Review

Cells of the synovium in rheumatoid arthritis

T lymphocytes

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Abstract

Recent findings have substantiated the importance of T lymphocytes to the pathogenesis of rheumatoid arthritis (RA). Here, we review emerging data regarding genetic predisposition, spontaneous animal models of arthritis, and cell-cell interactions that implicate T cells as driving synovial inflammation and joint destruction. Information regarding the proinflammatory role of interleukin-17-producing T cells and the functional state of regulatory T cells both in animal models and in patients with RA is also discussed. In light of the overwhelming evidence that disrupted T-cell homeostasis greatly contributes to joint pathology in RA, the therapeutic potential of targeting activators of pro-inflammatory T cells or their products is compelling.

Introduction

Our understanding of how T lymphocytes participate in the pathogenesis of rheumatoid arthritis (RA) is evolving rapidly with fundamental new insights into basic T-cell biology and the orchestration and regulation of immune responses. The simplistic notion of RA as a homogeneous, clonally driven, T cell-mediated autoimmune disease is outdated, as is the notion that the large numbers of T cells in RA synovium may be irrelevant bystanders. What is replacing these polarized hypotheses is a more integrated view of T cells as a central component of organ-focused immune-mediated pathology, capable of interactions not only with classical cells of the immune system but with tissue-specific cell populations that contribute to inflammation and tissue destruction. RA is emerging as a prototypic disease not only for the study of such interactions but also for the introduction of novel biologic therapies that inhibit these processes. This review will selectively focus on newer and topical aspects of T-cell biology in RA.

T cells and the genetics of RA

RA is a polygenic disease and its most important loci are in the major histocompatibility complex (MHC). The concept of the RA shared epitope, a peptide sequence common to disease-associated human leukocyte antigen-DR (HLA-DR) alleles, remains valid, but the precise mechanism of how the shared epitope predisposes individuals to RA is not yet established. Multiple possibilities have been proposed, most of which focus on recognition of antigen by mature T cells and/or T-cell repertoire differentiation [1]. Recent analyses of the full range of DRB1 alleles have emphasized that sequence variations at amino acids 67 to 74 can encode either susceptibility to or protection from RA and can influence disease severity as well as susceptibility [2-4]. It would be attractive to link protection from RA to immunoregulatory mechanisms, but evidence for such a link is not yet available.

An important epidemiologic study has linked smoking, the shared epitope, and seropositive RA [5]. In this Scandinavian population, the relative risk of seropositive (rheumatoid factor-positive) RA in individuals who smoked and were homozygous for the shared epitope was 15.7. In seronegative RA, neither smoking nor the shared epitope was a risk factor.

Antibodies to citrullinated proteins have become established as an RA feature that is more specific than rheumatoid factor,

APC = antigen-presenting cell; CCP = cyclic citrullinated peptide; CIA = collagen-induced arthritis; CTLA-4 = cytotoxic T lymphocyte antigen-4; FLS = fibroblast-like synoviocytes; Foxp3 = forkhead box p3; HLA-DR = human leukocyte antigen-DR; ICAM-1 = intercellular adhesion molecule-1; ICOS = inducible costimulator; IFN-γ = interferon-gamma; IL = interleukin; IL-1Ra = interleukin-1 receptor antagonist; LFA = lymphocyte function-associated antigen; MHC = major histocompatibility complex; NF-κB = nuclear factor-kappa B; PBMC = peripheral blood mononuclear cell; PD-1 = programmed death-1; PTPN22 = protein tyrosine phosphatase non-receptor type 22; RA = rheumatoid arthritis; RANK = receptor activator of nuclear factor-kappa B; SNP = single-nucleotide polymorphism; TCR = T-cell receptor; TGF-β = transforming growth factor-beta; TNF = tumor necrosis factor; Treg = regulatory T cell; ZAP-70 = zeta-associated protein of 70 kDa.

but information about the role of T-cell responses and genetic factors in this intriguing form of autoimmunity is just beginning to emerge. Auger and colleagues [6] reported that both citrullinated and non-citrullinated fibrinogen peptides bound to a range of HLA-DR molecules, both RA-associated alleles and non-associated alleles, but that T-cell proliferative responses were much more common in RA. These data argue that the shared epitope is not the sole factor governing development of T-cell autoreactivity to citrullinated proteins. Nonetheless, production of antibodies to citrullinated fibrinogen was more common in RA patients who carry HLA-DRB1*0404, a shared epitope-containing allele. Analysis of US and Dutch cohorts with RA found clear linkage of the shared epitope to anti-cyclic citrullinated peptide (CCP)-positive RA but not to anti-CCP-negative RA [7]. The presence of anti-CCP antibodies appeared to fully account for the greater disease severity observed in shared epitope-positive RA. Based on analysis of a cohort of patients with recent-onset inflammatory arthritis, the provocative suggestion has been advanced that the sole role of the shared epitope is to provide the genetic basis for stimulation of T-cell help in anti-CCP antibody formation and that it does not otherwise contribute to the development of RA [8]. Additional studies in cohorts of various ethnicities will help to further test this concept.

