Review

Regulatory T cells in rheumatoid arthritis

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Abstract

Apart from the deletion of autoreactive T cells in the thymus, various methods exist in the peripheral immune system to control specific human immune responses to self-antigens. One of these mechanisms involves regulatory T cells, of which CD4⁺CD25⁺ T cells are a major subset. Recent evidence suggests that CD4⁺CD25⁺ T cells have a role in controlling the development of autoimmune diseases in animals and in humans. The precise delineation of the function of CD4⁺CD25⁺ T cells in autoimmune inflammation is therefore of great importance for the understanding of the pathogenesis of autoimmune diseases. Moreover, the ability to control such regulatory mechanisms might provide novel therapeutic opportunities in autoimmune disorders such as rheumatoid arthritis. Here we review existing knowledge of CD4⁺CD25⁺ T cells and discuss their role in the pathogenesis of rheumatic diseases.

Introduction

The development of autoimmune diseases requires the breakdown of immunologic self-tolerance that usually controls self and non-self discrimination [1]. The primary mechanism that leads to tolerance to self-antigens is the thymic deletion of self-reactive T cells (‘negative selection’). However, because some self-reactive T cells escape this process physiologically and autoreactive CD4⁺ T cells are present in the peripheral circulation of healthy individuals, where they retain their capacity to initiate autoimmune inflammation [2], negative selection in the thymus is not sufficient to prevent the activation of self-reactive T cells in the periphery [3]. Thus, regulatory mechanisms in the peripheral immune system are required to protect against both the generation of self-directed immune responses and the consequence of this, namely the initiation of autoimmune diseases. It is likely that one such mechanism of peripheral tolerance involves the active suppression of T cell responses by CD4⁺ T cells with regulatory capacity, of which a major subset are the CD4⁺CD25⁺ regulatory T cells.

Phenotype and function of mouse regulatory T cells

Regulatory T cells were first discovered in experimental animal models and were subsequently identified in humans. In 1971, a unique subpopulation of T cells was described that was capable of downregulating or suppressing the functions of other cells [4]. These regulatory (‘suppressor’) T cells had the capacity to transfer antigen-specific tolerance to naive animals. However, the concept of active suppression by T cells lost acceptance because of several technical problems. For example, it was not possible to identify specific cell-surface markers associated with suppressor T cells. Further, when T cell receptor genes were analyzed, suppressor T cells did not seem to have functional gene rearrangements [5]. Most remarkably, soluble suppressor factors, which were believed to be the molecular mechanism of action of suppressor T cells, were thought to be encoded by the murine I–J locus of the major histocompatibility complex (MHC) region. But when molecular studies with hybrid DNA technology failed to identify the I–J region within the MHC [6], the concept of T cell suppression was discarded.

Nevertheless, various experimental observations remained difficult to interpret without postulating an active form of downregulation during an immune response [7]. For many years it was not clear whether distinct specialized T cells exerted this regulatory function or whether this phenomenon was a function of ‘non-specialized’ T cells. In the mid-1990s a phenotypic description of regulatory T cells eventually became available. Sakaguchi and colleagues [8] showed that injection of CD4⁺ T cells from Balb/c mice that had been depleted of the fraction of cells coexpressing CD25 (the IL-2 receptor α-chain) into athymic Balb/c mice resulted in the development of various organ-specific autoimmune diseases such as thyroiditis, gastritis, colitis and insulin-dependent autoimmune diabetes. Furthermore, co-transfer of CD4⁺CD25⁺...
with the pathogenic CD4+CD25− T cells prevented the development of experimentally induced autoimmune diseases [9,10]. These data implied that murine CD4+CD25+ T cells are actively able to regulate the responsiveness of autoreactive T cells that have escaped central tolerance, which distinguishes them from other mechanisms of peripheral tolerance including T cell depletion [11], T cell anergy [12] and immunologic ignorance [13].

CD4+CD25+ T cells are characterized by a low proliferative capacity after triggering with polyclonal or allogeneic stimulation, and by their ability to suppress CD4+ and CD8+ immune responses by means of cell-contact dependent mechanisms [14]. CD4+CD25+ T cells have therefore been named regulatory T cells (Tregs). They are typified by the expression of an array of surface molecules, of which several have been implicated in contributing to the suppressive function of Tregs. Although not unique to Tregs, the array of these surface molecules makes it possible to identify Tregs phenotypically. For example, CTLA4 and CD25, which are upregulated on naive and memory T cells after activation, are constitutively expressed on the surface of Tregs. In mice, an important role of CTLA4 in the function of Tregs can be inferred from the ability of CTLA4-specific antibodies to abrogate the CD25+ T cell-mediated protection of autoimmune gastritis [15] and the CD45RB low T cell-mediated inhibition of colitis in the appropriate animal model [16]. However, it is as yet uncertain whether these findings can be explained by the concept that CTLA4 transduces ‘negative’ signals to activated effector T cells.

