The Immunopathogenesis of Rheumatoid Arthritis

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Abstract
Rheumatoid arthritis is a chronic inflammatory polyarthritis whose etiology remains uncertain. Recently we have learned that autoimmunity to citrullinated protein antigens has specificity for rheumatoid arthritis and defines a clinically and genetically distinct form of the disease. Multiple genes contribute to disease susceptibility, with the HLA locus accounting for 30% to 50% of overall genetic risk. Five risk loci have been identified and validated: HLA-DRB1, PTPN22, STAT4, a region in 6q23, and the TRAF1/C5 locus. Also, there is renewed interest in the contribution of T cells to ongoing inflammation in rheumatoid arthritis. Autoantibodies to citrullinated protein epitopes are specific for rheumatoid arthritis, are associated with a more aggressive disease course, and are pathogenic in an animal model of autoimmune arthritis. There is a strong association between shared-epitope-expressing HLA-DRB1 alleles and the development of rheumatoid arthritis associated with autoimmunity to citrullinated protein antigens.
GENERAL CONSIDERATIONS

Rheumatoid arthritis (RA) is a chronic inflammatory polyarthritis that can affect any synovial-lined diarthrodial joint, although it has a predilection for the wrist and small joints of the hand. The typical natural history of RA is one of progressive articular damage leading to joint deformities and disability. Recently, there has been considerable progress in elucidating the mechanisms that mediate synovitis and articular damage. Indeed, recognition that tumor necrosis factor alpha (TNF-α) plays an important role in joint inflammation and damage has led to the introduction of therapies that target TNF-α and that have had a major impact on the care of patients with RA (1, 2).

Although RA has long been considered an autoimmune disease, there is no consensus as to the critical autoantigen(s) involved or the environmental factors that trigger the autoimmune process. An important insight into RA stems from the appreciation that autoimmunity to citrullinated proteins is highly specific for RA and may be of pathogenic significance. Autoantibodies to citrullinated protein antigens are present in ~70% of RA patients (3). Clinically these autoantibodies (known as anti-CCP antibodies) are detected by enzyme-linked immunosorbent assay using synthetic cyclic citrullinated peptides (CCPs) (3, 4). Anti-CCP-positive RA has a more aggressive clinical course than anti-CCP-negative RA, and it appears to have a different genetic risk profile (5, 6). It is likely that RA, which remains a clinical diagnosis, is not a single entity. From both a clinical and pathogenic perspective, the dichotomy of anti-CCP-positive versus anti-CCP-negative RA appears to be a more valid distinction than that based on the presence or absence of rheumatoid factor and serves to define two forms of the disease.

This review focuses upon evidence that the adaptive immune system plays a central role in the initiation and propagation of inflammation in RA. I review the genetic predisposition to RA, the possible roles played by Th17 cells and regulatory T cells (Treg cells), and the evidence that autoimmunity to citrullinated protein epitopes is pathogenic.

GENETIC SUSCEPTIBILITY

Estimate of the Genetic Contribution to Rheumatoid Arthritis Susceptibility

Genetic factors have a substantial impact on susceptibility to RA (7). The prevalence of RA in the general population is in the range of 0.24% to 1.0%, but it increases to 2% to 4% among siblings of RA probands (7). The overall genetic contribution to the risk of developing RA has been estimated through studies of monozygotic and dizygotic twin pairs. Cross-sectional twin studies performed on a national scale in Finland and in the United Kingdom found concordance rates for RA of 12.3% and 15.4%, respectively, for monozygotic twins, compared to 3.5% and 3.6% for dizygotic twins (8). Using these data, MacGregor et al. (8) estimated the heritability of RA—defined as the extent to which genetic variation can explain the variation in liability to RA—to be approximately 60%.

Multiple loci contribute to the genetic risk for RA (9). The HLA (human leukocyte antigen) locus is the most important of these and accounts for 30% to 50% of overall genetic susceptibility to RA (7, 9, 10). Within the HLA locus, the strongest association is with alleles of HLA-DRB1, which encodes the β-chain of the class II molecule HLA-DR, but recent evidence indicates that other HLA genes also contribute to genetic risk (10). Outside the HLA locus, the strongest association is with alleles of HLA-DRB1, which encodes the β-chain of the class II molecule HLA-DR, but recent evidence indicates that other HLA genes also contribute to genetic risk (10). Outside the HLA locus, the strongest association identified thus far is with a polymorphism in the protein tyrosine phosphatase nonreceptor 22 (PTPN22) gene, which encodes the protein tyrosine phosphatase Lyp (11). Recent genome-wide surveys have identified three additional RA risk loci (Table 1) (9).

HLA-DRB1

A link between RA and serologically defined HLA-DRw4 was reported in 1978 by Stastny


A recent analysis of HLA-DRB1 alleles in French patients with RA led Tezenas du Mochet et al. (14) to propose a classification scheme of HLA-DRB1 alleles that incorporates gradation of risk of developing RA based on amino acids 70–74 (Table 2). They noted that all HLA-DRB1 alleles associated with RA encode the sequence Arg-Ala-Ala at positions 72–74 and proposed that amino acids at positions 70 and 71 modulate the risk of RA (14). Thus, at position 71, Lys confers the highest risk of RA, whereas Arg is associated with intermediate risk and Ala and Glu with lower risk (14). At position 70, Gln or Arg carries a higher risk than does Asp (14). Subsequent analyses of HLA-DRB1 alleles in Caucasian RA patients and in Caucasian controls from France and the United Kingdom are consistent with the hierarchy of risk proposed by the Tezenas du Mochet classification scheme (15–17).

