) might initiate a series of events that eventuated in rheumatoid inflammation (Figure 1).



The Search for Antigen Reactive T Cells

Based upon the idea that rheumatoid arthritis might be driven by specific clones of antigen reactive CD4+ T cells, a number of investigators set out to detect clonal expansion of such T cells in synovial fluid and synovial tissue. A number of different approaches were employed, including Southern blot analysis to detect oligoclonal populations (16,17), polymerase chain reaction (PCR) amplification of T cell receptor alpha and beta chains (18-28), and identification of T cells expressing specific receptors with monoclonal antibodies (29-31). Although a number of investigators demonstrated apparent oligoclonality of T cells within individual patients, attempts to identify shared clones among patients has been largely unsuccessful. There are a number of potential explanations for these discrepant results, including differences in patient selection, disease duration, HLA-DR phenotype and technical aspects of the determination. Despite these caveats, most of the data are consistent with the conclusion that the T cell infiltrate in established rheumatoid arthritis is very heterogenous, with little evidence of clonal expansion of T cells that is common to a majority of patients. Moreover, in all of the analyses, a large degree of polyclonality was observed in each patient, with an occasional overrepresented clone, confirming the conclusion that marked heterogeneity and not oligoclonality is characteristic of the T cells in the rheumatoid synovium. However, the number of antigen specific T cells in an antigen initiated T cell infiltrate might be quite small. Therefore, analysis of the antigen binding third complementarity determining region (CDR3) of the T cell receptor has also been carried out (32-34). The results have suggested that within the heterogeneity, there might be the possibility of preferential expansion of T cells expressing TCR with specific antigen binding motifs.

Besides examination of potentially skewed TCR usage in rheumatoid synovium, more recent studies have suggested the possibility that the peripheral TCR repertoire may also be abnormal in both the CD4+ and CD8+ subsets (33-37). This remains a matter of controversy, however, as analysis of monozygotic twins discordant for rheumatoid arthritis revealed that there may be a genetic basis for T cell receptor expression, but this does not appear to be influenced by the presence of rheumatoid arthritis (38,39). Rather, the presence of specific HLA-DR molecules in the thymus may influence the distribution of TCR expressed by naive T cells (40,41).

In summary, despite great effort, the elusive antigen-specific CD4+ T cell has not emerged from molecular analysis of TCR utilization in patients with rheumatoid arthritis. Although the results are not conclusive, because genetically or phenotypically heterogeneous patients were examined and only few studies analyzed patients with very early disease, the findings have called into question the hypothesis that initiation of rheumatoid arthritis involves recognition of a limited set of arthritogenic peptides presented by specific HLA-DR molecules to unique populations of CD4+ T cells.

Genetic Association

Initial reports indicated that rheumatoid arthritis was associated with a particular allele of the class II MHC locus, HLA-Dw4 (15). The HLA-DR4 family is now known to include approximately 20 variants with amino acid differences focused in position 57, 67, 70, 71, 74 and 86 of the HLA-DRβ1 chain (42). Not all of the HLA-DR4 variants are found at increased frequency among individuals with rheumatoid arthritis. Specifically, HLA-DRβ1*0401, β1*0404, β1*0405, and β1*0408 are

preferentially found in patients with rheumatoid arthritis (Figure 2).

Table 72-1. HLA-DR association with RA

HLA-DR	HLA-DRB1 Alleles	Amino acids*								Associated with RA
		67	68	69	70	71	72	73	74	
DR4/Dw4	0401	L†	L	E	Q	K	R	A	A	+
DR4/Dw14	0404/0408	–	–	–	–	R	–	–	–	+
DR4/Dw15	0405	–	–	–	–	R	–	–	–	+
DR1	0101	–	–	–	–	R	–	–	–	+
DR6/Dw16	1402	–	–	–	–	R	–	–	–	+
DR10	1001	–	–	–	R	R	–	–	–	+
DR4/Dw10	0402	I	–	–	D	E	–	–	–	–
DR4/Dw13	1301-1304	–	–	–	–	R	–	–	E	–

*Amino acid sequence of the third hypervariable region of the β-chains of HLA-DR alleles associated with rheumatoid arthritis.
†Single-letter amino acid code; identity with DR4/Dw4 at each position is indicated by a dash.

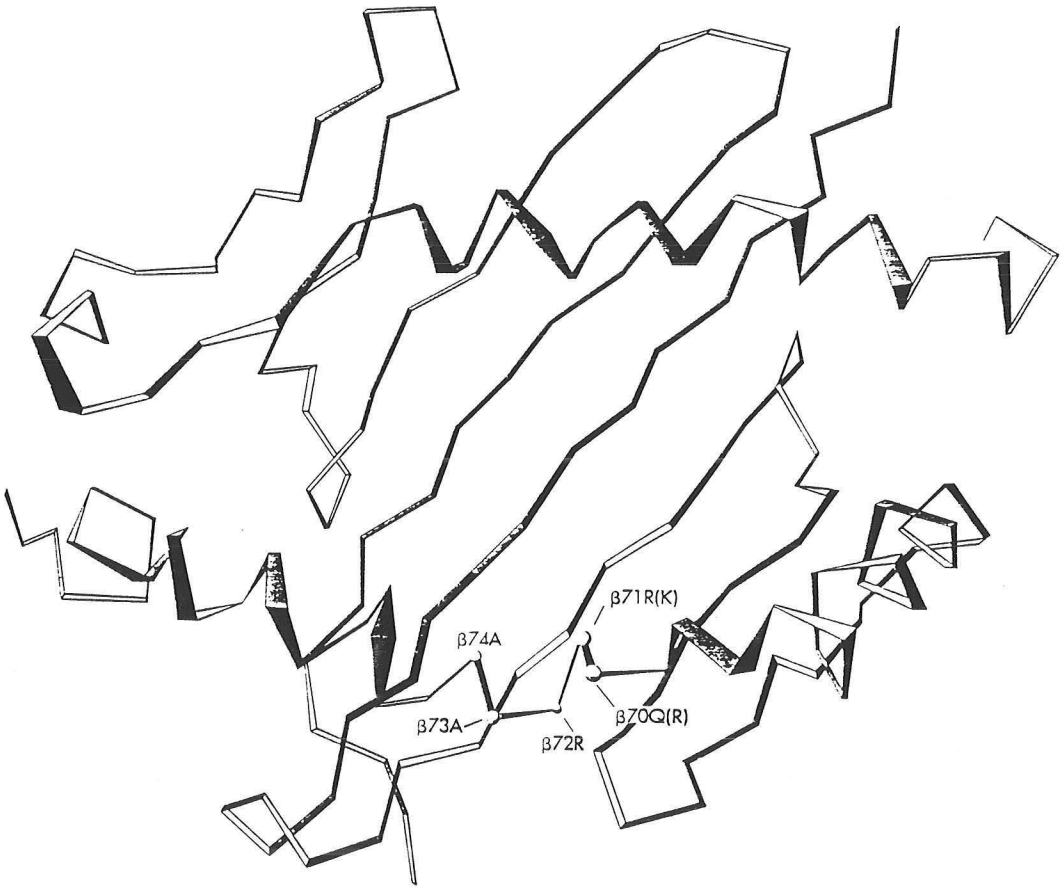


Fig. 72-1. Structure of the DR1 molecule, based on x-ray crystallography. The class II α_1 and β_1 domains of the DR1 molecule (DRB1*0101) are shown. This would correspond to a top view, as might be seen by an approaching T cell. The antigenic peptide would be held in the antigen binding groove, with the floor formed by a β sheet, and the sides formed by two helices. Amino acid residues for positions 70 through 74 are indicated in one letter code. Amino acid residues in parentheses indicate residues occurring at these positions in other class II molecules that are also associated with RA (Table 72-1). (Based on Brown JH, Jardetzky TS, Gorga JC et al: *Nature* 364:33, 1993.)

In contrast, HLA-DRβ1*0402 and β1*0403 have not been found to be increased in rheumatoid arthritis patient (43-46). These two sets of HLA-DR4 alleles vary in the third hypervariable region

of the beta chain of the molecule. Therefore, it has been suggested that the "shared epitope" between the susceptibility encoding HLA-DR4 molecules spanning amino acid positions 70-74 of the HLA-β1 chain represents the key genetic element in rheumatoid arthritis (44). As HLA-DR1, that contains the same amino acids in these positions, is also associated with rheumatoid arthritis in certain populations (44), the "shared epitope" hypothesis seemed reasonable. Since the major function of HLA-DR is to present peptides to CD4+ T cells during selection of the repertoire in the thymus and subsequently to present antigenic peptides to CD4+ T cells in the periphery and the "shared epitope" lies in a portion of the HLA-DR molecule that appears to be important for peptide binding (47), this association has suggested that presentation of specific arthritogenic peptides to CD4+ T cells plays a role in rheumatoid arthritis. In fact, HLA molecules expressing the "shared epitope" have been shown to bind a number of peptides (48,49), including those that could be involved in the pathogenesis of rheumatoid arthritis, such as peptides derived from collagen type II, 70 kD heat shock protein and cartilage glycoprotein 39 (50-52). Moreover transgenic animals expressing human HLA-DR molecules containing the "shared epitope" manifest increased susceptibility to arthritis induced by human type II collagen (53).

More recent analysis of HLA-DR4 and its association with rheumatoid arthritis, however, have suggested alternative interpretations (Table 2).

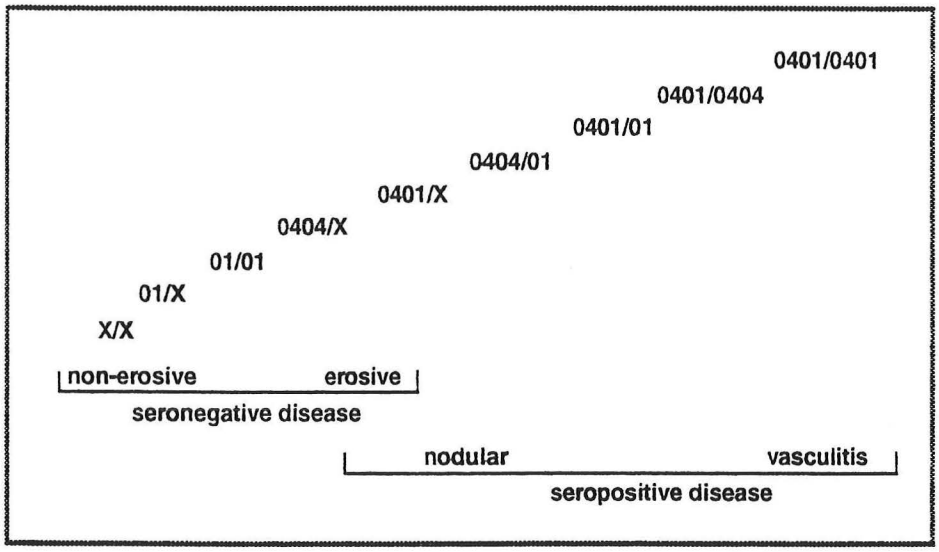
Table 2

HLA-DR β1*0401/0404 AND RHEUMATOID ARTHRITIS

- **Observations:**
  1. **Associated with rapidly progressive joint damage and extra articular manifestations**
  2. **No association with early disease**
  3. **Found in only a small number of patients in some ethnic groups**

First, in many populations, rheumatoid arthritis is not associated with the "shared epitope". Thus, in African Americans (54) and in certain ethnic groups in Southern Europe, the "shared epitope" appears to confer only a minor risk for rheumatoid arthritis (55,56). It should be emphasized that in some of these groups, certain manifestations of rheumatoid arthritis are different, in that the patients tend to have milder disease with few extraarticular manifestations, such as rheumatoid nodules and vasculitis. Secondly, a number of studies have suggested that early, self limited or minimally active rheumatoid arthritis is not associated with the presence of the "shared epitope" (57-62). Thus, individuals with early disease who meets criteria for rheumatoid arthritis, persons in primary care settings who meet criteria of rheumatoid arthritis or persons with intermittent or with minimally active inflammatory disease show no association with the "shared epitope". Only those persons with erosions after one year, an early manifestations of joint damage showed this association. An association with the "shared epitope" and rheumatoid arthritis has been reproducibly found predominantly in tertiary care centers who manage persons with aggressive disease. The

association of aggressive disease with the presence of the "shared epitope" has been reported in a number of studies (63-67). An hierarchy of disease association was noted (63,68,69) with erosive disease without extraarticular progression characteristically found in individuals who inherited a single copy of the "shared epitope" (Figure 3).