Glucocorticoid-induced tumor necrosis factor receptor family-related protein (GITR) is another membrane-associated receptor that was identified during the characterization of the phenotype and function of CD25+ Tregs [17]. GITR is the specific antigen of an antibody that was generated after stimulation of CD4+ T cells. Although antibodies against GITR abrogate CD25+CD4+ T cell-mediated suppression in vitro and in vivo [18], the mechanism behind these activities still remains to be determined. However, it should be emphasized that similarly to CD25 and CTLA-4, GITR is not Treg-specific and is upregulated on effector/memory cells after antigen-driven activation. Recently, LAG-3, an MHC class II-binding CD4 homologue was shown to be selectively upregulated on Tregs, and antibodies against LAG-3 inhibited suppression by Tregs, both in vitro and in vivo [19]. LAG-3 expression remains high on Tregs and decreases shortly after activation in memory T cells, indicating that LAG-3 might mark cells with regulatory activity and is not simply an activation marker. However, it is at present not clear whether LAG-3 selectively marks only certain Treg subsets analyzed in that study.

The transcription factor Foxp3 has been shown to be selectively expressed by Tregs. Foxp3 was first identified as the gene responsible for the defect in scurfy mice, which die early in life from CD4 T cell-mediated lymphoproliferative disease, and was subsequently shown to be important in murine Treg development and function [20]. Patients with the IPEX syndrome (for ‘immune dysregulation, polyendocrinopathy, enteropathy, and X-linked inheritance’), a clinical syndrome presenting with autoimmune diseases similar to that developing in mice after depletion of CD25+CD4+ regulatory cells, have mutations in Foxp3 [21,22]. This observation provided a first correlation between Tregs and T cell-mediated autoimmune diseases in humans and mice caused by a genetic defect in a defined transcription factor that is essential for the development of the function of Tregs.

However, despite these indications there is still a concern that CD4+CD25+ Tregs from mice that are kept in germ-free facilities with low levels of endogenous T cell activation are not identical with human CD4+CD25+ T cells [23]. In particular, it is at present unclear whether human CD4+CD25+ Tregs are able to suppress immune responses in vivo, as their counterparts do in the mouse.

**Phenotype and function of human CD4+CD25+ Tregs**

In humans, a population of CD4+CD25+ Tregs has been identified in the peripheral circulation [24-28] and in the thymus [29,30]. In general, the characteristics of human and mouse CD4+CD25+ T cells are very similar. As in mice, 5 to 15% of human peripheral blood CD4+ T cells constitutively express CD25. It has been proposed that the suppressive effects of human CD4+CD25+ T cells may reside in the CD25highCD4+ T cell fraction [28]; however, this finding is not uniformly accepted [31]. After isolation and in vitro allogeneic [25,26], polyclonal [27,29] or antigen-specific [32] stimulation, human CD4+CD25+ T cells do not proliferate – that is, they are anergic [33] – and when cultured with CD4+CD25− cells, CD4+CD25+ T cells suppress the CD4+CD25− T cell response in a cell-contact-dependent manner [25] (Fig. 1).

Although CD4+CD25+ cells are unresponsive to mitogenic stimulation, they do proliferate in the presence of exogenous IL-2 [34]. CD4+CD25+ T cells have a differentiated phenotype (CD45RA−RO+ in humans), indicating that they have been stimulated in their internal environment. Evidence suggests that, once these cells are activated, their suppressor function is antigen-nonspecific because CD4+CD25+ Tregs suppress not only T cells stimulated with the same antigens but also T cells activated by other antigens [35]. Thus, Tregs might be able to act as bystander suppressors through contact-dependent mechanisms.

Controversial data exist as to whether and which cytokines are produced by CD4+CD25+ Tregs. Whereas some investigators describe that these cells do not produce immunomodulatory cytokines [28], others demonstrate that they are able to produce IL-10 [27,29,36], transforming
growth factor-β (TGF-β) [26,36] and IL-4 [29]. As shown in Fig. 1, however, Tregs exert their inhibitory function independently of the production of potentially immunoregulatory cytokines. Nevertheless, it is widely accepted that Tregs do not produce IL-2.

