



Mini Review

FoxP3⁺ regulatory T cells in the peripheral blood and synovial fluid of patients with rheumatoid arthritis

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Human regulatory T (Treg) cells expressing the transcription factor forkhead box P3 (FoxP3) are indispensable for self-tolerance and immune homeostasis. Abnormal Treg cell counts and impaired function have been implicated in the development of various autoimmune diseases. The Treg cell counts in the peripheral blood of subjects with rheumatoid arthritis (RA) vary, probably due to heterogeneity of the Treg cells. Recent studies demonstrated that human FoxP3⁺ Treg cell populations actually include three functionally distinct subpopulations, and that CD45RA-FoxP3^{high} effector Treg cells are significantly decreased in the peripheral blood of patients with RA. On the other hand, it is generally agreed that the frequency of FoxP3⁺ Treg cells in synovial fluid is higher in patients with RA than in healthy controls. Although these findings were initially considered paradoxical, a recent study revealed that in RA patients, most of the cells in the FoxP3⁺ Treg population of the synovial fluid are actually non-suppressive cytokine-producing CD45RA-FoxP3^{low} non-Treg cells. The imbalance between FoxP3⁺ Treg cell subpopulations in the circulation and at inflammation sites may contribute to the pathogenesis of RA.

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Key words

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Introduction

Rheumatoid arthritis (RA) is a chronic systemic autoimmune disease characterized by synovial inflammation and hyperplasia, with T cells playing central pathogenic roles. Activated T cells infiltrate the pannus and synovial fluid, where they contribute to a series of inflammatory

processes that destroy cartilage and bone.

Human regulatory T (Treg) cells expressing the transcription factor forkhead box P3 (FoxP3) are indispensable for self-tolerance and immune homeostasis¹⁾, and low Treg cell counts may lead to the development of autoimmune diseases²⁻⁴⁾. This is best exemplified by the severe sys-



temic autoimmunity and lymphoproliferative disease observed in Treg cell-deficient mice (scurfy mice) and in humans carrying non-functional or hypomorphic alleles of the *FOXP3* gene, which cause immunodysregulation polyendocrinopathy enteropathy X-linked (IPEX) syndrome⁵. Other autoimmune diseases involving abnormal Treg cell counts or Treg cell activity include RA and systemic lupus erythematosus (SLE).

There is growing evidence that not all Treg cells are suppressive, and a recent study showed that human FoxP3+ Treg cells can be separated into three phenotypically and functionally distinct subpopulations based on their CD45RA and FoxP3 expression⁶. In this review, we consider the value of this classification of FoxP3+ Treg cells in the study of autoimmune diseases, and discuss what is currently known about the population and function of FoxP3+ Treg cells in human autoimmune diseases, including the role of Treg cells in the pathogenesis of human RA.

FoxP3+ Treg cells in autoimmune diseases

A natural first step in determining whether deficits in FoxP3+ Treg cells contribute to the development of autoimmune disease is to assess whether low Treg cell counts lead to impaired immune regulation. Studies in numerous animal models of autoimmunity have demonstrated that defects in FoxP3+ Treg cells contribute to the development of autoimmunity, which can be reversed by the adoptive transfer of FoxP3+ Treg cells. In mice, a lack of FoxP3+ Treg cells increases autoimmunity (scurfy mice), and the adoptive transfer of wild-type Treg cells prevents and reverses the autoimmunity⁷. In humans, a lack of functional Treg cells also leads to autoimmunity, as is seen in individuals who have IPEX syndrome due to mutations in the *FOXP3* gene. These individuals develop aggressive autoimmune disorders such as insulin-dependent diabetes, thyroiditis, and eczema. However, it is difficult to identify overt defects in Treg-mediated immune regulation in common human autoimmune diseases. Although several studies have reported that the frequency of FoxP3+ T cells in peripheral blood does not differ between patients with autoimmune diseases such as type 1 diabetes (T1D) and multiple sclerosis (MS), or between these patients and control subjects^{8,9}, a few reports disagree^{10,11}. On the other hand, CD25^{high} and FoxP3+ T cells are decreased in SLE patients, in whom the severity of this deficiency is correlated with the severity of the disease^{12,13}.

