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%, $P = 0.001$). 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 *ex 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 terms of $CD4^+CD25^+$ T cells, defined as having a fluorescence intensity of CD25 expression exceeding 100, SLE patients still had significantly lower levels than the normal controls, expressed as percentages of peripheral blood mononuclear cells (1.7 ± 1.3 vs. $3.7 \pm 1.3\%$, $P < 0.05$). 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

Treg = T-regulatory
RA = rheumatoid arthritis
IL = interleukin
SLE = systemic lupus erythematosus

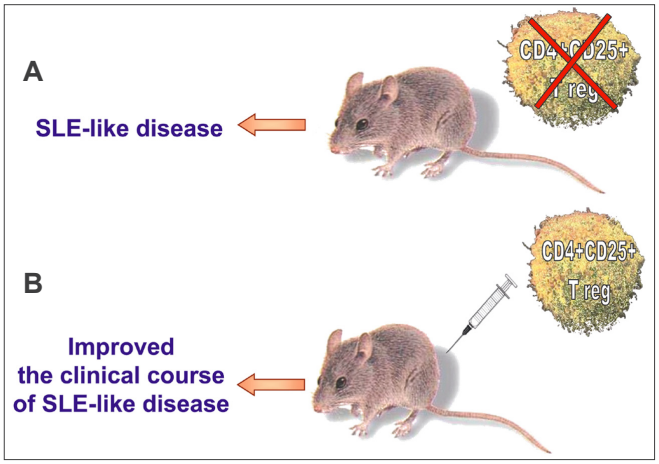

Figure 1. [A] When $CD4^+CD25^+$ Treg cells were deleted from mice, they developed a clinical picture of SLE-like syndrome, which included the appearance of antinuclear antibodies and markers of inflammation. **[B]** When $CD4^+CD25^+$ Treg cells were injected into NZB/W mice, SLE-like features such as lymphadenopathy, hemolytic anemia, and autoantibodies disappeared.

compared to patients with inactive SLE and to controls, and was inversely correlated with disease activity, as assessed by SLE Disease Activity Index 2000 scores (r = -0.59, P = 0.001) and serum anti-dsDNA levels (r = -0.65, P < 0.001) [12].

In another recent study, the transcription factor Foxp3 was measured in Treg cells of 43 patients with SLE [13]. Twenty of them comprised a group of newly admitted patients with the first manifestations of the disease, while the second group included patients who were treated with cytostatics and steroids. The results revealed a significant decrease in $CD4^+CD25^+$ and $CD4^+$CD25high T cell numbers in patients from group I compared to the control and group II patients. Co-expression of Foxp3 on Treg cells was significantly reduced in both groups regardless of the therapy. The ability of Treg cells to suppress the proliferation of autologous $CD8^+$ and $CD4^+$ T cells was significantly reduced in both groups of patients compared to the healthy donors.

Targeting T regulatory cells

The ability of various therapies to regulate the differentiation and function of Treg cells in autoimmune or allergic diseases has been the subject of many recent studies. In an earlier study, patients treated with glucocorticoids presented raised levels of CD25high cells, whereas untreated patients and those with antimalarial or immunosuppressive drugs had levels similar to those of the controls [14]. Although Treg cell percentage was not altered in non-steroid-treated SLE patients, glucocorticoid treatment increased their frequency, suggesting that this treatment could restore the suppressive function of Treg cells in SLE.

In line with this assumption, *in vitro* activation of $CD4^+$CD25high Treg cells from patients with active SLE increased the expression of FoxP3 and restored their suppressive function, suggesting that strategies to enhance the function of these cells might benefit patients with autoimmune diseases [15]. 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.

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 [17]. 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 infliximam 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 $CD25^+$ 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

TGFβ = tumor growth factor-beta
TNF = tumor necrosis factor

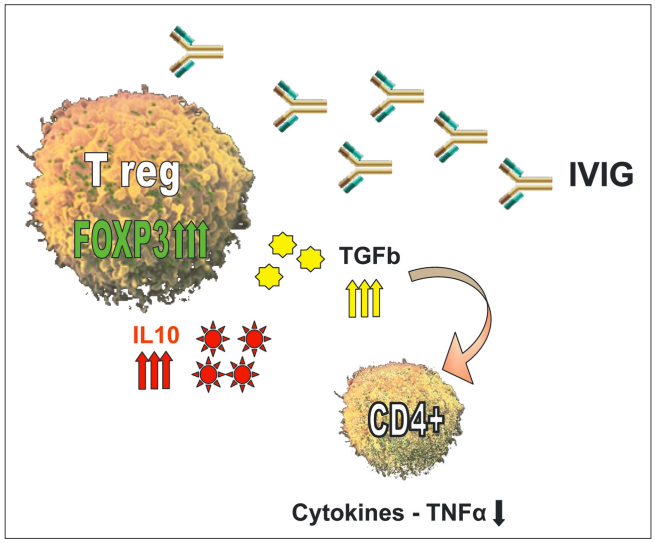

Figure 2. The incubation of IVIg with $CD4^+CD25^+$ Treg cells increased the expression of Foxp3 and the production of TGFβ and IL-10. The co-culture of IVIg with Treg cells also increased their suppressive ability and decreased TNFα production by $CD4^+CD25^-$ T cells.

study showed that treatment with glucocorticoids may promote or initiate differentiation toward Treg cells by a FoxP3-dependent mechanism. Thus, targeting these cells that aim to increase the expression of these molecules and their suppressive activity could become one of the tools by which self-tolerance is restored.

In agreement with all the above, we show for the first time that intravenous immunoglobulin was proven by a unique mechanism to enhance the suppressive activity of $CD4^+CD25^+$ Treg cells [21]. In this study we demonstrate that the addition of IVIg to $CD4^+$ cells increased intracellular expression of IL-10, TGFβ and FoxP3 when we gated on $CD4^+$CD2high T cells, suggesting that IVIg has the properties of directly affecting Treg cells. We then established that the addition of IVIg to the culture of cells increased the suppressive function of Treg cells by further attenuating TNF secretion by $CD4^+$ effector cells when IVIg was added to $CD25^+$ cells [Figure 2].

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

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.

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