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REVIEW

# Transcriptional Regulation of CD4+ T Cell Differentiation in Experimentally Induced Arthritis and Rheumatoid Arthritis

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**Rheumatoid arthritis (RA) is an autoimmune disorder characterized by chronic inflammation of the joint synovium and infiltration by activated inflammatory cells. CD4+ T cells form a large proportion of the inflammatory cells invading the synovial tissue, and are involved in the RA pathologic process. In general, CD4+ T cells differentiate into various T helper cell subsets and acquire the functional properties to respond to specific pathogens, and also mediate some autoimmune disorders such as RA. Because the differentiation of T helper cell subsets is determined by the expression of specific transcription factors in response to the cytokine environment, these transcription factors are considered to have a role in the pathology of RA. Treg cells control an excess of T cell–mediated immune response, and the transcription factor FoxP3 is critical for the differentiation and function of Treg cells. Treg cell dysfunction can result in the development of systemic autoimmunity. In this review, we summarize how the expression of transcription factors modulates T helper cell immune responses and the development of autoimmune diseases, especially in RA. Understanding the role of transcription factors in the pathogenesis of autoimmunity may lead to novel therapeutic strategies to control the differentiation and function of both T helper cells and Treg cells.**

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## Introduction

Rheumatoid arthritis (RA) is a chronic inflammatory disorder characterized by autoimmunity, infiltration of activated inflammatory cells into the joint synovium, synovial hyperplasia, neoangiogenesis, and progressive destruction of cartilage and bone. CD4+ T cells constitute a large proportion of the inflammatory cells invading the synovial tissue. Upon antigenic stimulation and cytokine signaling, naive CD4+ T cells activate and differentiate into various T helper cell subsets.

Classically, interferon-γ (IFNγ)–producing Th1 cells had been considered to play a predominant role in the development of RA. However, studies have demonstrated that the Th1 phenotype does not explain all of the mechanisms involved in RA (1).

The pathogenic role of interleukin-17 (IL-17)–producing Th17 cells has intrigued rheumatologists, because IL-17 is spontaneously produced by rheumatoid synovium (2), and Th17 cells are increased among peripheral blood mononuclear cells of RA patients compared with those of healthy control subjects (3). Th17 cells also appear to play a critical role in the generation of autoimmune arthritis in several experimental models. In addition, some studies have shown that the frequency of follicular helper T (Tfh) cells, which support high-affinity and long-term antibody response, is increased in the peripheral blood of RA patients and correlates with disease activity (4), suggesting that these cells also play a role in RA pathology. More recently, it was reported that PD-1$^{high}$CXCR5−CD4+ T cells were markedly expanded and activated in synovium, and appeared to be poised to promote B cell response and antibody production through expression of IL-21–like Tfh cells within pathologically inflamed nonlymphoid tissue in patients with RA (5).

Differentiation of naive CD4+ T cells into T helper cell subsets is dependent on the expression of specific transcription factors induced by specific cytokines. Each

T helper cell–specific transcription factor not only regulates the expression of effector molecules—e.g., cytokines and chemokine receptors specific for each T helper cell subset—but also negatively regulates the differentiation of other T cell subsets. Interestingly, CD4+ T cells overexpress *RORC* (encoding retinoic acid receptor–related orphan nuclear receptor γt [RORγt], a transcription factor), in RA patients but not in healthy subjects (3). Several studies using animal models of RA have highlighted T helper cell–specific transcription factors in the development of autoimmune arthritis, and we have previously described how the pathogenesis of murine autoimmune arthritis is regulated by T-bet and RORγt, which are specific transcription factors in Th1 and Th17 cells, respectively (6,7).

Treg cells control not only excess T cell–mediated immune responses against pathogens, but also autoreactive T cells, and thus they play a pivotal role in maintaining peripheral self tolerance. Transcription factor FoxP3 is needed to maintain the suppressive capacity of Treg cells (8). Previous studies stressed the importance of FoxP3+ Treg cells in the regulation of autoimmune arthritis in both human subjects and animal models, and our group reported that the balance between FoxP3+ Treg cells and Th17 cells in inflamed joints plays a critical role in the severity of arthritis (7).