HLA-DR molecules are heterodimers that present antigenic peptides to T lymphocytes.

### Table 1 Genetic susceptibility to rheumatoid arthritis

<table>
<thead>
<tr>
<th>Gene/locus</th>
<th>Odds ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>HLA-DRB1 SE alleles</td>
<td>Single SE ∼ 3 to ∼ 4.5; double SE ∼ 7 to ∼ 13</td>
</tr>
<tr>
<td>PTPN22 Arg620→Trp</td>
<td>∼ 1.6</td>
</tr>
<tr>
<td>STAT4</td>
<td>∼ 1.3</td>
</tr>
<tr>
<td>6q23</td>
<td>∼ 1.2</td>
</tr>
<tr>
<td>TRAF1/C5</td>
<td>∼ 1.3</td>
</tr>
</tbody>
</table>

Table based on information from References 6, 24–26, 10, 31, 14–36, and 71.

Abbreviations: PTPN, protein tyrosine phosphatase nonreceptor; SE, shared epitope; STAT, signal transducer and activator of transcription; TRAF, tumor necrosis factor–associated factor.

The peptide-binding groove is composed of two α-helical "walls" and a "floor" of β-pleated sheet (18). The shared epitope is in the α-helix wall of the peptide-binding groove (13). Structural studies provide evidence that the shared epitope can influence peptide binding as well as contact between HLA-DR and the T cell receptor. The crystallographic structures of shared-epitope-expressing HLA-DR1 (HLA-DRB1*0101) and HLA-DR4 (HLA-DRB1*0401) complexed with an influenza hemagglutinin peptide or with a peptide from human collagen II demonstrate that position 71 (Arg71 for DRB1*0101, Lys71 for HLA-DRB1*0401) interacts with the peptide amino acid in the P4 anchoring pocket (19–21). The side chains of the shared-epitope residues 72–74 either point away from the peptide-binding groove or are too far away from the peptides to make contact (21). Studies of the structure of a covalently stabilized complex of T cell receptor, HLA-DR1, and the influenza peptide reveal that the T cell receptor contacts Gln70 within the shared epitope (22) (Figure 1).

Although limited in scope, these structural studies point to an intriguing convergence with genetic epidemiological data. Residues 70 and 71 of the shared epitope—identified in epidemiological studies as modulating risk for RA—contact, respectively, the T cell receptor and the antigenic peptide. These results, therefore, suggest that the products of shared-epitope HLA-DRB1 alleles may predispose to RA by presenting peptides to T cells. The evidence for this, however, remains circumstantial.

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**Shared epitope:** a region of sequence similarity identified in HLA-DRB1 alleles that confer susceptibility to rheumatoid arthritis.
Table 2  Tezenas du Montcel classification system for HLA-DRB1 shared-epitope alleles

<table>
<thead>
<tr>
<th>HLA-DRB1 allele</th>
<th>Amino acid residue</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>70</td>
</tr>
<tr>
<td>Susceptibility alleles</td>
<td></td>
</tr>
<tr>
<td>High risk</td>
<td></td>
</tr>
<tr>
<td>*0401, *1303</td>
<td>Q</td>
</tr>
<tr>
<td>Intermediate risk</td>
<td></td>
</tr>
<tr>
<td>*0101, *0102, *0404, *0405,</td>
<td>Q/R</td>
</tr>
<tr>
<td>*0408, *1001, *1402, *1406</td>
<td></td>
</tr>
<tr>
<td>Low risk</td>
<td></td>
</tr>
<tr>
<td>*1501, *1502, *1503</td>
<td>Q</td>
</tr>
<tr>
<td>*1202, *16</td>
<td>D</td>
</tr>
<tr>
<td>Nonsusceptibility alleles</td>
<td></td>
</tr>
<tr>
<td>*03</td>
<td>Q</td>
</tr>
<tr>
<td>*0403, *0407</td>
<td>Q</td>
</tr>
<tr>
<td>*07</td>
<td>D</td>
</tr>
<tr>
<td>*08</td>
<td>D</td>
</tr>
<tr>
<td>*09</td>
<td>R</td>
</tr>
<tr>
<td>*14 (except *1402 and *1406)</td>
<td>R</td>
</tr>
</tbody>
</table>

Adapted from References 14 and 15.

Despite considerable progress in understanding the association of HLA-DRB1 with RA and the structure and function of HLA-DR molecules, the mechanisms by which inheritance of particular HLA-DRB1 alleles predisposes to the development of RA remain unknown. Proposed models include the selection of pathogenic T cells during thymic selection, the presentation of “arthritogenic peptides” to class II–restricted peripheral-effector T cells, and the failure to generate appropriate T reg cells (23).

PTPN22

The candidate-gene approach has led to recognition of an association between RA and the Arg620→Trp polymorphism in the PTPN22 gene, a polymorphism that has also been associated with a variety of other autoimmune diseases, including type 1 diabetes, Graves’ disease, Hashimoto thyroiditis, and systemic lupus erythematosus (11, 24–26). As is the case with HLA-DRB1 shared-epitope alleles, PTPN22 Arg620→Trp confers susceptibility for anti-CCP-positive RA, and there is no significant association with anti-CCP-negative RA (25). The effect size is more modest than that of HLA-DRB1 but is greater than that of the other non-HLA RA-susceptibility loci identified to date. The association with RA has been reproduced in multiple studies of populations of European ancestry; the Arg620→Trp variant is rare in Asian populations and accordingly has not been linked to RA susceptibility in those populations (9).