CD4+CD25+ Tregs in rheumatoid arthritis

The development of assays to evaluate the function of human CD4+CD25+ Tregs in vitro has provided the opportunity to analyze the role of Tregs in human autoimmune diseases such as rheumatoid arthritis (RA). A series of recent articles has focused on the role of Tregs in rheumatoid inflammation and has indicated that CD4+CD25+ T cells might function as potential regulators of immune responses in RA.

Phenotype of peripheral blood CD4+CD25+ T cells in RA

Controversy exists with regard to the frequency of CD4+CD25+ T cells in the peripheral circulation of patients with RA in comparison with healthy individuals [31,37,38]. The divergent results might be in part related to different definitions of CD4+CD25+ T cells, because some investigators focused on the CD25bright T cells [39], whereas others analyzed the total population of CD25+ T cells [31]. In patients with a different but related inflammatory joint disease, juvenile idiopathic arthritis (JIA), the frequency of CD25brightCD4+ cells in the peripheral blood was lower than in healthy controls [40]. Patients with a self-limiting form of JIA had an increased frequency of CD4+CD25+ T cells in comparison with healthy individuals [31,37,38]. In contrast to the situation in the peripheral blood, there is clear evidence that the frequencies of CD4+CD25+ T cells in the synovial fluid of patients with RA are elevated compared with those in the peripheral blood (Fig. 2) [31,39]. CD25brightCD4+ T cells are enriched in the synovial fluid not only in patients with RA but also in patients with spondyloarthropathies or with JIA [37,40].

Phenotype of synovial CD4+CD25+ T cells in RA

In contrast to the situation in the peripheral blood, there is clear evidence that the frequencies of CD4+CD25+ T cells in the synovial fluid of patients with RA are elevated compared with those in the peripheral blood (Fig. 2) [31,39]. CD25brightCD4+ T cells are enriched in the synovial fluid not only in patients with RA but also in patients with spondyloarthropathies or with JIA [37,40].
Several alternative mechanisms might contribute to the enrichment of CD4+CD25+ T cells in the synovial fluid of patients with rheumatic diseases. A preferential migration of these cells into the inflamed joint might be inferred from the observation that CD4+CD25+ T cells specifically express the chemokine receptors CXCR4, CCR4 and CCR8 [41]. The CCR4 ligands CCL17 and CCL22 are highly expressed in synovial tissue [42], and it has been suggested that dendritic cells are able to ‘chemoattract’ cells by the secretion of CCL17 and CCL22 [41]. However, it should be pointed out that although CCR4+ T cells can be detected in the peripheral blood of healthy individuals and in the synovial fluid of patients with RA, the vast majority of T cells in the rheumatoid synovial fluid do not express CCR4 [43], making the CCR4–CCL17-mediated recruitment of Tregs into the rheumatoid joint rather unlikely. The ligand for CXCR4, stromal-derived factor-1 (SDF-1), is expressed on synovial endothelial cells [44], and persistent expression of the chemokine receptor CXCR4 on CD4+ T cells mediates their active retention within the rheumatoid synovium [45]. Because human CD4+CD25+ Tregs traffic to and are retained in the bone marrow through interactions involving CXCR4 [46], it is also conceivable that CD4+CD25+ T cells are selectively recruited to and retained in the rheumatoid joint through interactions involving CXCR4.

In line with the hypothesis that CD4+CD25+ T cells are effectively recruited to sites of chronic inflammation, CD25+CD4+ T cells are found in inflammatory infiltrates of C57BL/6 mice infected with Leishmania major [47] and of Balb/c mice infected with Candida albicans [48]. The data therefore suggest that the accumulation of CD4+CD25+ T cells during an inflammatory immune response might be a physiologic control mechanism of potentially dangerous effector functions to prevent tissue damage.

A second mechanism leading to the accumulation of CD4+CD25+ T cells in the rheumatic joint might relate to the fact that inflammatory cytokines such as IL-2 and costimulatory molecules cause CD4+CD25+ T cells to revert to an anergic phenotype [34] (Fig. 1c). Because the synovial fluid contains high levels of inflammatory cytokines and of antigen-presenting cells that are able to engage costimulatory molecules on synovial T cells, CD25+CD4+ T cells might expand locally in the rheumatoid joint. However, in the rheumatoid synovium it was found that T cells display low proliferative responses [49], and in patients with JIA the T cells in the synovial fluid are not actively dividing [50].