FoxP3+ Treg cells in RA

As with other murine autoimmune models, the experimental mouse model of RA (collagen-induced arthritis) is exacerbated by Treg cell depletion. In humans, although most studies report normal Treg cell populations in the peripheral blood of RA patients^{14,15}, a modest decrease in Treg cell count was reported for untreated early RA patients¹⁶. These findings are in contrast with a previous report showing that the number of CD4+CD25^{high}FoxP3+ Treg cells was increased in RA patients compared with controls¹⁷. These conflicting results may be due to differences in race, disease activity, disease duration, medications, detection markers, gating strategies, or, importantly, the heterogeneity of Treg cells.

A novel classification approach for FoxP3+ Treg cells

Unlike murine FoxP3+ Treg cells, human FoxP3+ cells are not functionally homogenous. To understand the roles of FoxP3+ Treg cells in the pathogenesis of autoimmunity, it is necessary to distinguish functionally different subpopulations of FoxP3+ Treg cells and to examine how those subsets differentiate and interact in various disease states. In 2009, Miyara et al. showed that the human FoxP3+ Treg cell population can be separated into three functionally and phenotypically different subpopulations based on the FoxP3 expression, cell-surface phenotype, degree of DNA methylation of the *FOXP3* gene, DNA microarray profile, proliferation status in the physiological state, cytokine-secreting capacity, TCR repertoire, and *in vitro* suppressive activity. These subpopulations are (I) CD45RA+FoxP3^{low} naïve Treg cells and (II) CD45RA-FoxP3^{high} effector Treg cells, both of which are suppressive *in vitro*, and (III) non-suppressive cytokine-secreting CD45RA-FoxP3^{low} non-Treg cells (Table 1).

After dividing the FoxP3+ Treg cell population into these subpopulations, the authors described the dynamics of Treg cell differentiation *in vitro*, *in vivo*, and *ex vivo* in normal and disease states. Terminally differentiated CD45RA-FoxP3^{high} effector Treg cells quickly died, whereas CD45RA+FoxP3^{low} naïve Treg cells proliferated and converted into effector Treg cells *in vitro* and *in vivo*⁶. In fact, the relative proportions of the three subpopulations within the subset of CD4+ T cells in peripheral blood differed between patients with various immunological diseases. In patients with active sarcoidosis, the proportion of CD45RA-FoxP3^{high} effector Treg cells was significantly elevated, and the proportion of CD45RA+



Table 1 Subpopulations of human FoxP3+ Treg cells

	Surface and intracellular markers	Suppressive effects	Increase or decrease diseases	Reference#
Naïve Treg cells	CD45RA+FoxP3 ^{low}	suppressive	↑ SLE (active) ↓ sarcoidosis (active)	(6) (6)
Effector Treg cells	CD45RA-FoxP3 ^{high}	suppressive (express high levels of CTLA4)	↑ sarcoidosis (active) ↓ SLE (active) ↓ Behçet's disease ↓ RA (peripheral blood)	(6) (6) (18) (15)(18)
Non-Treg cells	CD45RA-FoxP3 ^{low} IL-17	non-suppressive (secrete IL-2, IFN-γ and IL-17)	↑ RA (synovial fluids) ↓ Stevens-Johnson syndrome ↓ toxic epidermal necrolysis	(24) (19) (19)