PTPN22 encodes Lyp, a lymphocyte intracellular tyrosine phosphatase that binds to the regulatory kinase Csk; the Lyp–Csk complex in turn inhibits Lck, a protein tyrosine kinase that plays a critical role in T cell receptor signaling (11). Thus Lyp acts in concert with Csk to downregulate the initiation of T cell activation. The physical binding of Lyp to Csk is mediated by interactions between four proline-rich motifs in Lyp and in the Src homology 3 (SH3) domain of Csk (11). Amino acid 620 in Lyp lies within one of these proline-rich sequences, and the substitution of Trp for Arg at
this residue disrupts the association with Csk (11). Somewhat surprisingly, this substitution results in a gain of function for Lyp (27, 28). Lyp-Trp620 has greater phosphatase activity than Lyp-Arg620, and T cells expressing Lyp-Trp620 produce less interleukin-2 (IL-2) in response to T cell receptor signaling than do T cells expressing Lyp-Arg620. Thus RA is associated with a polymorphism that renders Lyp a more potent negative regulator of T cell activation. Two models have been proposed to explain the apparent link between diminished T cell receptor signaling and susceptibility to RA. First, expression of Lyp-Arg620 early in T cell development may result in weaker T cell receptor signaling during thymic selection and thus a failure to delete autoreactive T cells. Alternatively, the inhibitory effect may be manifest at the level of T reg cells and may result in diminished activity of these cells (11, 27, 28).

**STAT4**

By examining candidate genes in a region of chromosome 2q that had previously been linked to RA in a genome-wide survey of >5700 single nucleotide polymorphism (SNP) markers, Remmers et al. (30) recently identified a SNP haplotype in the third intron of STAT4 (signal transducer and activator of transcription 4) that was associated with RA in a North American population (29, 30). The risk allele was present in 27% of chromosomes of the RA patients versus 22% of chromosomes from controls (odds ratio 1.32), and the association was replicated in Swedish and Korean RA populations (30, 31). The STAT4 haplotype was also associated with risk for systemic lupus erythematosus (30).

**STAT4** encodes STAT4, which is expressed primarily by lymphoid and myeloid tissues (32). Whether the RA-associated **STAT4** haplotype affects the function or expression of **STAT4** is not known. However, direct participation of **STAT4** in RA susceptibility is biologically plausible. **STAT4** plays a central role in the cellular responses to IL-12, IL-23, interferon (IFN)-α and IFN-β (32). When activated by the receptors for these cytokines, **STAT4** homodimerizes
and translocates to the nucleus, where it stimulates transcription of specific genes (32). It induces IFN-γ and plays a key role in the IL-12-mediated development of a Th1 response (32). By transducing signals for IL-23, it also plays a role in the survival and expansion of proinflammatory Th17 cells (32).

**Genome-Wide Association Studies**

Genome-wide studies have identified two additional risk loci for RA that have been validated in independent cohorts: a SNP in 6q23 and a SNP on chromosome 9 (34–36). The latter is in linkage disequilibrium with genes encoding tumor necrosis factor receptor–associated factor 1 (TRAF1) and complement component C5 (C5) (36).

**6q23**

The 6q23 locus was identified in the genome-wide association study performed by the Wellcome Trust Case Control Consortium, which analyzed 500,000 SNPs in 1860 RA cases and 2938 controls from the United Kingdom (33). The association of RA with the SNP in 6q23 was confirmed in a second British cohort of 5063 RA cases and 3849 controls; 6q23 was independently identified as a risk locus in a study of an American cohort (34, 35). The SNP maps between the genes oligodendrocyte lineage transcription factor 3 (OLIG3) and TNF-α-induced protein 3 (TNFAIP3) (34, 35). The latter is an attractive candidate for a RA-susceptibility gene because its product, A20, is a widely expressed cytoplasmic protein that inhibits nuclear factor–kappa B (NF-κB) activity and limits inflammation mediated by TNF-α and by Toll-like receptors (36–38).

**TRAF1-C5**

This risk locus was identified in a genome-wide survey of 297,086 SNPs in 1522 cases of anti-CCP-positive RA and 1850 controls (36). Both TRAF1 and C5 are biologically plausible contributors to RA susceptibility. The product of TRAF1 appears to act as a negative regulator of signals mediated through TNF receptors and through the T cell receptor (36). C5 is an important component of the complement pathways, which are thought to play a role in articular inflammation in RA.

**THE ROLE OF T CELLS IN THE INITIATION AND PROPAGATION OF RHEUMATOID ARTHRITIS**

It is striking that three genes—HLA-DRB1, PTPN22, and STAT4—linked to RA susceptibility have the potential to impact T cell biology through effects on selection of the T cell repertoire, the presentation of peptide antigens to mature T cells, thresholds for T cell activation, and differentiation into Th1 and Th17 effectors.