Apart from the MHC, the best-established genetic locus that influences RA is the gene *PTPN22* (protein tyrosine phosphatase non-receptor type 22), which encodes Lyp, a tyrosine phosphatase that is expressed in T lymphocytes and that regulates signal transduction from the T-cell receptor (TCR) [9,10]. Substitution of tryptophan for arginine at residue 620 results in a gain-of-function, leading to decreased TCR signaling and decreased production of interleukin (IL)-2 [11]. The current understanding is that this causes a failure to delete autoreactive T cells during thymic development and/or a decreased formation of regulatory T (Treg) cells. The combination of the shared epitope and the RA-associated PTPN22 allele (termed PTPN22*R620W) was found to predispose to formation of autoantibodies to type II collagen in early RA, which implies concurrent T-cell autoreactivity to this cartilage autoantigen [12].

Another set of single-nucleotide polymorphisms (SNPs) implicated in susceptibility to RA has been found in the gene encoding programmed death-1 (PD-1), a molecule that regulates T-cell homeostasis through apoptosis [13-15]. Although different PD-1 SNPs have been identified in RA patients from distinct ethnic backgrounds and some but not all SNPs of PD-1 have also been linked to susceptibility toward systemic lupus erythematosus, these polymorphisms most likely have in common a defective activity of PD-1, leading to decreased elimination of autoreactive T cells. Interestingly, a very recent study showed that PD-1 and its ligand are overexpressed in synovial cells from patients with RA and that an alternatively spliced variant of PD-1 leading to formation of an inhibitory soluble form of the protein was abundant in serum and synovial fluid of patients with RA [16]. These data suggest that soluble PD-1 may protect autoreactive T cells from undergoing apoptosis and corroborate the idea that ineffective PD-1 signaling is an important contributor to RA susceptibility.

It appears likely that several genes will also be validated as being linked to RA severity but not to susceptibility. Although the notion of RA as a Th1 disease as opposed to a Th17 disease is currently in flux (see below), it remains clear that RA is not a Th2 disease, and it is plausible that the production or function of Th2 cytokines or both are deficient in RA. In this context, a report that associates a hypofunctional allele of the IL-4 receptor with increased radiographic damage in RA is of particular interest [17]. Given the ability of IL-4 to regulate Th17 responses (as well as Th1 responses), reduced responsiveness of the IL-4 receptor would be expected to worsen the outcome of RA.

Overall, recent advances in our knowledge concerning the genetics of RA not only reinforce the importance of T cells in both susceptibility to and outcome of this disease but also emphasize the complex and interdependent roles of T cells in the context of the entire immune response.

T cells in spontaneous animal models of arthritis

Over the past several decades, many inductive models of inflammatory polyarthritis, such as collagen-induced arthritis (CIA) and adjuvant-induced arthritis, have been employed to study immune responses in arthritis. These animal models of arthritis have contributed significantly to our understanding of cellular and molecular events that may be relevant to RA. Recently, several models of spontaneous arthritis have been identified due to perturbations in the TCR and alterations of cytokine regulation. This section will focus on new findings related to T cells in four of these recently identified models of spontaneous arthritis: SKG, K/BxN-transgenic, gp130 (IL-6R) mutant, and IL-1 receptor antagonist (IL-1Ra)-deficient mice.

An intrinsic defect in TCR signaling or alteration of the cytokine milieu can lead to T cell-dependent arthritis in mice. Sakaguchi and colleagues [18,19] have generated mice with a point mutation in the COOH-terminal SH2 domain of zeta-associated protein of 70 kDa (ZAP-70) which develop spontaneous arthritis and demonstrate extra-articular manifestations found in RA, including interstitial pneumonitis, subcutaneous nodules, and vasculitis. The role of T cells in the SKG mutant model has been demonstrated by the predominant infiltration of a Vβ-restricted subset of $CD4^+$ T cells in the inflamed synovium [20]. Adoptive transfer of splenic or lymph node ZAP-70 mutant T cells or thymocytes leads to chronic arthritis in syngeneic, nude, or severe combined immunodeficient mice [18,20]. As a result of the mutation, ZAP-70 expression is not altered but ZAP-70 does

not bind normally to the TCR. This probably leads to signaling abnormalities of the TCR in the thymus, resulting in positive selection of self-reactive T-cell clones that would otherwise be eliminated. In addition to providing strong evidence for the ability of autoreactive T-cell clones to initiate arthritis, this model is also dependent on the proinflammatory cytokines IL-6, IL-1β, and tumor necrosis factor (TNF)-α, which are highly implicated in RA synovial pathology [19]. The dependence on proinflammatory cytokines in this model was further substantiated by a report that spontaneous arthritis in SKG mice did not occur in specific pathogen-free housing conditions but was inducible with the fungal glucan, zymosan A, a dectin-1 and toll-like receptor 2 agonist that stimulated IL-1β and IL-6 production in the model [21,22]. The pattern of cytokine expression in zymosan-treated SKG mice is highly correlated with the conditions required to drive a Th17 response; moreover, dependence of this model on IL-17 has recently been established [23].