A third alternative method for the enrichment of CD4+CD25+ T cells in the rheumatoid joint is related to the observation that synovial T cells are actively inhibited from undergoing apoptosis, thereby expanding their lifespan compared with their peripheral counterparts. An integrin–ligand interaction is involved in the fibroblast-mediated survival of synovial T cells [51]. Fibroblast-secreted IFN-β is also able to inhibit apoptosis, and in particular that of CD4+CD25+ T cells [24].

A final explanation for the increased frequencies of CD25+ T cells in the synovium derives from the characteristic of CD25 to be upregulated on activated T cells. Thus, the sole determination of CD25 does not make it possible to discriminate Tregs from activated effector cells. Because synovial T cells express an array of activation markers and effector functions, it is likely that most CD25-expressing T cells from the synovial fluid constitute an effector population actively engaged in driving synovial inflammation.

Recent evidence suggests that the CD4+CD25+ Tregs from the synovial fluid are different from those in the peripheral circulation. CD25brightCD4+ T cells from the synovial fluid in RA contain higher frequencies of cells expressing CTLA-4 and GITR than those from the peripheral blood of healthy donors and of patients with RA [31,37]. Tregs from synovial fluid also display an activated phenotype with a higher expression of CD69 and MHC class II than CD4+CD25+ cells in the peripheral blood of matched individuals.

Intermittent flares in disease activity are typical of RA. Whether the frequency of regulatory CD25brightCD4+ T cells fluctuate over time or are correlated with disease activity is therefore of considerable interest. Although the frequency of synovial CD25brightCD4+ T cells varies between patients, the numbers of these cells do not vary significantly over time in a single joint [39]. Similar stable frequencies of synovial CD25brightCD4+ T cells over time were also observed in patients with JIA, psoriatic arthritis and spondyloarthropathies [37]. Moreover, the frequencies of synovial CD25brightCD4+ T cells in patients with RA was not correlated with clinical parameters such as disease duration, the presence of rheumatoid factor, the level of C-reactive protein and the presence of erosions [31,37]. In addition, no association was
found between the use of methotrexate, corticosteroids or anti-TNF therapy and the frequency of CD4+CD25+ T cells in the synovial fluid [31]. These data suggest that the presence of CD4+CD25+ T cells in the rheumatoid synovium is a function of the disease and is characteristic of a particular patient but unrelated to treatment, clinical course and disease activity. These results might therefore question the importance of CD4+CD25+ Tregs in the regulation of synovial inflammation.

Together, the data suggest that CD4+CD25+ T cells in chronically inflamed rheumatoid joints might enrich and persist as a result of preferential recruitment, rescue from cell death and activation by their specific antigen. Consequently, the determination of frequencies of CD25+ T cells in the synovial fluid without complementary functional studies does not make it possible to draw meaningful conclusions about the role of CD4+CD25+ Tregs in rheumatoid inflammation.

**Function of synovial CD4+CD25+ T cells in RA**

When examined in conventional *in vitro* assays, synovial CD4+CD25bright T cells are able to suppress the proliferation of autologous CD4+CD25- (responder) T cells of synovial and peripheral origin [31,37,39]. Synovial CD4+CD25+ T cells display an even increased suppressive capacity compared with blood CD4+CD25+ T cells in RA [31] and in JIA [40]. It is of interest that CD4+CD25intermediate T cells enhance rather than suppress the proliferation of synovial responder CD4+CD25- T cells, which might suggest that CD25intermediate T cells represent effector T cells.

The major question that these results immediately bring up is why inflammation occurs in the rheumatoid joints despite elevated frequencies of apparently functional CD4+CD25+ T cells with an even enhanced suppressive capacity in assays *in vitro*.

One possible explanation for this seeming paradox might be an active inhibition of the function of Tregs in the rheumatoid joint. For example, several constituents of the inflamed synovial environment, such as IL-2 and IL-7, have been shown to abrogate the function of Tregs [34,52], suggesting that Tregs are inhibited at sites of inflammation from performing their regulatory function by pro-inflammatory cytokines. Similarly, although shown only for peripheral blood, it has been suggested that CD4+CD25+ T cells display functional differences before and after treatment with anti-TNF [38]. CD4+CD25high cells isolated from the peripheral blood of patients with active RA suppress the proliferative response of responder CD4 T cells but not the secretion of inflammatory cytokines such as IFN-γ and TNF. In contrast, CD4+CD25high cells isolated from the patients’ blood after anti-TNF therapy suppress (like CD4+CD25high cells in healthy individuals) not only the proliferation but also the secretion of these cytokines from responder CD4 T cells derived from anti-TNF-treated patients. Thus, these findings indicate a functional deficit of CD4+CD25high T cells from patients with active RA with regard to their ability to suppress pro-inflammatory cytokine production that reverts after treatment with TNF-neutralizing agents. Additional evidence for an inhibitory function of TNF on Tregs in RA derives from experiments in which the depletion of CD4+CD25high T cells from peripheral blood mononuclear cells (PBMC) from patients with active RA did not alter the frequency of cells producing TNF or IL-10 in a 2-day cell culture, whereas an increase in TNF-secreting cells and a reduction in IL-10-secreting cells occurred in the culture of PBMC derived from anti-TNF-treated patients with RA that were depleted of Tregs [38]. Together, these data might underline the potential role of cytokines in maintaining chronic inflammation *in vivo*.