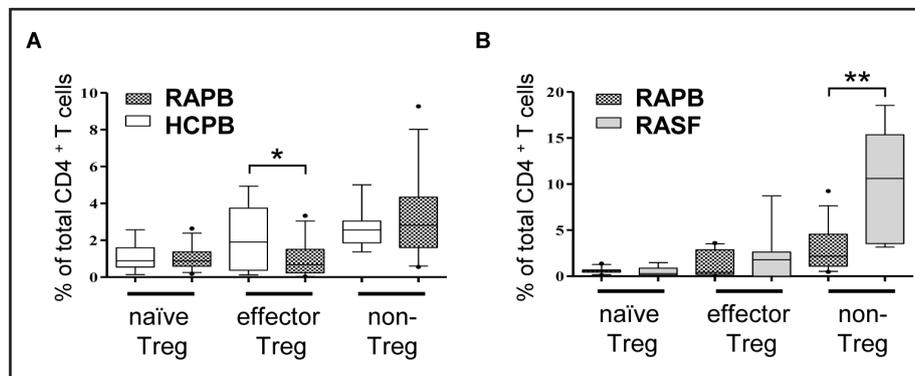


Fig.1 In patients with RA, effector Treg cells are decreased in the peripheral blood and non-Treg cells are increased in the synovial fluid

Peripheral-blood CD4+ T cells from RA patients (RAPB) and healthy controls (HCPB), and synovial-fluid CD4+ T cells from RA patients (RASB) were classified into three distinct subpopulations (naïve, effector, and non-Treg) according to their CD45RA and FoxP3 expression. The frequency of each FoxP3+ T cell subpopulation within the total CD4+ T cell population was analyzed in RAPB, HCPB, and RASB^{15, 24}.

FoxP3^{low} naïve Treg cells was significantly decreased. In contrast, in patients with active SLE, the proportion of CD45RA-FoxP3^{high} effector Treg cells decreased, while the proportion of CD45RA+FoxP3^{low} naïve Treg cells increased⁶. The proportion of CD45RA-FoxP3^{high} effector Treg cells was also decreased in patients with Behçet's disease¹⁸. In addition, the frequency of CD45RA-FoxP3^{low} non-Treg cells in the acute stage of Stevens-Johnson syndrome (SJS) and toxic epidermal necrolysis (TEN) was significantly decreased, but that in the resolution stage of SJS/TEN was significantly higher than that of the healthy controls¹⁹ (Table 1).

Recent research, including our own, demonstrates a significant decrease in the frequency of CD45RA-FoxP3^{high} effector Treg cells in the peripheral blood of patients with

RA (Fig. 1)^{15, 18}. We phenotypically classified the peripheral blood CD4+ T cells from RA patients into six novel, functionally distinct subsets according to the presence of CD45RA, CCR7, CD27, and CD28, and found that the proportions of total FoxP3+ Treg cells and CD45RA-FoxP3^{high} effector Treg cells in the CCR7+CD45RA-CD27+CD28+ central memory T cell subset were significantly lower in RA patients. We also showed that the percentage of CD45RA+FoxP3^{low} naïve Treg cells in peripheral blood was negatively correlated with the RA disease activity (DAS-28)¹⁵. Our results suggest that decreased numbers of CD45RA-FoxP3^{high} effector Treg cells may contribute to the pathogenesis of RA.

FoxP3+ Treg cells in the synovial fluid of patients with RA

In some diseases, FoxP3+ Treg cell counts may be normal in the peripheral blood but abnormal at the site of disease pathology. This situation can be assessed by counting Treg cells in the affected region. In the NOD mouse model of diabetes, T1D progression was associated with a decreased Treg:effector T cell ratio in the inflamed islets but not in the pancreatic lymph nodes²⁰.

As the rheumatic joint is both accessible and highly cellular, researchers can study immune responses at the site of inflammation and destruction in RA. Although conclusions vary regarding the Treg cell frequency in the peripheral blood of RA patients, it is generally agreed that the frequency of FoxP3+ Treg cells in synovial fluid is higher in RA patients than in controls^{21, 22}. Similarly, FoxP3+ Treg cells are increased in the cerebral spinal fluid of MS patients²³. Since FoxP3+ Treg cells are generally considered suppressive, these findings seem paradoxical, and raise the question of whether FoxP3+ T cells are functional regulatory cells, or T cells that transiently upregulate FoxP3. In fact, in the synovial fluid from RA patients, the suppressive function of FoxP3+ Treg cells is impaired²¹.

