

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

著者別名	住田 孝之, 瀬川 誠司, 松本 功, 近藤 裕也, 坪井 洋人
journal or publication title	Arthritis & rheumatology
volume	70
number	5
page range	653-661
year	2018-05
権利	(C) 2017 The Authors. Arthritis & Rheumatology published by Wiley Periodicals, Inc. on behalf of American College of Rheumatology. This is an open access article under the terms of the Creative Commons Attribution-NonCommercial License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited and is not used for commercial purposes.
URL	http://hdl.handle.net/2241/00152011

doi: 10.1002/art.40398

REVIEW

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

Yuya Kondo, Masahiro Yokosawa, Shunta Kaneko, Kotona Furuyama, Seiji Segawa, Hiroto Tsuboi, Isao Matsumoto, and Takayuki Sumida

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.

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 (IFN γ)-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

Supported by the Research Program for Intractable Diseases, Health and Labor Sciences Research Grants from the Ministry of Health, Labor and Welfare, Japan, and Grants-in Aid for Scientific Research (Grant-in-Aid for Scientific Research [C]) from the Ministry of Education, Culture, Sports, Science and Technology and Japan Society for the Promotion of Science.

Yuya Kondo, MD, PhD, Masahiro Yokosawa, MD, PhD, Shunta Kaneko, MD, Kotona Furuyama, Seiji Segawa, PhD, Hiroto Tsuboi, MD, PhD, Isao Matsumoto, MD, PhD, Takayuki Sumida, MD, PhD: University of Tsukuba, Tsukuba, Japan.

Address correspondence to Takayuki Sumida, MD, PhD, Department of Internal Medicine, Faculty of Medicine, University of Tsukuba, 1-1-1 Tennodai, Tsukuba, Ibaraki 305-8575, Japan. E-mail: tsumida@md.tsukuba.ac.jp.

Submitted for publication July 14, 2017; accepted in revised form December 5, 2017.

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 γ t [ROR γ t], 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 ROR γ t, 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 ROR γ t 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 IFN γ 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 (TGF β) (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	IFN γ	–	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 TGF β	–	–	8, 18–22

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

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

T cell type/ cytokine	Findings	References
Th1/IFN γ	T cell clones from RA synovium produce large amounts of IFN γ ; IFN γ inhibits bone resorption mediated by suppression of osteoclast formation; monoclonal antibody to IFN γ 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 IFN γ /STAT-1 signaling pathway (23–25). T-bet directly activates the production of IFN γ (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 ROR γ t induced by TGF β and the IL-6/STAT3 pathway (28–30). ROR γ t is also up-regulated in human Th17 cells (31), and TGF β with IL-1 β and IL-6, with IL-1 β and IL-21, or with IL-1 β and IL-23 can induce ROR γ t and IL-17 expression in naive human CD4+ T cells (32,33). Down-regulation of ROR γ t inhibits Th17 cell differentiation, suggesting that ROR γ t 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 ROR γ t (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 ROR γ t by interacting with RUNX-1, which induces the expression of ROR γ t and IL-17 (41), and the

indirect suppression of STAT-3 phosphorylation via the IFN γ /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/IFN γ			
Deficiency	CIA	Exacerbation	50
Deficiency of IFN γ 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 IFN γ , and IFN γ 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 IFN γ (48), and the lack of efficacy of monoclonal antibodies to IFN γ in most patients (49) (Table 2). In animal models of RA, IFN γ also exhibits antiinflammatory properties during the development of autoimmune arthritis; the severity of collagen-induced arthritis (CIA) is exacerbated in both IFN γ -deficient mice and IFN γ 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 ROR γ t, resulting in inhibition of differentiation of antigen-reactive Th17 cells, and that the regulatory effect was independent of IFN γ (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 IFN γ 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/ROR γ t, transgenic	CIA	Suppression	Accumulation of ROR γ t+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; ROR γ t = retinoic acid receptor-related orphan nuclear receptor γ t; 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 ROR γ t 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^{fllox/fllox} 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 ROR γ t (ROR γ t antagonists) have also been developed recently (68), and their therapeutic potential in autoimmunity has been assessed in at least some animal models. In CIA, ROR γ t antagonism ameliorated the severity of arthritis (69), and our own data (70) confirmed that ROR γ t 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