As noted above, a potential mechanism by which either shared-epitope-expressing HLA-DRB1 alleles or the PTPN22 Arg620→Trp variant could confer susceptibility to RA is through effects on thymic selection, leading to a T cell repertoire that contains potentially autoreactive cells (11, 27, 28). Although experimental evidence for or against this possibility is lacking in humans, there is precedence for this mechanism in a mouse model of arthritis. A spontaneous mutation in BALB/c mice gave rise to a strain, designated SKG, that develops chronic polyarthritis (39). The mutation is in the C-terminal SH2 domain of the protein tyrosine kinase ZAP-70 and affects the interaction of ZAP-70 with the T cell receptor ζ-chain (39). This impairs signal transduction through the T cell receptor, reduces thresholds for thymic deletion of autoreactive T cells, and leads to the selection of CD4+ T cells with arthritogenic potential (39).

The inflamed RA synovium contains large numbers of T cells (23). Nonetheless, it has proved difficult to delineate the role T cells play in the propagation of articular inflammation. Indeed, trials with monoclonal antibodies to CD4, CD5, and CD52 failed to demonstrate clinical benefit in RA despite substantial depletion of the targeted T cells in peripheral...
blood (but not necessarily in the synovium) (40). The subsequent therapeutic success of abatacept, however, provides empiric evidence for a role for T cells in ongoing synovial inflammation in RA (41). Abatacept is a fusion protein composed of the extracellular domain of human cytotoxic T cell–associated antigen–4 (CTLA-4) linked to the hinge and the CH2 and CH3 domains of human immunoglobulin G1 (IgG1) (41). The CTLA-4 domain enables abatacept to bind CD80 and CD86 molecules on antigen-presenting cells, thereby preventing their interactions with T cell CD28 (41). Although designed to block CD28-mediated T cell costimulation, the T cell–inhibitory effects of abatacept may also be mediated through modulation of tryptophan metabolism in antigen-presenting cells (42). Abatacept has efficacy for RA that remains active despite standard therapy with methotrexate. Moreover, approximately 50% of patients with disease refractory to anti-TNF therapy have a clinically meaningful response to abatacept (41).

IFN-γ and other Th1 cytokines can be detected in RA synovial tissue, but at far lower levels than those of proinflammatory cytokines such as TNF-α, IL-6, and IL-1 (23). Although the concept of RA as a Th1-mediated disease has failed to gain traction, the identification of the Th17 pathway has renewed interest in a proinflammatory role for T cells in RA.

**Th17 Cells**

Th17 is a T cell–effector lineage distinct from Th1 and Th2 (43). Th17 differentiation, which is orchestrated by the nuclear receptor RORγt, is initiated by IL-6 acting in concert with transforming growth factor beta (TGF-β) and is enhanced by TNF-α and IL-1β (43, 44). IL-23, a member of the IL-12 family, plays an important role in Th17-mediated tissue damage, probably by promoting Th17 expansion and survival (43, 44). The effects of IFN-γ on Th17 cells are inhibitory (43, 44).

IL-17—the signature proinflammatory cytokine produced by Th17 cells—acts on multiple cell types found in inflamed rheumatoid joints: monocytes, macrophages, fibroblasts, osteoclasts, and chondrocytes (43, 45). IL-17 induces a wide range of effector molecules that have been implicated in joint damage, including proinflammatory cytokines (e.g., IL-1β, IL-6, and TNF-α), multiple chemokines, cyclooxygenase-2, prostaglandin E2, and matrix metalloproteinases (43, 45). IL-17 upregulates RANK (receptor activator of NF-κB) ligand on chondrocytes and osteoblasts, thereby promoting the development of osteoclasts, which are the destructive element in the bony erosions of RA (46). Thus the cellular targets and biological effects of IL-17 are consistent with the hypothesis that Th17 cells have a key role in mediating synovitis and articular damage (Figure 2). Studies in several

**Figure 2**

The differentiation of naive CD4 T cells into Th17 effectors. Transforming growth factor beta (TGF-β) and interleukin (IL)-6 promote the differentiation of activated naive CD4 T cells into Th17 cells, whose signature cytokine, IL-17, can act upon multiple cell types within rheumatoid synovium to promote inflammation and joint damage. IL-23 appears to enhance Th17 survival and expansion, thereby promoting Th17-mediated synovitis.
animal models indicate that this is the case (47, 48); the evidence in human RA remains circumstantial.

Compelling data indicate that T\(17\) cells mediate arthritis in the SKG mouse model (47). T\(17\) cells develop spontaneously in SKG mice but not in IL-6-deficient SKG mice, which are protected from arthritis (47). Moreover, CD\(4\) T cells from SKG mice, but not from IL-17-deficient SKG mice, can transfer arthritis to RAG\(2^{−/−}\) BALB/c recipients (47). Similarly, collagen-induced arthritis (CIA) in the mouse, which was initially thought to be IL-12 dependent and a T\(1\)-mediated disease, appears instead to depend upon IL-23 and T\(17\) cells (48). Neutralizing antibodies to the p40 subunit of IL-12 inhibit CIA, but IL-12 and IL-23 share p40 (48). By targeting the genes for specific subunits of IL-12 (p35) and IL-23 (p19), Murphy et al. (48) demonstrated that the specific absence of IL-23 protects against CIA, whereas the selective absence of IL-12 promotes disease, probably due to the absence of the inhibitory effects of T\(1\)-derived IFN-\(\gamma\) on T\(17\) cells.