We recently demonstrated that most of the FoxP3+ Treg cells in the synovial fluid of RA patients are non-suppressive CD45RA-FoxP3^{low} non-Treg cells, and that these non-Treg cells are significantly more frequent in synovial fluid than in the peripheral blood (Fig. 1). In addition, we phenotypically classified the synovial fluid CD4+ T cells into six functionally distinct subsets based on their CCR7, CD45RA, CD27, and CD28 expression, and showed that the CCR7-CD45RA-CD27+CD28+ effector memory T cell subset was significantly increased in synovial fluid compared with the peripheral blood. Moreover, the frequency of total FoxP3+ Treg cells and CD45RA-FoxP3^{low} non-Treg cells in the CCR7-CD45RA-CD27+CD28+ effector memory T cell subset was significantly increased in the synovial fluid of patients with RA²⁴.

Our findings that non-suppressive CD45RA-FoxP3^{low} non-Treg cells are increased in the synovial fluid in RA shed light on the seemingly paradoxical observation that FoxP3+ Treg cells are increased in the synovial fluid of RA patients. When strongly activated by pro-inflammatory cytokines, Treg cells concomitantly lose the ability to suppress T cells and gain the capacity to secrete IL-17^{25, 26}. Our results suggest that the pro-inflammatory environment in RA joints may induce non-suppressive cytokine-secreting non-Treg

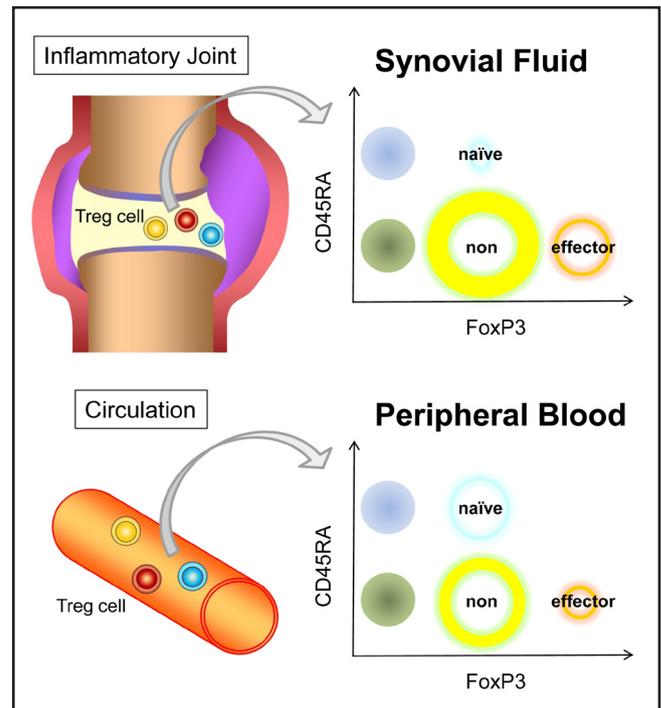


Fig.2 FoxP3+ Treg cell subpopulations in the synovial fluid and peripheral blood of patients with RA

Diagram showing the increased numbers of non-Treg cells in the joints and decreased population of effector Treg cells in the blood circulation; this imbalance may be involved in the pathogenesis of RA.

cells in synovial fluid.

Conclusions

It is clear that FoxP3+ Treg cells are instrumental in controlling autoimmunity, and numerous studies have reported anomalous frequencies and/or functions of Treg cells in systemic autoimmune diseases. On the other hand, human Treg cells are heterogeneous in phenotype and function, and a recent study revealed that human FoxP3+ Treg cell populations include three functionally distinct subpopulations. We recently demonstrated that in RA patients, effector Treg cells are decreased in the peripheral blood, and non-Treg cells are increased in synovial fluid (Fig. 2). These findings should help elucidate the pathogenesis of RA and may be useful in the development of novel Treg cell-based RA therapies.

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Conflict of interests

None declared.