In this review, we summarize the latest research findings on transcription factors in the differentiation, function, and roles of CD4+ T cells in the development of autoimmune arthritis. In particular, we focus on the effects of T-bet and RORγt expression in autoimmune arthritis based on our previous findings in murine autoimmune arthritis. Furthermore, we focus on transcription factors as a potential target of new therapies for autoimmune arthritis based on modulation of CD4+ T cell differentiation.

## Distinct role of CD4+ T cells in immune response

CD4+ T helper cells are divided into several subsets based on their function, cytokine profile, and chemokine receptor expression (Table 1). Th1 cells produce IFNγ and play an important role in immunity against intracellular pathogens, whereas Th2 cells produce IL-4, IL-5, and IL-13, and are essential for defense against parasites and extracellular pathogens (9). Furthermore, Th17 cells produce IL-17, IL-21, and IL-22, and are involved in immunity against bacterial and fungal infections (10–12). Tfh cells are also a subset of CD4+ T cells and play a critical role in humoral immune response. IL-21, produced by Tfh cells, supports B cell proliferation and differentiation of plasma cells in germinal centers (13,14). Localization of T helper cells in inflammatory conditions depends mainly on chemokines and their receptor expression. T helper cells characteristically express specific chemokine receptors—Th1, Th2, Th17 and Tfh cells express CXCR3, CCR4 and CCR8, CCR6, and CXCR5, respectively—and migrate into sites of inflammation in response to the chemokine ligands (15). Thus, the expression patterns of chemokine receptors are recognized as markers to standardize immunophenotyping of human T helper cells, and are used to isolate viable T helper subpopulations (by cell sorting) to analyze gene expression (16).

Treg cells suppress not only excess T cell-mediated immune responses against pathogens, but also autoreactive T cells. Thus, these cells play an important role in maintaining peripheral self tolerance (17). Breakdown of self tolerance contributes to the development of autoimmune diseases, such as antibody responses against citrullinated self proteins in RA. Transcription factor FoxP3 is required in order to maintain the suppressive properties of CD4+CD25+ Treg cells (8). FoxP3+ Treg cells are divided into thymus-derived Treg cells and Treg cells derived peripherally. The former type is generated through the recognition of self peptide and major histocompatibility complex complexes in the thymus, and is thus important for self tolerance (18). FoxP3+ Treg cells exert their immunosuppressive functions through a variety of effector mechanisms, such as up-regulation of CTLA-4 (19), consumption of IL-2 (20), and production of immunosuppressive cytokines, such as IL-10 (21) and transforming growth factor β (TGFβ) (22).

**Table 1.** Characteristics of T helper and Treg cells in normal immune response*

| T cell type | Function | Cytokine | | Chemokine receptor | References |
|---|---|---|---|---|---|
| | | Major | Minor | | |
| Th1 | Immunity against intracellular pathogens | IFNγ | – | CXCR3+CCR6− | 9, 15 |
| Th2 | Immunity against parasites and extracellular pathogens | IL-4, IL-5, and IL-13 | IL-10 | CCR4+CCR8+ | 9, 15 |
| Th17 | Immunity against bacterial and fungal infections | IL-17 and IL-22 | IL-21 | CXCR3−CCR6+ | 10–12, 15 |
| Tfh | Humoral immune response by supporting B cell proliferation and plasma cell differentiation | IL-21 | IL-4 and IL-10 | CXCR5+ | 13–15 |
| Treg | Maintaining peripheral self-tolerance | IL-10 and TGFβ | – | – | 8, 18–22 |

* IFNγ = interferon-γ; IL-4 = interleukin-4; Tfh = T follicular helper cell; TGFβ = transforming growth factor β.

