# Review
# Developments in the scientific understanding of rheumatoid arthritis

Jörg J Goronzy and Cornelia M Weyand

Lowance Center for Human Immunology and Rheumatology, Department of Medicine, Emory University School of Medicine, Atlanta, GA 30322, USA

Corresponding author: Jörg J Goronzy, jgoronz@emory.edu

## Abstract

Rheumatoid arthritis (RA) is recognized to be an autoimmune disease that causes preclinical systemic abnormalities and eventually leads to synovial inflammation and destruction of the joint architecture. Recently identified genetic risk factors and novel insights from animal models of spontaneous arthritis have lent support to the concept that thymic selection of an autoreactive T-cell repertoire is an important risk factor for this disease. With advancing age, defects in the homeostatic control of the T-cell pool and in the setting of signaling thresholds lead to the accumulation of pro-inflammatory T-effector cell populations and loss of tolerance to neo-antigens, such as citrullinated peptides. As the breakdown of tolerance to modified self-antigens can precede synovitis by decades, repair of homeostatic defects may open a unique window of opportunity for preventive interventions in RA. The end result of RA, destruction of cartilage and bone, appears to be driven by cytokine- and cell contact-induced activation of synoviocytes and monocytic cells, some of which differentiate into tissue-destructive osteoclasts. Targeting mediators involved in this process has greatly improved the management of this chronic inflammatory syndrome.

## Introduction

Understanding of the chronic inflammatory disease rheumatoid arthritis (RA) has evolved considerably during the past decade. Introduction of novel therapeutic strategies has had a major impact not only on how we treat affected patients but also on how we conceptualize the disease process [1]. RA has served as a model to enhance our knowledge of the pivotal role played by cytokines during the effector stages of human disease; has been instrumental in clarifying the place of cytokines in the maintenance and chronicity of inflammation; and has been instrumental in deciphering the involvement of cytokine networks in tissue damage [2,3].

This enormous progress was made possible by the introduction of cytokine-directed therapies, the prototype of which is the neutralization of tumor necrosis factor (TNF)-α activity [4]. Inhibition of IL-6, another apparently effective treatment, is entering clinical application [5], and additional cytokine inhibitors are currently in clinical studies [6]. The availability of this therapeutic armamentarium has fundamentally changed the management of RA and has re-emphasized the primarily inflammatory character of this autoimmune syndrome. In support of the concept that cytokine-driven inflammation and not uncontrolled proliferation of synoviocytes is the primary disease process, inflammatory markers have emerged as the best predictors of clinical outcome [1].

As much as we have learned about the cytokines that are involved in the disease process and can be therapeutically targeted, our understanding of the upstream mechanisms that eventually lead to a destructive inflammatory reaction has received less attention. However, there is agreement within the scientific community that changing RA from a manageable into a curable disease entity will eventually require identification of etiologic factors and initiating pathways. RA is not a prototypic autoimmune disease such as type 1 diabetes mellitus or autoimmune thyroid disease, in which a failure in tolerance to a tissue-specific antigen results in selective and organ-destructive immune responses. Although the synovial inflammation is clinically prominent, the disease is systemic at all stages. The two most characteristic auto-antibodies, rheumatoid factor and antibodies to citrullinated peptides, are directed at common antigens widely expressed outside of the joint; their presence can precede synovial inflammation by decades [7,8]. Systemic complications manifest themselves as rheumatoid nodules, rheumatoid vasculitis, Felty's syndrome, or interstitial lung disease.

Interestingly, major organ manifestations of RA have become less frequent in clinical practice [9]. This decline in incidence

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HLA = human leukocyte antigen; hTERT = human telomerase reverse transcriptase; IFN = interferon; IL = interleukin; MHC = major histocompatibility complex; NKG2D = natural-killer group 2, member D; RA = rheumatoid arthritis; RANK = receptor activator of nuclear factor-κB; SNP = single nucleotide polymorphism; TCR = T-cell receptor; TNF = tumor necrosis factor; TREC = T-cell receptor excision circle.

started in the 1980s, before aggressive treatment of RA was introduced and the advent of biologics, suggesting that not only treatment but also changes in lifestyle and environment influence the clinical pattern of RA. As we move from successful palliative management to the goal of curative and preventive interventions, it is important to understand the mechanisms that initiate the disease and to identify the endogenous and environmental determinants that cause pathology upstream of synovial inflammation.

## Clues to RA pathogenesis
### Genetic risk factors in humans
Genetic factors have a substantial influence on determining the susceptibility to develop RA. Twin studies have demonstrated a fourfold higher concordance rate in monozygotic (15%) than in dizygotic (3.6%) twins [10]. The risk in siblings of patients compared with that in a 'normal' population has been estimated at between two- and 17-fold greater [11]. It is now clear that the relative risk for each genetic polymorphism is rather minor, making it unlikely that individual genetic polymorphisms will gain value in RA diagnosis or in identifying healthy individuals at risk. Also, preliminary studies, mostly of anti-TNF-treated patients, have indicated that large cohorts will be necessary to identify genetic polymorphisms that correlate with treatment response and that predictive power in individual cases will be small [12]. The primary promise of identifying disease-associated genes lies in the potential to define pathways that are important in disease pathogenesis. Recent advances made in linkage and in genome-wide association studies and the availability of large RA cohorts have allowed several novel risk genes to be identified. Although none of them was an obvious candidate gene, it is of interest to note that all of the confirmed disease-associated genes represent genes that are involved in immune responses, again emphasizing the immune pathogenesis of the disease.