Another spontaneous arthritis model that is contributing to our understanding of the role of T cells in arthritis is the IL-1Ra-deficient mouse strain [24]. IL-1Ra is an endogenous, natural inhibitor of IL-1 bioactivity through binding and blockade of the IL-1 receptor. An important finding was that arthritis fails to develop in IL-1Ra-deficient mice in the absence of mature T cells [25]. Transfer of T cells from IL-1Ra-deficient mice into nude mice resulted in arthritis, further substantiating the role of T cells in this model [25]. Cytokines, especially IL-17 and TNF-α, also play an important role in this model of arthritis [25,26].

Recently, IL-6 in conjunction with transforming growth factor-beta (TGF-β) has been implicated in the generation of Th17 cells. A mutation in the tyrosine residue, at the phosphatase-binding site, of the gp130 subunit of the IL-6 receptor has been shown to result in spontaneous arthritis in mice [27,28]. This mutation leads to an increase in receptor signaling through STAT3 (signal transducer and activator of transcription 3), resulting in both increased IL-7-dependent proliferation as well as impaired Fas ligand expression and decreased T-cell apoptosis. Development of arthritis in the gp130 mutant model is dependent on $CD4^+$ T cells despite the finding that mutation of gp130 in nonhematopoietic cells is sufficient to drive disease. These data suggest that arthritogenic T cells are usually regulated by nonhematopoietic cells through a mechanism that can be overridden by increased signaling through gp130.

The K/BxN transgenic mouse is another example of spontaneous arthritis in the mouse, resulting from recognition of self-antigens and breakdown of tolerance [29]. F1 offspring (K/BxN) of nonobese diabetic mice crossed with KRN TCR transgenic mice, which have specificity for a glucose-6-phosphate isomerase peptide in the context of $I\text{-}A^{g7}$, develop spontaneous arthritis. Despite low cell numbers, there was enrichment of $CD4^+$ T cells in the synovial compartment with high levels of expression of the KRN transgene. Administration of anti-CD4 T-cell antibody before, but not during or after, the onset of arthritis blocked arthritis development. This suggests that T cells are important in only the early pathogenesis of arthritis in K/BxN mice. A subsequent study demonstrated that passive transfer of serum from K/BxN arthritic mice resulted in arthritis in various mouse strains which was dependent on engagement of the innate immune system [30,31]. These data suggest that the main pathogenic role of autoreactive T cells in the K/BxN model is to drive the development of autoantibodies. In contrast, Schubert *et al*. [32] have more recently reported a model, based on immunization with glucose-6-phosphate isomerase, that is T cell-dependent at both initiating and effector phases and that establishes disease in DBA/1 mice only.

Recent data from these animal models emphasize that inflammatory arthritis can be engendered by T-cell autoreactivity through pathways that also require participation of other arms of both the innate and adaptive immune responses, ranging from production of autoantibodies by B cells to elaboration of proinflammatory cytokines.

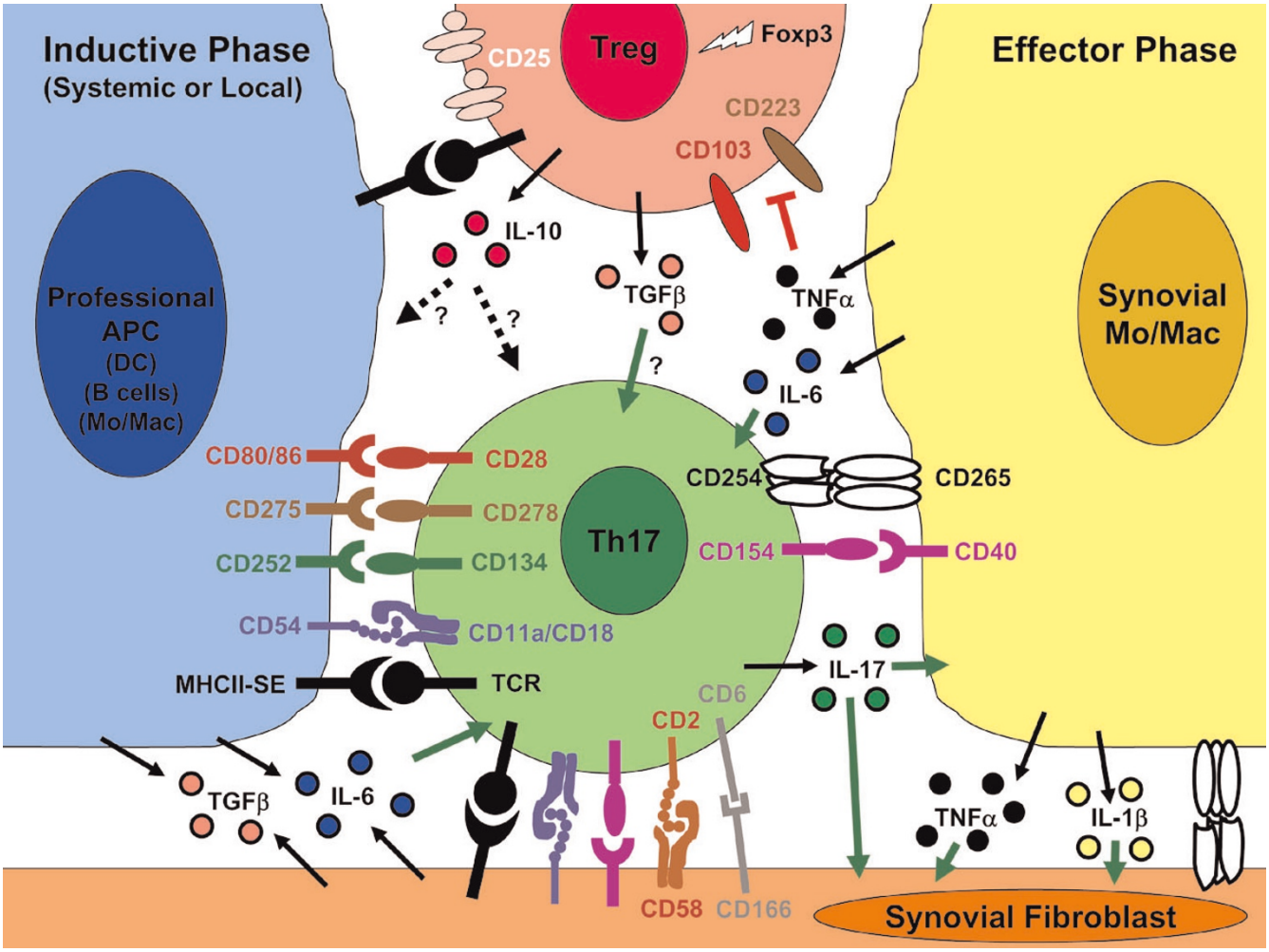
Cell-cell interactions important to T-cell function in RA

