

## Arthritis therapy: a role for regulatory T cells?

*“...a combination of different approaches will be required to establish a more specific immune suppression of autoimmunity, only enhancing Treg function at sites of ongoing inflammation.”*

**KEYWORDS:** chronic inflammation ■ FOXP3<sup>+</sup> regulatory T cells ■ HDAC inhibitors ■ HSP60 ■ immune regulation ■ immune suppression ■ T-cell plasticity

In the last decades, regulatory T cells (Tregs), particularly the CD4<sup>+</sup>CD25<sup>+</sup> Treg expressing the transcription factor FOXP3, have been extensively investigated in autoimmunity. Tregs have been shown to suppress autoimmunity in several murine models; for instance, for Type 1 diabetes, inflammatory bowel disease, arthritis and multiple sclerosis (MS) [1-5]. The most obvious example showing the importance of Tregs for immune suppression in humans is immunodysregulation polyendocrinopathy enteropathy X-linked syndrome, a lethal inflammatory syndrome caused by a mutated *FOXP3* gene, leading to dysfunctional FOXP3 [6,7]. Furthermore, in several human autoimmune disorders, such as in juvenile idiopathic arthritis (JIA) and rheumatoid arthritis (RA), diminished Treg function or decreased numbers have been related to a more severe disease course [8]. Therefore, Tregs appear to be potent suppressors of autoimmunity. However, so far, this has not led to a direct application of Treg-mediated therapy in arthritis patients.

Currently used effective therapies in arthritis do not primarily target Tregs. However, it is possible that they could also work partly through a promotion of Treg numbers or function. For instance, anti-TNF- $\alpha$  (infliximab) increases Treg numbers threefold in RA patients, probably by induction of new FOXP3<sup>+</sup> Tregs from CD4<sup>+</sup>CD25<sup>-</sup> T cells, which was demonstrated *in vitro* [9,10]. In addition, membrane-bound TNF- $\alpha$  diminishes Treg function and anti-TNF- $\alpha$  treatment decreases membrane-bound TNF- $\alpha$  on Tregs both *in vitro* and in RA patients, thereby restoring Treg function [11].

Furthermore, steroid-mediated immune suppression may be enhanced by increasing Treg frequency or function. For instance, methylprednisolone pulses can increase the frequency

of Tregs in patients with MS [12,13] and myasthenia gravis [14,15]. In patients with myasthenia gravis, the reduced suppressive function of Tregs was restored by prednisolone treatment [15]. This suggests that glucocorticoids can induce tolerogenic responses through Tregs and thereby diminish disease. A proposed mechanism of glucocorticoid induction of tolerance is through inhibition of dendritic cell maturation [15-18]. Immature dendritic cells produce TGF- $\beta$  and IL-10 [15] and are able to induce Tregs [19]. Another mechanism is induction of cell death by glucocorticoids, specifically in effector T cells but not in Tregs. Dexamethasone-treated mice demonstrated increased apoptosis of CD4<sup>+</sup>CD25<sup>-</sup> T cells, but not of CD4<sup>+</sup>CD25<sup>+</sup> T cells, thereby increasing the percentage of CD4<sup>+</sup>CD25<sup>+</sup> T cells, which were suppressive *in vitro* [20]. Obviously, the effects of treatment of patients with anti-TNF- $\alpha$  or corticosteroids cannot be solely attributed to Treg-mediated mechanisms. However, the increase of Tregs due to treatment has to be taken into consideration when drawing conclusions on the relevance of Tregs in disease regulation in patient groups receiving these therapeutics.

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The question is what could be a realistic and feasible way to directly promote Treg function and numbers in arthritis? Several methods of promoting Treg function have been described, mostly in experimental models. Of particular interest is (antigen-specific) peripheral induction of new Tregs from naive T cells or peripheral



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expansion of natural Tregs. For instance, we showed recently that HSP60, a self-antigen that is highly expressed in the synovial lining tissue of inflamed joints of JIA patients, is able to induce functional FOXP3<sup>+</sup> Tregs [21]. Since the antigen (HSP60) recognized by the Treg is only expressed extracellularly at the inflammatory site, HSP60-specific Tregs will most likely specifically suppress inflammation locally at the time of inflammation, without unwanted side effects.