The recent identification of a T\(17\) haplotype associated with susceptibility to RA is especially intriguing in light of the biology of T\(17\) cells and the key role played by T\(17\)4 in transducing signals for IL-23. Nonetheless, it remains uncertain whether the haplotype affects T\(17\) expression or function. Mechanistic studies on the role of T\(17\) cells and IL-17 in mediating articular inflammation in human RA are necessarily limited. IL-17 has been identified in synovial fluid of RA patients, but not in controls with osteoarthritis (49). In an intriguing prospective study, Kirkham et al. (50) examined the ability of synovial membrane cytokine expression to predict joint damage in 56 RA patients. At enrollment, each patient underwent synovial biopsies of the knee. Levels of IL-17 and other cytokines in the synovial tissue were assessed by quantitative reverse transcription-polymerase chain reaction and then were used to predict the progression of articular damage, as determined by comparing radiographic and magnetic resonance imaging studies at the time of biopsy and 2 years later. Most patients in the study received active treatment of their RA, and many had little or no progressive joint destruction. Biopsies from only 28% of RA patients expressed IL-17, whereas ≥98% had detectable TNF-\(\alpha\), IL-1\(\beta\), IL-10, IL-16, and IFN-\(\gamma\) (50). Nonetheless, multivariate analysis demonstrated that levels of IL-17 (as well as of TNF-\(\alpha\), IL-1\(\beta\), and IL-10) predicted joint damage (50). The effects of IL-17 and TNF-\(\alpha\) were synergistic and were most evident in those patients who had RA of more recent onset (50). The effect of IL-1\(\beta\) was more important in disease of longer duration. IFN-\(\gamma\) had a protective effect on joints—an interesting observation in light of IFN-\(\gamma\)'s ability to inhibit T\(17\) cells (50). The study was limited by (a) the possible impact of treatment, which was not uniform and which could have affected cytokine expression, and (b) the absence of progression of joint damage in a number of patients. Nonetheless, the results are consistent with the possibility that IL-17, and by implication T\(17\) cells, have a proinflammatory role in at least a subset of RA patients.

### Regulatory T Cells

Naturally occurring Treg cells are a unique population of CD\(4^{+}\)CD\(25^{+}\) T cells whose development requires the transcription factor FoxP3 (51). These Treg cells suppress CD4 and CD8 T cell responses though cell-cell contact and play an essential role in maintaining peripheral tolerance to self (51). Because of the importance of Treg cells in suppressing organ-specific and systemic autoimmunity in rodent models, an attractive hypothesis is that quantitative or qualitative abnormalities in Treg cells contribute to inflammation in RA (51).

There are conflicting data with respect to the possibility of quantitative abnormalities of Treg cells in peripheral blood in patients with RA; these discrepancies may reflect the difficulties in the phenotypic identification of Treg cells without admixture of effector T cells, which also can express CD25 (51). In contrast, there is remarkable agreement that Treg cells, defined phenotypically and functionally, are
enriched in synovial fluid of RA patients relative to peripheral blood (51–53). This phenomenon of synovial enrichment begs the question as to why synovial inflammation persists despite the presence of Treg cells (51).

Natural Treg cells in the peripheral blood of patients with active RA appear to be functionally abnormal (54–56). Ehrenstein et al. (54) observed that peripheral-blood Treg cells, isolated as CD4+CD25hi T cells from patients with very active RA, suppressed proliferation of CD4+CD25− T cells as well as Treg cells from healthy controls did. A defect emerged, however, in the authors’ analysis of inhibition of cytokine production: In contrast to the control Treg cells, the RA Treg cells failed to inhibit production of TNF-α and IFN-γ by CD4+CD25− T cells (54). The defect resided in the RA Treg cells and was not due to resistance of RA CD4+CD25− T cells to suppression (54). Interestingly, treatment with the anti-TNF-α monoclonal antibody infliximab reversed this Treg cell defect in patients who had a significant clinical response to infliximab, but not in patients who were nonresponders (54). Infliximab responders had significantly higher numbers of peripheral-blood CD4+CD25hi T cells than did nonresponders (54). These observations, therefore, indicate that there is a Treg cell functional defect in patients with active RA and raise the possibility that reversal of that defect may contribute to the therapeutic efficacy of anti-TNF treatments.

Valencia et al. (55) also observed a functional defect in peripheral-blood CD4+CD25hi Treg cells from RA patients that reversed following treatment of these patients with infliximab. In contrast to the study by Ehrenstein et al. (54), this Treg cell defect included an inability to suppress proliferation by CD4+CD25− T cells. Importantly, Valencia et al. (55) provided evidence that TNF-α can act directly on Treg cells to impair their function. They observed that 20% of peripheral-blood CD4+CD25hi Treg cells isolated from healthy individuals express the TNF receptor type II and that this proportion increased to 100% following stimulation in vitro with anti-CD3 (55). The addition of TNF-α to CD4+CD25hi T cells led to downregulation of FoxP3 expression and to a diminished ability to suppress proliferation by CD4+CD25− T cells (55). The concentration (50 ng ml−1) of TNF-α was high in these in vitro experiments, but it fell within the ranges reported in serum of RA patients and was likely achieved within inflamed tissues (55). Thus, these results suggest a model in which high levels of TNF-α within inflamed rheumatoid synovium limit the activity of Treg cells. Inhibition of TNF-α, however, restores the function of Treg cells, which then can act to suppress T cell–effector responses.

