EDITORIAL

Regulatory T Cell Defects in Rheumatoid Arthritis

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Regulatory T (Treg) cells have emerged as a distinct subset of lymphocytes that are responsible for limiting immune responses and avoiding pathologic autoimmunity. Evidence is accumulating that defects in Treg function are important in immune-mediated diseases such as rheumatoid arthritis (RA). We discuss herein the biology of Treg cells and emphasize what is known about Treg dysfunction in RA.

Treg cells, a component of immune homeostasis

Specific immunologic tolerance to self antigens requires the immune system to discriminate between self and nonself. Elimination of self-reactive T cells in the thymus during T cell development leads to central tolerance. Peripheral tolerance is designed both to control responses to foreign antigens encountered in the periphery and to maintain tolerance to self antigens. While tolerance is mediated by a variety of mechanisms, work over the last 12 years has established a critical role for a specific subset of T cells known as regulatory T cells.

How are Treg cells identified?

Interest in regulatory T cells represents a reincarnation of immunologic concepts that were first proposed decades ago. The initial reports of what were then called T suppressor cells appeared in the 1970s, but these cells were difficult to characterize, and they fell out of favor by the late 1980s, despite intriguing evidence

of defective T suppressor cell dysfunction in systemic rheumatic diseases. Subsequent experiments performed by Sakaguchi et al (1) reinvigorated interest in what were renamed regulatory T cells, and focused greater attention on the regulation of T cell function by a subset of CD4+ cells, in contrast to an earlier focus on the suppression of antibody production by CD8+ suppressor cells. Sakaguchi showed that adoptive transfer of CD4+,CD25– T cells into athymic nude mice resulted in autoimmune disease in the recipient animals, which was ameliorated by transfer of CD4+, CD25+ T cells. CD25, the interleukin-2 receptor α-chain (IL-2Rα), is also expressed by activated T cells, but the CD4+,CD25^{high} cells among the CD4+,CD25+ subset are considered to be Treg cells. In general, the characteristics of mouse and human CD4+,CD25+ Treg cells are similar. Typically, 8–12% of CD4+ T cells are CD25+ Treg cells.

How do Treg cells develop?

Current data suggest that there are 2 subsets of Treg cells, the CD4+,CD25+ natural Treg (nTreg) cells, which mediate central tolerance, and induced Treg (iTreg) cells, which mediate peripheral tolerance. Natural Treg cells develop in the thymus, have a repertoire similar to that of conventional T cells but more skewed toward recognition of autoantigens, undergo clonal expansion upon antigen exposure, and yet maintain their suppressive phenotype. The thymic cellular events underlying the development and maturation of nTreg cells are not fully understood. It is possible that some thymocytes receive a strong signal via their T cell receptor (TCR) and CD28, escape negative selection, and differentiate into nTreg cells. The nTreg cells then migrate to the periphery and suppress autoreactive T cells, thus suppressing autoimmunity. Stimulation through the CD28 molecule is also required for the survival and proliferation of nTreg cells in the periphery. These cells express CD25, forkhead box P3 (FoxP3)/winged-helix transcription factor, cytotoxic T lymphocyte–associated

protein 4 (CTLA-4), and glucocorticoid-induced tumor necrosis factor receptor (GITR). (GITR is not a true tumor necrosis factor receptor [TNFR] despite its name, but rather, it is a member of the TNFR family.) In addition to nTreg cells, subsets of γ/δ T cells and NK1.1 T cells also develop in the thymus that have a suppressive phenotype in the periphery (2).

Induced Treg cells are generated in the periphery after antigen recognition by CD4+,CD25– T cells. These cells acquire FoxP3 and CD25 expression and have suppressive functions. The biology of iTreg cells is complex and less well understood than that of the nTreg cells. The type and amount of antigen, as well as the route of antigen administration, can influence the generation of iTreg cells in the periphery. Intranasal or oral administration of antigens has been shown to induce iTreg cells, and they can also be generated by low-affinity antigen recognition or altered TCR signaling. The repertoire of the iTreg TCR is not known. Intact CD28 signaling may not be necessary for the generation of iTreg cells in the periphery. The iTreg cells are made up of different subsets, each with a unique cytokine profile or surface phenotype. These are generated after exposure of naive T cells to antigen and a variety of other signals, such as IL-10, IL-4, transforming growth factor β (TGFβ), vitamin D$_3$, or plasmacytoid dendritic cells. In addition to CD4+ Treg cells, a subset of mature CD8+ T cells has regulatory function (3).

How do Treg cells function?