When considering the use of FOXP3<sup>+</sup> Tregs for therapy it has to be noted that only sustained FOXP3 expression establishes functional Tregs and, while ectopic expression of FOXP3 in murine CD4<sup>+</sup>CD25<sup>-</sup> T cells can induce a suppressive phenotype [22,23], Tregs can lose FOXP3 and suppressive capacity in an inflammatory environment [24–27]. Over the last few years, several protocols have been established for differentiating T cells into specific T-cell subtypes *in vitro*. IFN- $\gamma$  and IL-12 are required to develop Th1 cells, IL-4 induces Th2 cells, TGF- $\beta$ , IL-1 and IL-23 induce Th17 cells [24] and TGF- $\beta$  and IL-2 induce Tregs [28]. Each T-cell subtype has its own master transcription factor, which drives T-cell differentiation and maintains T-cell subtypes. For example, Tregs express FOXP3, Th17 expresses RORC2 (ROR $\gamma$ T in mice), Th1 expresses T-bet [29] and Th2 expresses GATA3 [30] and IFN regulatory factor 4 (IRF-4) [31]. It has recently been demonstrated that human FOXP3<sup>+</sup> Tregs can coexpress RORC2 and produce IL-17 *ex vivo* [32]. In mice, FOXP3<sup>+</sup> Treg can also coexpress T-bet [33] or IRF-4 [34], which enables them to specifically suppress Th1 or Th2 responses. However, Tregs can also differentiate into effector T cells. Human FOXP3<sup>+</sup> Tregs can, under inflammatory conditions, convert to IL-17-producing, RORC2-expressing Th17 cells [25–27]. This is of particular importance, since IL-17 is a pathogenic cytokine and highly expressed at inflammatory sites in several human autoimmune diseases, such as MS [35,36], RA [37], Crohn's disease [38] and psoriasis [39]. Moreover, numbers of IL-17-producing T cells in synovial fluid of RA patients correlates with rapid joint damage progression [40]. Furthermore, in murine models it has been shown that Tregs can lose FOXP3 and convert to Th2 [41] or to effector T cells producing IL-17 and IFN- $\gamma$  [42]. This phenomenon is called T-cell 'plasticity' [43–45] and is also observed in Th cells; for example, Th2 can convert to Treg [46] and Th17 to Th1 [47]. T-cell plasticity provides flexibility in T-cell responses, which may be required to generate an appropriate immune response upon a different type of

infection [45]. This plasticity evidently forms a great challenge when considering cell therapy with Tregs.

Taken together, induced FOXP3<sup>+</sup> Tregs with transient FOXP3 expression may not only lose Treg phenotype, but may even differentiate into pathogenic effector T cells once they are in an inflammatory environment. Therefore, it is important to carefully evaluate Treg stability to ensure that Tregs are safe for treatment or to promote FOXP3 expression in order to prevent conversion of Tregs to effector T cells, for example by utilizing histone deacetylase (HDAC) inhibitors. HDAC inhibitors promote acetylation of FOXP3, thereby prevent degradation of FOXP3 [48] and, subsequently, may prevent conversion of Tregs to Th17 under inflammatory conditions *in vitro* [25].

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In humans, peripheral induction of Tregs is probably important for regulation of inflammation [49,50]. This would suggest that Tregs are either absent or not functional in patients with autoimmune disease. However, in synovial fluid from inflamed joints of JIA and RA patients, large numbers of FOXP3<sup>+</sup> Tregs are found [8] and Tregs from JIA synovial fluid are functional *ex vivo* assays [51]. It is likely that the chronic inflammatory environment *in vivo* either causes local dysfunction of Tregs or removes activated cells' susceptibility to Treg-mediated suppression [52–54]. Thus, to create a therapeutic window for Treg-targeted therapies, it may be important to first dampen chronic inflammation, for instance by using anti-TNF- $\alpha$  therapy for a short period of time [8,55]. Thereafter, Treg function could be induced by, for instance, HSP60 epitopes inducing HSP60-specific Tregs, which will suppress actively and exclusively at sites where HSP60 is present, such as the inflamed synovial tissues in joints of arthritis patients [56]. Furthermore, in order to maintain Treg function and prevent conversion into effector T cells, HDAC inhibitors, such as nicotinamide, can enhance FOXP3 stability [25,48]. However, this still has to be tested more extensively in *in vivo* model systems. Thus, a combination of different approaches will be required to establish a more specific immune suppression of autoimmunity, only enhancing Treg function at sites of ongoing inflammation.

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