An alternative explanation for persistent synovial inflammation despite enriched numbers of CD4+CD25+ T cells with enhanced suppressive capacity *in vitro* is provided by the finding that synovial responder T cells express a decreased susceptibility to the regulatory effect of CD4+CD25+ Tregs in comparison with peripheral blood responder T cells, thereby ‘compensating’ for the enhanced regulatory capacity of the synovial Tregs [31]. IL-6, which is known to be found in large amounts in the rheumatoid synovium [53], has been shown to enhance the resistance of T effector cells to the suppressive effects of Tregs [54]. Finally, although suppression by Tregs is probably not antigen-specific but might involve neighboring T cells in a ‘bystander’ fashion [35], Tregs require activation through their T-cell antigen receptor to deliver their regulatory function. Thus, if the specific antigen for the synovial Tregs is not presented either in the secondary lymphoid organs or in the inflamed synovia, or, alternatively, if Tregs in RA express an altered threshold for antigen-specific activation, synovial Tregs, although present, will not become activated and will therefore fail to inhibit ongoing inflammation.

Together, these arguments indicate that rheumatoid inflammation occurs in the presence of Tregs that express an impaired regulatory function *in vivo*, despite their enhanced regulatory capacity *in vitro*. Although it is tempting to speculate that synovial inflammation is the consequence of an inadequate ability of synovial Tregs to downmodulate local inflammation, several observations indicate clearly that synovial Tregs are functional and actively dampen the inflammatory immune response *in vivo*. For example, in JIA the frequencies of CD4+CD25+ synovial T cells are inversely correlated with the clinical outcome, and the expression of FoxP3 mRNA, a ‘marker’ for Treg function, is elevated in mild cases in comparison with severe forms of the disease [50]. In collagen-induced arthritis, depletion of CD4+CD25+ T cells accelerates the onset of severe disease, and transfer of syngeneic CD4+CD25+ T cells into Treg-depleted mice reverses the increased severity [55]. Thus, the local expansion in the CD4+CD25+ Treg cell population in the rheumatoid synovium might reflect a mechanism for resolving the inflammatory immune response. Although not sufficient to
prevent inflammatory activity in the joint, the CD4+CD25+ Tregs in the inflamed rheumatoid synovium might nevertheless be important for a downmodulation of the inflammation, thereby delaying further tissue damage and impeding erosive inflammation. These findings might be of relevance in validating and fostering the development of clinical applications of in vitro-generated Tregs in autoimmune diseases in the near future by means of personalized cellular therapy.

It should be noted that other subsets of CD4 T cells have been identified that are capable of suppressing specific immune responses. The most prominent of these are termed Treg 1 (Tr1) and T helper type 3 (Th3) cells. Th3 cells produce predominantly TGF-β. They are generated in vivo by immunization through an oral or other mucosal route [56], and have been detected in patients with multiple sclerosis after oral administration of myelin basic protein [56]. Groux and colleagues first isolated mouse and human Tr1 cells that have immune-regulatory activities both in vitro and in vivo [57, 58]. These regulatory CD4+ T cells secrete IL-10 and have been generated in vitro by repeated antigenic stimulations of human and murine CD4+ cells in the presence of IL-10 [26, 59, 60] or by activation through immature antigen-presenting cells that lack potent costimulatory activity [61].

However, comprehensive analyses of Tr1 and Th3 cells in humans are not available, so the precise role of these subsets in human autoimmune disease has not been defined.