Treg cells are anergic and require IL-2 or T cell receptor ligation for their proliferation and suppressive function. Their effector function is not restricted by histocompatibility antigens, and they can suppress both CD4+ and CD8+ T cells in an antigen-nonspecific manner (2). Treg cells can selectively suppress various aspects of T effector function, such as proliferation, cytokine production, chemokine receptor expression, or cytolytic function. The precise mechanisms underlying suppression mediated by Treg cells are controversial. It is possible that Treg cells suppress immunologic responses in multiple ways, which may involve contact-dependent processes, with direct contact between Treg cells and T effector cells, or may involve Treg interactions with antigen-presenting cells (APCs), which then suppress T effector cells. Other mechanisms may include cytotoxic killing of target cells by Treg cells, production of suppressive cytokines, or induction of other regulatory cells.

The contact-dependent suppression is mediated by the interaction of CTLA-4 on Treg cells with CD80/CD86 on T effector cells, leading to down-regulation of T effector function. Alternatively, CTLA-4 on Treg cells could interact with CD80/CD86 on APCs and induce indoleamine dioxygenase (IDO) in the APCs. IDO causes the depletion of tryptophan and the formation of toxic metabolites, resulting in local suppression of T cell proliferation. Treg cells can also express granzyme A and kill T effector cells in a perforin-dependent manner. IL-10 has immunosuppressive effects in vivo, and both nTreg and iTreg cells have been shown to mediate suppression via expression of this cytokine. TGFβ is another immunosuppressive cytokine, and studies have shown that it may be responsible for both the expansion of Treg cells and Treg cell–mediated suppression of CD8 T cells. Most of these mechanisms have been studied in vitro and some of them (e.g., cytotoxic killing) may not be occurring in vivo. The current notion is that the nTreg cells mediate suppression via a contact-dependent mechanism and the iTreg cells mediate suppression in a contact-independent, cytokine (IL-10 and TGFβ)–mediated manner (4).

What is FoxP3, and why is it important?

FoxP3, a transcription factor uniquely expressed by Treg cells, is critically important in the development of these cells. Humans with defective/mutated FoxP3 develop immune dysregulation, polyendocrinopathy, enteropathy, and X-linked syndrome. In addition to FoxP3, CD28, CD40, and IL-2 are also important in the development of Treg cells. IL-2 receptor–knockout mice lack both nTreg and iTreg cells, implying that IL-2 is absolutely necessary for their development. Treg cells also express GITR, CTLA-4, CD103, CD62L, OX40L, TNFRII, TGFβ receptor type I (TGFβRI), 4-1BB, programmed death 1, neuropilin, and lymphocyte activation gene 3 (LAG-3). Ligands of some of these molecules are expressed on APCs (i.e., GITR ligand and 4-1BB ligand). There is some degree of differential expression of the surface molecules on different subsets of Treg cells. However, the functional roles of these surface molecules are yet to be fully understood. All of these structures are also expressed on conventional activated T cells except FoxP3, neuropilin, and LAG-3.

LAG-3, a class II major histocompatibility complex (MHC)–binding CD4 analog, is expressed selectively on Treg cells, and antibodies against LAG-3 inhibit the suppressive function of these cells. Neuropilin-1, a receptor involved in axon guidance,

angiogenesis, and activation of T cells, is constitutively expressed on CD4+,CD25+,FoxP3+ T cells and is down-regulated in activated T effector cells (3,5,6).

What do we know about Treg cells in RA?

Few studies have been conducted to evaluate the role of Treg cells in RA. There is controversy regarding the relative number of CD4+,CD25+ T cells in the peripheral blood of patients with RA. Most studies concur that there are *increased* numbers of Treg cells in RA synovium. CD4+,CD25+ T cells from the inflamed joint express higher levels of CTLA-4 and GITR and have an activated phenotype, which is characterized by the expression of CD69 and class II MHC molecules. Thus, there is ongoing inflammation and joint damage in the presence of increased numbers of CD4+,CD25+ cells in the RA joint. Studies have shown that CD4+,CD25+ Treg cells from patients with RA have a defective ability to suppress the production of TNFα and interferon-γ (IFNγ) by CD4+ T cells or monocytes, even though they can suppress the proliferation of T effector cells (7,8).

TNF is abundantly present in the sera and joints of patients with RA. It has been shown that TNF inhibits the suppressive function of nTreg cells and TGFβ1-induced Treg cells. CD4+,CD25+ cells express TNFRII, and signaling through this receptor by TNF results in decreased FoxP3 expression and an associated decrease in suppressive function. Treg cells from patients with active RA have reduced expression of FoxP3 and demonstrate blunted suppression of cytokine production and proliferation of T effector cells. In patients who undergo anti-TNF therapy (infliximab), there is an increased number of CD4+,CD25+ T cells, increased FoxP3 expression, and restoration of the cytokine-suppressive function of Treg cells (8). Furthermore, IL-6, which has been shown to be abundantly present in the rheumatoid synovium, has also been shown to render T effector cells resistant to Treg-mediated suppression (9).

