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$CD4^+CD25^+$ regulatory T cells as a therapeutic target in rheumatoid arthritis

QING YU, MAN XU, FANG YU, YONG JIN

Anhui Medical University, Hefei, Anhui, PR China

Abstract

$CD4^+CD25^+$ T cells are regulatory T cells ($CD4^+CD25^+$ Tregs), which can strengthen immune tolerance. They play a critical role in controlling the development of autoimmune diseases in animals and humans. Rheumatoid arthritis (RA) is a chronic autoimmune disease that affects the peripheral joints and eventually leads to joint destruction. Although the pathogenesis of RA remains unknown, it is supposed to be affected by autoreactive T cells and antibodies. At the same time, to make the $CD4^+CD25^+$ T cells active and to increase the number of the cells are responsible in the therapy of RA in recent studies. Now, many techniques about expansion of Tregs in vitro have been established to overcome the problem of their limited numbers in vivo. It is important to carry out a study of induction or amplification of Tregs in vitro. Here, we review our current understanding of $CD4^+CD25^+$ T cells in RA and the targeting of these cells in RA therapy.

Key words: *CD4+CD25+ regulatory T cells, autoimmune diseases, immune tolerance, rheumatoid arthritis.*

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Introduction

Recent studies have revealed a special class of T cells' subset which is called $CD4^+CD25^+$ regulatory T cells (Tregs). Tregs have a unique function of immune regulation and more specifically are identified by Foxp3 [1]. Tregs not only inhibit the development of autoimmune disease but also restrain antitumor immune response. Rheumatoid arthritis (RA) is a systemic inflammatory disease characterized by chronic synovial inflammation resulting in cartilage and bone damage and the eventual joint destruction. It is often chronic and debilitating, and current therapies are not satisfactory for all patients. Meanwhile, in recent years, a study has found that activated Tregs can inhibit non-specific immune response, the activation and proliferation of effector T cells and the osteoclast formation. These characteristics are beneficial in the treatment of arthritis, and therefore imply that Tregs are potential drug targets for the treatment of RA. At present, research mainly focus on how to induce the generation of Tregs and promote the function of Tregs to treat RA or its animal model. Research has found that $CD4^+CD25^+$ Tregs play a crucial role in usual autoimmune diseases [2-5]. In this review, we mainly concentrate on the roles of $CD4^+CD25^+$ Tregs and their possible therapeutic value in autoimmune disease of RA.

Expression and function of CD4+CD25+ Tregs in rheumatoid arthritis peripheral blood and synovial fluid

Most research uses flow cytometry method (FCM) to calculate the populations of $CD4^+CD25^+$ Tregs and uses the European League Against Rheumatism (EULAR) criteria to determine the clinical response. Compared to healthy controls, the number of $CD4^+CD25^+$ Tregs in peripheral blood (PB) of RA patients is controversial [6, 7]. One study uses infliximab therapy ($n = 44$) and a robust 8-gene predictor model (applying this model to an independent validation set of RA patients) and observes a significantly higher number of $CD4^+CD25^+$ Tregs in whole blood of the responder group, as compared to the non-responder group at baseline [8]. Meanwhile, another research reported that the frequency of PB Tregs in RA patients was obviously lower than that of healthy controls and the number of $CD4^+CD25^+$ Tregs was the lowest in patients with active RA [9]. The above results resemble those obtained in both animal models and *in vitro* models of RA. Ohsugi *et al.* [10] used Tax transgenic mice as subjects and found out that $CD4^+CD25^+$ Tregs are significantly decreased in arthropathic Tax transgenic mice. McHugh *et al.* [11] used *in vitro* model systems to study

Correspondence: Yong Jin, Anhui Medical University, 81 Meishan Road, 230032 Hefei, Anhui, PR, China, e-mail: 89789389@gg.com

the function of these potent suppressor cells and came to the conclusion that CD4⁺CD25⁺ Tregs also protect the animals from a large spectrum of organ-specific autoimmune diseases. However, one research [12] shows that there are extant CD4⁺CD25⁺ Tregs in the peripheral blood of severe RA patients, while the gene expression of Foxp3 is absent. According to Ryder *et al.* [13], RA patients express more full-length Foxp3 than healthy controls in peripheral blood, and there is an increased number of Tregs in RA patients. These results indicate that it remains uncertain whether the gene expression of Foxp3 in CD4⁺CD25⁺ Tregs is a conclusive element. However, another study [2] shows that the alterations in Foxp3 expression may affect the functional stability of Tregs. A supplementary study demonstrates that although not capable to prevent the disease onset, transferring exogenous Tregs can modify the development of arthritis [14].

Also, previous reports [15, 16] show no significant differences as to the Tregs frequency between the RA patients and the controls. Why does this happen? This diversity may be partly due to the different marker methods used for determining Tregs.