**Table 2.** Association of T helper cells and their cytokines with RA*

| T cell type/ cytokine | Findings | References |
|---|---|---|
| Th1/IFNγ | T cell clones from RA synovium produce large amounts of IFNγ; IFNγ inhibits bone resorption mediated by suppression of osteoclast formation; monoclonal antibody to IFNγ is less effective in RA | 1, 48, 49 |
| Th2/IL-4 | SNPs in the coding region of IL-4R modulate the course and severity of RA via the magnitude of IL-17 production | 53, 54 |
| Th17/IL-17 | IL-17 is spontaneously produced by the RA synovium; higher proportion of Th17 cells among peripheral blood mononuclear cells in RA compared with healthy subjects; correlation with RA disease activity | 2,3 |
| Tfh/IL-21 | High proportion of circulating Tfh-like cells in peripheral blood of RA patients; correlation with RA disease activity; significantly high levels of serum IL-21 in RA | 4, 71 |
| Treg/– | RA risk SNPs overlap with epigenetically activated H3K4me3 peaks in Treg cells | 74 |

* RA = rheumatoid arthritis; SNPs = single -nucleotide polymorphisms; IL-4R = IL-4 receptor; H3K4me3 = histone H3 trimethyl lysine 4 (see Table 1 for other definitions).

## Regulation of CD4+ T cell differentiation and function by transcription factors

Differentiation of naive CD4+ T cells into each type of T helper cell subset depends on the expression of specific transcription factors induced by specific cytokines (Table 2). For example, differentiation of naive CD4+ T cells to Th1 cells depends on the expression of the transcription factor T-bet, which is induced by T cell receptor (TCR) stimulation, the IL-12/STAT-4 signaling pathway, and the IFNγ/STAT-1 signaling pathway (23–25). T-bet directly activates the production of IFNγ (23). On the other hand, differentiation of Th2 cells is dependent on the induction of transcription factor GATA-3 by the IL-4/STAT-6 pathway (26,27). Similarly, Th17 cell differentiation in mice is determined by the expression of transcription factor RORγt induced by TGFβ and the IL-6/STAT3 pathway (28–30). RORγt is also up-regulated in human Th17 cells (31), and TGFβ with IL-1β and IL-6, with IL-1β and IL-21, or with IL-1β and IL-23 can induce RORγt and IL-17 expression in naive human CD4+ T cells (32,33). Down-regulation of RORγt inhibits Th17 cell differentiation, suggesting that RORγt is a master transcription factor in Th17 cell differentiation. Other transcription factors such as RUNX-1 and aryl hydrocarbon receptor (Ahr) are also known to enhance Th17 differentiation dependent on or independent of RORγt (34–36). In Tfh cells, Bcl-6 has been identified as the transcription factor involved in the induction of differentiation (37,38). Furthermore, Bcl-3 was also recently reported to enhance the differentiation of human Tfh cells (39).

Previous studies have demonstrated that these transcription factors negatively regulate the differentiation of other T cell subsets by direct co-interaction and/or an indirect effect of cytokine production by each T cell subset. T-bet interacts directly with GATA-3, and inhibits transcriptional activity (40). T-bet also inhibits the expression of RORγt by interacting with RUNX-1, which induces the expression of RORγt and IL-17 (41), and the indirect suppression of STAT-3 phosphorylation via the IFNγ/STAT-1/SOCS-3 pathway (42). Moreover, we recently reported that T-bet regulates Th17 differentiation through inhibition of Ahr expression (43).

The suppressive capacity of Treg cells requires the presence of FoxP3. FoxP3+ Treg cells can also undergo stimulus-specific differentiation, which is regulated by the expression of transcription factors typically associated with the differentiation of conventional CD4+ T helper cells (44). These mechanisms affect the migratory and functional features of effector Treg cells matched to the environment that induced the initial response. Thus, FoxP3+ Treg cells are phenotypically and functionally diverse, and closely parallel the differentiation state of conventional T cells that occupy the same regulatory environment and tissue niche.