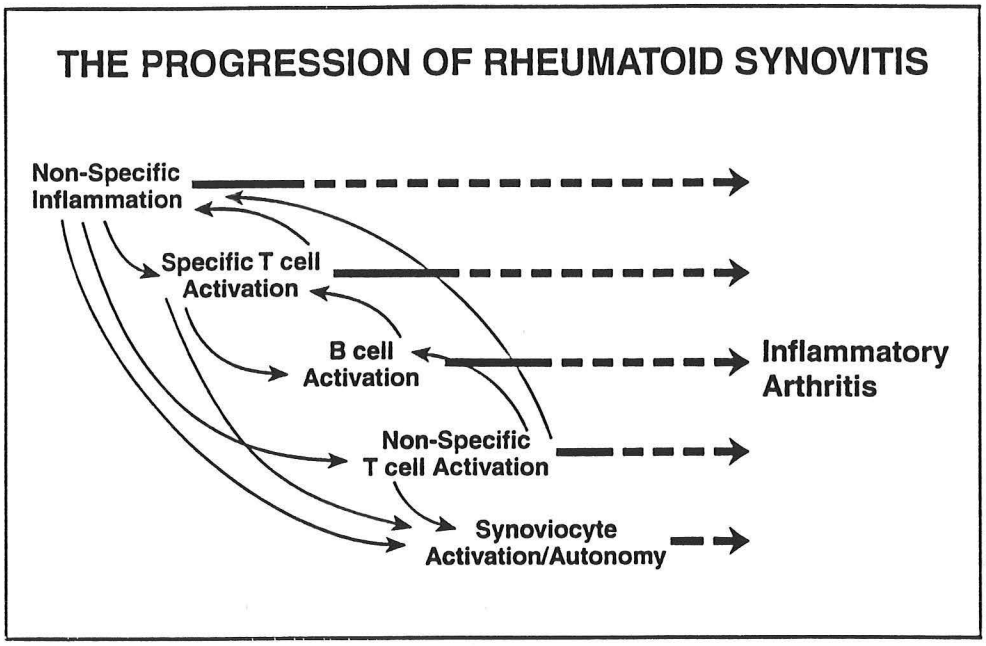
Inheritance of two "shared epitope" alleles was associated with development of rheumatoid nodules, whereas rheumatoid vasculitis was associated with HLA-DRβ1*0401/*0404 heterozygosity or HLA-DRβ1*0401/*0401 homozygosity. Homozygosity for HLA-DRβ1*0401 appeared to be the strongest predictor for progression of disease to rheumatoid vasculitis. These results strongly suggested that the "shared epitope" is not a disease susceptibility gene, but rather is associated with disease progression.

The association between the "shared epitope" and the production of rheumatoid factor is controversial (63), as the capacity to produce rheumatoid factor appears to be an independent risk for severe rheumatoid arthritis (64,65,70-74). However, the ability to make rheumatoid factor is

tightly associated with erosive disease, extraarticular manifestations and the increased motality associated with rheumatoid arthritis (75-77). Therefore, one interpretation of these data is that the presence of the "shared epitope" and, especially, HLA-DRβ1*0401 may be associated with the development of a sufficient magnitude of T cell help for B cell responses to ensure production of high titers of rheumatoid factor that, in turn, may play a role in the evolution of aggressive rheumatoid arthritis. In this regard, HLA-DR4-expressing individuals have been noted to have a bias in the production of antibodies (78).

These findings suggest that susceptibility to rheumatoid arthritis may not be related to presentation of a specific peptide, as would be implied from an association of all disease with the "shared epitope", but rather that during the evolution of the disease, the quality of T cell help, which may be dependent on expression of the" shared epitope" could influence the evolution of the disease by facilitating the production of the autoantibody, rheumatoid factor. While supporting a role that CD4+ T cells in rheumatoid arthritis, this contention moves the role of these T cells from the point of initiating disease by recognizing a specific antigen in all patients to defining the quality of T cell responses in some patients that manifest progressive disease. These findings, therefore, imply that the role of CD4+ T cells may be more complex than initially anticipated, functioning to influence the regulation of the immune response after its inception (Figure 4).

Figure 4



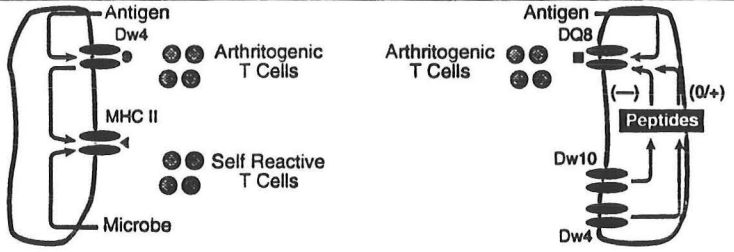
Additional studies have called into question the function of the "shared epitope" in rheumatoid arthritis. Thus, it has been suggested that the "shared epitope" might not function to bind and present specific antigenic peptides, but might itself, be the source of a peptide that is recognized by T cells after presentation by a class II MHC molecule (79). In this regard, the reactivity might be amplified by a form of molecular mimicry (80-85). The "shared epitope" (QKRAA) has been noted to be encoded not only by HLA-DR β1*0401, but also by the DnaJ heat shock protein of *E. coli*, *L. lactis* and *B. ovis,* as well as the gp110 protein of Epstein-Barr virus. T cell reactivity to peptides containing this epitope has been noted in persons with rheumatoid arthritis, but not in normal individuals expressing the "shared epitope" (81,85). This has suggested the possibility that the "shared epitope" may serve as an antigenic peptide, presented by another class II MHC molecule. The presence of this "shared epitope" in the thymus during T cell ontogeny might serve to alter the expressed CD4+ T cell repertoire (79,81), as has been suggested from analysis of the TCR expressed by naive T cells of persons expressing the "shared epitope" (39-41). Cross-reactivity between these T cells and bacterially derived proteins expressing a similar epitope might expand these CD4+ T cells in the periphery and trigger ongoing autoimmune diseases (Figure 5). Of note, it has been

suggested that HLA-DQ molecules might uniquely present these peptides (81).

Figure 5

ROLE OF CLASS II MHC MOLECULES IN SUSCEPTIBILITY TO RHEUMATOID ARTHRITIS

	Proposed Susceptibility Gene DRβ1*0401	DQβ1*0302 (8)
Proposed function	a) Present arthritogenic peptide b) Source of auto-stimulatory peptide	Present arthritogenic peptide
Source of arthritogenic peptide	Collagen II/ Cartilage gp39 / unknown	Collagen II / unknown
Regulation	Peptides derived from microbes with cross-reactive epitopes	Peptides derived from MHC II lacking shared epitope



The possibility that HLA-DQ may bind and present an arthritogenic peptide and, thus, be a disease susceptibility gene has also been suggested (86). In this regard, it has been suggested that HLA-DQ may actually be the disease susceptibility allele, that HLA-DR may encode for peptides that can modulate the development of arthritis. Thus, HLA-DR alleles that are not associated with rheumatoid arthritis, such as HLA-DR4 Dw10 (DRβ1*1502) or may even be protective against rheumatoid arthritis in some populations, such as HLA-DR2Dw12 (DRβ1*0402) may serve to alter the recognition of arthritogenic peptides presented by HLA-DQ (86). By contrast, peptides derived from HLA-DR molecules derived from rheumatoid arthritis associated molecules, such as HLA-DR4 Dw4 (DRβ1*0401) may have no such blocking activity.

Two HLA-DQ alleles (HLA-DQw8, DQβ1*0302; and HLA-DQw7, DQβ1*0301) are in linkage disequilibrium with most HLA-DR4 haplotypes in North America and Europe. Most HLA-DR4+ patients with rheumatoid arthritis express one of these alleles. However, association with rheumatoid arthritis may not be seen because of the modifying influences of HLA-DR. HLA-DQW8

has been shown to be a susceptibility factor that predisposes animals made transgenic for this molecule to intense inflammation following immunization with type II collagen (87). Moreover, peptides derived from rheumatoid arthritis resistant individuals, but not rheumatoid arthritis susceptible individuals, block collagen arthritis in this model (88,89). Based upon these findings, it has suggested that HLA-DR derived molecules may be permissive or resistant to the development of arthritis mediated by presentation of peptides by HLA-DQ molecules (Figure 5). As a result, linkage with rheumatoid arthritis might not be seen because the HLA-DQ molecule of relevance is found in both individuals who are susceptible and resistant to rheumatoid arthritis. The development of arthritis, however, may be dependent on the HLA-DQ molecule, but regulated by the disease modifying influence of the HLA-DR molecule. As different HLA-DR peptides might differ in their ability to block disease, various influences on disease might be noted with some HLA-DR molecules having no influence, some mitigating, but not blocking disease and others permitting aggressive disease. In this regard, HLA-DQ4, DQ7, DQ8 and DQ9 alleles have a common DQA1*0301α chain, whereas their DQB1 β chains (DQB1*0401, DQB1*0301, DQB1*0302 and DQB1*0303, respectively) are likely to be similar in the structure that influences antigenic peptide binding. Moreover, in different ethnic group these HLA-DQ molecules are associated with HLA-DR alleles that are associated with rheumatoid arthritis (90-93). This suggests that these specific HLA-DQ molecules may actually be the genetic element associated with rheumatoid arthritis. Based on these considerations, rheumatoid arthritis patients may be classified into two groups: an HLA-DQ4/7/8/9 population predisposed to severe arthritis, and an HLA-DQ5 population predisposed to milder arthritis and the absence of rheumatoid factor (96,97). These differences may be related to the variation in the mitigating influences of HLA-DR4 (or 9) on the one hand and HLA-DR10 on the

other. Finally, the association between homozygocity of two non-protective HLA-DRβ1 alleles and severe disease (63,68,69) might relate to the lack of any mitigating influences.

Whether this regulatory mechanism plays a role in the initiation of disease or subsequently is currently not known. However, the association of alleles of HLA-DR and disease progression (57,59,63,68,69) suggests that if HLA-DQ is playing a role in rheumatoid arthritis, it is likely to involve propagation of previously established disease.

In summary, each of these considerations about the HLA associations with rheumatoid arthritis suggests that simple antigen recognition of a single arthritogenic peptide presented to clones of CD4+ T cells by HLA-DR molecules expressing the "shared epitope" cannot explain the entire pathophysiology of rheumatoid arthritis. Rather, more complex immunoregulatory roles of CD4+ T cells appear to be involved in the pathogenesis of rheumatoid arthritis. Despite an incomplete understanding of the precise nature of the peptides and the MHC class II restricting elements involved and their precise function, all available information implies that the role of CD4+ T cells is likely to be far more complex than hitherto appreciated and may vary as the disease progresses (Table 3).

