Targeting T Regulatory Cells in Autoimmune Diseases

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Abstract
Among the several mechanisms that play a role in maintaining peripheral self-tolerance is the existence of a unique CD4+CD25+ population of naturally occurring regulatory T cells, which actively prevent both the activation and the effector function of autoreactive T cells that have escaped different mechanisms of tolerance. Many studies have shown the benefit of targeting this cell population by restoring self-tolerance. Therapies that could possibly increase the suppressive ability of T regulatory cells were proven to improve the course of autoimmune diseases.

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Many studies, both in mice and humans, have confirmed the importance of the T regulatory CD4+ cell subset (approximately 10% of all CD4+ T cells) in the pathogenesis of many autoimmune diseases [1-3]. These CD4+ T cells co-expressing the interleukin-2 receptor alpha-chain (CD25) appear to play a pivotal role in the control of T cell homeostasis by suppressing the proliferation of effector T cells, thus maintaining self-tolerance. Experimental in vivo studies have demonstrated that the absence of regulatory T cells allows organ and non-organ-specific autoimmune diseases such as thyroiditis, gastritis, rheumatoid arthritis and systemic lupus erythematosus to occur, while the addition of this T cell population can prevent or delay these diseases [4,5] [Figure 1].

T regulatory cells and autoimmune diseases
The investigation of the status of CD4+CD25+ Treg cells in active RA is more than relevant, due to the crucial role that this subset of cells plays in maintaining immune homeostasis. Alterations in the apoptotic properties of Treg cells may favor the ratio of responder CD4+CD25– T cells to suppressor CD4+CD25+ T cells, thus leading to the breakdown of self-tolerance and permitting excessive inflammation and autoimmunity [6]. The exact mechanism by which alterations in Treg function can play a role in the development of active human RA is still not clear. In murine models, impaired secretion of tumor growth factor-beta and IL-10 seem to be essential; however, the role of these cytokines in human RA is not yet well established [7]. The suppressive function of CD4+CD25+ T cells in autoimmune diseases has been shown to be mediated in part through the expression of the CTLA-4 molecule on their surface [8].

The hypothesis that there is a deficit in the CD4+CD25+ Treg cells in early rheumatoid arthritis either in size or in functional activity was investigated [9]. There was a smaller proportion of Treg cells in the peripheral blood of early active RA patients compared to that in healthy controls (4.25% versus 5.3%, \(P = 0.0011\)). Frequencies in stable well-controlled RA were not significantly different from early active RA or controls. However, there were no differences in suppressor function of Treg cells between the groups. Higher frequencies of CD4+CD25+ Treg cells were found in synovial fluid compared to that in the peripheral blood of RA patients. In this respect, a recent study was undertaken to investigate the regulatory capacity of autologous peripheral blood Treg cells in contact with synovial tissue cell cultures and to evaluate their presence in peripheral blood, synovial tissue and synovial fluid of patients with RA [10]. RA synovial tissue cell cultures exhibited spontaneous expression of interferon-gamma that was abrogated by the depletion of CD3+ T cells and specifically reduced by co-culture with autologous peripheral blood Treg cells. The amount of Foxp3 transcripts, however, was lower in the synovial membrane than in peripheral blood or synovial fluid. The T-beta/Foxp3 ratio correlated with both the higher grade of synovial tissue lymphocyte infiltration and higher disease activity. This study shows the efficacy of autologous Treg cells in reducing the inflammatory activity of synovial tissue cell cultures in vivo in human RA.

In addition, many studies investigated the status of Treg cells in patients with systemic lupus erythematosus. In one of the first studies, the level of CD4+CD25+ T cells was evaluated in the peripheral blood of patients with SLE [11]. The study population comprised 94 SLE patients, 52 patients with RA and 50 age and gender-matched healthy individuals who served as controls. In this study, the level of CD4+CD25+ T cells was significantly lower than that in healthy controls (4.25% versus 5.3%, \(P = 0.0011\)). There was no significant difference in the percentage of Treg cells in healthy controls (5.3%) and healthy controls (4.25%) [11]. No significant differences could be found between RA patients and normal controls. In this study, although decreased CD4+CD25+ T cells were found in SLE patients, no correlation was found between the levels of Treg cells and disease activity in SLE. Contrary to these results, a later study was able to demonstrate that the frequency of CD4+CD25+ Treg cells was significantly decreased in patients with active SLE.
Treatment with anti-TNF antibody inhibited the proliferation and cytokine production of CD4+ T effector cells and restored the suppressive function of Treg cells isolated from patients with active RA [18]. In another recent study, infliximab therapy increased Foxp3 mRNA and protein expression by CD4+CD25+ Treg cells and restored their suppressive function [17].

The ability of tumor necrosis factor to inhibit the suppressive function of both naturally occurring CD4+CD25+ Treg cells and TGFβ1-induced CD4+CD25+ Treg cells was previously reported [11]. TNF-mediated inhibition of suppressive function was related to a decrease in Foxp3 mRNA and protein expression by Treg cells. Notably, CD4+CD25+ Treg cells isolated from patients with active RA expressed reduced levels of Foxp3 mRNA and protein and poorly suppressed the proliferation and cytokine secretion of CD4+ effector T cells in vitro. Treatment with anti-TNF antibody (infliximab) increased Foxp3 mRNA and protein expression by CD4+CD25+ Treg cells and restored their suppressive function.