Cell-cell contact is necessary both at the inductive phase of T-cell activation in RA and at the effector phase, in which T cells indirectly mediate autoantibody production, joint inflammation, and bone resorption [33]. The schematic diagram in Figure 1 summarizes many of the cell-cell and cell-cytokine interactions that have been implicated in RA and arthritis animal models. In the inductive phase, TCR binding to antigen/MHC on antigen-presenting cells (APCs) is a critical first step for T-cell activation and might form part of the basis for the importance of MHC alleles that contain the shared epitope. However, it is likely that the nature of accessory signals received from APCs and the local environment during TCR stimulation determines the type of T-cell response and governs disease progression. This section will focus on recent advances in our understanding of accessory interactions between T cells, APCs, and synovial cells.

Costimulation of naïve T cells through ligation of CD28 by B7-1 (CD80) or B7-2 (CD86) is perhaps the most important secondary signal to drive T-cell proliferation and differentiation [34]. Once activated, the T cell upregulates expression of cytotoxic T lymphocyte antigen-4 (CTLA-4), an inhibitory receptor that has a higher affinity for CD80 and CD86, in order to modulate activation. Use of a CTLA-4-immunoglobulin fusion protein, which blocks the interaction of CD28 with B7 ligands, has yielded promising results as a treatment for RA and demonstrates the importance of this cell-cell interaction in immune-mediated disease [35].

Other CD28/B7 family interactions have been shown to mediate important interactions between T cells and other

Figure 1



Schematic diagram of the putative interactions of pathogenic Th17 cells in the synovial microenvironment. Induction of T-cell responses in rheumatoid arthritis (RA) is initiated by T-cell receptor (TCR) interaction with shared epitope major histocompatibility complex class II (MHCII-SE) and peptide on antigen-presenting cells (APCs) either systemically or in the synovium. Accessory molecules expressed by APCs, including ICAM-1 (intercellular adhesion molecule-1) (CD54), OX40L (CD252), inducible costimulator (ICOS) ligand (CD275), B7-1 (CD80), and B7-2 (CD86), participate in T-cell activation by binding lymphocyte function-associated antigen (LFA)-1 (CD11a/CD18), OX40 (CD134), ICOS (CD278), and CD28. Activated fibroblast-like synoviocytes (FLS) may also participate in antigen presentation and have additional accessory molecules such as LFA-3 (CD58) and ALCAM (activated leukocyte cell adhesion molecule) (CD166) which interact with T cell-expressed CD2 and CD6, respectively. Cytokines interleukin (IL)-6 and transforming growth factor-beta (TGF-β), most likely derived from activated APCs, signal the T cell to differentiate into IL-17-producing Th17 cells. IL-17 has independent and synergistic effects with other proinflammatory cytokines (tumor necrosis factor-alpha [TNF-α] and IL-1β) in the synovium to induce further cytokine release, matrix metalloproteinase production, RANK/RANK ligand (CD265/CD254) expression, and osteoclastogenesis. CD40L (CD154) interaction with CD40 also leads to activation of synovial monocytes/macrophages (Mo/Mac), FLS, and B cells. Although present in the synovia of most patients with RA, $CD4^{+}CD25^{hi}$ regulatory T (Treg) cells are ineffective at controlling inflammation and may be deactivated by synovial TNF-α. IL-10 is abundant in synovial fluid but its effect on Th17 regulation has yet to be determined. Expression of accessory molecules on Th17 cells, as denoted in the figure, are speculative and are inferred from expressions found on non-subdivided T-cell populations in animal models. Further investigation is necessary to directly demonstrate expression of these structures on the Th17 cell subset in human RA synovium. DC, dendritic cell; RANK, receptor activator of nuclear factor-kappa B.