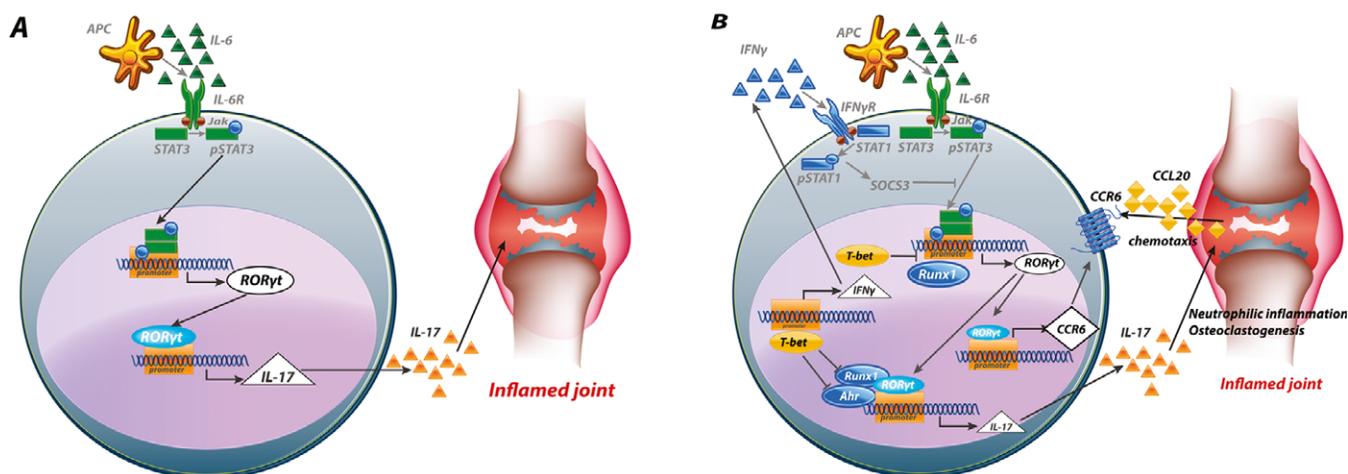


Figure 1. Regulation of transcription factors involved in the pathogenesis of experimentally induced autoimmune arthritis. The potential relationships among various transcription factors involved in the pathology of rheumatoid arthritis (left) and experimentally induced autoimmune arthritis (right) are shown. APC = antigen-presenting cell; IL-6R = interleukin-6 receptor; ROR γ t = retinoic acid receptor-related orphan nuclear receptor γ t; IFN γ R = 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 ROR γ t transgenic mice, the majority of FoxP3⁺ Treg cells highly expressed ROR γ t 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.

ACKNOWLEDGMENT

We thank Dr. F. G. Issa for the critical reading of the manuscript.

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.