Table 3

UNRESOLVED QUESTIONS ABOUT THE ROLE OF CD4+T CELLS IN RHEUMATOID ARTHRITIS

- **Are they important in early disease and/or late disease?**
- **What antigenic peptides do they recognize?**
- **Do they recognize the same peptides early in disease and late in disease?**

Cytokine Production by T Cells

Cytokines are thought to play an essential role in the immunopathogenesis of synovial inflammation, as well as the initiation of damage to cartilage and bone (94-96). It was reasoned that if T cells were playing a central role in rheumatoid inflammation, they would be likely to produce large amounts of cytokines. However, initial studies quantitating the amount of T cell derived cytokines, such as interferon-γ and interleukin-2, in rheumatoid inflammation indicated that they were found in small amounts and were much less abundantly produced than cytokines derived from macrophages and synovial fibroblasts (97,98). This was initially interpreted as indicating that T cells played only a bystander role in rheumatoid inflammation (97). However, recent studies using more sensitive techniques of immunohistology and quantitative PCR amplification of cytokine mRNAs have clearly shown the presence of T cell derived cytokines in rheumatoid synovitis (99-104). The most prominent cytokine appears to be interferon-γ that may be produced by as much as two to ten percent of synovial membrane CD4+ T cells in rheumatoid arthritis (101). Even though the number of cytokine producing CD4+ T cells in the rheumatoid synovium is comparable to that found in other chronic inflammatory diseases (105), their paucity has suggested to some that CD4+ T cells may

not be sufficiently activated to drive the intense inflammation characteristic of rheumatoid arthritis. Additional features of rheumatoid synovial CD4+ T cells were interpreted to substantiate the contention that they were effete cells with little function (97). Thus, signaling defects in rheumatoid synovial T cells have been demonstrated that relate to an altered redox state and altered phosphorylation of the MAP kinase, p38, and the zeta chain of the T cell receptor (114,115). Moreover, synovial T cells appear to have a limited potential to grow in culture, related to their tendency to undergo apoptosis *in vitro* (116). There is evidence to support the conclusion that these cells are functionally altered by virtue of their exposure to oxidative and other stress within the synovium (114,117).

Despite perturbation of their function, synovial T cells exhibit an activated phenotype as gauged by the expression of a variety of activation markers and upregulation of a number of functional activities (118-127). For example, synovial CD4+ T cells have a markedly enhanced capacity to provide help for B lymphocytes (117). Part of this activity relates to the constitutive expression on the TNF receptor family member that is essential for contact dependent activation of B cells and macrophages by T cells, CD40 ligand/CD154 (128). By virtue of the upregulation of CD40 ligand/CD154, synovial CD4+ T cells not only can directly induce immunoglobulin production by B cells, but can also upregulate cytokine production by myeloid cells in a contact dependent manner (128). These findings are consistent with the conclusion that the function of synovial CD4+ T cells may be somewhat perturbed, but they remain capable of providing both membrane signals and cytokines responsible for the inflammation characteristic of rheumatoid synovitis.

It has also become apparent that synovial CD4+ T cells are markedly enriched in a subpopulation of highly differentiated memory T cells characterized by the phenotype, $CD45RB^{dim}$, $CD27^{-}$ (119). These cells have the capability of producing cytokines rapidly after stimulation, providing intense help for B cells and co-stimulating macrophage activation, but limited capability for proliferation (129). Thus, when compared to a similar population of peripheral CD4+ memory T cells, functional capabilities of synovial CD4+ T cells are reasonably intact. However, they appear to be markedly biased toward production of the pro-inflammatory cytokine, interferon-γ, and markedly diminished in the capacity to produce the anti-inflammatory cytokine, interleukin-4 (99,101,103,104,130).

Table 4

EVIDENCE AGAINST AN ESSENTIAL ROLE FOR T CELLS IN RHEUMATOID ARTHRITIS

1. **Few cytokine producing T cells in rheumatoid synovium**
2. **Synovial T cells proliferate poorly and produce small amounts of cytokines in vitro**
3. **Autonomous growth of synovium in immunodeficient mice**
4. **Lack of clinical response to depleting monoclonal antibodies to CD4**

Pro-inflammatory Activity of Multiple Cell Types in the Rheumatoid Synovium

Many cell types in the rheumatoid synovium express an activated phenotype and appear to contribute to the inflammatory milieu. Thus, synovial fibroblasts and macrophages are activated to produce

a variety of cytokines and tissue damaging enzymes (131-135). Synovial fibroblasts express an increased density of adhesion molecules, have up-regulated a number of oncogenes associated with cellular proliferation and produce increased amounts of matrix metalloproteinases (131-133), whereas mononuclear phagocytes in both bone marrow (136), peripheral circulation (138), and synovial space (134,135) are functionally and phenotypically activated, producing increased amounts of proinflammatory cytokines. The pro-inflammatory capabilities of macrophage-derived cytokines are indicated by their capacity to induce marked inflammation when expressed in the synovial tissue of laboratory animals (139-140). Antigen presenting dendritic cells are also markedly activated in the synovial tissue and synovial fluid and highly enhanced in their capacity for antigen presentation (141,142). Whether any of these abnormalities represent fundamental etiologic events in rheumatoid arthritis or are secondary to stimulation by cytokines or other inflammatory mediators produced in the local milieu and systemically is a matter of intense debate. Some have suggested that acquired abnormalities is proliferative regulation may render synovial fibroblasts primarily pathogenic (133). Thus, grafts of human synovium or isolated cultured synovial fibroblasts in immunodeficient SCID mice have been shown to have the capacity to induce degradation of cartilage, even though inflammatory cells have apparently been deleted (143,144,144a). Whether this reflects a primary alteration resulting in unregulated aggressive behavior or is the end point of growth and selection in the cytokine containing synovium remains unknown. In this regard, alterations of p53 activity have been noted in rheumatoid synovial fibroblasts that could contribute to their "partially transformed" phenotype (133) .

Similarly, macrophages appear to be abnormally stimulated in the bone marrow, peripheral blood

and in the synovial tissue. In the tissue, they produce intense amounts of cytokines, including IL-6, TNFα, IL-1 and IL-10 (97,98,145-148). The ongoing production of these cytokines by macrophages suggests the possibility that there might be a loss of the normal down-regulation of macrophage cytokine production in the rheumatoid synovium or, alternatively an imbalance between the production of pro-inflammatory cytokines and their natural antagonists. One influence that normally appears to down-regulate macrophage production of pro-inflammatory cytokines is interleukin-4 (149-151), that is absent from rheumatoid synovium (151). Therefore, a deficiency in interleukin-4 may contribute to the ongoing macrophage production of cytokines. A systemic effect of such stimulation of these cytokines is suggested by the dysregulation of macrophage maturation in the bone marrow (136) and the presence of myeloid cells in the peripheral blood expressing increased levels of cytokine mRNA constitutively (138). Again, whether the systemic activation of macrophages is a primary event or secondary to other events, including production of interferon-γ by CD4+ T cells in the synovium, as well as the local production of immune complexes (152) remains to be determined conclusively.

Finally, the local differentiation of antigen presenting dendritic cells within the rheumatoid synovium appears to relate to the increased cytokine concentrations in the environment, although a primary defect related to other signals stimulating dendritic cell differentiation is also possible (141,142).

The plethora of activated cell types within the synovium, as well as the local differentiation of B lymphocytes into plasma cells secreting the autoantibody, rheumatoid factor, with the subsequent

local production of immune complexes (152) has caused some to question the primary role of CD4+ T cells in driving the inflammation of rheumatoid arthritis. In this regard, recent successful therapeutic interventions with monoclonal antibodies to TNFα, a soluble TNFα receptor -IgG fusion protein, the interleukin-1 receptor antagonist and monoclonal antibodies to interleukin 6 have emphasized the important role of these cytokines in the pathogenesis of rheumatoid arthritis (153-160). However, therapy directed at these macrophage derived cytokines clearly decreases disease activity only temporarily, with little suppression of disease after the therapeutic intervention is discontinued. This suggests that macrophage-derived cytokines are important effector molecules, but are not primary initiators of rheumatoid inflammation.

Treatment of Rheumatoid Arthritis with Antibodies to T Cells and T Cell Subsets

With the initial documentation of the participation of CD4+ T cells in rheumatoid inflammation, a number of biotechnology companies rapidly developed monoclonal antibodies to a variety of T cell determinants with the expectation that depleting T cells or interfering with their function should ameliorate rheumatoid inflammation. Monoclonal antibodies were employed that were directed toward CD4, CD5 and CD52 (161). Although there was initial enthusiasm from the results of non-controlled trials, subsequent controlled trials showed no clinical efficacy (161-172). There are a number of possible interpretations of these results (Table 5).

Table 5

TREATMENT OF RHEUMATOID ARTHRITIS WITH ANTI-T CELL MONOCLONAL ANTIBODIES

Observation: **Peripheral depletion of CD4(+) T cells is insufficient to affect the course of rheumatoid arthritis**

Implications:

1. **Rheumatoid arthritis is not driven by CD4(+) T cells**
2. **Depletion of tissue CD4(+) T cells is not achieved**
3. **Inappropriate subsets are depleted**
4. **Generation of new T cells reinstitutes the pathogenic immune response**

Some have interpreted these results to indicate that T cells and especially CD4+ T cells play no role in rheumatoid synovitis. However, emerging information about the impact of these interventions on synovial versus peripheral T cells and the nature of the T cell subsets differentially effected by these therapies has called into question the validity of this interpretation. It appears that more has been learned about the correct way to do a clinical trial than about rheumatoid inflammation from these interventions (168). However, compared to the therapeutic impact of therapies aimed at blocking macrophage-derived cytokines, such as TNFα, IL-1 and IL-6, the results have caused some to question the "T cell paradigm" of the immunopathogenesis of rheumatoid arthritis and to suggest that primary abnormalities of syniovocytes and/or macrophages might lead to manifestations of rheumatoid arthritis (173).

The Impact of a Non-depleting Anti-CD4 Monoclonal Antibody in Rheumatoid Arthritis

In experimental autoimmune disease, monoclonal antibodies to CD4 have been successfully used, not only to prevent the induction of disease (174-176), but also to inhibit further disease development when given after initial inflammation has already become apparent (177,178). Moreover, antigen specific unresponsiveness (anergy) to soluble antigens or tissue allografts can be induced by anti-CD4 monoclonal antibodies in animals (179,180). In man, uncontrolled pilot studies with murine monoclonal antibodies to CD4 yielded some initial promising results in several refractory autoimmune disorders, including rheumatoid arthritis (162,163,164,168). Short term treatment with relatively low doses of these monoclonal antibodies to CD4 was not sufficient, however, to result in long-term clinical benefit. Moreover, the development of host antibody responses to the therapeutic murine monoclonal antibody limited repeat applications.

More recently, placebo controlled trials with chimerized depleting monoclonal antibodies to CD4 have shown no clinical efficacy, despite significant peripheral CD4+ T cell depletion and a marked but transient reduction in synovial cellular infiltration (165,166). This result is consistent with finding from animal models of autoimmune diseases, indicating that depletion of CD4+ T cells is not the mechanism underlying anti-CD4 monoclonal antibody efficacy and, moreover, that short term therapy with such monoclonal antibodies is insufficient to effect disease progression in animal models of autoimmune disease (181,182). In contrast to the effects of depleting anti-CD4 monoclonal antibodies, there are numerous reports documenting the immunosuppressive actions of non-depleting monoclonal antibodies to CD4 in animals (182-185). Moreover, non-depleting

monoclonal antibodies to CD4, which modulate and/or block T cell functions, were found to be more effective than depleting monoclonal antibodies at inducing anergy in different experimental situations in the mouse, such as the transfer of β cells in non-obese diabetic mice or allogeneic skin grafting (185). Finally, sustained suppression of autoimmunity in NZB/NZW F1 mice was shown to depend on continuous inhibition, but not depletion of CD4+ T cells (177,181). Taken together, these observations are all consistent with the hypothesis that successful treatment of rheumatoid arthritis might require long-term inhibition of peripheral T cell functions by a non-depleting monoclonal antibody to CD4.

To test this hypothesis, a placebo controlled randomized multicenter double-blind study of a non-depleting anti-CD4 monoclonal antibody was initiated in patients with rheumatoid arthritis, refractory to standard therapy with disease modifying anti-rheumatic drugs. To reduce the immunogenecity of the monoclonal antibody and to minimize the host immune response, a therapeutic monoclonal antibody was employed that was generated by a grafting complementary determining regions of a murine monoclonal antibody to CD4 onto the variable regions of a heavy and a light chain of a human IgG4/κ immunoglobulin (186). The resulting humanized anti-CD4 monoclonal antibody was administered in repeated cycles in concentrations that resulted in saturation of CD4 on peripheral T cells for up to two weeks. Marked clinical benefit was noted in this blinded trial. Benefit lasted as long as the monoclonal antibody was present in the circulation. Clinical benefit was not associated with depletion of CD4+ T cells, as no depletion was noted. Moreover, no changes in the circulating number of monocytes, B cells or CD8+ T cells was observed. Of importance, improvement with the non-depleting monoclonal antibody to CD4 was

associated with a marked decrease in plasma levels of C-reactive protein (Table 6).