## Pivotal roles of T helper and Treg cells in the pathogenesis of RA and experimentally induced arthritis

The importance of CD4+ T cells in the pathogenesis of RA has been demonstrated in some studies, including investigations on the infiltration of T cells in inflammatory synovial tissue, the association of HLA genes with susceptibility to RA (45), and the effectiveness of CTLA-4 immunoglobulin Fc fusion protein and of abatacept in RA (46). In particular, the HLA alleles associated with susceptibility to RA are HLA–DR4 (DRB1*0401, *0404, and *0405), HLA–DR1 (DRB1*0101 and *0102), and HLA–DR10 (DRB1*0101), all of which share a conserved 5-residue motif ([Q/R]-[R/K]-R-A-A) in their peptide-binding groove, called the shared epitope (47). Thus, susceptibility-associated HLA alleles are likely to affect antigen presentation, especially in citrullinated antigens (47).

The precise role of CD4+ T cell subpopulations and their secreting cytokines in RA remains unclear. In addition, it is uncertain how transcription factors regulate

**Table 3.** Association of T helper cells and their cytokines with murine autoimmune arthritis models*

| T cell type/cytokine, experimental system | Disease | Phenotype | Reference |
|---|---|---|---|
| Th1/IFNγ | | | |
| Deficiency | CIA | Exacerbation | 50 |
| Deficiency of IFNγ receptor | CIA | Exacerbation | 51 |
| Th2/IL-4, treatment with IL-4 | CIA | Amelioration | 55 |
| Th17/IL-17 | | | |
| Deficiency | CIA | Amelioration | 57 |
| Blocking antibody | CIA | Amelioration | 58 |
| Blocking antibody | GIA | Amelioration | 59 |
| Th17/IL-22, deficiency | CIA | Amelioration | 61 |
| Tfh/IL-2, blocking antibody | CIA | Amelioration | 72 |
| Treg/– | | | |
| Depletion of CD25+ cells | CIA | Exacerbation | 76 |
| Transfer of CD4+CD25+ Treg cells | K/BxN arthritis | Amelioration | 77 |

* CIA = collagen-induced arthritis; GIA = glucose-6-phosphate isomerase–induced arthritis (see Table 1 for other definitions).

the differentiation and function of T helper cells and Treg cells in RA. However, several new findings have been revealed in studies of RA patients and of animal models of autoimmune arthritis that focused on the functional role of Th17 and Tfh cells in RA. Herein we summarize the role of cytokine and transcription factor expression in each T cell subpopulation in the pathology of human and experimental RA.

**Th1 cells.** Classically, Th1 cells have been considered to play a predominant role in inflammatory RA, because most synovium-infiltrating CD4+ T cells express IFNγ, and IFNγ activates macrophages and induces production of the proinflammatory cytokine tumor necrosis factor (TNF) (1). However, a number of studies have demonstrated that the Th1 phenotype does not explain all of the mechanisms involved in RA, such as the inhibition of bone resorption mediated by the suppression of osteoclast formation by IFNγ (48), and the lack of efficacy of monoclonal antibodies to IFNγ in most patients (49) (Table 2). In animal models of RA, IFNγ also exhibits antiinflammatory properties during the development of autoimmune arthritis; the severity of collagen-induced arthritis (CIA) is exacerbated in both IFNγ-deficient mice and IFNγ receptor–deficient mice (50,51) (Table 3).

We previously reported significant suppression of CIA in CD2-specific, T-bet transgenic mice, that were cultured for Th17 cell differentiation (6). In the same study, we also found that T-bet overexpression suppressed the expression of RORγt, resulting in inhibition of differentiation of antigen-reactive Th17 cells, and that the regulatory effect was independent of IFNγ (6). Another study showed that T-bet deficiency did not affect the development of arthritis in a transfer model of arthritogenic KRN TCR transgenic T cells in TCR-deficient mice (52) (Table 4). These data suggest the possibility that Th1 cells induced by T-bet and their major cytokine IFNγ might not only facilitate the inflammation of RA but also have a protective role in the pathology of RA.