The only genetic region that has emerged in linkage and in genome-wide association studies in all ethnic groups is the major histocompatibility complex (MHC) region [13]. The strength of the association varies significantly, depending upon the ethnic group [14], but the shared epitope hypothesis - first formulated during the 1980s [15] - has held up. Human leukocyte antigen (HLA)-DRB1 alleles expressing the amino acid sequence stretch Q/R-K/R-R-A-A at positions 70 to 74 are the major risk factor within the MHC region in individuals of diverse ethnic origin; for example, *HLA-DRB1*0101*, *\*0401*, and *\*0404* in individuals of European ancestry or *\*0405* and *\*0901* in Asians. In addition to disease-associated alleles, a disease protective HLA-DRB1 polymorphism (DERAA) may exist; however, this notion of an active protective mechanism versus the absence of a disease risk gene is difficult to ascertain. HLA alleles appear to be more closely associated with the presence of antibodies to IgG Fc or to citrullinated peptides than with RA itself [16,17], suggesting that the polymorphisms primarily predispose to autoantibody production and that seronegative RA is funda-

mentally different from seropositive RA. Only *DRB1\*0401* and *\*0405* carry relative risks greater than 3; all other epitope-positive alleles contribute only a minor risk. Overall, it has been estimated that HLA polymorphisms account for 30% to 50% of the genetic load [18].

All other disease risk genes identified so far confer relative risks of about 1.3 to 1.5. Although these disease risk genes have been confirmed in independent studies, their association is not universal but occurs only within the context of particular ethnic backgrounds. A polymorphism within the *PTPN22* gene has been unequivocally associated with RA in several studies in Canada, Europe, and the USA [19-21]. The polymorphism is responsible for an amino acid exchange from an arginine to a tryptophan within the coding region of the gene. This polymorphism represents a minor allele that is infrequent in healthy control individuals as well as in the RA population (8.7% versus 14.4%) [22]. A disease association in the Japanese population has not been found [23]; in fact, the polymorphism does not exist in Asians [24]. The PTPN22 protein is a tyrosine phosphatase that exerts negative feedback regulation in T-cell receptor (TCR) signaling [25]. The phosphatase binds to the regulatory kinase Csk; the complex of PTPN22 and Csk is responsible for terminating TCR signaling by phosphorylating Lck at position 505 and dephosphorylating Lck at position 394. The genetic polymorphism acts by directly modifying the phosphatase activity of PTPN22 and/or controlling its binding to Csk [26].

Surprisingly, studies have shown that the polymorphism is a gain-of-function mutation [27] (the carriers of the polymorphism are more likely to terminate TCR signaling), which is counterintuitive as a risk factor for an autoimmune disease. It has therefore been proposed that the underlying mechanism does not involve signaling of peripheral T cells, but that the signaling defect impairs negative thymic selection, resulting in the selection of an autoreactive repertoire. In this model, a defect in central tolerance sets the stage for the eventual development of a chronic inflammatory disease. This model not only applies to RA but also to a number of autoimmune syndromes, including type 1 diabetes mellitus, systemic lupus erythematosus, juvenile idiopathic arthritis, Graves' disease and vitiligo, each of which has been found to be associated with the *PTPN22* polymorphism [28].

A genetic polymorphism of peptidylarginine deiminase 4 (*PADI4*) is important in the Asian population [29-31]. This polymorphism could very well play a role in the citrullination of proteins and therefore influence the development of antibodies to citrullinated antigens, which are among the autoimmune hallmarks of RA. Although this polymorphism also exists in Caucasian populations, an association with RA could not be demonstrated [32-35]. Because antibodies to citrullinated antigens are a general phenomenon in RA, independent of ethnicity, the meaning of this discrepancy is currently unclear.

Three additional risk regions have been identified during the past year. All three of these genetic regions have in common that they confer a 50% risk increase and represent a single nucleotide polymorphism (SNP) close to an immune response gene. The functional implications of these disease risk regions are unclear, and it is therefore premature to develop pathogenetic models. Linkage studies and subsequent SNP mapping identified a region on chromosome 1q in the third intron of the *STAT4* gene [36]. The association originally identified in a North American study was confirmed in a Swedish and in a Korean cohort [37]. An influence of the polymorphism on *STAT4* transcription or function could have implications for signal calibration of a number of cytokine receptors, including type I IFN, IL-12, and IL-23. Whole-genome association studies identified two additional regions, one on chromosome 6q23 and one on chromosome 9q33-34. One SNP on chromosome 6q23 is between the genes encoding oligodendrocyte lineage transcription factor 3 and TNF-α-induced protein 3 [38,39]. TNF-α-induced protein 3, if confirmed to be the relevant variant, would be of interest because it functions as a negative regulator of nuclear factor-κB activation in response to Toll-like receptors, and mice deficient for TNF-α-induced protein 3 develop an auto-inflammatory syndrome [40-42]. The second region, on chromosome *9q33-34*, was confirmed in independent candidate gene studies and maps between the complement 5 gene and the TNF receptor-associated factor 1 [43-45]. The latter functions as a signaling molecule of receptors of the TNF receptor superfamily, including type 2 TNF receptor and CD40 ligand. Again, it remains to be determined whether functional polymorphisms can be identified. CD40 has also been identified as a disease-associated gene [46].

The common theme that emerges from these genetic linkage and association studies is the possible involvement of signaling pathways transmitting activation signals into cells of the immune system (Figure 1). The major genetic risk factor continues to be shared epitope-expressing HLA-DRB1 alleles, which function in triggering the TCR. The minor genetic risk factors identified thus far are mostly related to signal calibrations, either to antigen recognition by TCRs or B-cell receptors, or in response to certain cytokines. The genetic polymorphisms are neither necessary nor sufficient for disease development because they are too infrequent and the associated risk is low; however, they indicate that these pathways are of importance in rendering an individual susceptible to RA development.