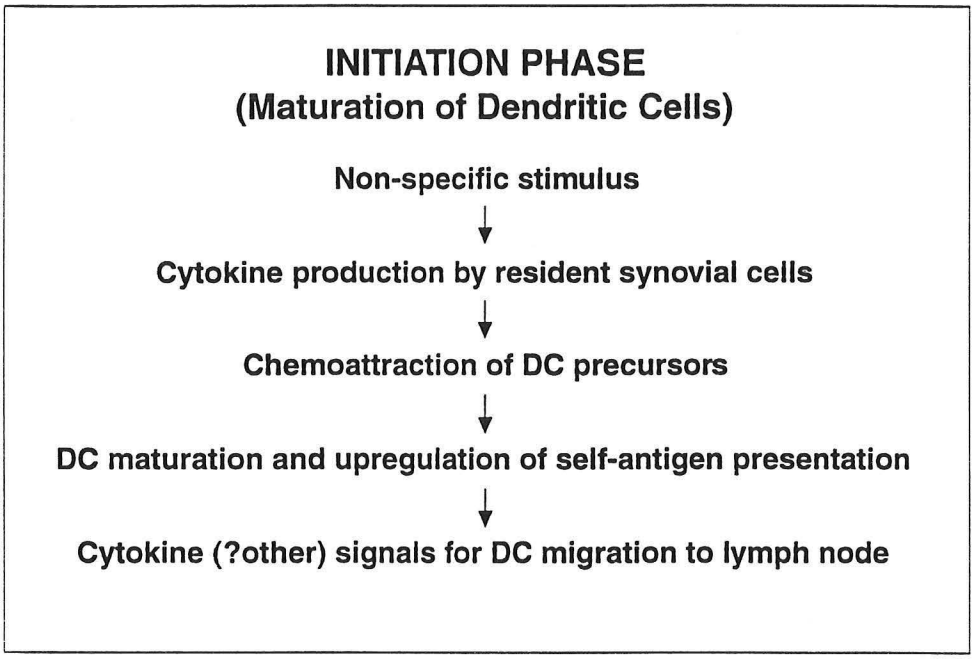
C-Reactive Protein Levels Following Treatment With A Non-Depleting Anti-CD4 Monoclonal Antibody

	C Reactive Protein (mg/L)					
	First Course		Second Course		Third Course	
Patient #	Wk 0, Day 1	Wk 1	Wk 7, Day 1	Wk8	Wk 14, Day 1	Wk 15
	OKTcdr4a		**OKTcdr4a**		**Placebo**	
1	6.5	<0.8	2.3	<0.8	0.9	<0.8
2	6.1	<0.8	9.1	1.1	12.3	NA
4	2.6	<0.8	<0.8	0.9	2.4	1.5
5	4.4	1.0	3.5	<0.8	5.9	NA
7	1.2	<0.8	1.2	<0.8	1.0	2.5
8	1.2	<0.8	1.1	<0.8	1.2	1.7
	Placebo		**Placebo**		**OKTcdr4a**	
3	<0.8	<0.8	<0.8	<0.8	0.9	<0.8
6	4.1	5.5	6.4	4.9	2.7	<0.8
10	1.0	0.9	0.8	<0.8	<0.8	<0.8
11	4.6	5.6	4.2	3.9	5.3	4.5

Since C-reactive protein is produced in the liver after stimulation by a variety of proinflammatory cytokines (187), a profound and rapid decline in CRP levels following therapy with the non-depleting monoclonal antibody to CD4 suggested that activated CD4+ T cells play a necessary role in production of these cytokines. Since the major source of CRP inducing cytokines is from macrophages and synoviocytes in the synovium (97,98), these results suggest that CD4+ T cells are playing a role in the production of these proinflammatory cytokines. This would tend to place CD4+ T cells upstream of macrophage effector cells in rheumatoid inflammation. Finally, this trial involved rheumatoid arthritis patients with long-standing disease, supporting the conclusion that CD4+ T cells play an important role in driving rheumatoid inflammation, even after years of disease activity.

Three Phases of Rheumatoid Arthritis

Initiation. Together, the data suggest that the first phase of rheumatoid synovitis would involve nonspecific inflammation (Figure 6), and nonspecific production of cytokines, such as TNFα in the joint.



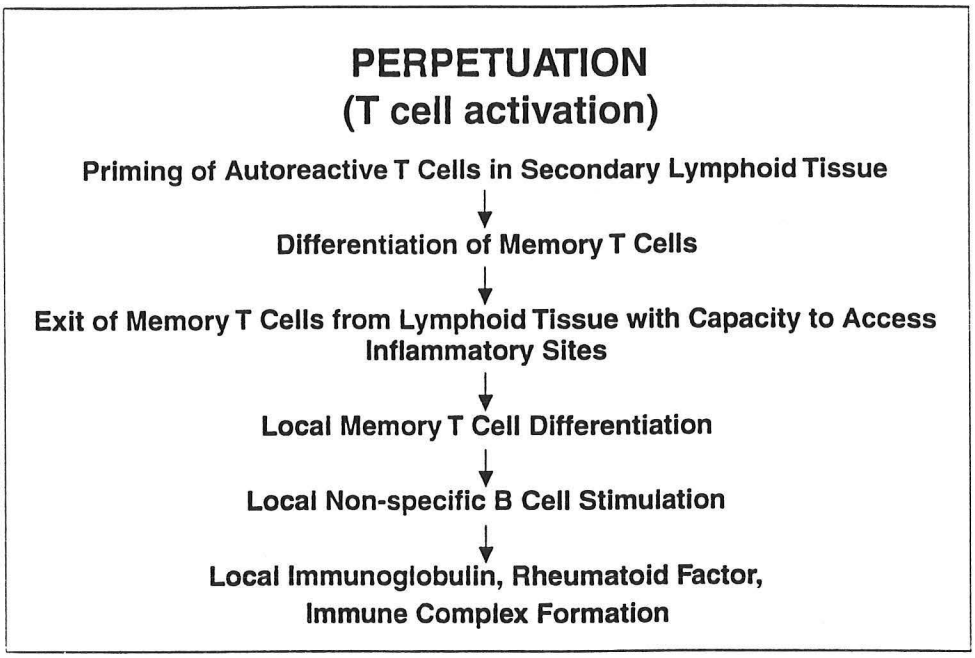
Many nonspecific events within the synovium, including minor trauma, infections, allergic or vaccination reactions, or deposition of immune complexes, might initiate local inflammation and cytokine production, leading to the stimulation of macrophages and other cells within the synovial lining layer. This could account for the frequent report of various "precipitating" events preceding the onset of or exacerbations of rheumatoid inflammation. An important event during this phase of rheumatoid arthritis is the local activation and differentiation of professional antigen presenting dendritic cells (141,142). Repeated local nonspecific stimulation might eventually create a milieu

either locally or in draining lymph nodes. This latter event appears to be much more likely to occur in individuals with the "shared epitope".

This scenario is consistent with a number of features of rheumatoid arthritis. The onset of rheumatoid arthritis is most commonly indolent (1,2), perhaps reflecting the need for recurrent nonspecific damage in order to induce dendritic cell differentiation and/or priming or activation of self-reactive CD4+T cells. Indeed, there is no association with the shared epitope with early inflammatory polyarthritis (57-62), as would be expected if this phase of the disease were not T cell dependent. In accordance with this is the lack of effect of anti-CD4 monoclonal antibody therapy in patients with early rheumatoid arthritis (162). These findings are all consistent with a model in which the earliest phases of rheumatoid arthritis are not T cell dependent, but rather involve repeated insults to the synovium, with the eventual differentiation of professional antigen presenting dendritic cells. This phase of the disease can be quite prolonged, but is likely to be followed by a phase of activation and differentiation of autoreactive T cells, which occurs most readily in individuals who express the "shared epitope".

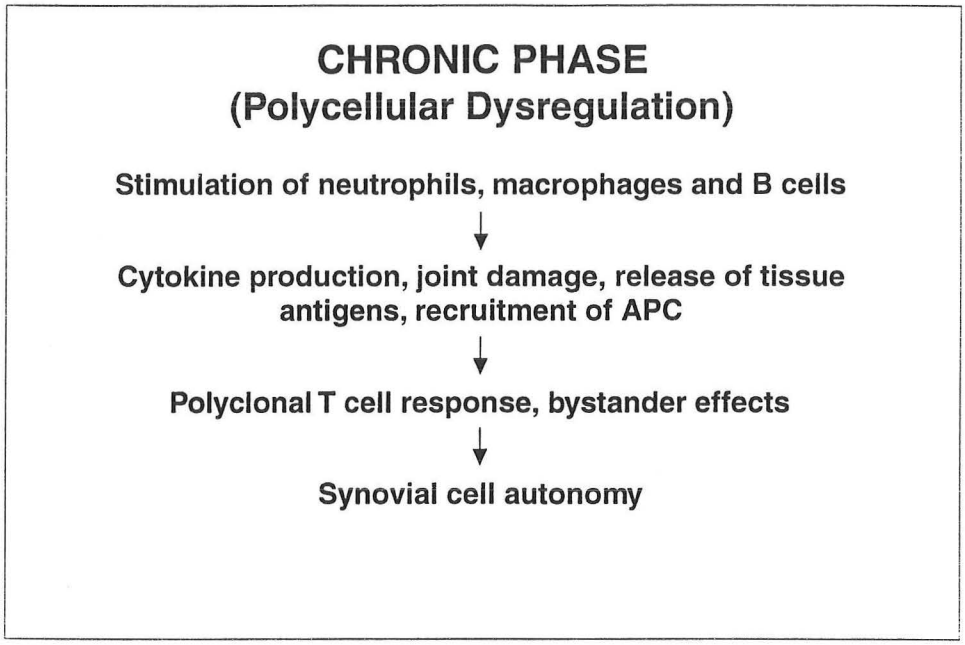
Perpetuation. The activation of CD4+ T cells in rheumatoid arthritis may be necessary to induce aggressive disease with rapid joint destruction and extraarticular features (Figure 7). CD4+ T cells may stimulate macrophages and synovial fibroblasts to secrete cytokines and B cells to produce immunoglobulin and the autoantibody rheumatoid factor via a contact-dependent mechanism (128). CD4+ T cells also facilitate rheumatoid inflammation by producing the pro-inflammatory cytokine, interferon-γ, but not the anti-inflammatory cytokine, interleukin-4 (99,101,103,104,130).

Figure 7



Chronic phase. In the chronic phase of rheumatoid arthritis (Figure 8), the autoimmune response eventually drives the recruitment of other effector cells, leading to polycellular dysregulation. Thus, many tissue-derived self antigens could become available as a result of tissue damage. The T cell response may thus become polyclonal in response to the number of antigens presented by locally differentiating antigen presenting cells. In addition, since the recruitment of memory T cells into the joint is not antigen specific (188), polyclonal T cell activation may occur as a result of bystander effects. The polyclonal nature of T cells infiltrating the synovial tissue of patients with established rheumatoid arthritis has been demonstrated (18-28). Finally, well-established disease may be characterized by synovial cell autonomy. At this stage of disease, the autoimmune response of CD4+ T cells may be less important in terms of tissue damage within the joint than are the actions of other cells, such as macrophages and synovial fibroblasts that secrete degradative enzymes and/or cytokines. However, CD4+ T cells still appear to influence inflammatory synovitis at this stage, as evidenced by the clinical impact of an intervention with a non-depleting monoclonal antibody to CD4.