**Th2 cells.** The Th1/Th2 paradigm, in which Th2 cells and their cytokines, such as IL-4 and IL-10, suppress Th1 cells, had been used to understand the pathology of RA. However, recent studies have focused on the relationships between Th2 cells and Th17 cells. A single-nucleotide polymorphism (SNP) in the coding region of *IL-4R*, governing the presence of isoleucine versus valine at position 50 in the amino acid sequence, affects the course and severity of RA (53) (Table 2). In addition, this polymorphism in *IL-4R* controls the magnitude of IL-17 production (54) (Table 2). In animal models of RA, IL-4 also suppresses CIA disease activity (55) (Table 3).

**Table 4.** Association of genetic modulation of transcription factors in CD4+ T cells with murine autoimmune arthritis models*

| T cell type/transcription factor, experimental system | Disease | Phenotype | Mechanism | Reference |
|---|---|---|---|---|
| Th1/T-bet | | | | |
| Transgenic | CIA | Suppression | Inhibition of Th17 cell differentiation | 6 |
| Deficiency | KRN T cell transfer | Not affected | Knockout of T-bet expression did not inhibit induction of arthritis | 52 |
| Th2/GATA-3, transgenic | Antigen-induced arthritis | Suppression | Inhibition of Th17 cell differentiation | 56 |
| Th17/RORγt, transgenic | CIA | Suppression | Accumulation of RORγt+CCR6+FoxP3+ Treg cells in inflamed joints | 7 |
| Th17/Ahr, conditional knockout (CD4+ cells) | CIA | Suppression | Decrease in Th17 cells | 63 |
| Tfh/Bcl6, conditional knockout (CD4+ cells) | KRN T cell transfer | Suppression | Reduction in anti-GPI IgG titer due to loss of Tfh cell differentiation | 73 |
| Treg/FoxP3, scurfy mutation | K/BxN arthritis | Acceleration | Earlier autoantibody production | 79 |

* CIA = collagen-induced arthritis; RORγt = retinoic acid receptor–related orphan nuclear receptor γt; Ahr = aryl hydrocarbon receptor; Tfh = T follicular helper cell; anti-GPI = anti–glucose-6-phosphate isomerase.

There are few reports concerning the commitment of Th2 cell–specific transcription factors to RA and its animal models. In mouse models of autoimmune arthritis, GATA-3 expression protects against joint inflammation and destruction by reducing the differentiation of Th17 cells (56) (Table 4). Accordingly, Th2 cells induced by GATA-3 may regulate the pathology of RA via inhibition of Th1 and Th17 cells.

**Th17 cells.** As described above, a number of studies have shown the importance of Th17 cells in RA and its animal models. A large proportion of IL-1–positive CD4+ T cells (Th17 cells) are found among peripheral blood mononuclear cells in RA patients compared with healthy control subjects, and their proportion correlates with systemic disease activity both at disease onset and during the progression of RA (3) (Table 2). Similarly, IL-17–producing Th17 cells appear to play a critical role in the development of various forms of experimental autoimmune arthritis; IL-17–deficient mice are resistant to CIA (57), and blockade of IL-17 ameliorates the severity of proteoglycan G1 domain–induced arthritis (58) and CIA (59) (Table 3). Moreover, Th17 cells induce osteoclastogenesis through the up-regulation of RANKL on their surface, and of osteoblasts and synovial fibroblasts via IL-17 (60). IL-17 also induces the production of inflammatory cytokines—such as TNF and IL-6—from synovial fibroblasts and macrophages and enhances the accumulation of inflammatory cells. IL-22 is produced by Th17 cells, and IL-22–deficient mice have also shown amelioration of CIA severity via a reduction in germinal center formation in the spleen, along with a decline in germinal center B cells (61).