cells involved in RA pathogenesis. Inducible costimulator (ICOS) (CD278) is more highly expressed on activated T cells found in patients with RA than in healthy individuals [36]. The ligand for ICOS, CD275, is expressed by professional APCs and has been shown to be expressed in synovial tissue [36,37]. Blockade of CD275/CD278 binding by antibodies was shown to diminish proinflammatory cytokine production, autoantibody formation, and inflammation in the CIA model [37]. Another CD28 family member, OX40 (CD134), was shown to be inducible on T cells by

TNF-α in the IL-1Ra-deficient mouse model, and blockade of CD134/CD252 interaction protected mice from developing spontaneous arthritis [25].

The integrin lymphocyte function-associated antigen (LFA)-1 (CD11a/CD18) is expressed on activated T cells and binds to intercellular adhesion molecule-1 (ICAM-1) (CD54) found on the surface of many cell types. Previously, it was thought that the main role of LFA-1/CD54 in inflammation had to do with lymphocyte homing given the importance of this adhesion axis in tight binding of lymphocytes to blood vessel walls and their subsequent extravasation. However, the cellular translocation of LFA-1 and CD54 to points of contact between T cells and APCs also suggests an important role in maintaining cell-cell contact during antigen presentation. In rodent models of arthritis, disruption of the LFA-1/CD54 interaction has consistently decreased the severity of inflammation, results that have led to the testing of LFA-1/CD54 blockade for treatment of RA in clinical trials [38,39].

In addition to antigen presentation mediated by professional APCs, our laboratory has been studying the ability of activated fibroblast-like synoviocytes (FLS) to present antigens to T cells. FLS, T cells, and macrophage-like synoviocytes are the three most abundant cell types in RA synovia. Interferon-gamma (IFN-γ)-treated FLS lines from patients with RA express MHC class II molecules and are able to stimulate T cells to respond to superantigens *in vitro* [40]. We have recently extended these observations by stimulating IL-2 production from T-cell hybridoma lines that are HLA-DRB1*0401-restricted and arthritogenic peptide-specific [41]. FLS lines do not express CD80 or CD86 but do express other molecules that have receptors or ligands on T cells, including ICAM-1 (CD54), VCAM-1 (vascular cell adhesion molecule-1) (CD106), CD40, LFA-3 (CD58), ALCAM (activated leukocyte cell adhesion molecule) (CD166), and a novel CD6 ligand termed 3A11 [38,42]. At the cell surface, FLS also express fractalkine, which has recently been shown to be involved in activation of $CD28^{null}CD4^{+}$ 'senescent' T cells from patients with RA [43]. The relative importance of activated FLS versus professional APCs in antigen presentation in RA synovium has not been determined.

The effector functions of arthritogenic T cells are carried out in the synovial lining and intra-articular space of the joints. Upon activation, T cells upregulate surface expression of CD40 ligand (CD154), which stimulates APCs through interaction with CD40. In B cells, ligation of CD40 in combination with cytokine activation stimulates antibody synthesis and isotype switching. Ligation of CD40 also induces CD80, CD86, and CD54, as well as production of proinflammatory cytokines, including IL-6, IL-8, MIP-1 (macrophage inflammatory protein-1), TNF-α, and IL-12, by APCs [44,45]. These cytokines are known to participate in joint inflammation and act reciprocally on T cells to drive production of other cytokines and surface molecules involved in the effector phase of joint inflammation.

The population of effector T cells in the joint may not be limited to antigen-stimulated T cells. Brennan *et al*. [46] found that TNF-α production in RA synovia was T cell-dependent and that synovial T cells from patients with RA were able to stimulate TNF-α production from peripheral blood monocytes. Interestingly, using blocking reagents for nuclear factor-kappa B (NF-κB) and PI3 (phosphoinositol 3) kinase, it was found that the RA synovial T cells more closely resembled normal T cells activated by IL-2, IL-6, and TNF-α than T cells activated through the TCR. These data suggest that these 'cytokine-activated' bystander T cells (Tck) in the cytokine milieu of the joint may become non-specifically activated and contribute to joint pathology.

Another important effector function of synovial T cells involves upregulation of receptor activator of NF-κB (RANK) ligand (CD254) on the cell surface [47]. $CD254^{+}$ T cells interact with synovial monocytes, leading to osteoclast differentiation. These monocyte-derived osteoclasts then mediate bone destruction.

T cells require many receptor-ligand interactions to become activated and to carry out their tissue-destructive role in RA. Disruption or modification of these cell-cell interactions may prove to be an effective strategy for treatment of RA. Recent data regarding the abilities of methotrexate and leflunomide to interrupt T-cell interactions with FLS and APCs may partially explain the efficacy of these medications and highlight the importance of cell-cell contact to the pathogenesis of RA [48-50].