Figure 8



Summary

The emerging data strongly suggest that rheumatoid synovitis involves the activity of CD4+ T cells. Although the initial idea that CD4+ T cells recognized a single arthritogenic epitope that initiated the disease seems unlikely, the demonstration that established rheumatoid arthritis can be ameliorated by altering the function of CD4+ T cells strongly implies a role for these cells in the propagation of ongoing inflammation. That role is likely to be immunoregulatory in nature and to develop after disease is initiated. The absence of an association with class II MHC alleles in early disease supports the idea that non-specific inflammation may be an initiating event. Afterward, however, CD4+ T cells recognizing multiple antigenic peptides in the synovium may amplify the disease. Unresolved production of interferon-γ by differentiated CD4+ effector T cells may facilitate the ongoing inflammatory nature of the disease. Expression of a variety of cell surface molecules by synovial CD4+ T cells, including CD40 ligand/CD154, may play an important role in perpetuating inflammation. The role of the CD4+ T cell is clearly more complex than initially

anticipated, but remains essential for the evolving inflammation characteristic of rheumatoid arthritis.

References

1. Lipsky PE: Rheumatoid Arthritis. In: Harrison's Principles of Internal Medicine, 14th edition. Edited by AS Fauci, E Braunwald, KJ Isselbacher, JD Wilson, JB Martin, DL Kasper, SL Hauser and DL Longo. McGraw Hill, New York, pp. 1880-1888, 1997

2. Harris E.D. Reumatoid Arthritis. W.B. Saunders. Philadelphia, PA, 1997

3. Jain R and Lipsky PE: Treatment of rheumatoid arthritis. In: The Medical Clinics of North America. Advances in Rheumatology. Edited by R. Snyderman and B.F. Haynes. W.B. Saunders, Philadelphia, pp. 57-84, 1997

4. Yelin E, Meenan R, Nevitt M, and Epstein W: Work disability in rheumatoid arthritis: effects of disease, social and work factors. Ann Int Med. 93:551-556, 1980

5. Wolfe F, Mitchell DM, Sibley JT, Fries JF, Bloch DA, Williams CA, Spitz PW, Haga M, Kleinheksel SM and Cathey MA: The mortality of rheumatoid arthritis. Arthritis Rheum 37:481-494, 1994

6. Pincus T, Brooks RH, Calahan LF: Prediction of long-term mortality in patients with rheumatoid arthritis according to simple questionnaire and joint count measures. Ann Intern Med 120:26-34,

1994

7. Rose, HM, Ragan C., Pearce E, Lipman MO: Differential agglutination of normal and sensitized sheep erythrocytes by sera of patients with rheumatoid arthritis. Proc Soc Exp Biol Med 68: 1-6, 1948

8. Waaler E: On the occurrence of a factor in human serum activation specific agglutination of sheep blood corpuscles. Acta Pathol Microbiol Scand 17:172-188, 1940

9. Singer JM and Plotz CM: The latex fixation test 1: Application to the serologic diagnosis of rheumatoid arthritis. Am J Med 21:888-892, 1956

10. Klemperer P, Pollack AD and Baehr G: Diffuse collagen disease: Acute disseminated lupus erythematosus and diffuse scleroderma. JAMA 119:331-334, 1942

11. Van Boxel JA and Paget SA: Predominantly T cell infiltrate in rheumatoid synovial membranes. N Engl J Med 293:517-520, 1975

12. Janossy G, Panay GS, Duke O, Bofill M, Poulter LW, and Goldstein G: Rheumatoid arthritis: a disease of T lymphocyte/macrophage immunoregulation. Lancet 2:839-842, 1981

13. Kurosaka M and Ziff M: Immunoelectron microscopic study of the distribution of T cell subsets in rheumatoid synovium. J Exp Med 158:1191-210, 1983

14. Cush JJ and Lipsky PE: Phenotypic analysis of synovial tissue and peripheral blood lymphocytes isolated from patients with rheumatoid arthritis. Arthritis Rheum 31:1230-1238, 1988

15. Stastny P: Association of the B-cell alloantigen DRw4 with rheumatoid arthritis. N Engl J Med 298:869-871, 1978

16. Stamenkovic I, Stegagno M, Wright KA, Krane SM, Amento EP, Colvin RB, Duquesnoy RJ, Kurnick JT: Clonal dominance among T-lymphocyte infiltrates in arthritis. Proc Nat'l Acad Sci USA 85:1179-1183, 1988

17. Duby AD, Sinclair AK, Osborne-Lawrence SL, Zeldes W, Kan L, Fox DA: Clonal heterogeneity of synovial fluid T lymphocytes from patients with rheumatoid arthritis. Proc Natl Acad Sci USA 86:6206-6210, 1989

18. Cooper SM, Dier DL, Roessner KE, Budd RC, Nicklas JA: Diversity of rheumatoid synovial tissue T cells by T cell receptor analysis: oligoclonal expansion in interleukin-2-responsive cells. Arthritis Rheum 34:537-546, 1991

19. Korthäuer U, Hennerkes B, Menninger H, Mages HW, Zacher J, Potocnik AJ, Emmrich F, Kroczek RA: Oligoclonal T cells in rheumatoid arthritis: identification strategy and molecular characterization of a clonal T-cell receptor. Scand J Immunol 36:855-863, 1992

20. Williams WV, Fang Q, Demarco D, VonFelt J, Zurier RB, Weiner DB: Restricted heterogeneity of T cell receptor transcripts in rheumatoid synovium. J Clin Invest 90:326-333, 1992

21. Howell MD, Diveley JP, Lundeen KA, Esty A, Winters ST, Carlo DJ, Brostoff SW: Limited T-cell receptor β-chain heterogeneity among interleukin 2 receptor-positive synovial T calls suggests a role for superantigen in rheumatoid arthritis. Proc Natl Acad Sci USA 88:10921-10925, 1991

22. Davey MP, Munkirs DD: Patterns of T cell receptor variable β gene expression by synovial fluid and peripheral blood T cells in rheumatoid arthritis. Clin Immunol Immunopathol 68:79-87, 1993

23. Lunardi C, Marguerie C, So AK: An altered repertoire of T cell receptor V gene expression by rheumatoid synovial fluid T lymphocytes. Clin Exp Immunol 90:440-446, 1992

24. Jenkins RN, Nikaein A, Zimmerman A, Meek K, Lipsky PE: T cell receptor Vβ gene bias in rheumatoid arthritis. J Clin Invest 92:2688-2701, 1993

25. Cooper SM, Roessner KD, Naito-Hoopes M, Howard DB, Gaur LK, Budd RC: Increased usage of Vβ2 and Vβ6 in rheumatoid synovial fluid T cells. Arthritis Rheum 37:1627-1636, 1994

26. Alam A, Luié J, Coppin H, Lambert N, Maziéres B, de Préval C, Cantagrel A: T-cell receptor variable region of the β-chain gene use in peripheral blood and multiple synovial membranes during rheumatoid arthritis. Hum Immunol 42:331-339, 1995

27. Fischer D-C, Opalka B, Hoffmann A, Mayr W, Haubeck H-D: Limited heterogeneity of rearranged T cell receptor V_α and V_β transcripts in synovial fluid T cells in early stages of rheumatoid arthritis. Arthritis Rheum 39:454-462, 1996

28. Paliard X, West SG, Lafferty FA, Clements JR, Kappler JW, Marrack P, Kotzin BL: Evidence for the effects of a superantigen in rheumatoid arthritis. Science 253:325-329, 1991

29. Bröker BM, Korthäuer U, Heppt P, Weseloh G, de la Camp R, Kroczek RA, Emmrich F: Biased T cell receptor V gene usage in rheumatoid arthritis: oligoclonal expansion of T cells expressing $V_\alpha 2$ genes in synovial fluid but not in peripheral blood. Arthritis Rheum 36:1234-1243, 1993

30. Zagon G, Tumang JR, Li Y, Friedman SM, Crow MK: Increased frequency of Vβ17-positive T cells in patients with rheumatoid arthritis. Arthritis Rheum 37:1431-1440, 1994

31. Travaglio-Encinoza A, Chaouni I, Dersimonian H, Jorgensen C, Simony-Lafontaine J, Romagné F, Sany J, Duput D'Angeac A, Breenner MB, Réme T: T Cell receptor distribution in rheumatoid synovial follicles. J Rheumatol 22:394-399, 1995.

32. Struyk L, Hawes GE, Chatila MK, Breedveld FC, Kurnick JT, van den Elsen PJ: T cell receptors in rheumatoid arthritis. Arthritis Rheum 38:577-589, 1995

33. Ikeda Y, Masuko K, Nakai Y, Kato T, Hasanuma T, Yoshino S-I, Mizushima Y, Nishioka K, Yamamoto K: High frequencies of identical T cell clonotypes in synovial tissues of rheumatoid

arthritis patients suggest the occurrence of common antigen-driven immune responses. Arthritis Rheum 39:446-453, 1996

34. Alam A, Lambert N, Lulé J, Coppin H, Mazaiéres B, de Préval C, Cantagrel A: Persistence of dominant T cell clones in synovial tissues during rheumatoid arthritis. J Immunol 156:3480-3485, 1996

35. Goronzy JJ, Bartz-Bazzanella P, Hu W., Jendro MC, Walser-Kuntz DR, Weyand CM: Dominant clonotypes in the repertoire of peripheral $CD4^+$T cells in rheumatoid arthritis. J Clin Invest 94:2068-2076, 1994

36. González-Quintial F, Baccalá R, Pope RM, Theofilopoulos AN: Identification of clonally expanded T cells in rheumatoid arthritis using a sequence enrichment nuclease assay. J Clin Invest 97:1335-1343, 1996

37. Hingorani H, Monteiro J, Furie R, Chartash E, Navarrete C, Pergolizzi R, Gregersen PK: Oligoclonality of Vβ3 TCR chains in the $CD8^+$ T cell population of rheumatoid arthritis patients. J Immunol 156:852-858, 1996

38. Kohsaka H, Taniguchi A, Chen PP, Ollier WER, Carson DA: The expressed T cell receptor V gene repertoire of rheumatoid arthritis monozygotic twins: rapid anaylsis by anchored polymerase chain reaction and enzyme-linked immunosorbent assay. Eur J Immunol 23:1895-1901, 1993

39. Nanki T, Kohsaka H, Mizushima N, Ollier WER, Carson D, Miyasaka N: Genetic control of T cell receptor BJ gene expression in peripheral lymphocytes of normal and rheumatoid arthritis monozygotic twins. J Clin Invest 98:1594-1601, 1996

40. Walser-Kuntz DR, Weyand CM, Weaver AJ, O'Fallon WM, Goronzy JJ: Mechanisms underlying the formation of the T cell receptor repertoire in rheumatoid arthritis. Immunity 2:597-605, 1995

41. Kohsaka H, Nanki T, Ollier WER, Miyasaka N, Carson DA: Influence of the rheumatoid arthritis-associated shared epitope on T-cell receptor repertoire formation. Proc Assoc Am Phys 108:323-328, 1996

42. Nepom BS and Nepom GT: Polyglot and polymorphism, an HLA update. Arthritis Rheum 38:1715-1721, 1995

43. Winchester R: Genetic determination of susceptibility and severity in rheumatoid arthritis. Ann Intern Med 117:869-871, 1992

44. Gregersen PK, Silver J, Winchester RJ: The shared epitope hypothesis: an approach to understanding the molecular genetics of susceptibility to rheumatoid arthritis. Arthritis Rheum 30:1205-1213, 1987

45. Winchester RJ, Gregersen PK: The molecular basis of susceptibility to rheumatoid arthritis: the conformational equivalence hypothesis. Springer Semin Immunopathol 10:119-139, 1988

46. Nepom GT and Nepom BS: Prediction of susceptibility to rheumatoid arthritis by human leukocyte antigen genotyping. Rheum Dis Clin North Am 18:785-792, 1992

47. Stern LJ, Brown JH, Jordetzky TS, et al: Crystal structure of the human class II MHC protein HLA-DR1 complexed with an influenza virus peptide. Nature 368:215-221, 1994