## Mouse models of arthritis
Several mouse models with arthritis of spontaneous onset have become available during the past decade. Earlier animal models were based on the premise that RA results from an adaptive immune response to a joint-specific antigen. Models such as collagen-induced or proteoglycan-induced arthritis have been very helpful in providing evidence for the paradigm that autoimmunity to joint-specific antigens can lead to

**Figure 1**
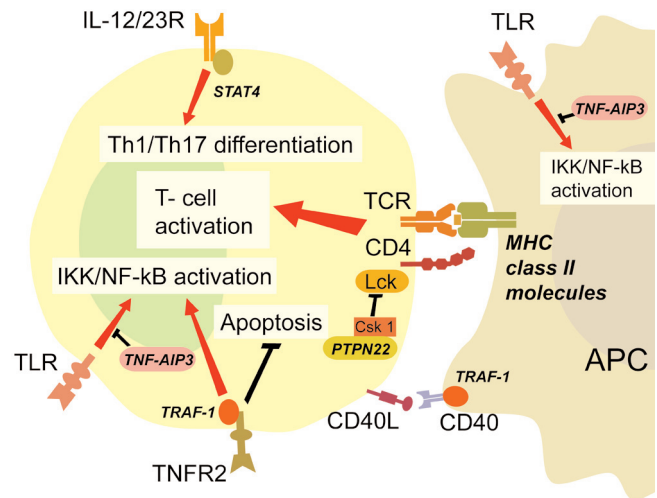


Shown is a T cell-APC interaction to illustrate biologic pathways involving rheumatoid arthritis-associated genes (indicated in italics). APC, antigen-presenting cell; IKK, IκB kinase; MHC, major histocompatibility complex; NF-κB, nuclear factor-κB; TCR, T-cell receptor; Th, T-helper; TLR, Toll-like receptor; TNFR2, type 2 TNF receptor.

arthritis [47,48]; these models have allowed investigators to study effector mechanisms in the arthritic process and to test therapeutic interventions. In contrast to spontaneously occurring arthritis models, models of induced arthritis are already built upon the notion that synovial inflammation is mediated by a response to a particular autoantigen, and therefore they do not permit study of upstream mechanisms. One of the first models that exhibited spontaneous onset of arthritis was the TNF-α transgenic mouse [49]. The finding that the over-production of TNF-α alone is sufficient to induce erosive arthritis emphasizes the sensitivity and response of synoviocytes to circulating cytokines, a concept that was first introduced by Feldman and Maini [4] and is now the basis for the treatment of human disease with anti-TNF inhibitors.

Four recently discovered mouse strains provide opportunities to decipher mechanisms upstream of synoviocyte activation. The spontaneous occurrence of arthritis in these models was unexpected, but all four models point toward T-cell repertoire selection as a critical determinant in initiating and sustaining arthritis (Figure 2). In the first model, Mathis and colleagues [50] crossed a TCR transgene onto the NOD background. This TCR transgene happened to recognize a ubiquitously expressed protein, namely glucose-6 phosphate isomerase, but thymic negative selection failed to purge this autoreactive receptor from the T-cell repertoire [51]. The mice, known as K/B×N mice, develop an early onset, rapidly progressive arthritis that is mediated by autoantibodies that bind glucose-6 phosphate isomerase. Arthritis can be transferred by anti-bodies, clearly demonstrating that the generation of a

**Figure 2**
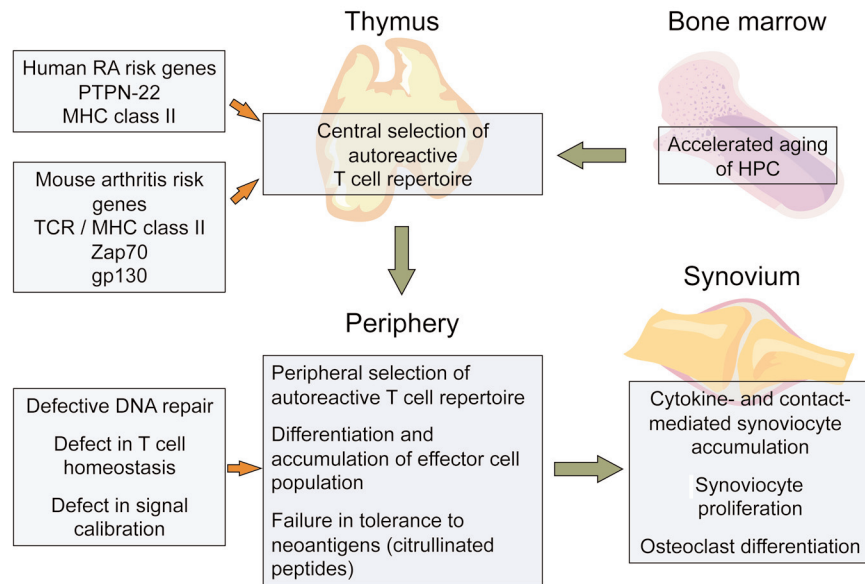


Central and peripheral T-cell selection and differentiation as risk factors for synovial inflammation. HPC, hematopoietic progenitor cells; MHC, major histocompatibility complex; TCR, T-cell receptor.

particular autoantibody due to defective thymic selection can induce disease. Unfortunately, autoantibodies to glucose-6 phosphate isomerase do not appear to play a role in RA, therefore limiting the applicability of this model beyond the notion that thymic selection may be important.

Similar conclusions can be drawn from a second TCR transgene model. Caton and colleagues [52] engineered mice that expressed an influenza hemagglutinin antigen in combination with a transgene for hemagglutinin-reactive TCR. Different strains were constructed carrying TCR with varying affinity for the antigen [52,53]. Mice that expressed the low-affinity TCR failed negative selection and developed erosive arthritis, again illustrating the notion that inclusion of autoreactive TCR in the T-cell repertoire can eventually lead to synovial inflammation, mimicking the conditions in RA.

Whereas the investigator teams led by Mathis and Caton used TCR transgenic mice to study central tolerance mechanisms and unexpectedly observed RA-like disease, investigators at the laboratory of Hirano [54] engineered mice that lacked a negative feedback loop in gp130 signaling, creating conditions of unopposed cytokine signaling. gp130 is a necessary constituent of a class of cytokine receptors that bind IL-6, leukemia-inhibiting factor, oncostatin M, and IL-11. A single point mutation at position 759 of gp130 prevents recruitment of negative regulatory molecules, such as SHP-2 and SOCS-3, thus causing sustained signaling. Mice transgenic for this gp130 variant develop an erosive arthritis. Defective cytokine signal calibration as a risk factor for

arthritis would be consistent with synovial fibroblasts being highly sensitive to cytokine action, similar to the TNF-hyper-producing mice. However, subsequent studies have shown that the pathogenesis in the gp130 mutant transgenic mice is dependent on T cells, because arthritis does not occur in RAG-deficient mice and includes polyclonal T-cell and B-cell stimulation with the production of rheumatoid factor and anti-nuclear antibodies. Subsequent studies of TCR transgenic mice expressing the gp130 mutant again described a defect in negative thymic selection.