Unlike the results from PB, there is clear evidence that the ratios of CD4⁺CD25⁺ Tregs in the synovial fluid (SF) of RA patients are enhanced. It has been found out that CD4⁺CD25⁺ Tregs are functional in RA patients and the suppressive activity of CD4⁺CD25⁺ Tregs in SF is more visible than in PB [17]. This is mostly due to the response of joint destruction and it is to help resist the disease of RA. Meanwhile, this phenomenon further proves the importance of CD4⁺CD25⁺ Tregs in preventing joint destruction.

Mechanism of CD4⁺CD25⁺ regulatory T cells in immunosuppression

Although research on CD4⁺CD25⁺ Tregs and immune regulation varies, the mechanism is still not fully understood. There are several theories about the role of CD4⁺CD25⁺ Tregs in immunosuppression, but the cytokine theory and the direct contact between cells theory are more reliable than others.

Tregs can secrete cytokines such as interleukin (IL) 10, IL-35, transforming growth factor β (TGF-β), which have a valid effect on immunosuppression. In a vivo study [18], it has been found that Tr1 cell subset can inhibit the function of macrophages through the secretion of IL-10. In turn, Th3 cell subset produces TGF-β to inhibit the immune reaction mediated by Th1 cells.

The direct contact between cells is the main theoretical mechanism that clearly describes how the CD4⁺CD25⁺ Tregs act in autoimmune diseases. Meanwhile, CD4⁺CD25⁺ Tregs need to be activated by T cell receptor (TCR) and auxiliary signals. It has been found that transmission of a suppressive signal by CD4⁺CD25⁺ Tregs requires the participation of B7 molecule expressed on target T cells. Meanwhile, the response of T cells from B7-deficient mice is resistant to suppression *in vitro* and these cells lead to a deadly disease in mice despite the presence of Tregs. Also, the surface of Tregs in humans and mice can express CTLA-4, which can combine with CD80 and CD86. Then, the signal is passed to effector cells to play the role of inhibition [19]. At the same time, findings from the research strongly suggest that CD4⁺CD25⁺ T cells exert immunosuppression by a cell-cell interaction involving cell surface TGF-β1 [18].

The effects of immunosuppressive drugs on CD4⁺CD25⁺ regulatory T cells

Although tumor necrosis factor α (TNF-α) antagonist biologics have been clinical therapy drugs of RA for over twenty years, the mechanism of their action is not entirely clear. Tumor necrosis factor α is an important proinflammatory cytokine which is involved in the pathogenesis of RA. Tumor necrosis factor α participates in joint inflammation, promotes the formation of osteoclasts and leads to the destruction of bone and cartilage. Tumor necrosis factor α expression is elevated in RA patients with joint space. When compared to healthy controls, RA patients showed an obvious increase in peripheral Th17 frequencies, elevated levels of Th17-related cytokines (IL-17, IL-23, IL-6, TNF-α), and a significant decrease in Tregs frequencies and Treg-related cytokine (TGF-β1) levels [20]. Tumor necrosis factor α antagonists may suppress Th17 by inhibiting generated IL-1 and IL-6, thereby promote Tregs-suppressive function. Nie *et al.* [21] found that the abnormal dephosphorylation of Foxp3 in rheumatoid arthritis was due to the ubiquitous enzyme protein phosphatase 1 (PP1), the expression of which was induced by TNF-α through the IKK-NF-κB pathway. In the synovium of individuals with rheumatoid arthritis, TNF-α keeps T cells and pathogenic T17 and T1 cells in balance through Foxp3 dephosphorylation. Treatment of patients with rheumatoid arthritis with a TNF-α antagonist decreased PP1 expression, increased Foxp3 phosphorylation and restored Tregs suppressive function. Methotrexate (MTX), a traditional antifolate and disease-modifying antirheumatic drug administered weekly, either alone or as a combination therapy, is the first-line disease-modifying agent for the treatment of RA worldwide. Methotrexate has excellent long-term efficacy, tolerability and safety. Early initiation of MTX in patients with RA controls joint destruction and slows disease progression. Methotrexate potentially acts via antiproliferative, anti-inflammatory, and/or immunosuppressive means. Low-dose MTX probably serves as a potent inducer of specific immunotolerance but not of nonspecific immunosuppression in the treatment of RA [22, 23]. In a study of Lina *et al.* [24], 20 active RA patients were given a stable weekly dose of MTX alone and other ten patients received a combined therapy of etanercept and MTX. Percentages of Th17 among CD4$^{(+)}$ T cells