48. Woulfe SL, Bono CP, Zacheis ML, Kirshmann DA, Baudino TA, Swearingen C, Karr RW, Schwartz BD: Negatively charged residues interacting with the p4 pocket confer binding specificity to DRB1*0401. Arthritis Rheum 38:1744-1753, 1995

49. Kirschmann DA, Duffin KL, Smith CE, Welply JK, Howard SC, Schwartz BD, Woulfe SL: Naturally processed peptides from rheumatoid arthritis associated and non-associated HLA-DR alleles. J Immunol 155:5655-5662, 1995

50. Hammer J, Gallazzi F, Bono E, Karr RW, Guenot J, Valsasnini P, Nagy ZA, Sinigaglia F: Peptide binding specificity of HLA-DR4 molecules: correlation with rheumatoid arthritis association. J Exp Med 181:1847-1855, 1995

51. Auger I, Escola JM, Gorvel JP, Roudier J: HLA-DR4 and HLA-DR10 motifs that carry susceptibility to rheumatoid arthritis bind 70-kD heat shock proteins. Nature Med 2:306-310, 1996

52. Verheijden GFM, Rijnders WM, Bos E, Coenen-de Roo CJJ, van Staveren CJ, Miltenburg, AMM,

Meijerink JH, Boots AMH, Organon NV, Elewaut D,de Keyser F, Veys E: Human cartilage glycoprotein-39 as a candidate autoantigen in rheumatoid arthritis. Arthritis Rheum 40:1115-1125, 1997

53. Rosloniec EF, Brand DD, Myers LK, Whittington KB, Gumanovskaya M, Zaller DM, Woods A, Altmann, DM, Stuart JM, Kang AH: An HLA-DR1 transgene confers susceptibility to collagen-induced arthritis elicited with human type II collagen. J Exp Med 185:1113-1122, 1997

54. McDaniel DO, Alarcón GS, Pratt PW, Reveille JD: Most African-American patients with rheumatoid arthritis do not have the rheumatoid antigenic determinant (epitope). Ann Intern Med 123:181-187, 1995

55. Yelamos J, Garcia-Lozano JR, Moreno I, Aguilera I, Gonzalez MF, Garcia A, et al: Association of HLA-DR4-Dw15 (DRB1*0405) and DR10 with rheumatoid arthritis in a Spanish population. Arthritis Rheum 36:811-4. 1993

56. Salvarani C, Macchioni P, Mantovani W, Rossi F, Veneziana M, Boiardi L, et al: Extraarticular manifestations of rheumatoid arthritis and HLA antigens in northern Italy. J Rheumatol 19:242-6, 1992

57. Gough A, Faint J, Salmon M, Hassell A, Wordsworth P, Pilling D, Emery P: Genetic typing of patients with inflammatory arthritis at presentation can be used to predict outcome. Arthritis Rheum 37:1166-1170, 1994

58. DeJongh BM, Van Romunde LKJ, Valkenburg HA, De Lange GG, Van Rood JJ: Epidemiological study of HLA and GM in rheumatoid arthritis and related symptoms in an open Dutch population. Ann Rheum Dis 43:613-619, 1984

59. Van Zeben D, Hazes JMW, Zwinderman AH, Cats A, Schreuder GMT, D'Amaro J, Breedveld FC: Association of HLA-DR4 with a more progressive disease course in patients with rheumatoid arthritis: results of a followup study. Arthritis Rheum 34:822-830, 1991

60. Salmon M, Wordsworth P, Emery P, Tunn E, Bacon PA, Bell JI: The association of HLA DRB alleles with self-limiting and persistent forms of early symmetrical polyarthritis. Br J Rheumatol 32:628-630, 1993

61. Fisher LR, Kirk A, Awad J, Festenstein H, Alonso A, Perry JD, Shipley M: HLA antigens in palindromic rheumatism and palindromic onset rheumatoid arthritis. Br J Rheumatol 23:345-348, 1986

62. Puttick AJ, Briggs DC, Welsh KI, Vanghan R, Williamson EA, Boyce M, Jacoby RK, Jones VE: Genes associated with rheumatoid arthritis and mild inflammatory arthritis. I. Major histocompatibility complex class I, II and III allotypes. Ann Rheum Dis 49:219-224, 1990

63. Weyand CM, McCarthy TG, Goronzy JJ: Correlation between disease phenotype and genetic heterogeneity in rheumatoid arthritis. J Clin Invest 95:2120-2126, 1995

64. Perdriger A, Chalés, Semana G, Guggenbuhl P, Meyer O, Quillivic F, Pawlotsky Y: Role of HLA-DR-DR and DR-DQ associations in the expression of extraarticular manifestations and rheumatoid factor in rheumatoid arthritis. J Rheum 24:1272-6, 1997

65. Wagner U, Kaltenhäuser S, Sauer H, Arnold S, Seidel W, Häntzschel H, Kalden JR, Wassmuth R: HLA markers and prediction of clinical course and outcome in rheumatoid arthritis. Arthritis Rheum 40:341-351, 1997

66. Jawaheer D, Thomson W, MacGregor AJ, Carthy D, Davidson J, Dyer PA, Silman AJ, Ollier WER: "Homozygosity" for the HLA-DR shared epitope contributes the highest risk for rheumatoid arthritis concordance in identical twins. Arthritis Rheum 37:681-686, 1994

67. van Zeben D, Hazes JMW, Zwinderman AH, Vandenbroucke JP, Breedveld FC: Factors predicting outcome of rheumatoid arthritis: results of a followup study. J Rheum 20:1288-1296, 1993

68. Weyand CM, Hicok KC, Conn D, et al: The influence of HLA-DRB1 genes on disease severity in rheumatoid arthritis. Ann Intern Med 117:801-806, 1992

69. Weyand CM, Xie C, Goronzy JJ: Homozygosity for the HLA-DRB1 allele selects for extra-articular manifestations in rheumatoid arthritis. J Clin Invest 89:2033-2039, 1992

70. Gran JT, Husby G, Thorsby E: The prevalence of HLA-DR4 and HLA-DR3 in healthy persons

with rheumatoid factor. Scand J. Rheum 14:79-82, 1985

71. Olsen NJ, Callahan LF, Brooks RH, Nance EP, Kaye JJ, Statsny P and Pincus T: Associations of HLA-DR4 with rheumatoid factor and radiographic severity in rheumatoid arthritis. Am J Med 84:257-264, 1988

72. Jaraquemada D, Ollier W, Awad J, Young A, Festenstein H: HLA and rheumatoid arthritis: susceptibility or severity? Dis Markers 4:43-53, 1986

73. Walker DJ and Griffiths ID: HLA associations are with severe rheumatoid arthritis. Dis Markers 4:131-132, 1986

74. Calin A, Elswood J, Clouda PT: Destructive arthritis, rheumatoid factor, and HLA-DR4: susceptibility versus severity, a case-control study. Arthritis Rheum 32:1221-1225, 1989

75. van Zeben D, Hazes JMW, Zwinderman AH, Cats A, van der Voort EAM, Breedveld FC: Clinical significance of rheumatoid factors in early rheumatoid arthritis: results of a follow up study. Ann Rheum Dis 52:1029-1035, 1992

76. Jacobsson LTH, Knowler WC, Pillemer S, Hanson RL, Pettitt DJ, Nelson RG, del Puente A, McCance DR, Charles MA and Bennett PH: Rheumatoid arthritis and mortality: a longitudinal study in Pima Indians. Arthritis Rheum 36: 1045-1053, 1993

77. van Schaardenburg D, Hazes JMW, de Boer A, Zwinderman AH, Meijers KAE, Breedveld BC: Outcome of rheumatoid arthritis in relation to age and rheumatoid factor at diagnosis. J Rheumatol 20:45-52, 1993

78. Limpany P, Kemeny DN, Welsh KI, Lee TH: An HLA-associated nonresponsiveness to mellitin: a component of bee venom. J Allergy Clin Immunol 86:160-170, 1990

79. Thomas R and Lipsky: Could endogenous self-peptides presented by dendritic cells initiate rheumatoid arthritis? Immunol Today 17:559-564, 1996

80. Albani S, Keystone EC, Nelson JL, Ollier WER, La Cava A, Montemayor AC, Weber DA, Montecucco C, Martini A, and Carson DA: Positive selection in autoimmunity: Abnormal immune responses to a bacterial dnaJ antigenic determinant in patients with early rheumatoid arthritis. Nature Med 1:448-452, 1995

81. Albani S and Carson DA: A multistep molecular mimicry hypothesis for the pathogenesis of rheumatoid arthritis. Immunol Today 17:466-470, 1996

82. Albani S, Ravelli A, Massa M, De Benedetti F, Andree G, Roudier J, Martini A and Carson DA: Immune responses to the *E. Coli* dnaJ heat shock protein in juvenile rheumatoid arthritis correlate with disease activity. J Pediatr 124:561-565, 1994

83. Roudier J, Sette A, Lamont A, Albani S, Karras JG and Carson DA: Tolerance to a self peptide

from the third hypervariable region of the Es beta chain. Implications for molecular mimicry models of autoimmune disease. Eur J Immun 21:2063-2067, 1991

84. Roudier J, Peterson J, Rhodes GH, Lukas J, and Carson DA: Susceptibility to rheumatoid arthritis maps to a T-cell epitope shared by the HLA Dw4 DR beta 1 chain and the Epstein-Barr virus glycoprotein gp110. Proc Nat'l Acad Sci, USA 86:5104-5108, 1989

85. Salvat S, Auger I, Rochelle L, Begovich A, Geburher L, Sette A, and Roudier J: Tolerance to a self-peptide from the third hypervariable region of HLA DRB1*0401 in rheumatoid arthritis patients and normal subjects. J Immunol 152:5321-5329, 1994

86. Zanelli E, Gonzalez-Gay MA, and David CS: Could HLA-DRB1 be the protective locus in rheumatoid arthritis? Immunol Today 16:274-278, 1995

87. Nabozny GH, Baisch JM, Cheng S, Cosgrove D, Griffiths MM, Luthra HS, and David CS: HLA-DQ8 transgenic mice are highly susceptible to collagen-induced arthritis: A novel model for human polyarthritis. J Exp Med 183:27-37, 1996

88. Gonzalez-Gay MA, Zanelli E, Khare SD, Krco CJ, Zhou P, Inoko H, Griffiths MM, Luthra HS, and David CS: Human Leukocyte Antigen-DRB1*1502 (DR2Dw12) Transgene Reduces Incidence and Severity of Arthritis in Mice. Human Immunol 50:54-60, 1996

89. Zanelli E, Krco CJ, Baisch JM, Cheng S, and David CS: Immune response of HLA-DQ8

transgenic mice to peptides from the third hypervariable region of HLA-DRB1 correlates with predisposition to rheumatoid arthritis. Proc Natl Acad Sci USA 93:1814-1819, 1996

90. Doherty DG, Vaughan RW, Donalson PT, and Mowat AP: HLA DQA, DQB, and DRB genotyping by oligonucleotide analysis: Distribution of alleles and haplotypes in British caucasoids. Human Immunol 34:53-63, 1992

91. Maeda H, Juji T, Mitsui H, Sonozaki H, and Okitsu K: HLA DR4 and rheumatoid arthritis in Japanese people. Ann Rheum Dis 40:299-302, 1981

92. González A, Nicovani S, Massardo L, Bull P, Rodriguez L, and Jacobelli S: Novel genetic markers of rheumatoid arthritis in Chilean patients, by DR serotyping and restriction fragment length polymorphism analysis. Arthritis Rheum 35:282-289, 1992

93. De Juan MD, Belmonte I, Barado J, Martinez Laso J, Figueroa M, Arnaiz-Villena A, and Cuadrado E: Differential associations of HLA-DR antigens with rheumatoid arthritis (RA) in Basques: High frequency of DR1 and DR10 and lack of association with HLA-DR4 or any of its subtypes. Tissue Antigens 43:320-323, 1994

94. Lipsky PE, Davis LS, Cush JJ, and Oppenheimer0Marks N: The role of cytokines in the pathogenesis of rheumatoid arthritis. Springer Semin Immunopathol 11:123-162, 1989