REFERENCES

1. Furst DE, Emery P. Rheumatoid arthritis pathophysiology: update on emerging cytokine and cytokine-associated cell targets. *Rheumatology (Oxford)* 2014;53:1560–9.
2. Chabaud M, Durand JM, Buchs N, Fossiez F, Page G, Frappart L, et al. Human interleukin-17: a T cell-derived proinflammatory cytokine produced by the rheumatoid synovium. *Arthritis Rheum* 1999;42:963–70.
3. Leipe J, Grunke M, Dechant C, Reindl C, Kerzendorf U, Schulze-Koops H, et al. Role of Th17 cells in human autoimmune arthritis. *Arthritis Rheum* 2010;62:2876–85.
4. Ma J, Zhu C, Ma B, Tian J, Baidoo SE, Mao C, et al. Increased frequency of circulating follicular helper T cells in patients with rheumatoid arthritis. *Clin Dev Immunol* 2012;2012:827480.
5. Rao DA, Gurish MF, Marshall JL, Slowikowski K, Fonseka CY, Liu Y, et al. Pathologically expanded peripheral T helper cell subset drives B cells in rheumatoid arthritis. *Nature* 2017;542:110–4.
6. Kondo Y, Iizuka M, Wakamatsu E, Yao Z, Tahara M, Tsuboi H, et al. Overexpression of T-bet gene regulates murine autoimmune arthritis. *Arthritis Rheum* 2012;64:162–72.
7. Kondo Y, Yao Z, Tahara M, Iizuka M, Yokosawa M, Kaneko S, et al. Involvement of ROR γ t-overexpressing T cells in the development of autoimmune arthritis in mice. *Arthritis Res Ther* 2015;17:105.
8. Hori S, Nomura T, Sakaguchi S. Control of regulatory T cell development by the transcription factor Foxp3. *Science* 2003;299:1057–61.
9. Mosmann TR, Coffman RL. TH1 and TH2 cells: different patterns of lymphokine secretion lead to different functional properties. *Annu Rev Immunol* 1989;7:145–73.
10. Park H, Li Z, Yang XO, Chang SH, Nurieva R, Wang YH, et al. A distinct lineage of CD4 T cells regulates tissue inflammation by producing interleukin 17. *Nat Immunol* 2005;6:1133–41.
11. Harrington LE, Hatton RD, Mangan PR, Turner H, Murphy TL, Murphy KM, et al. Interleukin 17-producing CD4⁺ effector T cells develop via a lineage distinct from the T helper type 1 and 2 lineages. *Nat Immunol* 2005;6:1123–32.
12. Huang W, Na L, Fidel PL, Schwarzenberger P. Requirement of interleukin-17A for systemic anti-Candida albicans host defense in mice. *J Infect Dis* 2004;190:624–31.
13. Vogelzang A, McGuire HM, Yu D, Sprent J, Mackay CR, King C. A fundamental role for interleukin-21 in the generation of T follicular helper cells. *Immunity* 2008;29:127–37.