were significantly higher, while $CD4^+CD25^{high}Foxp3^+$ Tregs were significantly lower in RA patients compared with healthy controls. After 12 weeks of therapy of single MTX or a combination of MTX and etanercept, the circulating Th17/Tregs ratio significantly decreased. Etanercept in combination with MTX ameliorates RA activity by normalizing the distribution of Th17 and Tregs and their related cytokines. This finding may partly explain the mechanism of combined therapy of etanercept plus MTX in RA treatment. Researchers [25-27] investigated the effects of various disease-modifying anti-rheumatic drugs (DMARDs) on Tregs function. They found that each DMARD had a different effect on Tregs function. Sulfasalazine (SSZ) and leflunomide (LEF) inhibited the anti-proliferative function of Tregs on co-cultured Teffs and reduced Treg expression of Foxp3 mRNA, whereas MTX and INF did not. In a special research on MTX [28], it has been found that the MTX therapy is connected with evident decreases in anti-CCP and IgM RF, IgA RF antibodies in good responders to therapy. The specific reason is still not clear, and larger clinical studies with longer follow-up are needed to more thoroughly assess the efficacy of immunosuppressive drugs on Tregs.

Proliferation of $CD4^+CD25^+$ regulator T cells to treat rheumatoid arthritis

There are defects of Tregs in RA, and both the increased number and recovery function of Tregs will contribute to the treatment of the disease. Clinical application is, however, frustrated by their scarcity, anergic status, and lack of defined specificity. Moreover, expanded Tregs are superior to fresh Tregs in suppressing T cell responses against alloantigens [29]. Thus, it is important to carry out a study on induction or amplification of a large number of Tregs *in vitro*.

Currently, many techniques about expansion of Tregs *in vitro* have been established to overcome the problem of their limited cell numbers *in vivo*. At present, *in vitro* $CD4^+CD25^+$ T cells expanded with anti-CD3/CD28 beads plus IL-2, which is the focus of current studies and expand Tregs with extensive. Yet another problem with this therapy is polyclonality of expanded Tregs. The expanded Tregs have a similar phenotype and Foxp3 expression to fresh Tregs, expanded Tregs show a higher tendency than freshly isolated Tregs to proliferate under normal assay conditions. However, $CD4^+CD25^+$T cells gained by this method have poor antigen specificity of the Tregs. Animal model studies show that antigen-specific Tregs are better than polyclonal Tregs in controlling autoimmunity [30]. So, most scholars are more likely to pay their attention to expanding antigen-specific Tregs. Dendritic cells (DCs) are likely to play a crucial role in reestablishing immune tolerance and long-time suppression via the expansion and/or induction of $CD4^+CD25^+$ Tregs. It can expand $CD4^+CD25^+$ T cells and recent studies suggest that antigen-specific Tregs expanded by DCs showed superior immunosuppression in comparison with polyclonal Tregs expanded by anti-CD3/CD28Ab, which is the focus of current studies. In addition, experiments show that $CD4^+CD25^-$ T cells can be converted into antigen-specific $CD4^+CD25^+$ Tregs with the participation of TGF-α and costimulatory molecules. It has also been demonstrated that $CD4^+CD25^-$ T cells can convert to $CD4^+CD25^+$ Tregs in the periphery under the influence of TGF-α and retinoic acid and concluded that to modulate the immune response by plasmacytoid DCs may provide us with novel immune-based therapies in autoimmune diseases [31, 32].

Currently, cell-based therapies in animal models showed some efficacy, but conditional limitation reports *in vivo* are relatively small, and many problems still need to be answered. In 2009, Trzonkowski *et al.* [33] for the first time treated chronic GVHD by making patients receive adoptive transfer of $CD4^+CD25^+CD127^-$ Tregs taken from the donor and expanded *ex vivo* beforehand. They achieved not only a significant reduction in the dose of immunosuppressants, but also alleviation of some adverse effects of immunosuppressants. They successfully treated chronic GVHD, in whom routine approved immunosuppression was ineffective in stopping the progress of GVHD. Their trial proved that the adoptive transfer of expanded Tregs might be a good option as an adjuvant therapy in chronic and acute GVHD. Another study shows that infusion of *ex vivo* expanded Tregs after double umbilical cord blood (UCB) transplantation, there was a significantly reduced incidence of GVHD compared with those patients without Tregs. This indicates that the infusion of *ex vivo* expanded Tregs can reduce the incidence of GVHD, which is a common complication after UCB transplantation [34].

Discussion

In summary, as discussed in the report, populations of $CD4^+CD25^+$ Tregs may play a decisive role in RA. The development of therapeutic targeting $CD4^+CD25^+$ Tregs could result in helpful, innovative therapies for RA. Meanwhile, further studies are required, especially to find out a suitable way to enhance the populations of $CD4^+CD25^+$ Tregs to cure the disease of RA.

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