95. Feldmann M, Brennan FM, and Maini RN: Role of cytokines in rheumatoid arthritis. Ann Rev

Immunol 14:397-440, 1996

96. Koch AE, Kunkel SL, Strieter RM: Cytokines in rheumatoid arthritis. J Invest Med 43:28-38, 1995

97. Firestein GS, Xu WD, Townsend K, Broide D, Alvaro-Gracia J, Glasebrook A, and Zvaifler NJ: Cytokines in chronic inflammatory arthritis. I. Failure to detect T cell lymphokines (IL-2 and IL-3) and presence of macrophage colony-stimulating factor (CSF-1) and a novel mast cell growth factor in rheumatoid synovitis. J Exp Med 168:1573-1586, 1988

98. Firestein GS, AlvaroGarcia JM, and Maki R: Quantitative analysis of cytokine gene expression in rheumatoid arthritis. J Immunol 144:3347-3353, 1990

99. Simon AK, Seipelt E, and Sieper J: Divergent T-cell cytokine patterns in inflammatory arthritis. Proc Natl Acad Sci, USA 91:8562-8566, 1994

100. Brennan FM, Field M, Chu CQ, Feldmann M, and Maini RN: Cytokine expression in rheumatoid arthritis. Brit J Rheumatol 30:(suppl 1):76-80, 1991

101. Dolhain RJEM, van der Heiden AN, ter Haar NT, Breedveld FC, Miltenburg AMM: Shift toward T lymphocytes with a T helper 1 cytokine-secretion profile in the joints of patients with rheumatoid arthritis. Arthritis Rheum 39:1961-1969, 1997

102. Isomäki P, Luukkainen R, Toivanen P and Punnonen J: The presence of interleukin-13 in rheumatoid synovium and its antiinflammatory effects on synovial fluid macrophages from patients with rheumatoid arthritis. Arthritis Rheum 39:1693-1702, 1996

103. Kotake S, Schumacher Jr HR, Yarboro CH, Arayssi TK, Pando JA, Kanik KS, Gourley MF, Klippel JH and Wilder RL: In vivo gene expression of type 1 and type 2 cytokines in synovial tissues from patients with early stages of rheumatoid, reactive, and undifferentiated arthritis. Proc Assn Amer Phy 109:286-301, 1997

104. Bucht A, Larsson P, Weisbrot L, Thorne C, Pisa P, Smedegard G, Keystone EC and Grönberg: Expression of interferon-gamma (IFN-γ), IL-10, IL-12 and transforming growth factor-beta (TGF-β) mRNA in synovial fluid cells from patients in the early and late phases of rheumatoid arthritis (RA). Clin Exp Immunol 103:357-367, 1996

105. Wagner AD, Bjornsson J, Bartley GB, Goronzy JJ, and Weyand CM: Interferon gamma producing T cells in giant cell vasculitis represent a minority of tissue infiltrating cells and are located distant from the site of pathology. Amer J Pathol 148:1925-1933, 1996

106. Nykanen P, Bergroth V, Raunio P, Nordstrom D, and Kontinen YT: Phenotypic characterization of [^{3}H]thymidine incorporating cells in rheumatoid arthritis synovial membrane. Rheumatol Int 6:269-271, 1986

107. Haraoui B, Wilder RL, Malone DG, Allen JB, Katona IM, and Wahl SM: Immune function in

severe, active rheumatoid arthritis: a relationship between peripheral blood mononuclear cell proliferation to soluble antigens and mononuclear cell subset profiles. J Immunol 133:697-701, 1984

108. Kingsley GH, Pitzalis C and Panayi GS: Abnormal lymphocyte reactivity to self-major histocompatibility antigens in rheumatoid arthritis. J Rheumatol 14:667-673, 1987

109. Verwilghen J, Vertessen S, Stevens EAM, Dequeker J, and Ceuppens JL: Depressed T cell reactivity to recall antigens in rheumatoid arthritis. J Clin Immunol 10:90-98, 1990

110. Keystone EC, Poplonski L, Miller RG, Gorczynski R, Gladman D, and Snow K: Reactivity of T cells from patients with rheumatoid arthritis to anti-CD3 antibody. Clin Immunol Immunopathol 48:325-337, 1988

111. Pope RM, McChesney L, Talal N, and Fischbach M: Characterization of the defective autologous mixed lymphocyte response in rheumatoid arthritis patients. Arthritis Rheum 27:1234-1244, 1984

112. Mirza NM, Relias V, Yunis EJ, Pachas WN, and Dasgupta JD: Defective signal transduction via T-cell receptor-CD3 structure in T cells from rheumatoid arthritis patients. Hum Immunol 36:91-98, 1993

113. Allen ME, Young SP, Michell RH, and Bacon PA: Altered T lymphocyte signaling in rheumatoid arthritis. Eur J Immunol 25:1547-1554, 1995

114. Maurice MM, Nakamura J, van der Voort EAM, van Vliet AI, Staal FJT, Tak PP, Breedveld FC and Verweij CL: Evidence for the role of an altered redox state in hyporesponsiveness of synovial T cells in rheumatoid arthritis. J Immunol 158:1458-1465, 1997

115. Maurice MM, Lankester AC, Bezemer AC, Geertsma MF, Tak PP, Breedveld FC, van Lier RAW and Verweij CL: Defective TCR-medicated signaling in synovial T cells in rheumatoid arthritis. J Immunol 159:2973-2978, 1997

116. Salmon M, Scheel-Toellner D, Huissoon AP, Pilling D, Shamsadeen N, Hyde H, D'Angeac AD, Bacon PA, Emery P and Akbar AN: Inhibition of T cell apoptosis in the rheumatoid synovium. J Clin Invest 99:439-446, 1997

117. Thomas R, McIlraith M, Davis L and Lipsky PE: Rheumatoid synovium is enriched in $CD45RB^{dim}$ memory T cells that are potent helpers for B cell differentiation. Arthritis Rheum 35:1455-1465, 1992

118. Cush JJ and Lipsky PE: Phenotypic analysis of synovial tissue and peripheral blood lymphocytes isolated from patients with rheumatoid arthritis. Arthritis Rheum 31:1230-1238, 1988

119. Kohem CL, Brezinschek RI, Wisbey H, Tortorella C, Lipsky PE and Oppenheimer-Marks N: Enrichment of differentiated $CD45RB^{dim}$, CD27- memory T cells in the peripheral blood, synovial fluid, and synovial tissue of patients with rheumatoid arthritis. Arthritis Rheum 39:844-854, 1996

120. Hemler ME, Glass D, Coblyn JS, and Jacobson JG: Very late activation antigens on rheumatoid synovial T lymphocytes. Association with stages of T cell activation. J Clin Invest 78:696-702, 1986

121. Takahashi H, Söderström K, Nilsson E, Kiessling R, and Patarroyo M: Integrins and other adhesion molecules on lymphocytes from synovial fluid and peripheral blood of rheumatoid arthritis patients. Eur J Immunol 22:2879-2885, 1992

122. Afeltra A, Galeazzi M, Ferri GM, Amoroso A, De Pita O, Porzio F, and Bonomo L: Expression of CD69 antigen on synovial fluid T cells in patients with thrumatoid arthritis and chronic synovitis. Ann Rheum Dis 52:457-460, 1993

123. Laffon A, Garcia Vicuna R, Humbria A, Postigo AA, Corbi AL, de Landazuri MO, and Sanchez Madrid F: Upregulated expression and function of VLA-4 fibronectin receptors on human activated T cells in rheumatoid arthritis. J Clin Invest 88:546-552, 1991

124. Sfikakis PP, Zografou A, Viglis V, Iniotaki-Theodoraki A, Piskontaki I, Tsokos GC, Sfikakis P and Choremi-Papadopoulou H: CD28 expression on T cell subsets in vivo and CD28-mediated T cell response in vitro in patients with rheumatoid arthritis. Arthritis Rheum 38:649-644, 1995

125. Kelleher D, Murphy A, Hall Nicola, Omary MB, Kearns G, Long A and Casey EB: Expression of CD44 on rheumatoid synovial fluid lymphocytes. Ann Rheum Dis 54:566-570, 1995

126. Yokota A, Murata N, Saiki O, Shimizu M, Springer TA and Kishimoto T: High avidity state of leukocyte function-associated antigen-1 on rheumatoid synovial fluid T lymphocytes. J Immunol 155:4118-4124, 1995

127. Isomäki P, Aversa G, Cocks BG, Luukkainen R, Saario R, Toivanen P, de Vries JE and Punnonen J: Increased expression of signaling lymphocytic activation molecule in patients with rheumatoid arthritis and its role in the regulation of cytokine production in rheumatoid synovium. J Immunol 159:2986-2993, 1997

128. MacDonald KPA, Nishioka Y, Lipsky PE and Thomas R: Functional CD40 ligand is expressed by T cells in rheumatoid arthritis. J Clin Invest, 1997, in press

129. Tortorella C, Schulze-Koops H, Thomas R, Splawski JB, Davis LS, Picker LJ and Lipsky PE: Expression of CD45RB and CD27 identifies subsets of CD4+ memory T cells with different capacities to induce B cell differentiation. J Immunol 155:149-162, 1995

130. Schulze-Koops H, Lipsky PE, Kavanaugh AF and Davis LS: Elevated Th1 or Th0-like cytokine mRNA in peripheral circulation of patients with rheumatoid arthritis. Modulation by treatment with anti-ICAM-1 correlates with clinical benefit. J Immunol 155:5029-5037, 1995

131. Zvaifler NJ and Firestein GS: Pannus and Pannocytes: Alternative Models of joint Destruction in Rheumatoid Arthritis. Arthritis Rheum 37:783-789, 1994

132. Xue C, Takahashi M, Hasumuna T, Aono H, Yamamoto K, Yoshino S, Sumida T, and Nishioka K: Characterisation of fibroblast-like cells in pannus lesions of patients with rheumatoid arthritis sharing properties of fibroblasts and chondrocytes. Annal Rheum Dis 56:262-267, 1997

133. Firestein GS: Invasive fibroblast-like synoviocytes in rheumatoid arthritis: passive responders or transformed aggressors? Arthritis Rheum 39:1781-1790, 1996

134. Mulherin D, Fitzgerald O, and Bresnihan B: Synovial tissue macrophage populations and articular damage in rheumatoid arthritis. Arthritis Rheum 39:115-124, 1996

135. Burmester GR, Stuhlmüller B, Keyszer G, and Kinne RW: Mononuclear phagocytes and rheumatoid synovitis. Arthritis Rheum 40:5-18, 1997

136. Hirohata S, Yanagida T, Itoh K, Nakamura H, Yoshino S, Tomita T, and Ochi T: Accelerated generation of CD14+ monocyte-lineage cells from the bone marrow of rheumatoid arthritis patients. Arthritis Rheum 39:836-843, 1996

137. St. Clair EW, Wilkinson WE, Lang T, Sanders L, Misukonis MA, Gilkeson GS, Pisetsky DS, Granger DL, and Weinberg JB: Increased expression of blood mononuclear cell nitric oxide synthase type 2 in rheumatoid arthritis patients. J Exp Med 184:1173-1178, 1996

138. Schulze-Koops H, Davis LS, Kavanaugh AF and Lipsky PE: Elevated cytokine mRNA levels in the peripheral blood of patients with rheumatoid arthritis suggest different degrees of myeloid cell

activation. Arthritis Rheum 40:639-647, 1997

139. Butler DM, Malfait A-M, Mason LJ, Warden PJ, Kollias G, Maini RN, Feldmann M, and Brennan FM: DBA/1 mice expressing the human TNF-α transgene develop a severe, erosive arthritis. J Immunol 159:2867-2876, 1997

140. Ghivizzani SC, Kang R, Georgescu HI, Lechman ER, Jaffurs D, Engle JM, Watkins SC, Tindal MH, Suchanek MK, McKenzie LR, Evans CH, and Robbins PD: Constitutive intra-articular expression of human IL-1β following gene transfer to rabbit synovium produces all major pathologies of human rheumatoid arthritis. J Immunol 159:3604-3612, 1997

