Tregs in immunotherapy: opportunities and challenges

"...we are faced with the opportunity to develop immunotherapies for human autoimmune diseases by using ... knowledge ... [that] allows us to increase Treg ratios over other T cells subsets."

KEYWORDS: apoptosis ■ autoimmunity ■ Foxp3 ■ immune tolerance ■ TGF-β ■ Th17 ■ Tregs

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The emergence and characterization of Tregs expressing the transcription factor Foxp3 has presented opportunities to develop more effective and specific immunotherapies for human diseases, including autoimmune diseases and chronic inflammation. Here, I will provide a personal perspective on the progress and challenges of such therapies.

Despite the variable symptoms and different organs affected in individual autoimmune diseases, the main underlying mechanisms that account for the pathogenesis of human autoimmune diseases are surprisingly similar. In this regard, compelling evidence has indicated that the autoimmune diseases are attributable to loss of immune tolerance within the patients' immune systems, which leads to the patients' own immune cells overreacting or incorrectly attacking 'self' antigens in vital organs. The main trend in current therapies is to rely heavily on nonspecific immunosuppressive drugs such as steroids. However, these drugs relieve only some of the symptoms, and often have serious side effects particularly if they are used for the long term, due to their indiscriminate immunosuppressive function. Thus, the search for more specific and effective therapies for aberrant inflammation with no or reduced side effects is the 'holy grail' in the fields of immunology and clinical medicine.

The emergence and characterization of $CD4^+CD25^+Foxp3^+$ Tregs has brought about a new hope and the opportunity to develop a novel immunotherapy for human autoimmune diseases and chronic inflammation [1–6]. Tregs are instrumental in maintaining immune tolerance and in preventing and curtailing the aberrant immune activation that leads to autoimmunity and chronic inflammation. Deficiency in the number or function of Tregs leads to uncontrolled systemic inflammation and autoimmune-like diseases in mice and also humans. Tregs show potent immunoregulatory effects on T responder cells and also most other types of immune cells, in cultures and also *in vivo* in experimental animals. However, thus far their clinical application has been limited. Besides the incomplete understanding of their mechanisms of suppression, an inability to identify antigen specificity of Tregs and difficulty in expanding these cells for transfer into mouse and human, has limited their clinical use. The recent breakthrough that TGF-β, one of the most potent immunoregulatory cytokines, induces $CD4^+Foxp3^+$ Tregs from naive $CD4^+$ T cells (called iTregs) *in vitro* and *in vivo* and maintains the homeostasis of natural Tregs (nTregs, or thymically derived Tregs) *in vivo* [7–9], has brought new hope to induce antigen-specific Tregs (iTregs) for therapy in autoimmune diseases. Indeed, these iTregs show potent immunosuppression of effector T-cell activation *in vitro* and also *in vivo* when transferred into mice [10]. Despite these promising results, so far most published studies are limited to the prevention of the diseases by preinjection of the Tregs into the naive mice before the disease is induced. Although these studies have provided experimental evidence for the potential application of Tregs in the treatment of autoimmune diseases, there is a huge difference in the immune status between an unmanipulated, naive mouse and a mouse with an established autoimmune disease. This problem is particularly salient in the clinical setting in which patients present with an already dysregulated immune system and established autoimmune or inflammatory disease. In an animal in which an autoimmune disease is already established, the immune system is activated, which creates a monumental

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barrier to the application of Tregs to treat autoimmune diseases. Recent evidence has started to reveal that Tregs may be converted into proinflammatory cells such as IFN-γ-producing Th1 cells and/or IL-17-producing Th17 cells in inflammatory settings, thus transferred Tregs could also potentially contribute to the autoimmune disease [11,12]. In a recent study, Hafler and his colleagues described a higher frequency of Th1-like IFN-γ-secreting Foxp3$^+$ T cells in untreated subjects with relapsing–remitting multiple sclerosis as compared with healthy control individuals [13]. These Th1-like Tregs show reduced suppressive activity and can be acquired from human Tregs from healthy subjects. By contrast, other studies have indicated that some populations of nTregs are stable even under inflammatory conditions in mice [14].

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In facing these challenges, investigators have started to explore the possibility of inducing antigen-specific Tregs or expanding nTregs *in vivo*, in hope of overcoming the disadvantages seen with the transfer of *in vitro* induced/expanded Tregs. In this regard, several lines of experimental approaches have been explored, including continuous infusion of low doses of peptides [8] and the administration of antigens bound to dendritic cell-specific antibodies [15]. Although these approaches could induce Tregs *in vivo*, the barrier remains, as the experimental approach is reliant on the mice being naive and disease free, and has not been sufficiently demonstrated in more relevant mice with established autoimmune diseases. In addition, the disappointing results of early attempts to treat patients with orally administered peptides or altered peptide ligands, both of which worked effectively in naive mice and even in some disease models, further highlight the difficulty of treating autoimmune diseases with peptides alone.