14. Nurieva RI, Chung Y, Hwang D, Yang XO, Kang HS, Ma L, et al. Generation of T follicular helper cells is mediated by interleukin-21 but independent of T helper 1, 2, or 17 cell lineages. *Immunity* 2008;29:138–49.
15. DuPage M, Bluestone JA. Harnessing the plasticity of CD4⁺ T cells to treat immune-mediated disease. *Nat Rev Immunol* 2016;16:149–63.
16. Maecker HT, McCoy JP, Nussenblatt R. Standardizing immunophenotyping for the Human Immunology Project. *Nat Rev Immunol* 2012;12:191–200.
17. Sakaguchi S, Sakaguchi N, Asano M, Itoh M, Toda M. Immunologic self-tolerance maintained by activated T cells expressing IL-2 receptor α -chains (CD25): breakdown of a single mechanism of self-tolerance causes various autoimmune diseases. *J Immunol* 1995;155:1151–64.
18. Jordan MS, Boesteanu A, Reed AJ, Petrone AL, Hohenbeck AE, Lerman MA, et al. Thymic selection of CD4⁺CD25⁺ regulatory T cells induced by an agonist self-peptide. *Nat Immunol* 2001;2:301–6.
19. Wing K, Onishi Y, Prieto-Martin P, Yamaguchi T, Miyara M, Fehervari Z, et al. CTLA-4 control over Foxp3⁺ regulatory T cell function. *Science* 2008;322:271–5.
20. De la Rosa M, Rutz S, Dorninger H, Scheffold A. Interleukin-2 is essential for CD4⁺CD25⁺ regulatory T cell function. *Eur J Immunol* 2004;34:2480–8.
21. Maynard CL, Harrington LE, Janowski KM, Oliver JR, Zindl CL, Rudensky AY, et al. Regulatory T cells expressing interleukin 10 develop from Foxp3⁺ and Foxp3⁻ precursor cells in the absence of interleukin 10. *Nat Immunol* 2007;8:931–41.
22. Li MO, Wan YY, Flavell RA. T cell-produced transforming growth factor- β 1 controls T cell tolerance and regulates Th1- and Th17-cell differentiation. *Immunity* 2007;26:579–91.
23. Szabo SJ, Kim ST, Costa GL, Zhang X, Fathman CG, Glimcher LH. A novel transcription factor, T-bet, directs Th1 lineage commitment. *Cell* 2000;100:655–69.
24. Afkarian M, Sedy JR, Yang J, Jacobson NG, Cereb N, Yang SY, et al. T-bet is a STAT1-induced regulator of IL-12R expression in naive CD4⁺ T cells. *Nat Immunol* 2002;3:549–57.
25. Kanhere A, Hertweck A, Bhatia U, Gokmen MR, Perucha E, Jackson I, et al. Tbet and GATA3 orchestrate Th1 and Th2 differentiation through lineage-specific targeting of distal regulatory elements. *Nat Commun* 2012;3:1268.
26. Zheng W, Flavell RA. The transcription factor GATA-3 is necessary and sufficient for Th2 cytokine gene expression in CD4 T cells. *Cell* 1997;89:587–96.
27. Skapenko A, Leippe J, Niesner U, Devriendt K, Beetz R, Radbruch A, et al. GATA-3 in human T cell helper type 2 development. *J Exp Med* 2004;199:423–8.
28. Ivanov II, McKenzie BS, Zhou L, Tadokoro CE, Lepelley A, Lafaille JJ, et al. The orphan nuclear receptor ROR γ t directs the differentiation program of proinflammatory IL-17⁺ T helper cells. *Cell* 2006;126:1121–33.
29. Nishihara M, Ogura H, Ueda N, Tsuruoka M, Kitabayashi C, Tsuji F, et al. IL-6-gp130-STAT3 in T cells directs the development of IL-17⁺ Th with a minimum effect on that of Treg in the steady state. *Int Immunol* 2007;19:695–702.
30. Yang XO, Pappu BP, Nurieva R, Akimzhanov A, Kang HS, Chung Y, et al. T helper 17 lineage differentiation is programmed by orphan nuclear receptors ROR α and ROR γ . *Immunity* 2008;28:29–39.
31. Unutmaz D. RORC2: the master of human Th17 cell programming. *Eur J Immunol* 2009;39:1452–5.
32. Manel N, Unutmaz D, Littman DR. The differentiation of human T_H-17 cells requires transforming growth factor- β and induction of the nuclear receptor ROR γ t. *Nat Immunol* 2008;9:641–9.
33. Volpe E, Servant N, Zollinger R, Bogiatzi SI, Hupe P, Barillot E, et al. A critical function for transforming growth factor- β , interleukin 23 and proinflammatory cytokines in driving and modulating human T_H-17 responses. *Nat Immunol* 2008;9:650–7.