A defect in thymic function has been postulated also to cause the arthritis in the SKG mouse model. SKG mice have a spontaneously incurred loss-of-function mutation in the *Zap70* gene [55]. TCR signaling is therefore attenuated. Using appropriate TCR transgenic mouse systems, positive, as well as negative, selection in the thymus was found to be impaired. Both defects may contribute to the emergence of peripheral autoimmunity [56]. Defective negative selection would bias the TCR repertoire toward autoreactivity. Defective positive selection may cause lymphopenia, which has been shown to be a risk factor for autoimmunity [57,58]. Peripheral T cells in the SKG mouse continue to be hypo-responsive, but adoptive transfer of these T cells into T/B-cell-deficient mice reproduces joint inflammation, clearly demonstrating that the T cells are sufficient to transfer disease. Given their low responsiveness, there must be a strong peripheral stimulus to overcome peripheral tolerance. In support of this notion, mice maintained in germ-free conditions do not develop disease. In fact, fungal infection

and the IL-6-mediated development of T-helper-17 response appear to be instrumental in disease development [56].

None of the genetic polymorphisms that cause disease in mice has been associated with RA. However, it is striking that all of these disease models involve TCR threshold calibration and thymic selection. Of the genes associated with RA, *HLA-DRB1* and *PTPN22* are also directly involved in TCR stimulation. In particular, the *PTPN22* polymorphism attenuates TCR signaling and may be associated with defective negative selection.

The mouse models have the potential to improve our understanding of how misguided T-cell responses translate into synovial inflammation and the other organ manifestations in RA patients. In the K/B×N model, this transition is made by the induction of autoantibodies to a joint nonspecific antigen; the disease can be rapidly transferred by glucose-6 phosphate-specific autoantibodies. For the SKG and the gp130 mutant models, specific autoantigens have not been identified. Instead, these mice have a broadly autoreactive repertoire. Although the TCR signaling capacity is low, T cells develop into polyclonal effector T cells that mediate arthritis. Based on these animal models, Cope and colleagues [59,60] have postulated that a similar mechanism is functional in RA and that autoreactive T cells that are generally low reactive, but can be activated to develop into very potent effector cells, hold the pathogenetic key to RA. One factor that calibrates the TCR threshold in these T cells and enables their differentiation into effector T cells may be lymphopenia and compensatory homeostatic proliferation [61].

### T-cell abnormalities in RA patients

In the majority of patients, RA occurs at an age when formation of the TCR repertoire has been concluded for many decades and thymic function is already severely reduced or has even completely ceased. Although possibly a predisposing factor, it is difficult to conceptualize how the process of central tolerance established early in life would only fail after many decades of disease-free survival. Rather, peripheral tolerance appears to be much more important in determining self/nonself distinction in a host older than 50 years (Figure 2).

The most remarkable finding in the T-cell compartment of RA patients is that the T cells exhibit a signature that is reminiscent of accelerated immune aging [62]. Of particular interest, this fingerprint of premature immune senescence is not limited to memory T cells but mostly affects antigen-inexperienced naïve T cells. One hallmark of immune aging is the loss of telomeric sequences. Telomeres are repeat sequences at the end of linear chromosomes that are being continuously shortened with each cycle of cellular division unless telomeric ends are replenished by telomerase. Telomeric sequences of proliferating cell populations decline with age; T cells, which are under explicit proliferative

demand, are no exception to this rule. During adulthood telomeres in T cells shorten by 50 to 100 base pairs per year [63]. In patients with RA, telomeric erosion in T cells is premature; with a loss of about 1,500 kilobases, RA T cells resemble control T cells that are 20 years older [64]. Possible mechanisms include an increased replicative history and accumulated DNA damage arising from a defective DNA repair response in RA. Of interest, age-inappropriate loss of telomeric ends in RA is not limited to T cells, but also involves the myeloid lineage and hematopoietic precursor cells, suggesting a defect in the homeostasis of bone marrow-derived progenitor cells [65,66].

Recent studies have uncovered a defect in telomeric repair in RA T cells. Specifically, naïve T cells undergoing priming typically upregulate telomerase to repair the chromosomal ends. This induction of telomerase is blunted in RA T cells because of transcriptional repression of the human telomerase reverse transcriptase (hTERT) component of the enzyme telomerase [67]. hTERT deficiency renders T cells from RA patients more susceptible to apoptosis, identifying a broader role for this enzyme in regulating T-cell fate. Knockdown of hTERT in healthy T cells impaired survival rates. Restoring telomerase activity in RA T cells rescued such cells from excessive apoptosis. In essence, telomeres and the telomeric surveillance machinery emerge as critical regulators of T-cell death and life. Inappropriate culling of T cells during the priming process potentially aggravates a vicious cycle of increased cell death, lymphopenia, compensatory homeostatic cell proliferation, and cellular senescence. Controlling nuclear integrity now emerges as a novel theme in assessing cell fate decisions in T cells, cells that are basically programmed to undergo cycles of expansion and contraction, with some of them living for extended time periods.

A recent study has shed light on defects in DNA repair mechanisms in RA T cells, linking the accumulation of damaged DNA to deficiency in the ataxia telangiectasia mutated (ATM) surveillance and repair pathway. Again, the inability of RA T cells to effectively repair DNA breaks was associated with increased cell death, straining T-cell regenerative mechanisms [68]. In support of this interpretation, TCR excision circles (TRECs) containing T cells are reduced in RA patients [64]. TRECs are DNA episomes generated during TCR rearrangement [69]. High numbers of TREC-positive T cells therefore reflect thymic activity, whereas decreased numbers are indicative of T-cell loss that is not compensated for by thymic production of new T cells [70]. Telomeric erosion, increased susceptibility to cell death due to defective telomerase activity and DNA repair mechanisms, as well as peripheral loss of TREC-positive cells, are all consistent with a model in which RA patients have a history of lymphopenia and accelerated homeostatic proliferation [61].