Interestingly, the gain in Treg cell function following anti-TNF therapy in RA may not be restoration of the function of “natural” Tregs, but rather may represent the differentiation of CD4+CD25− T cells into CD4+CD25hi T cells, which mediate suppression through TGF-β and IL-10 rather than through cell-cell contact (56).

### AUTOIMMUNITY TO CITRULLINATED PROTEIN ANTIGENS

A major limitation in our understanding of the role of the adaptive immune response of RA has been the paucity of information regarding target antigens relevant to the disease process. A breakthrough in this area is the recognition that autoimmunity to citrullinated protein antigens has specificity for RA and identifies a clinically and genetically distinct form of RA.

### Citrullination of Proteins

Citrullination of proteins is a posttranslational modification in which arginine residues are deiminated to citrulline. A posttranslational protein modification in which arginine residues are deiminated to citrulline. PAD: peptidylarginine deiminase.
Peptidylarginine Peptidylcitrulline

![Figure 3](417-434-1.psd)

Citrullination of proteins. Citrullination is a posttranslational modification of proteins mediated by the calcium-dependent enzyme peptidylarginine deiminase (PAD), which deiminates the side chain of arginine to form citrulline.

Figure 3

The physiological role of citrullination is uncertain. Constitutive citrullination of proteins occurs in normal epidermis and elsewhere (57, 58). Citrullinated proteins are also found in sites of inflammation, including the lining and sublining cells of inflamed rheumatoid joints (57, 58, 61). PAD2 and PAD4—isoforms expressed by hematologic cells—are present in the sublining of rheumatoid synovium and colocalize with citrullinated proteins (59, 60). Citrullinated proteins identified in rheumatoid synovium include the α- and β-chains of fibrin, vimentin, and probably α-enolase (57, 61). The presence of citrullinated proteins in joints, however, is not specific for RA and occurs in other forms of arthritis (57). Thus, the development of autoimmunity to citrullinated protein antigens results from a loss of tolerance that is specific for RA, rather than from disease-specific appearance of citrullinated proteins in rheumatoid joints.

Preclinical Autoimmunity in Rheumatoid Arthritis

Two independent studies have examined stored blood samples obtained from donors who developed RA subsequent to the sample collection (64, 65). Both studies establish that serum anti-CCP antibodies and rheumatoid factor can appear years prior to the onset of articular symptoms (64, 65). Indeed, in the majority of the anti-CCP-positive RA patients, anti-CCP antibodies were detected prior to the onset of clinical arthritis (64, 65). Thus, the course of RA is characterized by a period of preclinical autoimmunity that can be years in duration. Little is known regarding the evolution of the antibody response to citrullinated protein antigens during this preclinical phase. Of particular interest is whether epitope spreading occurs and whether there is an accumulation of autoantibodies to multiple targets, as has been observed with autoantibodies during the preclinical phase of systemic lupus erythematosus (66). The presence of anti-CCP antibodies prior to clinical arthritis argues against the possibility that autoimmunity to citrullinated protein antigens is a consequence of joint inflammation.

Are Antibodies to Citrullinated Protein Antigens Pathogenic?

The specificity of anti-CCP antibodies for RA, the strong association of anti-CCP-positive RA—but not anti-CCP-negative RA—with HLA-DRB1 shared-epitope alleles, the presence of citrullinated proteins in inflamed joints, and the appearance of anti-CCP antibodies prior to the onset of clinical arthritis argue in favor of the hypothesis that antibodies to citrullinated protein antigens are pathogenic. Additional circumstantial evidence stems from clinical observations that the anti-CCP antibodies are strong predictors of erosions and that anti-CCP-positive patients tend to have a more aggressive disease course (5, 67).

In studies of the CIA mouse model of arthritis, Kuhn et al. (68) observed that immunization
with type II collagen induced anti-CCP antibodies as well as antibodies to type II collagen. The antibodies developed in parallel and appeared prior to the onset of arthritis. To test the significance of the anti-CCP response, the authors tolerized animals with a citrulline-containing peptide epitope prior to collagen challenge. Tolerance induction decreased the extent of citrullinated epitopes recognized by serum antibodies following collagen immunization and significantly reduced the severity of the arthritis (68). Passive transfer of the monoclonal antibodies to citrullinated fibrinogen epitopes overcame the protective effect of tolerance induction and elicited arthritis (68). These monoclonal antibodies alone could not induce arthritis, but they significantly enhanced arthritis when coadministered with a submaximal dose of anticollegen antibodies (68). Taken together, these findings establish a central role for antibodies to citrullinated protein antigens in the CIA animal model of immune-mediated arthritis (Figure 4).

Although there is increasing evidence that antibodies to citrullinated protein antigens are pathogenic (Table 3), it is also clear that the mere presence of these antibodies is not sufficient to cause arthritis. Thus, passive administration of monoclonal antibodies to citrullinated proteins overcame the protective effect of tolerance induction and elicited arthritis (68). These monoclonal antibodies alone could not induce arthritis, but they significantly enhanced arthritis when coadministered with a submaximal dose of anticollegen antibodies (68). Taken together, these findings establish a central role for antibodies to citrullinated protein antigens in the CIA animal model of immune-mediated arthritis (Figure 4).