In one of our previous studies we demonstrated a higher sensitivity of Treg cells to undergo spontaneous apoptosis in patients with active RA [18]. Alterations in CD4+CD25+ cell apoptosis and cell count were found to correlate with RA disease activity. Here again, the reversal of these deviations from normal was documented in association with the beneficial outcome of infliximab therapy. In another recent study, infliximab therapy was shown to give rise to a CD4+CD25high Foxp3 Treg cell population, which mediated suppression via TGFβ and IL-10, and lacked CD62L expression, thereby distinguishing this Treg cell subset from natural Treg cells present in healthy individuals and patients with active RA [19]. In spite of potent suppressor capacity displayed by this CD62L+ Treg cell population, the natural CD62L+ Treg cells remained defective in infliximab-treated patients. These results suggest that anti-TNFα therapy in RA patients generates a newly differentiated population of Treg cells that compensates for the defective natural Treg cells.

Glucocorticoids were also reported to affect the activity of Treg cells on the basis of FoxP3 and cytokine expression [20]. FoxP3 mRNA expression was significantly increased in asthmatic patients receiving inhaled glucocorticoid treatment, systemic glucocorticoid treatment, or both. The frequency of CD4+ memory CD4+ T cells and transient FoxP3 mRNA expression by CD4+ T cells significantly increased after systemic glucocorticoid treatment. In addition, glucocorticoids induced IL-10 and FoxP3 expression in short-term and long-term cultures in vitro. This strengthened the idea of using various therapeutic regimens to restore self-tolerance, by improving the function of Treg cells.

B cell depletion may affect T cell activation and co-stimulation status in rituximab-treated patients with SLE. In this respect, increased expression of functional markers of Treg cells was found to occur following rituximab administration [16]. In the early phase of B cell depletion, mRNA levels of CD25, CTLA-4, GITR and Foxp3 increased significantly in all seven patients examined. In contrast, mRNA levels of the co-stimulatory/activation T cell molecule CD40L were profoundly reduced, while mRNA levels of TGFβ, a cytokine contributing to Treg induction, increased significantly in all patients. During follow-up, increased Foxp3 mRNA persisted in those patients in clinical remission, while subsequent decreases were noted in those patients with active disease.

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TGFβ = tumor growth factor-beta
TNF = tumor necrosis factor
Targeting Treg cells should be intensively investigated, and many therapeutic modalities that were proven to increase suppressive abilities of Treg cells should be applied in the treatment of many autoimmune diseases. The role of Treg cells in other fields such as malignancy should encourage us to try and target these cells in cancer patients as well.

References


Figure 2. The incubation of IVIg with CD4+CD25+ Treg cells increased the expression of Foxp3 and the production of TGFβ and IL-10. The co-culture of IVIg with Treg cells also increased their suppressive activity and decreased TNFα production by CD4+CD25+ T cells.

The mechanisms by which IVIg could possibly affect the function of Treg cells is still not sufficiently clear. Increased expression of intracellular IL-10 in Treg cells could inhibit the production of pro-inflammatory cytokines by Th1 such as TNFα. In this regard, IVIg treatment resulted in the down-regulation of the Th1-type cytokine TNFα, and the up-regulation of the Th2-type cytokine IL-10 [22]. As supported by several experimental studies, IVIg regulates crucial steps of T cell-mediated immune responses. These effects involve the modulation of activation, proliferation, differentiation, apoptosis, and effector mechanisms of T cells. The pattern of IVIg-T cell interactions is complex, as IVIg may directly bind to regulatory structures on T cells or modulate T cell functions indirectly via soluble or cellular components of the immune system.

IVIg = intravenous immunoglobulin


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**Capsule**

**Anatomy of an immune response**

Intravital imaging techniques allow experimentally induced immune responses to be traced in real time. Nevertheless, the techniques have often relied on the transfer of no-physiological numbers of artificially labeled immune cells into animals. Khanna et al. report the use of in situ confocal microscopy of the spleen with a sufficient level of resolution to detect fine features of an immune response to a bacterial infection. Endogenous primary and secondary (memory) T cell responses could be compared, revealing unexpected relocalization within the spleen, as T cells underwent activation, expansion, and then migration out to peripheral anatomical sites.

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**Capsule**

**Drug craving and the insula**

An important factor that contributes to drug-seeking in addicted individuals is the negative feelings that result from abstinence. Such mood states are monitored by the interoceptive sensory system, and particularly by a brain area called the insular cortex, known to process emotional information. Contreras et al. observed that inactivation of the rat posterior granular insula reversibly disrupts the craving for amphetamine in animals repeatedly injected with amphetamine, as well as the behavioral signs of malaise induced by lithium administration. Thus, therapeutic interventions in the insula may help to alleviate drug cravings.

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**Capsule**

**Another case of cellular identity theft**

Solid tumors use a variety of crafty mechanisms to optimize their growth, invasion and metastasis. One unusual mechanism that has attracted much recent interest is a form of cellular identity theft whereby tumor cells morph into a different cell type or induce surrounding normal cells to do so. The best characterized of these phenotypic changes is EMT, or “epithelial-mesenchymal” transition, a process thought to endow tumor epithelial cells with migratory and invasive features and/or provide the tumor with a pool of activated fibroblasts that produce molecules required for metastasis. Zeisberg and co-workers describe a new variation on this theme. Using genetically marked transgenic mice, they show that proliferating endothelial cells can also morph into mesenchymal cells resembling activated fibroblasts and that the latter cells are present in at least two distinct tumor types in mice. This endothelial-mesenchymal transition is promoted by transforming growth factor-11, a cytokine that also promotes EMT and is abundant in many tumors. The next question is whether and how these intriguing cells contribute to tumor progression.

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