Conclusions

In conclusion, human CD4+CD25+ Tregs that are capable of suppressing CD4 T cell proliferation in vitro are enriched in the synovial fluid of patients with RA. Synovial Tregs express an increased regulatory capacity in comparison with Tregs derived from the peripheral blood, in assays in vitro. In the synovium, Tregs might be inhibited by different mechanisms such as inflammatory cytokines including TNF, or stimulation by antigen-presenting cells, which in concert might allow synovial inflammation to evolve and persist despite the enhanced frequencies of synovial Tregs. However, evidence suggests that synovial Tregs, although not sufficient to ameliorate disease activity completely, are involved in regulating synovial inflammation in vivo, future treatment strategies of autoimmune diseases can be envisaged in which Tregs generated and/or expanded in vitro will be employed in an attempt to control local and systemic autoimmune inflammation.

Competing interests

The author(s) declare that they have no competing interests.

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References

1. Bach JF, Chatenoud L: Tolerance to islet autoantigens in type 1 diabetes. Annu Rev Immunol 2001, 19:131-161.
2. Ota K, Matsui M, Miller JF, White EL, Malmstrom V, Powrie F, Whitesell L, Kelly TE, Saulsbury FT, Chance PF, Ochs HD: Haffner DA: T-cell recognition of an immunodominant myelin basic protein epitope in multiple sclerosis. Nature 1990, 346:183-187.
3. Sakaguchi S: Regulatory T cells: key controllers of immunologic self-tolerance. Cell 2000, 101:455-458.
4. Medzhitov R, Kondo H: Infectious immunological tolerance. Immunology 1971, 21:903-914.
5. Hedrick SM, Steinmetz M, Kobori J, Kraig E, Kapp JA, Pierce CW, Sorensen CM, Suzuki G, Tada T, Hood L: RNA transcripts for I-J polypeptides are apparently not encoded between the I-I subregion and I-E subregion of murine major histocompatibility complex. Proc Natl Acad Sci USA 1983, 80:5704-5708.
6. Janeway CA Jr: Do suppressor T cells exist? A reply. Scand J Immunol 1988, 27:621-623.
7. 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.
8. Miller JF, Shevach EM: Cutting edge: deletion of CD4+CD25+ regulatory T cells is necessary, but not sufficient, for induction of organ-specific autoimmune disease. J Immunol 2002, 168:5979-5983.
9. Mottet C, Uhlig HH, Powrie F: Cutting edge: cure of colitis by CD4+CD25+ regulatory T cells. J Immunol 2003, 170:3939-3943.
10. Miller JF, Basten A: Mechanisms of tolerance to self. Curr Opin Immunol 1996, 8:815-821.
11. Schwartz RH: Models of T cell anergy: is there a common molecular mechanism? J Exp Med 1996, 184:119.
12. Miller JF, Heath WR: Self-ignorance in the peripheral T-cell pool. Immunol Rev 1993, 133:131-150.
13. Thornton AM, Shevach EM: CD4+CD25+ immunoregulatory T cells are antigen nonspecific. J Immunol 2000, 164:183-190.
14. Takahashi T, Tagami T, Yamaizaki S, Uede T, Shimizu J, Sakaguchi N, Mak TW, Sakaguchi S: Immunologic self-tolerance maintained by activated T cells expressing CD25. Breakdown of a single mechanism of self-tolerance causes various autoimmune diseases. J Immunol 1995, 155:1151-1164.
15. Read S, Malmstrom V, Powrie F: Cytotoxic T lymphocyte-associated antigen 4 plays an essential role in the function of CD4+CD25+ regulatory T cells constitutively expressing IL-2 receptor alpha-chains (CD25). J Exp Med 2000, 192:303-310.
16. Read S, Malmstrom V, Powrie F: Cytotoxic T lymphocyte-associated antigen 4 plays an essential role in the function of CD4+CD25+ regulatory cells that control intestinal inflammation. J Exp Med 2000, 192:295-302.
17. Shevach EM, Collins M, Byrne MC: CD4+CD25+ immunoregulatory T cells: gene expression analysis reveals a functional role for the glucocorticoid-induced TNF receptor. Immunity 2002, 16:311-323.
18. Shimizu J, Yamaizaki S, Takahashi T, Ichihara Y, Sakaguchi S: Stimulation of CD25+CD4+ regulatory T cells through GITR breaks immunological self-tolerance. Nat Immunol 2002, 3:135-142.
19. Huang CT, Workman CJ, Flies D, Pan X, Marson AL, Zhou G, Hipkiss EL, Ravi S, Kowalski J, Levitsky HL, et al.: Role of LAG-3 in regulatory T cells. Immunity 2004, 21:503-513.
20. Horis S, Nomura T, Shimizu J, Kojima M, Suzuki Y, Sato S: Control of regulatory T cell development by the transcription factor Foxp3. Science 2003, 299:1057-1061.
21. Bennett CL, Christie J, Ramsdell F, Brunke ME, Ferguson PJ, Whitesell L, Kelly TE, Saulsbury FT, Chance PF, Ochs HD: The immune dysregulation, polyendocrinopathy, X-linked syndrome (IPEX) is caused by mutations of FOXP3. Nat Genet 2001, 27:20-21.
22. Gambineri E, Torgerson TR, Ochs HD: Immune dysregulation, polyendocrinopathy, enteropathy, and X-linked inheritance (IPEX), a syndrome of systemic autoimmunity caused by mutations of FOXP3, a critical regulator of T-cell homeostasis. Curr Opin Rheumatol 2003, 15:430-435.
23. Baecher-Allan C, Haffner DA: Suppressor T cells in human diseases. J Exp Med 2004, 200:273-276.
24. Taams LS, Smith J, Rustin MH, Salmon M, Poulter LW, Akbar AN: Human anergic/suppressive CD4+CD25+ T cells: a highly differentiated and apoptosis-prone population. Eur J Immunol 2001, 31:122-131.