Homeostatic proliferation of naïve CD4+ and CD8+ T cells is dependent on recognition of MHC class II and class I

molecules, respectively, and will therefore eventually be associated with peripheral selection of a T-cell repertoire with high affinity to self [71]. In support of this interpretation, the diversity of the naïve TCR repertoire in patients with RA is contracted by a factor of about 10 [72]. Thus, in addition to defective central thymic selection, peripheral selection over the years could set the stage for an autoimmune disposition. This model would also fit with the observation that the best characterized autoimmune responses in patients with RA are directed at neoantigens. A pathognomonic autoantibody in RA patients is that directed against citrullinated peptides, which are generated mostly in matrix molecules by converting an arginine to a citrullin [73]. Even the second hallmark of RA, namely the antibody response to the constant region of IgG measured as rheumatoid factor, may be directed to neoantigens because glycosylation differences of the Fc fragment have been shown to be important in autoantibody recognition [74].

Peripheral repertoire selection is only one of the mechanisms by which lymphopenia and compensatory homeostatic pro-liferation increase the risk of autoimmunity. In many spon-taneous animal models of autoimmunity, a transient, often minute state of lymphopenia is a prerequisite for developing autoimmune disease. This was first described in the NOD mouse model of immune-mediated diabetes [57]. Develop-ment of autoimmune phenomena in NOD mice, which are slightly lymphopenic at a young age, is dependent on IL-21-driven homeostatic proliferation. Similarly, Calzascia and coworkers [58] demonstrated that homeostatic proliferation, in this case in response to IL-7, released autoreactive CD4+ cells from inhibitory networks. Lymphocyte depletion largely enhanced the activity of CD4+ T cells to license dendritic cells and to initiate a cascade of CD4+ and CD8+ auto-reactive responses, eventually leading to disease. As one possible mechanism, homeostatic proliferation lowers the TCR threshold that antigen recognition must surpass to deliver an activating signal. Recent studies have provided direct evidence supporting a model in which TCR calibration is altered in RA patients. RA T cells have a spontaneously hyper-responsive Ras/Raf-MEK-ERK (Ras/Raf-mitogen-activated protein kinase kinase/extracellular signal-regulated kinase) module. As originally proposed by Germain and colleagues [75,76], increased extracellular signal-regulated kinase activity inhibits a negative feedback loop in response to TCR stimulation and therefore lowers the TCR activation threshold, eventually breaking tolerance. Hyperactivity of this pathway in healthy T cells can be induced by exposure to homeostatic cytokines [77]. Within the panel of homeostatic cytokines, IL-7 appears to be reduced in RA [78]; however, IL-15 and IL-21 are increased [79,80], and this increase appears to precede disease development.

Excessive proliferative turnover and premature senescence not only change the phenotype and function of naïve peripheral CD4+ cells, but also have consequences for the memory

subpopulations. Again, these appear to be global phenomena and not limited to a small fraction of expanded antigen-specific T cells. Telomeres in the RA memory population are shortened, and dominant oligoclonal T-cell populations are more frequently detected [64,81-83]. These populations have a phenotype of effector memory or even terminally differentiated effector cells. CD28 and CD27 are lost [84], expression of lymphocyte function-associated antigen-1 (LFA-1) is increased [85], and the chemokine receptor profile is consistent with the differentiation state of effector cells [86]. End-differentiated memory T cells in RA frequently acquire expression of the fractalkine receptor $CX_3CR1$ (chemokine [C-X$_3$-C motif] receptor 1) [87], as well as regulatory receptors that are usually found on natural killer cells, such as natural-killer group 2, member D (NKG2D) and killer immunoglobulin-like receptors [88-90]. In the periphery, these cells are high producers of effector cytokines and are capable of perforin-mediated cytotoxicity [91,92]. Their frequency in peripheral blood correlates with disease severity and the presence of extra-articular manifestations including co-morbidities such as cardiovascular disease [93-95]. Because of their phenotype and functional properties, these cells are prone to be tissue invasive and to be regulated by environmental cues (cytokines; stress-induced ligands binding to NKG2D; MHC class I molecules engaging killer immunoglobulin-like recep-tors) rather than the classical costimulatory signals.

It is conceivable and even likely that the forces that drive remodeling of the T-cell compartment also affect the frequency and function of regulatory T cells. Depletion or functional degeneration of regulatory T cells could cause a tolerance defect and favor inflammatory responses. The data on regulatory T cells in RA thus far are conflicting. The frequencies of these cells appear to be increased, but their function is compromised, possibly secondary to the effects of TNF-$\alpha$ [75-77,96].

In the synovial tissue, most T cells exhibit features of lympho-cyte exhaustion. Characteristic is a loss of the CD3 $\zeta$-chain [97]. Over-expression of PD1, which has been implicated in lymphocyte exhaustion with chronic viral infections [98], has not yet been described. Several factors probably contribute to the exhausted state of synovial T cells, including chronic TCR stimulation and the redox state in the synovial tissue [99,100]. It is also possible that synovial T cells are not truly exhausted but activated by cytokines. Cytokine activation generates an effector function profile that may in part be responsible for the synovial inflammation [101]. In fact, some of these features are reversible upon TNF withdrawal [102]. Importantly, T-cell exhaustion should not be mistaken for T-cell anergy; the two states have different transcriptional profiles [103].

## Characterization of novel autoantigens
Production of autoantibodies to the Fc portion of IgG, known as rheumatoid factors, has been the serologic hallmark of RA

for the past five decades. Despite considerable effort, attempts to identify autoantibodies to joint-related antigens have yielded inconsistent results. Antigens that are now recognized as relatively specific targets for autoantibodies include the perinuclear factor and keratin. In 1998, van Venrooij and colleagues [104] first reported that these antibodies were directed against deiminated peptides. Subsequent studies showed that the epitopes preferentially recognized in RA are citrullinated peptides of a number of different matrix proteins including fillaggrin, keratin, fibrinogen, and vimentin [73,105]. These antibodies can be measured through their recognition of cyclic citrullinated peptides, now commonly used in clinical practice. Based on these autoantibody profiles, RA patients fail to maintain or induce tolerance to post-translational modifications of common cellular proteins.