The expression levels of RORC in CD4+ T cells are higher in RA patients than healthy subjects (3) (Table 5). We have also demonstrated that overexpression of RORγt in CD4+ T cells induces high expression of CCR6 and facilitates the migration of CD4+ T cells into inflamed joints of RA patients via CCL20, a CCR6-specific chemokine ligand (Kaneko S, et al: unpublished observations). In addition, Nguyen et al (62) suggested that Ahr accelerates the differentiation of Th17 cells by regulating the expression of microRNAs (miRs), such as miR-212. In studies that examined the relationship between Ahr and the pathology of autoimmune arthritis in animal models, T cell–specific, Ahr-deficient mice (Lck-Cre $Ahr^{flox/flox}$ mice) showed significant suppression of CIA through a decrease in Th17 cells and an increase in Th1 cells in lymph nodes, suggesting that Ahr regulates the Th1/Th17 balance during the development of autoimmune arthritis (63) (Table 4). Collectively, these findings indicate that the pathology of RA might be strongly influenced by an imbalance of the differentiation and function of Th1 and Th2/Th17 cells.

Based on these immunomodulatory effects in RA and its animal models, IL-17 blockade has been considered as a therapeutic option for RA, and clinical trials exploring this possibility have been conducted. While the IL-17–blocking antibodies secukinumab and ixekizumab and their receptor brodalumab are reported to be therapeutically effective in RA, their efficacy appears to be inferior to that of preexisting biologics, such as anti-TNF agents (64,65). These results probably reflect the differences in the pathogenesis of RA and experimentally induced arthritis in laboratory animals, as well as the heterogeneity in the etiology of RA.

Moreover, several low-molecular weight compounds that target various transcription factors and signaling molecules have been synthesized and their therapeutic effects tested in autoimmune diseases. For example, tofacitinib, a JAK inhibitor, has been used for the treatment of RA, and its efficacy is reported to be better than or equal to that of biologic disease-modifying anti rheumatic drugs (66). The effectiveness of tofacitinib is reported to be due, at least in part, to the inhibition of Th1 and Th17 cells differentiation (67). Several synthetic ligands that bind to and inhibit RORγt (RORγt antagonists) have also been developed recently (68), and their therapeutic potential in autoimmunity has been assessed in at least some animal models. In CIA, RORγt antagonism ameliorated the severity of arthritis (69), and our own data (70) confirmed that RORγt antagonists can suppress autoimmune sialadenitis in mice with type 3

**Table 5.** Association of genetic modulation of transcription factors in CD4+ T cells with RA*

| T cell type/transcription factor | Findings | Reference |
|---|---|---|
| Th17/*RORC* | *RORC* overexpression in CD4+ T cells of RA patients | 3 |
| Th17/Ahr | Ahr accelerates differentiation of Th17 cells | 62 |
| Tfh/ Bcl3 | Significantly high Bcl-3 levels in RA patients; Bcl-3 up-regulates Bcl-6 expression and induces IL-21–producing CD4+ T cells | 39 |
| Treg/FoxP3 | In RA patients, FoxP3+ Treg cells lose their suppressive capacity by inhibition of transcriptional activity of FoxP3 through dephosphorylation by TNF | 78 |

* RA = rheumatoid arthritis; Ahr = aryl hydrocarbon receptor; TNF = tumor necrosis factor (see Table 1 for other definitions).

muscarinic acetylcholine receptor–induced sialadenitis. These findings add support to the concept that autoimmunity, especially in RA, can potentially be treated by targeting one or more transcription factors.

**Tfh cell and PD-1$^{high}$CXCR5– activated T cells.** Some studies have shown that Tfh cells also play an important pathogenic role in RA; a high proportion of circulating CXCR5+PD-1+CD4+ Tfh–like cells is found in patients with RA, and correlates positively with RA disease activity. Furthermore, serum IL-21 levels are significantly increased in RA patients and correlate with RA disease activity (4,71) (Table 2). Recent studies have also demonstrated the important role of Tfh cells in the pathology of autoimmune arthritis; blockade of IL-21 suppresses the development of CIA (72) (Table 3).