25. Jonuleit H, Schmitt E, Staassen M, Tuettenberg A, Knop J, Enk AH: Identification and functional characterization of human CD4+CD25+ T cells with regulatory properties isolated from peripheral blood. J Exp Med 2001, 193:1285-1294.

26. de Kleer IM, Wedderburn LR, Taams LS, Patel A, Varsani H, Klein 2004, 6:1213-1221.

27. Dieckmann D, Plottner H, Berchtold S, Berger T, Schuler G: The role of CCL19, and CCL17 by dendritic cells from patients with rheumatoid arthritis and regulation by Fc γ receptors. Eur J Immunol 2004, 34:3346-3354.

28. Baecher-Allan C, Brown JA, Freeman GJ, Hafler DA: Induction of circulating myelin basic protein and proteolipid protein-specific transforming growth factor-beta1-secreting Th3-type TGF-beta-secreting regulatory cells. J Immunol 2004, 172:3407-3416.

29. Weiner HL: Tolerance: immune mechanisms and the generation of Th3-type TGF-beta-secreting regulatory cells. Microbes Infect 2001, 3:947-954.

30. Nakamura K, Kitani A, Strober W: Cell contact-dependent immunosuppression by CD4+CD25+ regulatory T cells mediated by cell surface-bound transforming growth factor beta. J Exp Med 2001, 194:829-844.

31. Cao D, van Vollenhoven R, Klareskog L, Trolle C, Malmström V: CD25brightCD4+ regulatory T cells are enriched in inflamed joints of patients with chronic rheumatic disease. Arthritis Res Ther 2004, 6:R335-346.

32. 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-TNFα therapy. J Exp Med 2004, 200:277-285.

33. Stephans LA, Mottet C, Mason D, Powrie F: Human CD4+CD25+ thymocytes and peripheral T cells have immune suppressive activity in vitro. Eur J Immunol 2001, 31:1247-1254.

34. Annunziato F, Cosmi L, Liotta F, Lazenzi E, Manetti R, Vanini V, Rognagni M, Paggi E, Romagnani S, et al.: Antigen-specific T cell suppression by human CD4+CD25+ regulatory T cells. Eur J Immunol 2002, 32:1621-1630.

35. van Amelsfort JM, Jacobs KM, Bijlsma JW, Lafeber FP; Taams LS: CD4+CD25+ regulatory T cells in rheumatoid arthritis: different functions in the presence, phenotype, and function between peripheral blood and synovial fluid. Arthritis Rheum 2004, 50:2775-2786.

36. Taams LS, Vukmanovic-Stijeci M, Smith J, Dunne PJ, Fletcher JM, Plunkett FJ, Ebeling SB, Lombardi G, Rustin MH, Bijlsma JWJ, et al.: Antigen-specific T cell suppression by human CD4+CD25+ regulatory T cells. Eur J Immunol 2002, 32:1621-1630.

37. Cao D, van Vollenhoven R, Klareskog L, Trolle C, Malmström V: CD25brightCD4+ regulatory T cells are enriched in inflamed joints of patients with chronic rheumatic disease. Arthritis Res Ther 2004, 6:R335-346.

38. 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-TNFα therapy. J Exp Med 2004, 200:277-285.

39. van Amelsfort JM, Noordegraaf M, Bijlsma JWJ, Taams LS, Lafeber FPJG: Influence of the inflammatory milieu on the suppressive function of CD4+CD25+ T cells in rheumatoid arthritis. Arthritis Rheum 2004, 50:SO52.