Of note, another post-translational modification, glycosylation of IgG Fc, has been implicated in the generation of rheumatoid factors. IgG Fc glycosylation defects are not specific to RA but occur in a number of inflammatory conditions [106]. Similarly, citrullination is not specific for RA or for the synovium, but occurs in most individuals with aging to a varying degree and in numerous tissues. Quantitative difference in the degree of citrullination may play a role in the initiation of an immune response. The finding that Asian RA patients are more likely to have inherited an enzyme variant of PADI-4 (peptidylarginine deiminase 4), the enzyme that is responsible for arginine deimination and citrullination, is consistent with this notion. In addition, smoking, which has been proposed to represent an environmental risk factor for RA, has been correlated with increased citrullination in lung tissue and generation of citrullinated peptide-specific antibodies [107]. Smoking induced an anti-cyclic citrullinated peptide response only in individuals carrying a shared epitope allele, which fits with the immune response gene hypothesis of the HLA-DRB1 association of RA [108]. For reasons unclear, an impact of smoking was observed in Europe but not in the USA [107,109,110].

However, the primary defect in patients with RA appears not to be a defect in post-translational modification but a defect in inducing or maintaining peripheral tolerance, which is very much in line with the global changes in the T-cell compartment observed in patients with RA described above. If RA patients have a broad tolerance defect, then autoantibody responses to an increasing array of self-antigens must be expected. Indeed, Auger and colleagues [111] identified antibodies to PADI-4 and several signaling molecules, including BRAF (v raf murine sarcoma viral oncogene homologue B1 catalytic domain), PKCβ1 (protein kinase Cβ1), and PIP4K2C (phosphatylinositol 4 phosphate 5 kinase type II γ), using protein arrays. Goeb and colleagues [112] used mass spectrometry to identify antibodies to glycolytic enzymes and to chaperones. Confirmatory studies and epitope mapping are needed, but preliminary data indicate that some but not all

of these immune responses are once again directed against citrulline modifications.

## Translating systemic autoreactivity into synovitis

Most of the abnormalities in the adaptive immune system in RA are systemic in nature, but in patients with established disease synovial manifestations clearly dominate. The question of how systemic abnormalities are translated into inflammation of the synovium is one of the major challenges in elucidating RA pathogenesis. Antibodies to citrullinated peptides and rheumatoid factors can predate the onset of joint manifestations by more than a decade [7,8,113], clearly demonstrating that they are not a consequence of disease and alone are not sufficient to induce disease. This prodromal stage appears to be longer among those patients who develop disease later in life [114], again emphasizing the role played by time and aging in pathogenesis. Similar to autoantibodies, a case-control study from the Women's Health Study and the Nurses' Health Study [115] found that elevated serum levels of soluble TNF receptor II (as a proxy for TNF-α) and of IL-6 predated disease by up to 12 years. Similar conclusions apply to other cytokines, such as IL-15. In essence, autoimmunity and inflammation exist long before inflammatory lesions are established in the synovial membrane. Epidemiologic data currently do not support the notion of identifiable precipitating events, such as a trauma or an infection, which would turn systemic immune abnormalities into localized tissue inflammation. Rather, it appears that either cumulative changes or stochastically occurring instabilities precipitate the onset of symptoms, suggesting that there is a window of opportunity for preventive interventions.

What is the role played by antigen-specific responses in synovitis? Citrullinated antigens exist in the synovial tissues, but they are hardly specific. An immune response to citrullinated antigens can induce arthritis, as demonstrated in HLA-DR4-IE transgenic mice with citrullinated fibrinogen [116]. In contrast to RA, this arthritis was nonerosive. In the animal model of collagen-induced arthritis, the immune response to citrullinated antigens emerged as an important co-factor to amplify disease manifestations, but by itself it was not sufficient to induce disease [117]. Adoptive transfer of antibodies to citrullinated collagen frequently induced arthritis in naïve mice, however, only when co-administered with antibodies to unmodified collagen [118].

The best evidence for antigen-specific responses in synovial tissue comes from the synovial pathology. The synovial tissue is rich in dendritic cells, which can present antigen and support the activation of T cells [119,120]. About a quarter of the patients have lymphoid follicles with germinal centers, sophisticated structures that facilitate antigen recognition by B and T cells presented by follicular and myeloid dendritic cells [121]. Developing these structures may be a decisive step in sustaining an autoimmune response in the tissue [122]. Important mediators associated with synovial germinal

center formation are lymphotoxin-$\alpha_1\beta_2$, IL-7, a proliferation inducing-ligand (APRIL), and CXCL13 (chemokine [C-X-C motif] ligand 13) - cytokines that have also been implicated in the generation of secondary lymphoid structures [123]. Somatic hypermutation of immunoglobulin genes demonstrate the full functionality of these follicles [124]. The antigen recognized by T cells on myeloid dendritic cells and presented by follicular dendritic cells to B cells does not need to be locally produced, but can be taken up by follicular dendritic cells from the bloodstream and can be brought into the synovial tissue by migrating dendritic cells.

Most RA patients do not have germinal centers and do not exhibit unequivocal evidence of antigen recognition in the synovial tissue, although subdued antigen-specific stimulation, as is often seen with exhausted lymphocytes, remains possible. Lymphocytes are scattered in the synovial sublining layer, and T cell-derived cytokines, with the exception of TNF-$\alpha$ and IL-17, are not abundant. IL-17 was originally detected in human synovium from RA patients [125]. Its pathogenetic importance in chronic inflammation has been suggested in a variety of murine model systems. It is attractive to speculate that T cell-derived IL-17 drives the synovial fibroblast activation and cytokine secretion that are characteristic of the rheumatoid synovium [126]. The role played by IFN-$\gamma$ as a T cell-derived cytokine is less clear in RA. Many T cells isolated out of the environment of the rheumatoid synovitis are able to produce IFN-$\gamma$, and studies have shown that the survival of macrophage-like synoviocytes is dependent on IFN-$\gamma$ production [127]. Furthermore, in humans, contrary to mice, IL-17 and IFN-$\gamma$ are not mutually exclusive, and IFN-$\gamma$/IL-17 double-producing T cells are not infrequent. However, production of IFN-$\gamma$ *in situ* is difficult to demonstrate, and treatment of RA patients with IFN-$\gamma$ has at least not led to disease exacerbation. Synoviocytes are extremely sensitive to cytokine action. Given the multitude of pro- and anti-inflammatory cytokine activities in the synovial tissue, it is difficult to predict hierarchical organization. As recently reviewed, many different cytokines are or will soon be targeted in clinical studies, which will provide insights into the relative contributions made by individual cytokines to the disease process [2,3,6].