34. Zhang F, Meng G, Strober W. Interactions among the transcription factors Runx1, ROR γ t and Foxp3 regulate the differentiation of interleukin 17-producing T cells. *Nat Immunol* 2008;9:1297–306.
35. Veldhoen M, Hirota K, Westendorf AM, Buer J, Dumoutier L, Renaud JC, et al. The aryl hydrocarbon receptor links TH17-cell-mediated autoimmunity to environmental toxins. *Nature* 2008;453:106–9.
36. Trifari S, Kaplan CD, Tran EH, Crellin NK, Spits H. Identification of a human helper T cell population that has abundant production of interleukin 22 and is distinct from T_H-17, T_H1 and T_H2 cells. *Nat Immunol* 2009;10:864–71.
37. Yu D, Rao S, Tsai LM, Lee SK, He Y, Sutcliffe EL, et al. The transcriptional repressor Bcl-6 directs T follicular helper cell lineage commitment. *Immunity* 2009;31:457–68.
38. Kroenke MA, Eto D, Locci M, Cho M, Davidson T, Haddad EK, et al. Bcl6 and Maf cooperate to instruct human follicular helper CD4 T cell differentiation. *J Immunol* 2012;188:3734–44.
39. Meguro K, Suzuki K, Hosokawa J, Sanayama Y, Tanaka S, Furuta S, et al. Role of Bcl-3 in the development of follicular helper T cells and in the pathogenesis of rheumatoid arthritis. *Arthritis Rheumatol* 2015;67:2651–60.
40. Hwang ES, Szabo SJ, Schwartzberg PL, Glimcher LH. T helper cell fate specified by kinase-mediated interaction of T-bet with GATA-3. *Science* 2005;307:430–3.
41. Lazarevic V, Chen X, Shim JH, Hwang ES, Jang E, Bolm AN, et al. Tbet represses T_H17 differentiation by preventing Runx1-mediated activation of the gene encoding ROR γ t. *Nat Immunol* 2011;12:96–104.
42. Tanaka K, Ichiyama K, Hashimoto M, Yoshida H, Takimoto T, Takaesu G, et al. Loss of suppressor of cytokine signaling 1 in helper T cells leads to defective Th17 differentiation by enhancing antagonistic effects of IFN- γ on STAT3 and Smads. *J Immunol* 2008;180:3746–56.
43. Yokosawa M, Kondo Y, Tahara M, Iizuka-Koga M, Segawa S, Kaneko S, et al. Tbet over-expression regulates aryl hydrocarbon receptor-mediated T helper type 17 differentiation through an interferon (IFN) γ -independent pathway. *Clin Exp Immunol* 2017;188:22–35.
44. Cretney E, Kallies A, Nutt SL. Differentiation and function of Foxp3⁺ effector regulatory T cells. *Trends Immunol* 2013;34:74–80.
45. Deighton CM, Walker DJ, Griffiths ID, Roberts DF. The contribution of HLA to rheumatoid arthritis. *Clin Genet* 1989;36:178–82.
46. Kremer JM, Peterfy C, Russell AS, Emery P, Abud-Mendoza C, Sibilia J, et al. Longterm safety, efficacy, and inhibition of structural damage progression over 5 years of treatment with abatacept in patients with rheumatoid arthritis in the Abatacept in Inadequate Responders to Methotrexate trial. *J Rheumatol* 2014;41:1077–87.
47. Boissier MC, Semerano L, Challal S, Saidenberg-Kermanac'h N, Falgarone G. Rheumatoid arthritis: from autoimmunity to synovitis and joint destruction. *J Autoimmun* 2012;39:222–8.
48. Takahashi N, Mundy GR, Roodman GD. Recombinant human interferon- γ inhibits formation of human osteoclast-like cells. *J Immunol* 1986;137:3544–9.
49. Sigidin YA, Loukina GV, Skurkovich B, Skurkovich S. Randomized, double-blind trial of anti-interferon- γ antibodies in rheumatoid arthritis. *Scand J Rheumatol* 2001;30:203–7.
50. Chu CQ, Swart D, Alcorn D, Tocker J, Elkon KB. Interferon- γ regulates susceptibility to collagen-induced arthritis through suppression of interleukin-17. *Arthritis Rheum* 2007;56:1145–51.
51. Geboes L, de Klerck B, van Balen M, Kelchtermans H, Mitera T, Boon L, et al. Freund's complete adjuvant induces arthritis in mice lacking a functional interferon- γ receptor by triggering tumor necrosis factor α -driven osteoclastogenesis. *Arthritis Rheum* 2007;56:2595–607.
52. Hickman-Brecks CL, Racz JL, Meyer DM, LaBranche TP, Allen PM. Th17 cells can provide B cell help in autoantibody induced arthritis. *J Autoimmun* 2011;36:65–75.