The role of CD4+,CD25+ cells in the pathogenesis and regulation of arthritis has been best studied in a mouse model of RA, collagen-induced arthritis (CIA). Depleting Treg cells with anti-CD25 antibody before the onset of arthritis has been shown to result in increased cellular and humoral immune responses and increased arthritis severity (10). Adoptive transfer of CD4+,CD25+ T cells was shown to result in decreased severity of disease (11). These studies suggest that Treg cells are important in the immune imbalance that culminates in arthritis.

IFNγ is a Th1 cytokine that has both protective and deleterious effects in CIA. Experiments have shown that IFNγ receptor (IFNγR) deficiency did not change the number or in vitro suppressive function of CD4+,CD25+ T cells. However, CD4+,CD25+ T cells from IFNγR-deficient mice had impaired suppressive ability in vivo and developed worse arthritis. This was likely secondary to altered interaction of the Treg cells with APCs on an IFNγ-deficient background (12).

In this issue of *Arthritis & Rheumatism*, van Amelsfort and colleagues (13) provide additional insight into how Treg function might be thwarted in RA. They propose that activated monocytes interacting with Treg cells through both cell–cell contact and secreted cytokines could prevent Treg cells from executing their program of immune suppression. Arguing that RA synovial monocytes overexpress CD80 and CD86, the ligands for the prototypical T cell costimulatory molecule CD28, these investigators added an agonistic monoclonal anti-CD28 to cocultures of Treg cells and their T effector cell targets and observed a reduction in Treg function.

Interpretation of this experimental system is complex, since anti-CD28 could directly affect either the Treg cells or the target cells and/or alter contact between the two cell populations. Moreover, the extent to which anti-CD28 can be used as a proxy for the monocyte cell surface is a matter of debate. This work is interesting in the context of the recent clinical introduction of CTLA-4Ig as a biologic therapeutic for RA. CTLA-4Ig is believed to reduce the binding of CD28 ligands on monocytes and other cells to CD28 on T cells. If this action of CTLA-4 protects Treg function and inhibits T effector cell activation, a two-pronged beneficial effect on T cell homeostasis would be achieved. On the other hand, to the extent that CTLA-4 on Treg cells is necessary for the suppression of T effector cells through cell–cell contact between T effectors and Treg cells, CTLA-4Ig could theoretically interfere with Treg function. Analysis of the effects of CTLA-4Ig in vivo on the numbers, activation state, and function of Treg cells in RA patients would be both feasible and informative.

Other experiments conducted by van Amelsfort (13) examined the effects of several proinflammatory cytokines on the function of Treg cells. TNF and especially IL-7, but not IL-6, inhibited Treg function. These cytokines are appropriate for study since they are all present in the RA synovium and are proinflammatory. As the authors point out, the effect of IL-7 may be to

render T effector cells resistant to Treg cells, since the Treg cells themselves show minimal expression of the IL-7 receptor. The results with TNF provide yet another possible mechanism for favorable effects of TNF blockers in RA.

Their negative findings with IL-6 may require further experimental investigation with a wider range of IL-6 concentrations, since the concentrations of IL-6 used in vitro were no higher that the TNF concentrations used in these experiments, while the production and levels of IL-6 in vivo may be much higher. As noted in the article (13), IL-6 potently interferes with the function of mouse Treg cells, and the notion that human T cells are less responsive to IL-6 seems unconvincing. The potential importance of IL-6 as a therapeutic target in RA is further highlighted by the observation that IL-6 plus TGFβ are critical to the development of the recently described Th17 subset of CD4+ T cells, while TGFβ in the absence of IL-6 can both favor the development of Treg cells and mediate some of the functions of Treg cells (14–16). Th17 cells produce IL-17, which plays a critical role in many immune-mediated diseases in mouse model systems, and probably in RA as well. It is likely that these cells, as well as cytokines that promote their development, will become important targets in the treatment of RA and other human diseases.

Treg cells, the reincarnation of T suppressor cells, are here to stay—this time around. The complexity of the immune system and its regulatory pathways is daunting, but this should not discourage further attempts at understanding Treg cell function. After all, if nature has devised exquisite mechanisms for regulating autoimmunity while simultaneously preserving host defenses, should not our goal in the treatment of autoimmune disease be the restoration of this balance?

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