Complicating matters further, although TGF-β is considered a regulatory cytokine, it is constitutively expressed and recent studies have indicated that the presence of inflammatory cytokines is able to effectively abrogate the induction of Tregs by TGF-β. For example, IL-6 and TGF-β shut down Foxp3 and induce CD4$^+$ T cells to differentiate into the Th17 phenotype [16–19]. IL-4 hijacks TGF-β induction of Tregs and switches CD4$^+$ T cells into Th9 cells [20,21] or Th17 cells [22]. Finally, IFN-γ has been shown to force Tregs to become T effector cells [11]. Thus, it is extremely difficult, if not impossible, to induce sufficient numbers of functional Tregs to conquer disease in such an inflammatory environment that is present in autoimmunity. Therefore, the biggest challenge is how to create a microenvironment in the animals, and ultimately in patients, under which T cells are able to differentiate into Tregs in response to their specific antigen rather than into Th1, Th2, Th17 or Th9 effector cells. In other words, how could we create a transient milieu *in vivo* in which immunoregulatory cytokines such as TGF-β and/or IL-10 are dominant and proinflammatory cytokines subside or are absent?

The solution to this problem is still being sought. It may rest on discoveries and breakthroughs that create a permissible microenvironment(temporarily) under which Tregs are favorably generated and differentiated *in vivo*, despite the inflammatory conditions. The task to reach this goal is difficult, yet possible. Several lines of investigation have begun to explore this possibility. We and others have shown that phagocytes such as macrophages and immature dendritic cells preferentially produce immunosuppressive cytokines such as TGF-β and IL-10, but not proinflammatory cytokines, upon uptake and digestion of apoptotic cells *in vitro* and *in vivo* [23]. Administration of intact CD3-specific monoclonal antibodies depletes large numbers of T cells by inducing apoptosis, which in turn triggers professional phagocytes to produce TGF-β which, again in turn, influences Treg development and favors immune tolerance *in vivo* [24]. A transient, yet severe, systemic proinflammatory cytokine storm is induced by intact CD3-specific antibodies. However, this could be sufficiently overcome by new forms of CD3-specific antibodies which are nonmitogenic [25,26]. These modified nonmitogeneic CD3-specific antibodies substantially reduce the strength of TCR stimulation, yet still have the ability to induce apoptosis of T cells, production of TGF-β and resultant induction of Tregs, *in vivo*. One of the most important mechanisms by which nonmitogenic CD3-specific antibodies mediate immune tolerance is by increasing Tregs *in vivo* in a TGF-β-dependent manner [25,27]. Importantly, Chatenoud and her colleagues have translated this CD3 antibody treatment to the clinic and shown impressive therapeutic effects on patients with autoimmune diseases, particularly Type 1 diabetes [28]. Weiner and

his colleagues showed that oral administration of anti-CD3-suppressed murine systemic lupus erythematosus and inhibited Th1 and Th17 responses, but increased TGF-β/IL-10 expression and decreased IL-23/IL-6 expression by dendritic cells in human volunteers [29,30]. Other groups have attempted to selectively deplete activated effector T cells while preserving the naive or regulator T cells for a purpose of induction of immune tolerance in preventing transplantation settings [31]. This approach is attractive as it could selectively remove the inflammatory T cells and might reduce the potential risk of general immunosuppression. However, whether it is workable and feasible in the treatment of autoimmune diseases remains to be determined. Administration of T-cell-depleting CD4- and/or CD8-specific antibodies has been known for some time to induce immune tolerance in mouse models of autoimmunity and inflammation. Although the underlying mechanisms have not been thoroughly investigated, subsequent increases in Tregs have been seen; therefore, it would be interesting to revisit this area and explore the possible relationship between the tolerance and the Treg population. It is intriguing that CD4-specific antibody injection preferentially depletes non-Treg cells rather than Foxp3$^+$ Tregs, resulting in an increased ratio of Tregs to responder T cells *in vivo* [32] [Chen W *et al.*, Unpublished Data]. Important questions include whether Tregs are more resistant to the CD4 antibody-induced death, or the antibody treatment results in Treg generation. In line with this investigation, Waldmann and his colleagues have elegantly shown that non-T-cell-depleting CD4-specific antibody treatment could induce Foxp3$^+$ Treg cells *in vivo*, which occurs in a TGF-β dependent manner [33]. Moreover, recent studies have started to reveal that several steroid and nonsteroidal anti-inflammatory drugs such as aspirin, used conventionally for the treatment of autoimmune diseases and chronic inflammation, are linked to the immune tolerance program by altering the ratios of Tregs *in vivo* in experimental models of inflammation [34].

> *"By refocusing our investigation on creating a microenvironment to selectively generate antigen-specific Tregs ... it may become possible to develop novel immunotherapies to conquer autoimmune diseases and chronic inflammation."*

In summary, we are faced with the opportunity to develop immunotherapies for human autoimmune diseases by using the knowledge we obtained from recent studies, which allows us to increase Treg ratios over other T cells subsets. However, these are merely potential therapeutics, and we still face many challenges, thus we are tasked with making good on the opportunities presented and overcoming the many challenges. By refocusing our investigation on creating a microenvironment to selectively generate antigen-specific Tregs in addition to understanding the immunoregulatory mechanisms of Tregs, it may become possible to develop novel immunotherapies to conquer autoimmune diseases and chronic inflammation.

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