53. Prots I, Skapenko A, Wendler J, Mattyasovszky S, Yoné CL, Spriewald B, et al. Association of the IL4R single-nucleotide polymorphism I50V with rapidly erosive rheumatoid arthritis. *Arthritis Rheum* 2006;54:1491–500.
54. Wallis SK, Cooney LA, Endres JL, Lee MJ, Ryu J, Somers EC, et al. A polymorphism in the interleukin-4 receptor affects the ability of interleukin-4 to regulate Th17 cells: a possible immunoregulatory mechanism for genetic control of the severity of rheumatoid arthritis. *Arthritis Res Ther* 2011;13:R15.
55. Joosten LA, Lubberts E, Helsen MM, Saxne T, Coenen-de Roo CJ, Heinegard D, et al. Protection against cartilage and bone destruction by systemic interleukin-4 treatment in established murine type II collagen-induced arthritis. *Arthritis Res* 1999;1: 81–91.
56. Van Hamburg JP, Mus AM, de Bruijn MJ, de Vogel L, Boon L, Cornelissen F, et al. GATA-3 protects against severe joint inflammation and bone erosion and reduces differentiation of Th17 cells during experimental arthritis. *Arthritis Rheum* 2009;60:750–9.
57. Nakae S, Nambu A, Sudo K, Iwakura Y. Suppression of immune induction of collagen-induced arthritis in IL-17-deficient mice. *J Immunol* 2003;171:6173–7.
58. Iwanami K, Matsumoto I, Tanaka-Watanabe Y, Inoue A, Mihara M, Ohsugi Y, et al. Crucial role of the interleukin-6/interleukin-17 cytokine axis in the induction of arthritis by glucose-6-phosphate isomerase. *Arthritis Rheum* 2008;58:754–63.
59. Lubberts E, Koenders MI, Oppers-Walgreen B, van den Bersselaar L, Coenen-de Roo CJ, Joosten LA, et al. Treatment with a neutralizing anti-murine interleukin-17 antibody after the onset of collagen-induced arthritis reduces joint inflammation, cartilage destruction, and bone erosion. *Arthritis Rheum* 2004;50:650–9.
60. Kikuta J, Wada Y, Kowada T, Wang Z, Sun-Wada GH, Nishiyama I, et al. Dynamic visualization of RANKL and Th17-mediated osteoclast function. *J Clin Invest* 2013;123:866–73.
61. Corneth OB, Reijmers RM, Mus AM, Asmawidjaja PS, van Hamburg JP, Papazian N, et al. Loss of IL-22 inhibits autoantibody formation in collagen-induced arthritis in mice. *Eur J Immunol* 2016;46:1404–14.
62. Nguyen NT, Nakahama T, Nguyen CH, Tran TT, Le VS, Chu HH, et al. Aryl hydrocarbon receptor antagonism and its role in rheumatoid arthritis. *J Exp Pharmacol* 2015;7:29–35.
63. Nakahama T, Kimura A, Nguyen NT, Chinen I, Hanieh H, Nohara K, et al. Aryl hydrocarbon receptor deficiency in T cells suppresses the development of collagen-induced arthritis. *Proc Natl Acad Sci U S A* 2011;108:14222–7.
64. Tlustochowicz W, Rahman P, Seriole B, Krammer G, Porter B, Widmer A, et al. Efficacy and safety of subcutaneous and intravenous loading dose regimens of secukinumab in patients with active rheumatoid arthritis: results from a randomized phase II study. *J Rheumatol* 2016;43:495–503.
65. Genovese MC, Greenwald M, Cho CS, Berman A, Jin L, Cameron GS, et al. A phase II randomized study of subcutaneous ixekizumab, an anti-interleukin-17 monoclonal antibody, in rheumatoid arthritis patients who were naive to biologic agents or had an inadequate response to tumor necrosis factor inhibitors. *Arthritis Rheumatol* 2014;66:1693–704.