Meguro et al have reported that levels of transcription factor Bcl-3 were significantly higher in RA patients compared with healthy subjects and that Bcl-3 up-regulates the expression of Bcl-6—a master transcription factor of Tfh cells—and induces IL-21–producing CD4+ T cells (39) (Table 5). In a model using transfer of arthritogenic KRN TCR– transgenic T cells into TCR-deficient mice, conditional deletion of Bcl-6 in T cells (CD4-Cre Bcl-6$^{flox/flox}$ mice) blocked Tfh differentiation, resulting in inhibition of arthritis through a reduction in autoantibody formation. In contrast, conditional deletion of IL-17 in T cells (CD4-Cre IL-17a$^{flox/flox}$ mice) had no effect on the development of arthritis in the same model (73) (Table 4). These findings suggest that autoantibody formation in autoimmune arthritis is regulated mainly by Bcl-6–induced Tfh cells rather than by Th17 cells.

Recently, using mass cytometry, Rao et al identified PD-1$^{high}$CXCR5– T cells among activated T cells infiltrating RA synovium (5). Like Tfh cells, PD-1$^{high}$ CXCR5– activated T cells expressed some factors enabling B cell help, including IL-21 and CXCL13, and induced plasma cell differentiation in vitro. In addition, PD-1$^{high}$CXCR5– T cells may infiltrate chronically inflamed tissues, recruit both Tfh cells and B cells, and promote local autoantibody formation in ectopic lymphoid structure. The expression of transcription factor Bcl-6 is not elevated in PD-1$^{high}$CXCR5– T cells, while B lymphocyte–induced maturation protein 1, a transcription factor typically down-regulated in Tfh cells, is up-regulated. Thus, the transcriptional regulation of the differentiation of PD-1$^{high}$CXCR5– T cells was different from that of Tfh cells, although both cell types are related in autoantibody formation in RA (5).

Taken together, the above findings demonstrate that not only Tfh cells, but also PD-1$^{high}$CXCR5– T cells, have a central role in the pathology of RA via facilitation of B cell help to produce autoantibodies. The precise regulatory mechanism of these cells' differentiation, however, remains to be clarified.

**Treg cells.** A meta-analysis of genome-wide association in RA patients that evaluated ~10 million SNPs revealed that biologic RA risk genes segregate in a region where RA risk and SNP overlap with epigenetically activated H3K4 trimethylation peaks in Treg cells (74) (Table 2). These findings highlight the important role of Treg cells in the pathogenic process of RA, though the exact regulatory mechanisms of these cells also remain