A novel T-cell subset that secretes IL-17: relevance to RA

Until recently, T-cell responses were typically classified as either Th1 or Th2, based on the relative expression levels of cytokines, especially IFN-γ and IL-4. Although neither Th1 nor Th2 cytokines are present at high levels in the RA joint, IFN-γ consistently predominated over IL-4 and RA had been viewed as a Th1 disease. Recent evidence from mouse models has questioned the role of Th1 cells in RA and identified a new T-helper subset, Th17, with effector functions that make it a prime candidate for mediating joint pathology. Th17 cells are characterized by production of the highly inflammatory cytokine IL-17. The first evidence of the inflammatory role of IL-17 arose 10 years ago, when Fossiez and colleagues [51] cloned human IL-17 from activated memory T cells and showed that adding IL-17 to primary cultures of human RA synovial fibroblasts induced expression of IL-6, IL-8, prostaglandin E_2, and G-CSF (granulocyte colony stimulating factor). Furthermore, IL-17 synergized with TNF-α to induce high levels of IL-6 and GM-CSF (granulocyte-monocyte colony stimulating factor). Since then, the effects of IL-17 have been widely studied, resulting in a remarkable list of

Table 1

Effector molecules induced by IL-17 from human cells

Molecule produced	Cell source	Major functional effects	Reference
IL-1β	FLS, Mono/Mac, Chond/OC	Inflammation, fever, synergy with IL-17	[52,53,55,58]
IL-6	FLS, Mono/Mac, Chond/OC	Acute-phase reaction, B-cell stimulation, Th17 differentiation	[51-53,58]
IL-23	FLS	Inflammation, Th 17 cell stimulation	[56]
TNF-α	Mono/Mac	Inflammation, synergy with IL-17	[52,53]
CXCL1 (GRO-α)	FLS, Chond/OC	Leukocyte recruitment	[55,60]
CXCL5 (LIX)	Chond/OC	Leukocyte recruitment	[60]
CXCL8 (IL-8)	FLS	Leukocyte recruitment	[51-53,55]
CCL2 (MCP-1)	FLS, Chond/OC	Leukocyte recruitment	[55,60]
CCL20 (MIP-3α)	FLS	Leukocyte recruitment	[54]
G-CSF	FLS	Granulopoiesis	[51]
GM-CSF	FLS	Granulopoiesis	[51-53]
VEGF	FLS, Chond/OC	Angiogenesis	[57,59]
Cyclooxygenase-2	FLS, Mono/Mac, Chond/OC	Inflammation	[52,58]
Prostaglandin E_2	FLS, Mono/Mac, Chond/OC	Inflammation	[51-53]
RANK/RANK ligand	Chond/OC	Osteoclastogenesis and bone resorption	[53]
Nitric oxide	Chond/OC	Tissue destruction	[52,53,58]
Matrix metalloproteinases	Mono/Mac, Chond/OC	Cartilage and tissue destruction	[53,60]

Chond/OC, chondrocytes and osteoclasts; FLS, fibroblast-like synoviocytes; G-CSF, granulocyte colony stimulating factor; GM-CSF, granulocyte-monocyte colony stimulating factor; GRO, growth-regulated oncogene; IL, interleukin; MCP, monocyte chemotactic protein; MIP-3α, macrophage inflammatory protein-3 alpha; Mono/Mac, monocytes and macrophages; RANK, receptor activator of nuclear factor-kappa B; TNF-α, tumor necrosis factor-alpha; VEGF, vascular endothelial growth factor.

target cell types and downstream inflammatory mediators relevant to RA. Table 1 summarizes the IL-17-inducible factors produced by cell types relevant to RA synovium [51-60]. The downstream activities of these factors contribute to pathology through recruitment and activation of inflammatory cells, positive feedback of the IL-17 response, and destruction of tissue and bone.

One of the reasons that IL-17 can play an important role in the pathogenesis of multiple autoimmune and inflammatory diseases is the ubiquitous expression of the IL-17 receptor. IL-17 directly and indirectly augments both inflammatory mediator production and joint destruction. Early reports suggested that IL-17 had little effect on its own and acted primarily in synergy with IL-1β and TNF-α, but it is now known that IL-17 can be pathogenic independently of IL-1β and TNF-α. Although IL-17-induced TNF-α, IL-1β, and IL-6 can induce cartilage destruction and bone erosion, IL-17 itself has independent effects on cartilage and bone. IL-17 upregulates CD265 (RANK ligand) expression on chondrocytes and osteoblasts and acts on chondrocyte metabolism by reducing proteoglycan synthesis and enhancing cartilage degradation [52,53]. IL-17 enhances matrix breakdown, cartilage proteoglycan depletion, chondrocyte death, and cartilage and bone erosion in mice even under TNF- or IL-1-neutralizing conditions [52,53,61]. These results suggest that treatments designed to block IL-17 would not be redundant with treatments that block TNF-α or IL-1β and that combination therapy could be beneficial, especially for patients who do not respond to TNF blockade. In cultures of RA synovium, combining TNF blockade with agents that block IL-1 and IL-17 was more effective at controlling IL-6 production and collagen degradation than blocking TNF-α alone [62]. Similarly, combination blockade of TNF-α and IL-17 suppressed ongoing CIA and was more effective than neutralization of TNF-α alone [53].