In addition to cytokines, the inflammatory infiltrate influences resident synoviocytes through contact-dependent mechanisms (Figure 3). Dayer and colleagues [128] first reported that T cells regulate the production of inflammatory cytokines and metalloproteinases by fibroblasts through cell-to-cell contact. In parallel, direct T cell-synoviocyte interaction inhibits the production of matrix proteins. A number of receptor-ligand interactions in the inflamed synovium have been identified [79,129]. Some of these receptors are constitutively expressed on tissue-infiltrating inflammatory cells, and the mere presence of a cellular infiltrate is sufficient to elicit the responses. Others are activation dependent; however, even for T cells, the activation may not require antigen recognition, but merely cytokine exposure.

NKG2D and its ligands MIC-A and MIC-B contribute to the persistence of the inflammatory infiltrate [88]. Interaction of lymphocyte function-associated antigen-1 with intercellular adhesion molecule-2 influences synoviocyte fibroblastic activation and survival [85]. The fractalkine receptor expressed on cytotoxic effector and terminally differentiated CD4+ T cells binds to cell-bound fractalkine on synovial fibroblasts [87]. The interaction provides a reciprocal activation signal on T cells and synoviocytes, and the subsequent production of soluble fractalkine is a major growth factor for synovial fibroblasts [130]. Cytokine-activated T cells can also directly interact with synovial fibroblasts through membrane-integrated TNF-$\alpha$ expressed on the T cells [131]. Most important is the expression of receptor activator of nuclear factor-$\kappa$B (RANK) ligand on CD4+ T cells and other infiltrating cells that promote bone erosion through differentiation of monocytic cells into osteoclasts [132]. This list of receptor-ligand interactions is far from inclusive, but it illustrates how the interaction between inflammatory and resident cells develops an architecture that has the ability to be self-perpetuating and tissue damaging.

## How does synovitis cause joint destruction?

If not appropriately treated, RA progressively leads to articular destruction and functional disability. In contrast to many tissue-specific autoimmune diseases, the tissue injury is not directly immune-mediated by antigen-specific antibodies or T cells, but is an active remodeling process of the synovium in response to the inflammatory attack.

At least three components contribute to joint destruction: transformation of the synovium into a proliferative, tissue-invasive pannus; generation of osteoclasts that lead to local resorption of bone; and effects of cytokines on cartilage cell function and survival (Figure 3). The normal synovium is a thin layer of macrophage-like and fibroblast-like synoviocytes without an endothelial or epithelial layer and without a true basement membrane. Synovium produces extracellular matrix, ensures a low-resistance surface at joint interface, and possibly has a role in clearing debris. Cadherin-11 has been identified as a critical organizer in forming the synovial lining [133]. Cadherins mediate homotypic cell-to-cell adhesion and are expressed in fibroblast-like synoviocytes. Absence of cadherin in mice results in a hypoplastic synovium [134], whereas forced expression in fibroblasts *in vitro* produces synovial lining-like structures [135]. Of particular interest, targeting cadherin-11 suppresses arthritis [134]. Cadherin-11-deficient mice do not develop erosive disease; blocking of cadherin-11 by monoclonal antibodies or fusion protein constructs prevents or treats arthritis in the appropriate animal models.

Synovial fibroblasts are very responsive to a large number of stimuli, including cytokines and growth factors produced by the inflammatory infiltrate, and are also responsive to direct receptor-ligand interactions [133]. In addition, the chemokine