References

- 1) Sakaguchi S: Regulatory T cells in the past and for the future. *Eur J Immunol.* 2008; 38: 901-937.
- 2) Sakaguchi S, Sakaguchi N, Asano M, Itoh M, Toda M: Immunologic self-tolerance maintained by activated T cells expressing IL-2 receptor alpha-chains (CD25). Breakdown of a single mechanism of self-tolerance causes various autoimmune diseases. *J Immunol.* 1995; 155: 1151-1164.
- 3) Bonelli M, Savitskaya A, von Dalwigk K, Steiner CW, Aletaha D, Smolen JS, Scheinecker C: Quantitative and qualitative deficiencies of regulatory T cells in patients with systemic lupus erythematosus (SLE). *Int Immunol.* 2008; 20: 861-868.
- 4) Buckner JH: Mechanisms of impaired regulation by CD4(+)CD25(+)FOXP3(+) regulatory T cells in human autoimmune diseases. *Nat Rev Immunol.* 2010; 10: 849-859.
- 5) Campbell DJ, Koch MA: Phenotypical and functional specialization of FOXP3+ regulatory T cells. *Nat Rev Immunol.* 2011; 11: 119-130.
- 6) Miyara M, Yoshioka Y, Kitoh A, Shima T, Wing K, Niwa A, Parizot C, Taflin C, Heike T, Valeyre D, Mathian A, Nakahata T, Yamaguchi T, Nomura T, Ono M, Amoura Z, G Gorochov, Sakaguchi S: Functional delineation and differentiation dynamics of human CD4+ T cells expressing the FoxP3 transcription factor. *Immunity.* 2009; 30: 899-911.
- 7) Sakaguchi S, Ono M, Setoguchi R, Yagi H, Hori S, Fehervari Z, Shimizu J, Takahashi T, Nomura T: Foxp3+ CD25+ CD4+ natural regulatory T cells in dominant self-tolerance and autoimmune disease. *Immunol Rev.* 2006; 212: 8-27.
- 8) Putnam AL, Vendrame F, Dotta F, Gottlieb PA: CD4+CD25high regulatory T cells in human autoimmune diabetes. *J Autoimmun.* 2005; 24: 55-62.
- 9) Feger U, Luther C, Poeschel S, Melms A, Tolosa E, Wiendl H: Increased frequency of CD4+ CD25+ regulatory T cells in the cerebrospinal fluid but not in the blood of multiple sclerosis patients. *Clin Exp Immunol.* 2007; 147: 412-418.
- 10) Venken K, Hellings N, Broekmans T, Hensen K, Rummens JL, Stinissen P: Natural Naive CD4+CD25+CD127low Regulatory T Cell (Treg) Development and Function Are Disturbed in Multiple Sclerosis Patients: Recovery of Memory Treg Homeostasis during Disease Progression. *J Immunol.* 2008; 180: 6411-6420.
- 11) Kumar M, Putzki N, Limmroth V, Remus R, Lindemann M, Knop D, Mueller N, Hardt C, Kreuzfelder E, Grosse-Wilde H: CD4+CD25+FoxP3+ T lymphocytes fail to suppress myelin basic protein-induced proliferation in patients with multiple sclerosis. *J Neuroimmunol.* 2006; 180: 178-184.
- 12) Mellor-Pita S, Citores MJ, Castejon R, Tutor-Ureta P, Yebra-Bango M, Andreu JL, Vargas JA: Decrease of regulatory T cells in patients with systemic lupus erythematosus. *Ann Rheum Dis.* 2006; 65: 553-554.
- 13) Crispin JC, Martínez A, Alcocer-Varela J: Quantification of regulatory T cells in patients with systemic lupus erythematosus. *J Autoimmun.* 2003; 21: 273-276.
- 14) Ehrenstein MR, Evans JG, Singh A, Moore S, Warnes G, Isenberg DA, Mauri C: Compromised function of regulatory T cells in rheumatoid arthritis and reversal by anti-TNFalpha therapy. *J Exp Med.* 2004; 200: 277-285.
- 15) Matsuki F, Saegusa J, Miyamoto Y, Misaki K, Miura Y, Kurosaka M, Kumagai S, Morinobu A: CD45RA-Foxp3(high) activated/effector regulatory T cells in the CCR7+CD45RA-CD27+CD28+central memory subset are decreased in peripheral blood from patients with rheumatoid arthritis. *Biochem Biophys Res Commun.* 2013; 438: 778-783.
- 16) Lawson CA, Brown AK, Bejarano V, Douglas SH, Burgoyne CH, Greenstein AS, Boylston AW, Emery P, Ponchel F, Isaacs JD: Early rheumatoid arthritis is associated with a deficit in the CD4+CD25^{high} regulatory T cell population in peripheral blood. *Rheumatology (Oxford).* 2006; 45: 1210-1217.
- 17) Han GM, O'Neil-Andersen NJ, Zurier RB, Lawrence DA: CD4+CD25^{high} T cell numbers are enriched in the peripheral blood of patients with rheumatoid arthritis. *Cell Immunol.* 2008; 253: 92-101.
- 18) Kim J, Chae J, Kim S, Ha J: Subpopulations of Regulatory T Cells in Rheumatoid Arthritis, Systemic Lupus Erythematosus, and Behcet's Disease. *J Korean Med Sci.* 2012; 1009-1013.
- 19) Yoshioka N, Suto A, Abe R, Saito N, Murata J, Hayashi-Ujiie I, Hoshina D, Fujita Y, Shimizu H: Disturbed balance in three subpopulations of CD4(+)Foxp3(+) regulatory T cells in Stevens-Johnson syndrome and toxic epidermal necrolysis patients. *Clin Immunol.* 2013; 148: 89-91.