141. Thomas R, Davis LS and Lipsky PE: Rheumatoid synovium is enriched in mature antigen presenting dendritic cells. J Immunol 152:2613-2623, 1994

142. Thomas R and Lipsky PE: Presentation of self peptides by dendritic cells: Possible implications for the pathogenesis of rheumatoid arthritis. Arthritis Rheum 39:183-190, 1996

143. Geiler T, Kriegsmann J, Keyszer GM, Gay RE, Gay S: A new model for rheumatoid arthritis generated by engraftment of rheumatoid synovial tissue and normal human cartilage into SCID mice. Arthritis Rheum 37:1664-1671, 1994

144. Müller-Ladner L, Kriegsmann J, Franklin BN, Matsumoto S, Geiler T, Gay RE, Gay S: Synovial fibroblasts of patients with rheumatoid arthritis attach to and invade normal human human

cartilage when engrafted into SCID mice. Am J Pathol 149:1607-1615, 1996

145. Cush JJ, Splawski JB, Thomas T, McFarlin JE, Schulze-Koops H, Davis LS, Fujita K and Lipsky PE: Elevated interleukin-10 levels in patients with rheumatoid arthritis. Arthritis Rheum 38:96-104, 1995

146. Llorente L, Richaud-Patin Y, Fior R, Alcocer-Varela J, Wijdenes J, Fourrier BM, Galanaud P, and Emilie D: In vivo production of interleukin-10 by non-T cells in rheumatoid arthritis, Sjögren's syndrome, and systemic lupus erythematosus. Arthritis Rheum 37:1647-1655, 1994

147. Cohen SBA, Katsikis PE, Chu C-Q, Thomssen H, Webb LMC, Maini RN, Londei M, and Feldmann M: High level of interleukin-10 production by the activated T cell population within the rheumatoid synovial membrane. Arthritis Rheum 38:946-952, 1995

148. Isomäki P, Luukkainen R, Saario R, Toivanen P, and Punnonen J: Interleukin-10 functions as an antiinflammatory cytokine in rheumatoid synovium. Arthritis Rheum 39:386-395, 1996

149. Paul WE and Seder RA: Lymphocyte responses and cytokines. Cell 76:241-251, 1994

150. Miossec P, Chomarat P, and Dechanet J: Bypassing the antigen to control rheumatoid arthritis. Immunol Today 17:170-173, 1996

151. Miossec P, Naviliat M, Dupuy d'Angeac A, Sany J, and Banchereau J: Low levels of interleukin-4

and high levels of transforming growth factor beta in rheumatoid arthritis. Arthritis Rheum 33:1180-1187, 1990

152. Wernick R, Lipsky PE, Marban-Arcos E, Maliakkal JJ, Edelbaum D, Ziff M: IgG and IgM rheumatoid factor synthesis in rheumatoid synovial membrane cell cultures. Arthritis Rheum 28:742-752, 1985

153. Elliott MJ, Maini RN, Feldmann M, Long-Fox A, Charles P, Katsikis P, Brennan FM, Walker J, Bijl H, Ghrayeb J, and Woody JN: Treatment of rheumatoid arthritis with chimeric monoclonal antibodies to tumor necrosis factor α. Arthritis Rheum 36:1681-1690, 1993

154. Elliott MJ, Maini RN, Feldmann M, Kalden JR, Antoni C, Smolen JS, Leeb B, Breedveld FC, Macfarlane JD, Bijl H, and Woody JN: Randomised double-blind comparison of chimeric monoclonal antibody to tumour necrosis factor alpha (cA2) versus placebo in rheumatoid arthritis. Lancet 344:1105-1110, 1994

155. Tak PP, Taylor PC, Breedveld FC, Smeets TJM, Daha MR, Kluin PM, Meinders AE, and Maini RN: Decrease in cellularity and expression of adhesion molecules by anti-tumor necrosis factor α monoclonal antibody treatment in patients with rheumatoid arthritis. Arthritis Rheum 39:1077-1081, 1996

156. Paleolog EM, Hunt M, Elliott MJ, Fedlmann M, Maini RN, and Woody JN: Deactivation of vascular endothelium by monoclonal anti-tumor necrosis factor α antibody in rheumatoid arthritis.

Arthritis Rheum 39:1082-1091, 1996

157. Moreland LW, Baumgartner SW, Schiff MH, Tindall EA, Fleischmann RM, Weaver AL, Ettlinger RE, Cohen S, Koopman WJ, Mohler K, Widmer MB, and Blosch CM: Treatment of rheumatoid arthritis with a recombinant human tumor necrosis factor receptor (p75)-Fc fusion protein. New Engl J Med 337:141-147, 1997

158. Campion GV, Lebsack ME, Lookabaugh J, Gordon G, Catalano M, and The IL-1Ra Arthritis Study Group: Dose-range and dose-frequency study of recombinant human interleukin-1 receptor antagonist in patients with rheumatoid arthritis. Arthritis Rheum 39:1092-1101, 1996

159. Drevlow BE, Lovis R, Haag MA, Sinacore JM, Jacobs C, Blosche C, Landay A, Moreland LW, and Pope RM: Recombinant human interleukin-1 receptor type I in the treatment of patients with active rheumatoid arthritis. Arthritis Rheum 39:257-265, 1996

160. Wendling D, Racadot E, and Wijdenes J: Treatment of severe rheumatoid arthritis by anti-interleukin 6 monoclonal antibody. J Rheumatol 20:259-262, 1993

161. Moreland LW, Heck, Jr. LW, and Koopman WJ: Biologic agents for treating rheumatoid arthritis. Arthritis Rheum 40:397-409, 1997

162. van der Lubbe PA, Reiter Ch, Breedveld FC, Krüger K, Schattenkirchner M, Sanders ME, and Riethmüller G: Chimeric CD4 monoclonal antibody cM-T412 as a therapeutic approach to

rheumatoid arthritis. Arthritis Rheum 36:1375-1379, 1993

163. Moreland LW, Pratt PW, Bucy RP, Jackson BS, Feldman JW, and Koopman WJ: Treatment of refractory rheumatoid arthritis with a chimeric anti-CD4 monoclonal antibody. Arthritis Rheum 37:834-838, 1994

164. Moreland LW, Bucy RP, Tilden A, Pratt PW, LoBuglio AF, Khazaeli M, Everson MP, Daddona P, Ghrayeb J, Kilgarriff C, Sanders ME, and Koopman WJ: Use of a chimeric monoclonal anti-CD4 antibody in patients with refractory rheumatoid arthritis. Arthritis Rheum 36:307-318, 1993

165. Moreland LW, Pratt PW, Mayes MD, Postlethwaite A, Weisman MH, Schnitzer T, Lightfoot R, Calabrese L, Zelinger DJ, Woody JN, and Koopman WJ: Double-blind, placebo-controlled multicenter trial using chimeric monoclonal anti-CD4 antibody, cM-T412, in rheumatoid arthritis patients receiving concomitant methotrexate. Arthritis Rheum 38:1581-1588, 1995

166. van der Lubbe PA, Dijkmans BAC, Markusse HM, Nässander U and Breedveld FC: A randomized, double-blind, placebo-controlled study of CD4 monoclonal antibody therapy in early rheumatoid arthritis. Arthritis Rheum 38:1097-1106, 1995

167. Tak PP, van der Lubbe PA, Cauli A, Daha MR, Sheets TJM, Kluin PM, Meinders AE, Yanni G, Panayi GS and Breedveld FC: Reduction of synovial inflammation after anti-CD4 monoclonal antibody treatment in early rheumatoid arthritis. Arthritis Rheum 38:1457-1465, 1995

168. Ridgway W and Fathman CG: Anti-CD4 in the treatment of rheumatoid arthritis-A reappraisal. The Immunologist 4/6:203-206, 1996

169. Matteson EL, Yocum DE, St. Clair EW, Achkar AA, Thakor MS, Jacobs MR, Hays AE, Heitman CK and Johnston JM: Treatment of active refractory rheumatoid arthritis with humanized monoclonal antibody campath-1H administered by daily subcutaneous injection. Arthritis Rheum 38:1187-1193, 1995

170. Weinblatt ME, Maddison PJ, Bulputt KJ, Hazleman BL, Urowitz MB, Sturrock RD, Coblyn JS, Maier AL, Spreen WR, Manna VK and Johnston JM: Campath-1H, a humanized monoclonal antibody, in refractory rheumatoid arthritis. Arthritis Rheum 38:1589-1594, 1995

171. Ruderman EM, Weinblatt ME, Thurmond LM, Pinkus GS and Gravallese EM: Synovial tissue response to treatment with campath-1H. Arthritis Rheum 38:254-258, 1995

172. Olsen NJ, Brooks RH, Cush JJ, Lipsky PE, St Clair EW, Matteson EL, Gold KN, Cannon GW, Jackson CG, McCune WJ, Fox DA, The Xoma RA Investigator Group, B Nelson, Lorenz T and Strand V: A double-blind, placebo-controlled study of anti-CD5 immunoconjugate in patients with rheumatoid arthritis. Arthritis Rheum 39:1102-1108, 1996

173. Fox DA: The role of T cells in the immunopathogenesis of rheumatoid arthritis. New perspectives. Arthritis Rheum 40:598-600, 1997

174. Ranges GE, Sriram S, Cooper SM: Prevention of type II collagen-induced arthritis by in vivo treatment with anti-L3T4. J Exp Med 162:1105-1110, 1985

175. Wofsy D, Seaman WE: Reversal of advanced murine lupus in NZB/NZW F1 mice by treatment with monoclonal antibody to L3T4. J Immunol 1987: 3247-3253, 1987

176. Waldor MK, Sriram S, Hardy R, Herzenberg LA, Lanier L, Lim M, Steinman L: Reversal of experimental allergic encephalomyelitis with monoclonal antibody to a T-cell subset marker. Science 227:415-417, 1985

177. Wofsy D, Seaman WE: Successful treatment of autoimmunity in NZB/NZW F1 mice with monoclonal antibody to L3T4. J Exp Med 161:378-391, 1985

178. Shizuru JA, Taylor-Edwards C, Banks BA, Gregory AK, Fathman CG: Immunotherapy of the nonobese diabetic mouse: treatment with an antibody to T-helper lymphocytes. Science 240:659-662, 1988

179. Benjamin RJ and Waldmann H: Induction of tolerance by monoclonal antibody therapy. Nature 320:449-451, 1986

180. Shizuru JA, Alters SE, Fathman CG: Anti-CD4 monoclonal antibodies in therapy: creation of nonclassical tolerance in the adult. Immunol Rev 129:105-130, 1992

181. Gutstein NL and Wofsy D: Administration of F (ab′)2 fragments of monoclonal antibody to L3R4 inhibits humoral immunity in mice without depleting LeT4+ cells. J Immunol 137:3414-3419, 1986

182. Carteron NL, Wofsy D, Seaman WE: Induction of immune tolerance during administration of monoclonal antibody to L3T4 does not depend on depletion of L3T4+ cells. J Immunol 140:713-716, 1988

183. Brostoff SW and Mason DW: Experimental allergic encephalomyelitis: successful treatment in vivo with a monoclonal antibody that recognizes T helper cells. J Immunol 133;1938-1942, 1984

184. Burkhardt K, Charlton B, Mandel TE: An increase in the survival of murine H-2-mismatched cultured fetal pancreas allografts using depleting or nondepleting anti-CD4 monoclonal antibodies, and a further increase with the addition of cyclosporine. Transplantaion 47:771-775, 1989

185. Qin SX, Wise M, Cobbold SP, Leong L, Kong YC, Parnes JR, and Waldmann H: Induction of tolerance in peripheral T cells with monoclonal antibodies. Eur J Immunol 20:2737-2745, 1990

186. Pulito VL, Roberts VA, Adair JR, Rothermel AL, Collins AM, Varga SS, Martocello C, Bodmer M, Jolliffe LK, and Zivin RA: Humanization and molecular modeling of the anti-CD4 monoclonal antibody, OKT4A. J Immunol 156:2840-2850, 1996