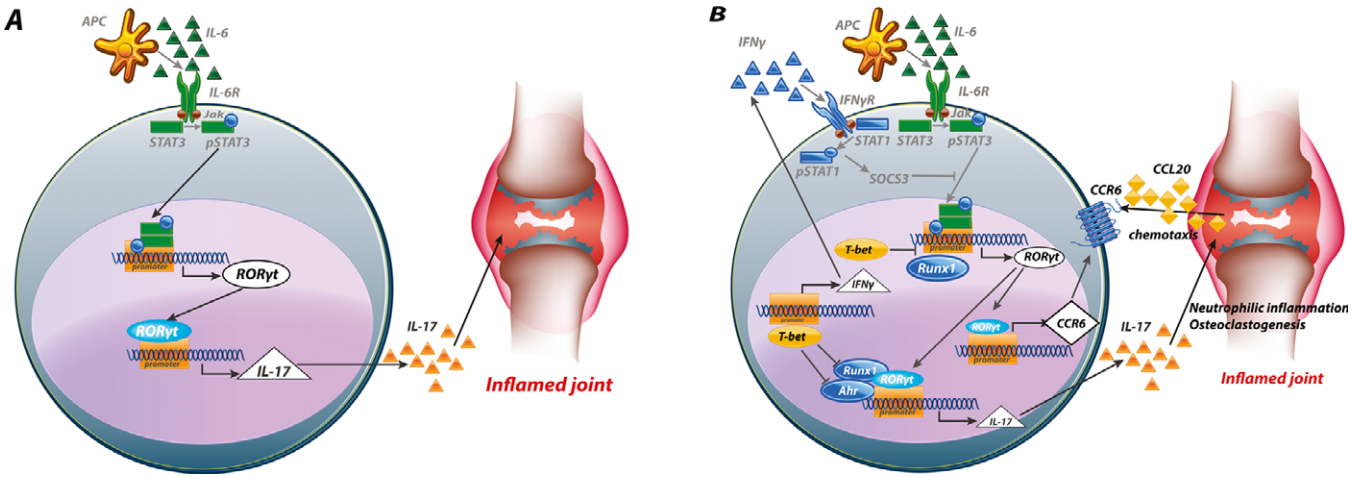


**Figure 1.** Regulation of transcription factors involved in the pathogenesis of experimentally induced autoimmune arthritis. The potential relationships among various transcription factors involved in the pathology of rheumatoid arthritis (left) and experimentally induced autoimmune arthritis (right) are shown. APC = antigen-presenting cell; IL-6R = interleukin-6 receptor; RORγt = retinoic acid receptor–related orphan nuclear receptor γt; IFNγR = interferon-γ receptor; SOCS3 = suppressor of cytokine signaling 3; Ahr = aryl hydrocarbon receptor.

elusive. While previous studies of the role of the Treg cell population in RA differed in their estimation of the proportion of circulating Treg cells, they consistently showed the presence of high numbers of these cells in the inflamed joints of RA patients (75). Treg cells appear to have regulatory roles in the development of murine autoimmune arthritis, because depletion of CD25+ cells exacerbates CIA (76), and transfer of CD4+ CD25+ Treg cells ameliorates the development of arthritis (77) (Table 3).

With regard to FoxP3 specifically, it is reported that FoxP3+ Treg cells in RA patients lose their suppressive capacity by inhibiting the transcriptional activity of FoxP3 through dephosphorylation by TNF (78) (Table 5). FoxP3-deficient KBx/N mice, which spontaneously develop autoimmune arthritis, have shown faster and more aggressive disease (79) (Table 4). In our studies of CIA in CD2-specific RORγt transgenic mice, the majority of FoxP3+ Treg cells highly expressed RORγt and CCR6, and accumulated in the inflamed joints. Moreover, Treg cells produced high amounts of IL-10, but not IL-17, resulting in the attenuation of severity of CIA compared with disease in wild-type mice (7) (Table 4). On the other hand, it has also been reported that $CD25^{low}$FoxP3+ T cells lose FoxP3 expression and redifferentiate into arthritogenic Th17 cells (exFoxP3 Th17 cells) (80).

These results suggest that the suppressive function of FoxP3+ Treg cells may be impaired by the inflammatory conditions of RA. Future research is needed to understand the mechanisms that regulate a balance between T helper cells and Treg cells in RA.

## Conclusions

In this review, we have summarized the latest research findings concerning the roles of transcription factors in the differentiation and function of CD4+ T cells in autoimmune arthritis. There is no doubt that the proportions and functional imbalances among various T helper cell subsets and between T helper and Treg cells play a critical role in RA pathogenesis. There is a need for more research into the role of transcription factors in human T helper cell differentiation: specifically, how the different transcription factors regulate arthritogenic T helper cells and the development of RA (Figure 1). Previous studies in mice have shown convincing evidence of the importance of transcription factors in the differentiation and function of both T helper and Treg cells. In addition, the available data confirm the role of transcription factors in the development of murine autoimmune arthritis through the regulation of differentiation of arthritogenic Th17 cells (Figure 1). Although more work is needed to determine how the expression of transcription factors in CD4+ T cells regulates the balance between T helper and Treg cells, especially in RA patients, we believe that new approaches that target specific transcription factors, and hence modulation of differentiation and function of CD4+ T cells, could likely yield efficacious therapies for RA.

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## AUTHOR CONTRIBUTIONS

All authors drafted the article, revised it critically for important intellectual content, approved the final version to be published, and take responsibility for the integrity of the data and the accuracy of the data analysis.

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