Clearly, IL-17 has the ability to induce inflammation and joint destruction when administered *in vitro* and *in vivo* in animal models, but the question remains: how relevant is it to RA? IL-17 is found in RA synovial fluid and in the T cell-rich area of RA synovial tissue [52,53]. In addition, IL-17 is over-expressed in serum and activated peripheral blood mononuclear cell (PBMC) cultures of patients with RA compared to healthy controls [45,63]. Experiments in multiple animal models of arthritis demonstrate a requirement for IL-17 at both early and late stages for full disease development. Both the incidence and severity of arthritis were significantly

reduced in mice deficient in IL-17 or IL-17 receptor during CIA and streptococcal cell wall arthritis [53,64]. In addition, spontaneous arthritis in IL-1Ra-deficient mice was completely blocked in the absence of IL-17 [53]. Several groups have also demonstrated that administering blocking antibody or soluble IL-17 receptor during either the induction or effector phase of experimental arthritis reduced inflammation and joint destruction [53]. Recent evidence suggests a similar correlation between IL-17 expression and joint damage progression in patients with RA. Using several different methods of statistical analysis, a 2-year prospective study of 50 patients with RA found that synovial membrane mRNA levels of IL-1β, TNF-α, IL-10, and IL-17 were consistently predictive of damage progression [65]. Moreover, IL-17 and TNF-α mRNA levels were synergistic as prognostic factors. This study provides important clinical corroboration of observations regarding the role of IL-17 in animal models of arthritis.

These and other studies have built a strong case that IL-17 is a key suspect in the pathogenesis of RA: it is overexpressed in RA synovium and blood, it induces and synergizes with many inflammatory mediators important in joint pathology, and it is both necessary and sufficient for joint inflammation in animal models. The Th1-type response mediated by IFN-γ, on the other hand, may have been falsely implicated. In CIA, knocking out the IFN-γ receptor or IL-12 actually exacerbated disease [53,66]. The protective role of IL-12 and IFN-γ is likely to stem from the ability of Th1 and Th2 cytokines to inhibit Th17 development. In addition, the same prospective study that found IL-17 to be predictive of joint damage progression in patients with RA also found that IFN-γ in the synovium was predictive of protection [65]. IFN-γ may in fact play dual roles, supporting inflammation in the early phase of the disease and inhibiting inflammation later. Administration of neutralizing anti-IFN-γ antibodies early in an experimental arthritis model was protective, whereas later administration exacerbated disease [67]. As evidenced by the IFN-γ dependence of the proteoglycan-induced arthritis model, the relative importance of IL-17 or IFN-γ may also depend on the method used to induce disease [67]. This leaves open the possibility that RA may not be as dependent on IL-17 as in mouse models or that distinct subsets of patients with RA may have different cytokine dependencies. These discrepancies will hopefully be resolved by continuing research into the role of human IL-17 in RA.

One of the important questions about IL-17 is the nature of the stimuli that cause IL-17 to be produced. Early studies found that IL-17 production by $CD4^+$ effector and memory T cells was augmented by IL-23, a heterodimeric cytokine composed of the IL-12 p40 subunit and a unique p19 subunit [52,53,68]. IL-23 stimulation of activated murine T cells induced production of IL-17, IL-17F (a close relative of IL-17), IL-6, TNF-α, and low levels of IFN-γ. In human T cells, IL-23 induced production of IL-17 and low levels of IL-10 and IFN-γ [69]. Notably, IL-23 has been found in RA synovial fluid and is produced by FLS [56]. Remarkably, three groups concurrently identified the combination of TGF-β and IL-6 as key initiators of Th17 differentiation in murine T-cell cultures [70-72]. IL-23 did not play a role in Th17 differentiation but did appear to be important for Th17 survival and expansion. The newly discovered role of TGF-β in the differentiation of a highly inflammatory T-cell subset seems paradoxical given that TGF-β is conventionally regarded as anti-inflammatory. However, there is also a documented role for TGF-β in exacerbating inflammatory responses and promoting autoimmunity. In rat models of arthritis, injection of TGF-β into the joint results in enhanced neutrophil recruitment, synovial inflammation, and hyperplasia, whereas injection of blocking antibody to TGF-β inhibits acute and chronic synovial inflammation [73]. The divergent effects of TGF-β may depend on systemic versus local expression and on the cytokine milieu. For example, *in vitro* stimulation of naïve $CD4^+$ T cells with TGF-β induces a regulatory phenotype, but when IL-6 is added to these cultures, T-cell differentiation is skewed toward Th17 instead [72]. Although many of these observations have not yet been confirmed in humans, they emphasize the potential importance and mechanisms of action of IL-6 blocking therapeutic strategies that are currently under evaluation in several rheumatologic diseases. Moreover, much of the current understanding of the role of TGF-β in arthritis and its suitability as a target in any immune-mediated disease needs to be re-evaluated in the context of what we now know about IL-17.