66. Kremer JM, Bloom BJ, Breedveld FC, Coombs JH, Fletcher MP, Gruben D, et al. The safety and efficacy of a JAK inhibitor in patients with active rheumatoid arthritis: results of a double-blind, placebo-controlled phase IIa trial of three dosage levels of CP-690,550 versus placebo [published erratum appears in *Arthritis Rheum* 2012;64:1487]. *Arthritis Rheum* 2009;60:1895–905.
67. Maeshima K, Yamaoka K, Kubo S, Nakano K, Iwata S, Saito K, et al. The JAK inhibitor tofacitinib regulates synovitis through inhibition of interferon- γ and interleukin-17 production by human CD4⁺ T cells. *Arthritis Rheum* 2012;64:1790–8.
68. Solt LA, Kumar N, Nuhant P, Wang Y, Lauer JL, Liu J, et al. Suppression of TH17 differentiation and autoimmunity by a synthetic ROR ligand. *Nature* 2011;472:491–4.
69. Chang MR, Lyda B, Kamenecka TM, Griffin PR. Pharmacologic repression of retinoic acid receptor-related orphan nuclear receptor γ is therapeutic in the collagen-induced arthritis experimental model. *Arthritis Rheumatol* 2014;66:579–88.
70. Tahara M, Tsuboi H, Segawa S, Asashima H, Iizuka-Koga M, Hirota T, et al. ROR γ t antagonist suppresses M3 muscarinic acetylcholine receptor-induced Sjogren's syndrome-like sialadenitis. *Clin Exp Immunol* 2017;187:213–24.
71. Wang J, Shan Y, Jiang Z, Feng J, Li C, Ma L, et al. High frequencies of activated B cells and T follicular helper cells are correlated with disease activity in patients with new-onset rheumatoid arthritis. *Clin Exp Immunol* 2013;174:212–20.
72. Ryu JG, Lee J, Kim EK, Seo HB, Park JS, Lee SY, et al. Treatment of IL-21R-Fc control autoimmune arthritis via suppression of STAT3 signal pathway mediated regulation of the Th17/Treg balance and plasma B cells. *Immunol Lett* 2015;163:143–50.
73. Block KE, Zheng Z, Dent AL, Kee BL, Huang H. Gut microbiota regulates K/BxN autoimmune arthritis through follicular helper T but not Th17 cells. *J Immunol* 2013;196:1550–7.
74. Okada Y, Wu D, Trynka G, Raj T, Terao C, Ikari K, et al. Genetics of rheumatoid arthritis contributes to biology and drug discovery. *Nature* 2014;506:376–81.
75. Miyara M, Gorochoff G, Ehrenstein M, Musset L, Sakaguchi S, Amoura Z. Human FoxP3⁺ regulatory T cells in systemic autoimmune diseases. *Autoimmun Rev* 2011;10:744–55.
76. Morgan ME, Suttmuller RP, Witteveen HJ, van Duivenvoorde LM, Zanelli E, Melief CJ, et al. CD25⁺ cell depletion hastens the onset of severe disease in collagen-induced arthritis. *Arthritis Rheum* 2003;48:1452–60.
77. Morgan ME, Flierman R, van Duivenvoorde LM, Witteveen HJ, van Ewijk W, van Laar JM, et al. Effective treatment of collagen-induced arthritis by adoptive transfer of CD25⁺ regulatory T cells. *Arthritis Rheum* 2005;52:2212–21.
78. Nie H, Zheng Y, Li R, Guo TB, He D, Fang L, et al. Phosphorylation of FOXP3 controls regulatory T cell function and is inhibited by TNF- α in rheumatoid arthritis. *Nat Med* 2013;19:322–8.
79. Nguyen LT, Jacobs J, Mathis D, Benoist C. Where FoxP3-dependent regulatory T cells impinge on the development of inflammatory arthritis. *Arthritis Rheum* 2007;56:509–20.
80. Komatsu N, Okamoto K, Sawa S, Nakashima T, Oh-hora M, Kodama T, et al. Pathogenic conversion of Foxp3⁺ T cells into TH17 cells in autoimmune arthritis. *Nat Med* 2014;20:62–8.