40. Belkaid Y, Piccirillo CA, Mendez S, Shevach EM, Sacks DL: CD4+CD25+ regulatory T cells control Leishmania major persistence and immunity. Nature 2002, 420:502-507.

41. Iellem A, Mariani M, Lang R, Recalde H, Panina-Bordignon P, Sini-Renoux J, Romagnani P, Maggi E, Romagnani S: Identification and functional characterization of human CD4+CD25+ regulatory T cells 1. Co-expression of FOXP3 and IL-10. J Immunol 2002, 169:8471-8480.

42. Buckley CD, Amft N, Bradford PF, Filling D, Ross E, Arenzana-Seisdedos F, Amara C, Curnow SJ, Lord JM, Scheel-Toellner D, et al.: Persistent induction of the chemokine receptor CXCR4 by TGF-beta 1 on synovial T cells contributes to their accumulation within the rheumatoid synovium. J Immunol 2000, 165:3423-3429.

43. Suzuki N, Nakajima A, Yoshino S, Matsushima K, Yagita H, Okumura K: Selective accumulation of CCR5+ T lymphocytes into inflamed joints of rheumatoid arthritis. Int Immunol 1999, 11:553-559.

44. Selker R, Zoubou V, Martin-Jaime A, Scalzotto F, Cassin D, et al.: Human CD25+ T regulatory cells suppress naive and memory T cell proliferation and can be expanded in vitro without loss of function. J Exp Med 2001, 193:1295-1302.

45. Dieckmann D, Pottner H, Berchtold S, Berger T, Schuler G: CD4+CD25+ regulatory T cells suppress naive and memory T cell proliferation and can be expanded in vitro without loss of function. J Exp Med 2001, 193:1295-1302.

46. Zou L, Barnett B, Safah H, Larussa VF, Evedeman-Hogan M, Mottram P, Wei S, David O, Curiel TJ, Zou W: Bone marrow is a reservoir for CD4+CD25+ regulatory T cells that traffic through CXCL12/CXCR4 signals. Cancer Res 2004, 64:8451-8455.

47. Petersen J, Andersen V, Ingemann-Hansen T, Halkjaer-Kristensen K, Albrechtsen F, Cunliffe WJ: Human anergic/suppressive CD4+CD25+ T cells with memory characteristics in patients with rheumatoid arthritis. Arthritis Rheum 2001, 45:847-853.

48. Belkaid Y, Piccirillo CA, Mendez S, Shevach EM, Sacks DL: CD4+CD25+ regulatory T cells control Leishmania major persistence and immunity. Nature 2002, 420:502-507.

49. Petersen J, Andersen V, Ingemann-Hansen T, Halkjaer-Kristensen K, Albrechtsen F, Cunliffe WJ: Human anergic/suppressive CD4+CD25+ T cells with memory characteristics in patients with rheumatoid arthritis. Arthritis Rheum 2001, 45:847-853.

50. Petersen J, Andersen V, Ingemann-Hansen T, Halkjaer-Kristensen K, Albrechtsen F, Cunliffe WJ: Human anergic/suppressive CD4+CD25+ T cells with memory characteristics in patients with rheumatoid arthritis. Arthritis Rheum 2001, 45:847-853.

51. Petersen J, Andersen V, Ingemann-Hansen T, Halkjaer-Kristensen K, Albrechtsen F, Cunliffe WJ: Human anergic/suppressive CD4+CD25+ T cells with memory characteristics in patients with rheumatoid arthritis. Arthritis Rheum 2001, 45:847-853.

52. Petersen J, Andersen V, Ingemann-Hansen T, Halkjaer-Kristensen K, Albrechtsen F, Cunliffe WJ: Human anergic/suppressive CD4+CD25+ T cells with memory characteristics in patients with rheumatoid arthritis. Arthritis Rheum 2001, 45:847-853.

53. Petersen J, Andersen V, Ingemann-Hansen T, Halkjaer-Kristensen K, Albrechtsen F, Cunliffe WJ: Human anergic/suppressive CD4+CD25+ T cells with memory characteristics in patients with rheumatoid arthritis. Arthritis Rheum 2001, 45:847-853.

54. Petersen J, Andersen V, Ingemann-Hansen T, Halkjaer-Kristensen K, Albrechtsen F, Cunliffe WJ: Human anergic/suppressive CD4+CD25+ T cells with memory characteristics in patients with rheumatoid arthritis. Arthritis Rheum 2001, 45:847-853.