In addition to the effects of TGF-β, IL-6, and IL-23, Stockinger and colleagues [70] found that IL-17 could be upregulated by IL-1β and TNF-α. These recent reports provide a clear explanation of how Th17 cells might differentiate and expand within the joint: TGF-β, IL-6, IL-23, IL-1β, and TNF-α are all found in RA synovium [52,53,56,73]. Upregulation of IL-17 by IL-6, IL-1β, and TNF-α, all of which are induced by IL-17, also creates a positive feedback loop. Thus, one can imagine how initially minor acute inflammation, in the right microenvironment and cytokine milieu, might escalate and ultimately lead to self-perpetuating chronic inflammation through IL-17-dependent pathways.

Treg cells in RA

Treg cells have become a major focus of immunologic research in the past decade due to their participation in controlling effector T-cell functions *in vitro* and to their potential for regulating autoimmune inflammatory responses *in vivo* [74]. Several phenotypically distinct subsets of $CD4^+$ T cells constitute the Treg cell repertoire, but some markers such as forkhead box p3 (Foxp3), neuropilin, LAG3 (lymphocyte activation gene 3) (CD223), CD103, and high surface expression of CD25 have emerged as specific markers of Treg cells [75-77]. Treg cells also produce high levels of TGF-β and IL-10 [78]. The precise mechanisms of suppression mediated by Treg cells are not fully understood. It is possible that Treg cells suppress immunological

responses in multiple ways, which may involve negative signals produced by inhibitory surface molecules, cytotoxic killing, downregulation of APC function, and/or induction of other regulatory cells.

Some studies have been performed to evaluate the role of Treg cells in RA, and there is controversy regarding the relative number and function of $CD4^+CD25^+$ Treg cells in RA [79,80]. Treg cells have been identified in peripheral blood and synovial tissue of patients with RA [80-82]. However, most studies have shown that the $CD4^+CD25^+$ Treg cells from patients with RA have a defect in suppression of TNF-α and IFN-γ production from $CD4^+$ T cells or monocytes, even though they can suppress the proliferation of effector T cells [81,83]. In other studies, it has been shown that effector T cells from peripheral blood of patients with RA were resistant to Treg-mediated suppression [84]. $CD4^+CD25^+$ cells express TNF receptor 2, and signaling through this receptor by TNF-α results in inhibition of suppressive function and decreased Foxp3 expression [85]. Treatment of RA patients with anti-TNF-α antibody leads to *in vivo* expansion of $CD4^+CD25^+$ Treg cells, increased Foxp3 expression, and restoration of cytokine suppressive function [81,85]. Interestingly, one study showed that PBMCs of MHC-shared epitope-positive, healthy individuals responded to the arthritis-associated autoantigen, HCgp39 (human cartilage glycoprotein of 39 kDa), by producing IL-10 whereas PBMCs of patients with RA tended to produce proinflammatory cytokines [86]. IL-10 production was attributed to Treg cells, which suggests that an important difference between healthy people and patients with RA is the ability to expand Treg cells specific for autoantigens.

The role of Treg cells has also been studied in the mouse CIA model. $CD4^+CD25^+$ cells are important in controlling the pathogenesis of CIA, and depletion of $CD25^+$ cells with anti-CD25 antibody led to aggravation of joint inflammation [87,88]. Adoptive transfer of $CD4^+CD25^+$ Treg cells during the initiation phase of arthritis resulted in decreased severity of disease, whereas the course of established arthritis remained unchanged [87,89,90]. Recent studies have demonstrated the ability to influence Treg development *in vivo* using oral collagen, immature dendritic cells, or vasoactive intestinal peptide as potential therapeutic agents to treat CIA [91-93]. Activation or reactivation of Treg cells in patients holds promise as a potential treatment for RA, but uncertainty persists concerning the effectiveness and durability of such strategies and the best way to mechanistically approach the *in vivo* manipulation of Treg cells.

Conclusion

Integration of the many compelling lines of evidence regarding the roles of T cells in RA remains a challenge for current and future research. A full description of the genetic loci that control RA will be essential, including further analyses in non-Caucasian populations, to provide a more comprehensive view of this complex disease and increased predictive and prognostic power. Once the interactions of T cells with other synovial cell populations are better understood, proof of their importance may ultimately be confirmed by application of new biologic therapies to the treatment of RA. Recent breakthroughs in the delineation of the novel Th17 subset have come primarily through animal studies, and data are urgently needed to assess which principles also apply to the human immune system and human disease. At the present time, it appears likely that the Th17 cells and their product, IL-17, will be attractive targets for therapy of RA and other immune-mediated human diseases. This therapy may come in the form of neutralizing inductive cytokines such as IL-6 and IL-23, blocking Th17-specific costimulatory signals, disruption of IL-17 signaling cascades, or direct targeting of antigen-specific Th17 cells for elimination. An interesting question is whether Treg cells can be mobilized to specifically regulate Th17-driven pathology. Progress in all of these areas should move us closer to the goal of re-establishing long-term physiologic regulation of immune responses in RA, using well-tolerated and more specifically targeted interventions.

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Cells of the synovium in rheumatoid arthritis
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Competing interests

The authors declare that they have no competing interests.

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