Interactions of Citrullinated Peptides with HLA-DR Molecules Expressing the Shared Epitope

The simplest model to explain the strong association between HLA-DRB1 and anti-CCP-positive RA is that shared-epitope-expressing HLA-DR molecules bind certain key citrullinated peptide antigens with higher affinity than do shared-epitope-negative HLA-DR molecules. Structural studies indicate that position 71 of the shared epitope contributes to the P4 peptide–anchoring pocket (19–21). The shared-epitope alleles that confer intermediate to high risk for anti-CCP-positive RA encode Arg or Lys at position 71, indicating that the resulting P4 pocket is positively charged (13, 14, 19–21). Hill et al. (72) reasoned that this positively charged P4 pocket would exclude Arg but not necessarily neutral citrulline. Using a predictive model for peptide–HLA affinity, the authors identified a vimentin peptide with favorable binding to HLA-DR4 (DRB1*0401)
Development of anti-CII antibodies and anti-Cit protein antibodies

a

Development of anti-CII antibodies and anti-Cit protein antibodies

Arthritis

b

Anti-CII antibodies

↓↓ anti-Cit protein antibodies

Arthritis

Tolerized to Cit peptide

Monoclonal antibodies to Cit peptides

Arthritis

Monoclonal antibodies to Cit peptides + monoclonal antibodies to CII

Arthritis

Tolerized to Cit peptide

Monoclonal antibodies to Cit peptides

Arthritis

Monoclonal antibodies to CII

No arthritis

Minimal arthritis

↑↑ Arthritis
Table 3 Observations favoring a pathogenic role for antibodies to citrullinated protein antigens in rheumatoid arthritis

- Specificity for rheumatoid arthritis
- Strong association with HLA-DRB1 shared-epitope alleles
- Appearance prior to onset of clinically evident arthritis
- Presence of peptidylarginine deiminases and citrullinated epitopes in rheumatoid synovium
- Association with joint erosions and more severe clinical course
- Ability to enhance tissue damage in the collagen-induced mouse model of arthritis

at the P1, P6, and P9 anchoring pockets, but with Arg binding at P4 (72). Vimentin was selected because citrullinated vimentin is present in rheumatoid joints and is recognized by antibodies to citrullinated protein antigens. As predicted, the citrullinated form of the peptide, but not its Arg-containing counterpart, elicited a CD4 T cell response in transgenic mice expressing DRB1*0401 (72). In binding studies in vitro, citrullination increased the affinity of the vimentin peptide 20- to 100-fold for shared-epitope-expressing HLA-DR molecules, but it did not affect binding to shared-epitope-negative HLA-DR (72).

In contrast to the example provided by the vimentin peptide, an extensive survey of multiple peptides derived from fibrinogen found promiscuous binding of peptides to HLA-DR molecules and failed to detect an effect of citrullination on binding (73). Thus, the model that citrullination facilitates peptide binding to shared-epitope-positive HLA-DR molecules is not universal. Nonetheless, citrullination of fibrinogen may influence its pathogenicity in vivo in the presence of HLA-DR molecule expressing the shared epitope. Immunization of transgenic mice expressing DRB1*0401 with citrullinated, but not unmodified, human fibrinogen elicited antibodies to an array of citrullinated epitopes in fibrinogen, vimentin, and other proteins, indicating either extensive epitope spreading or broad cross-reactivity (74). Thirty-five percent of the transgenic mice developed arthritis. Immunization of wild-type mice with citrullinated human fibrinogen led to a weaker antibody response to citrullinated epitopes, and these animals did not develop arthritis (74). Thus, both the arthritis and the robust antibody response to citrullinated epitopes required the DRB1*0401 transgene (74). An important caveat is that the effect of other DRB1 transgenes, including those that do not encode the shared epitope, has not been determined in this model.

In summary, citrullination of certain peptides increases their affinity for shared-epitope-expressing HLA-DR molecules and facilitates their presentation to T cells. However, it remains to be determined whether selective binding of citrullinated peptides by shared-epitope-expressing HLA-DR molecules plays a role in the pathogenesis of RA.

Figure 4

Antibodies to citrullinated protein epitopes are pathogenic in a mouse model of arthritis. Immunization with type II collagen (CII) induces pathogenic anti-CII antibodies and inflammatory arthritis in mice. Kuhn et al. (68) observed that immunization with CII also leads to the appearance of antibodies to citrullinated (Cit) proteins (a). Several lines of evidence indicate that the anti-Cit antibodies are pathogenic in this model. Tolerization of the mice with a Cit peptide prior to immunization with CII leads to a reduction in the level of anti-Cit antibodies and to less-severe arthritis (b). Passive administration of monoclonal antibodies to Cit peptides to the tolerized animals overcomes the effect of tolerization and enhances arthritis (b). Although administration of these anti-Cit monoclonal antibodies alone fails to induce arthritis, these antibodies can act in concert with submaximal doses of anti-CII antibodies to promote arthritis (c).
SUMMARY POINTS

1. Autoimmunity to citrullinated protein antigens has specificity for RA and defines a clinically and genetically distinct form of the disease.

2. Genetic factors have a substantial impact on susceptibility to RA. Heritability, estimated from the concordance rates for RA among monozygotic and dizygotic twin pairs, is approximately 60%. Multiple genes contribute to disease susceptibility, with the HLA locus accounting for 30% to 50% of overall genetic risk. With the exception of HLA-DRB1, the individual contributions of risk alleles are small.