**Figure 3**
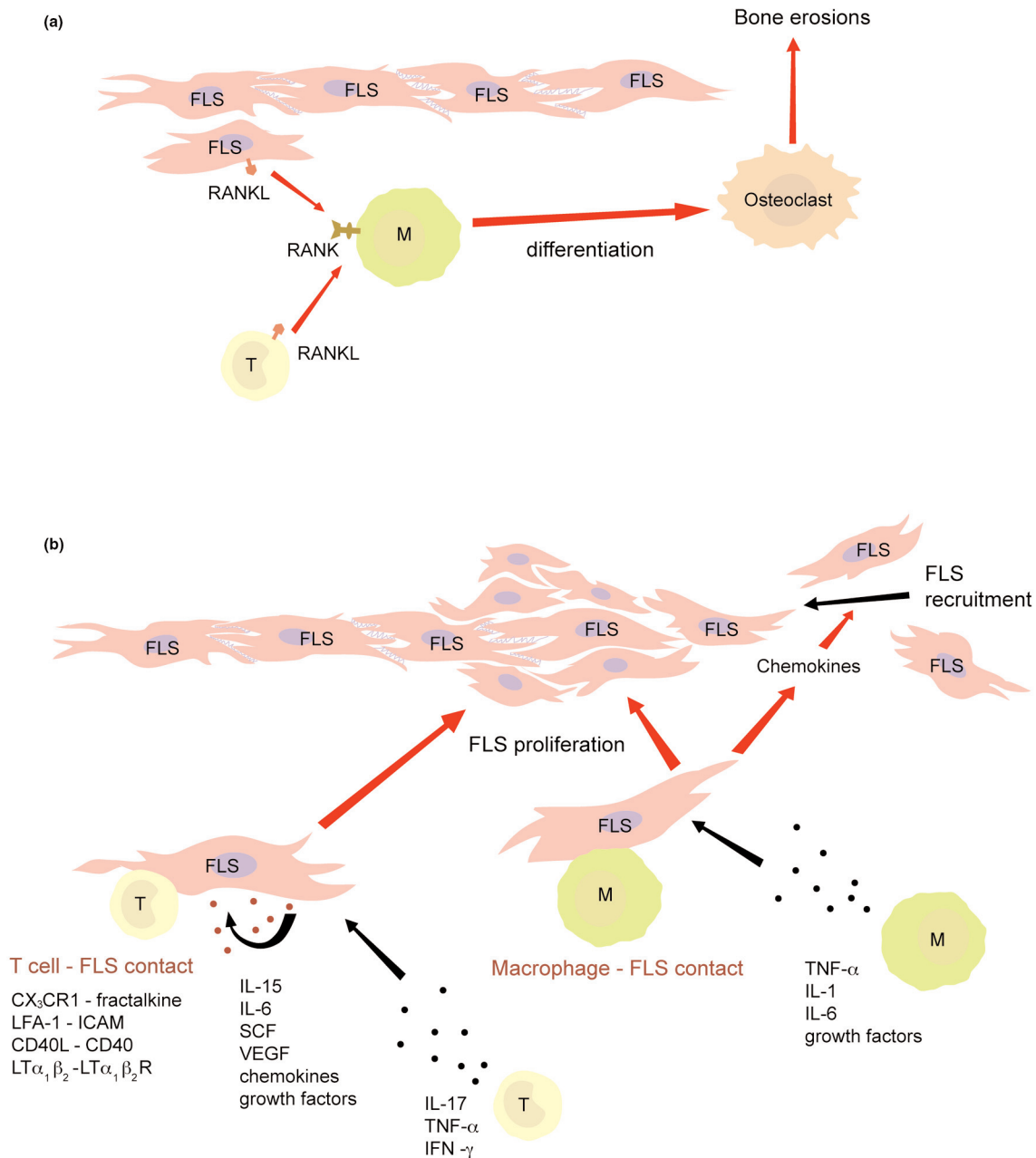


Major tissue destructive pathways in the rheumatoid joint. **(a)** Osteoclast differentiation and **(b)** fibroblast-like synoviocyte (FLS) proliferation. $CX_3CR1$, chemokine [C-$X_3$-C motif] receptor 1; FLS, fibroblast-like synoviocyte; HPC, hematopoietic progenitor cells; ICAM, intercellular adhesion molecule; LFA, lymphocyte function-associated antigen; LT, lymphotoxin; M, macrophage; MHC, major histocompatibility complex; RANKL, receptor activator of nuclear factor-$\kappa$B ligand; SCF, stem cell factor; TCR, T-cell receptor; TNF, tumor necrosis factor; VEGF, vascular endothelial growth factor.

milieu in the synovial inflammation allows for the recruitment of fibroblast-like synoviocytes, as was recently demonstrated in mice chimeric for green fluorescent protein expression in the bone marrow [136]. The synovium in these mice contained a large proportion of bone marrow-derived fibroblasts when arthritis was induced. The precise chemokines that control this recruitment are not known. Recruitment and local proliferation eventually form a hyperplastic membrane of syoviocytes that exhibits tissue-invasive character, targeting bone and cartilage. This neo-tissue has been termed 'pannus'. Several growth factors, including fibroblast growth factor, platelet-derived growth factor, transforming growth

factor-β, and fibronectin, promote synoviocyte proliferation. Studies in mouse models have shown that the tyrosine kinase inhibitor imatinib suppresses arthritis, presumably by inhibiting the platelet-derived growth factor receptor [137]. Because activated and proliferating synovial fibroblasts produce many of their growth factors, the inflammatory response in the synovial membrane induces a self-perpetuating cycle of synovial fibroblast activation and proliferation.

Activated synoviocytes, in particular in the pannus, produce matrix-degrading enzymes, such as aggrecanases and matrix metalloproteinases. Of particular relevance is the membrane type I matrix metalloproteinase, which has been shown to be a crucial promoter of synovial invasion [138]. Silencing of this enzyme reduced the invasiveness of synovial fibroblasts [139]. Matrix resorption and cartilage and bone invasion by synovial fibroblasts requires demineralization by osteoclasts [140]. Formation of osteoclasts is, therefore, an essential component of erosive RA. Osteoclast differentiation is in part driven by RANK ligand, which is expressed on tissue-residing CD4+ T cells and on synovial fibroblasts and is upregulated by a number of proinflammatory cytokines. By engaging RANK, RANK ligand induces the differentiation of monocytic cells into osteoclasts. Osteoclast differentiation can be inhibited by osteoprotegerin, which does not ameliorate the inflammatory signs of disease but can prevent structural damage to the joint.

## Conclusions

The success of anti-cytokine therapy in RA has revolutionized the management of this disease and has provided a paradigm for novel therapeutic avenues in a variety of other inflammatory syndromes. The fact that blocking the action of TNF-α inhibits synovial inflammation and its destructive consequences is conclusive evidence that, at least at the effector stage, excess cytokines are of critical importance in RA. The past decade has seen the identification and molecular characterization of a multitude of cytokines, all of which may make their own contribution to the inflammatory battlefield. The latest in this collection is IL-17, which may or may not prove to be a valuable therapeutic target. Clinical studies in the next decade will decide which of these cytokines is acting at pivotal junctures in synovial inflammation and tissue damage. A selective approach will be beneficial only if cytokines do not act in parallel, because combination therapy blocking several cytokines appears to be unlikely due to the risk for unacceptable side effects as well as cost reasons.

Preventive and curative interventions in RA will depend on identifying mechanisms upstream of the synovial inflammation. The most promising finding paving the way for potential preventive therapy relates to the more recent concept of a systemic prodromal stage preceding synovitis. Several immune pathologies appear to be characteristic for this preclinical phase of RA, including acceleration of immune

**The Scientific Basis of Rheumatology: A Decade of Progress**

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aging, loss of tolerance to neoantigens, and differentiation and accumulation of effector cells with high inflammatory capacity. The results from genetic association and linkage studies, as well as the recently described mouse models of spontaneous arthritis, suggest a role of signal calibration downstream of antigen recognition and triggering of cytokine receptors; understanding these abnormalities may inform novel strategies of stopping RA before it ever reaches its tissue targets.

## Competing interests

The authors declare that they have no competing interests.

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