- 20) Tang Q, Adams J, Penaranda C, Melli K, Piaggio E, Sgouroudis E, Piccirillo C, Salomon B, Bluestone J: Central role of defective interleukin-2 production in the triggering of islet autoimmune destruction. *Immunity*. 2008; 28: 687-697.
- 21) van Amelsfort J, Jacobs K, Bijlsma J, Lafeber F, Taams L: CD4(+)CD25(+) regulatory T cells in rheumatoid arthritis: differences in the presence, phenotype, and function between peripheral blood and synovial fluid. *Arthritis Rheum*. 2004; 50: 2775-2785.
- 22) Möttönen M, Heikkinen J, Mustonen L, Isomäki P, Luukkainen R, Lassila O: CD4+ CD25+ T cells with the phenotypic and functional characteristics of regulatory T cells are enriched in the synovial fluid of patients with rheumatoid arthritis. *Clin Exp Immunol*. 2005; 140: 360-367.
- 23) Feger U, Luther C, Poeschel S, Melms A, Tolosa E, Wiendl H: Increased frequency of CD4+ CD25+ regulatory T cells in the cerebrospinal fluid but not in the blood of multiple sclerosis patients. *Clin Exp Immunol*. 2007; 147: 412-418.
- 24) Matsuki F, Saegusa J, Nishimura K, Miura Y, Kurosaka M, Kumagai S, Morinobu A: CD45RA-Foxp3(low) non-regulatory T cells in the CCR7-CD45RA-CD27+CD28+ effector memory subset are increased in synovial fluid from patients with rheumatoid arthritis. *Cell Immunol*. 2014; 290: 96-101.
- 25) Koenen H, Smeets R, Vink P, Rijssen E, Boots A, Joosten I: Human CD25^{high} Foxp3^{pos} regulatory T cells differentiate into IL-17-producing cells. *Immunobiology*. 2008; 112: 2340-2352.
- 26) Komatsu N, Okamoto K, Sawa S, Nakashima T, Oh-hora M, Kodama T, Tanaka S, Bluestone JA, Takayanagi H: Pathogenic conversion of Foxp3+ T cells into TH17 cells in autoimmune arthritis. *Nat Med*. 2013; 1-9.