3. Two of the genes associated with risk for RA can impact T cell activation. HLA-DRB1 susceptibility alleles encode a similar sequence (the shared epitope) in the α-helical wall of the peptide-binding groove and therefore can influence the specificity of antigen recognition by T cells. The variant of PTPN22 associated with RA and other autoimmune disorders is a gain-of-function mutation that enhances the ability of its product, the protein tyrosine phosphatase Lyp, to inhibit T cell activation.

4. Compelling data establish a central role for Th17 cells and IL-17 in several mouse models of autoimmune arthritis. There is no conclusive evidence for the importance of Th17 cells in RA, but IL-17 can be detected in synovial fluid and tissue in RA.

5. CD4+CD25hi T reg cells are enriched in synovial fluid of RA patients relative to peripheral blood. Peripheral-blood CD4+CD25hi T reg cells in RA have a functional defect that impairs their ability to suppress CD4+CD25− T cells. The T reg defect reverses following a response to anti-TNF therapy, raising the possibility that improved T reg function contributes to the therapeutic efficacy of anti-TNF agents.

6. Serum anti-CCP antibodies can develop years prior to the onset of RA, indicating that RA is preceded by a period of preclinical autoimmunity. Anti-CCP antibodies are associated with erosions and a more severe clinical course, and citrullinated proteins are present in rheumatoid synovium. Antibodies to citrullinated protein antigens are pathogenic in the collagen-induced mouse model of arthritis.

7. The environmental factors that lead to the loss of tolerance to citrullinated proteins are not known. A striking relationship among smoking, the presence of HLA-DRB1 shared-epitope alleles, and the development of anti-CCP-positive RA in a Swedish cohort suggests that smoking may trigger autoimmunity to citrullinated protein epitopes. Studies of North American RA cohorts, however, do not find a strong association with smoking, thus pointing to the presence of additional environmental factors.

DISCLOSURE STATEMENT

The author is not aware of any biases that might be perceived as affecting the objectivity of this review.

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LITERATURE CITED


www.annualreviews.org • Rheumatoid Arthritis 451
33. The Wellcome Trust Consortium. 2007. Genome-wide association study of 14,000 cases of seven common diseases and 3000 shared controls. Nature 447:661–78


Contents

The First Fifty Years in Research
Peter A. Ward .............................................................. 1

Graft Vascular Disease: Immune Response Meets the Vessel Wall
Richard N. Mitchell ........................................................... 19

Molecular Pathology of Head and Neck Cancer: Implications for Diagnosis, Prognosis, and Treatment
Sara I. Pai and William H. Westra ........................................... 49

Mechanisms of Endothelial Dysfunction, Injury, and Death
Jordan S. Pober, Wang Min, and John R. Bradley ........................................... 71

The Pathogenesis of Pituitary Tumors
Sylvia L. Asa and Shereen Ezzat ........................................... 97

PTEN and the PI3-Kinase Pathway in Cancer
Nader Chalhoub and Suzanne J. Baker ........................................... 127

Pathogenesis of Classical and Lymphocyte-Predominant Hodgkin Lymphoma
Roland Schmitz, Jens Stanelle, Martin-Leo Hansmann, and Ralf Küppers ........................................... 151

Molecular Genetics of Acute Lymphoblastic Leukemia
Michael A. Teitell and Pier Paolo Pandolfi ........................................... 175

MicroRNAs in Cancer
Yong Sun Lee and Anindya Dutta ........................................... 199

Epigenetic Changes in Cancer
Christine A. Iacobuzio-Donahue ........................................... 229

Molecular Pathogenesis and Diagnostics of Bladder Cancer
Anirban P. Mitra and Richard J. Cote ........................................... 251

Ovarian Cancer
Kathleen R. Che and Ie-Ming Shih ........................................... 287


Drosophila Models of Neurodegenerative Diseases
Bingwei Lu and Hannes Vogel ................................................................. 315

Serrated Polyps and Colorectal Cancer: New Pathway to Malignancy
Amy E. Noffsinger .................................................................................. 343

Nod-Like Receptors: Role in Innate Immunity and Inflammatory Disease
Grace Chen, Michael H. Shaw, Yun-Gi Kim, and Gabriel Nuñez ............. 365

Tumor Suppressors, Chromosomal Instability, and Hepatitis C
Virus–Associated Liver Cancer
David R. McGivern and Stanley M. Lemon ........................................... 399

The Immunopathogenesis of Rheumatoid Arthritis
John B. Imboden .................................................................................... 417

The Pathology of Chronic Obstructive Pulmonary Disease
James C. Hogg and Wim Timens ............................................................. 435

Linking the Cellular Functions of BRCA Genes to Cancer
Pathogenesis and Treatment
Ashok R. Venkitaraman ........................................................................ 461

Regulation of Hepcidin and Iron-Overload Disease
Pauline L. Lee and Ernest Beutler .......................................................... 489

The Brainstem and Serotonin in the Sudden Infant Death Syndrome
Hannah C. Kinney, George B. Richerson, Susan M. Dymecki, Robert A. Darnall, and Eugene E. Nattie .......................................................... 517

Molecular Pathogenesis of Cutaneous Melanocytic Neoplasms
Nageatte Ibrahim and Frank G. Haluska ................................................. 551

Indexes

Cumulative Index of Contributing Authors, Volumes 1–4 ...................... 581

Cumulative Index of Chapter Titles, Volumes 1–4 .................................. 583

Errata

An online log of corrections to Annual Review of Pathology, Mechanisms of Disease articles may be found at http://pathol.annualreviews.org