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Recent Advances Show That Abnormal T-Regulatory Cell Function Perpetuates Chronic Inflammatory Arthritis

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The principal mechanism governing immune central tolerance is regulated by T-cells that reside in a pathway wherein the death of immature T-cells is coupled to the development of $CD4^+$ regulatory T (Treg) cells. In that regard, Treg cells undergo development in the thymus or peripheral tissues upon recognition of self-antigens.

The main function(s) of Treg cells is to suppress activation of other T-cell members that are specific for those self-antigens [1,2]. Thus, negative selection is the main mechanism which produces central tolerance and as such negative selection affects self-reactive $CD4^+$ and $CD8^+$ cells that recognize self-peptides in the context of class II MHC and class I MHC, respectively. In contrast, activation of peripheral tolerance as distinct from central tolerance occurs when T-cells that have reached maturity recognize self-antigens. This event results in T-cell inactivation (aka anergy) or even cell death when these T-cells are acted on by Treg cells. In that regard, it is obvious that in order to prevent T-cell responses to self-antigens that are not thymus-related, the activity of Treg cells becomes crucial for allaying autoimmune responses especially when central tolerance is compromised. Furthermore, it can now be shown that loss of Treg function(s) is a significant contributor to autoimmunity as was shown to be the case in rheumatoid arthritis (RA) [3-7] as well as in other autoimmune disorders, such as inflammatory bowel disease (IBD) [8] and psoriasis [9]. For example, in inflammatory arthritis, IBD, multiple sclerosis, Type 1 diabetes, etc. effector T-helper cells classified as regulatory T cells consists of 2 types, mainly $FoxP3^+$-expressing- and Type 1 Regulatory (TR1) cells. TR1 cells are identified by 2 molecular markers, namely, lymphocyte activation gene 3 and CD49b, and also express interleukin-10 [10]. Both regulatory cell types behave as termination of immune response cells [8]. Importantly, the TR1-type regulatory T-cells are decreased and their function impaired in autoimmune diseases.

The study of Treg cell function(s) has led us to understand their role in RA pathophysiology. This includes how various immune cell types involved in the progression of inflammatory arthritis in a validated animal model of arthritis induced by proteoglycan (PGIA) was associated with B-cell-mediated suppression of immune regulatory function (s) traced back to dysfunctional Treg cells. Thus, Hamel et al. [11] showed that the interaction between B-cells and Treg cells in PGIA was essential for enhancing effector T cell activity together with suppression of Treg cell function, although B-cell depletion also inhibited autoreactive T cell responses.

In the past few years, we have advanced our understanding of how dysfunctional Treg cells influence the pathogenesis and progression of RA [12,13]. For example, dysregulation of microRNA expression results in impaired Treg cell function. This finding was implicated in RA as well as IBD, psoriasis and diabetes [14]. Another facet of Treg cell dysfunction in inflammatory arthritis is based on the results reported by Hashimoto [15] who implied that in animal models of RA, Th17 cells “coordinate and promote joint destruction” that cannot be regulated by Treg cells in the setting of an inflammatory joint milieu. Importantly, neutrophils, macrophages, synovial fibroblasts and osteoclasts whose destructive activities are boosted by pathogenic Th17 cells cannot be efficiently controlled by Treg cells in these animal models of RA.

IL-23 which is also elevated in RA was shown to play a key role in the commitment of Th17 cells to undergo differentiation [16]. Th17 cells also synthesize retinoic acid-related orphan receptor (ROR)-γt, signal transducer and activator of transcription-3 (STAT3) and interferon related-factor-4 [17]. IL-17 secretion was also shown to be enhanced by the T-cell subset, Th9 via IL-9 [18]. These findings are important to understand the changing scope of altered immune function(s) in RA also called "immune cell plasticity." Thus, there was a report in early-onset RA patients showing that the reduction in circulating Th17 cells may result from a shift in the number of Th17 cells to Th1 or Treg cells [19]. This finding suggested that to some extent, the number of Treg cells ranging from early-onset to established RA was stable or even increased and their function(s) may even be retained eliciting some level of immune suppressive activity [20,21].

The elevated levels of tumor necrosis factor-α (TNF-α) reported in autoimmune inflammatory arthritis has also been implicated in Treg cell dysregulation [22]. Thus, TNF-α stimulates the destruction of RA synovial joints mainly through the capacity of TNF-α to interact with both membrane-receptors, TNFR1 and TNFR2 [15], each of which can promote cell death or cell proliferation [23]. Of note, effector T cells, including Treg cells become less responsive after exposure to chronically elevated levels of TNF-α, although they do retain some level of suppressive activity [21]. Of note, Jung et al. [24] have recently shown that Treg cells acquire the ability to produce TNF-α under inflammatory conditions, most prominently when the host is infected with acute viral hepatitis. This observation stresses the point that under certain conditions Treg cells are also capable of exhibiting Th17-like activity further implicating these Treg cells directly in the progression of autoimmune arthritis and systemic autoimmunity [25].

The mechanism(s) governing the function(s) of Treg cells in inflammatory arthritis may also be further altered by the action of newly discovered pro-inflammatory cytokines, such as IL-38 [26]. Thus, IL-38 was shown to use several receptors for engaging cells, including, IL-36R, interleukin-1 receptor accessory protein-like-1 (IL-1RAPL1) and interleukin-1R1. Using these cytokine receptors blocks access to cells via other pro-inflammatory cytokines [26]. In fact, the elevated production of IL-38 in RA and, other autoimmune disorders, was shown to positively affect Treg cells (as well as Th1s, Th17s) via the IL-38/IL-36R and IL-38/IL-1RAPL1 axis. This finding indicated a potentially therapeutic use for IL-38 in these disorders. The same result can be also implied from the activity of transforming growth factor-β (TGF-β) because of its inhibitory effects on cytotoxic T-cells, and Th2 cell differentiation [27]. TGF-β also promotes the generation of Treg cells as well as other cells of the T helper cell lineage that lead to their residence in peripheral tissues.

The critical role that dysfunctional Treg cells play in RA pathogenesis and progression of disease led to studies that examined whether drugs employed in the medical therapy of RA restored Treg cell function (s) [13,28,29]. The drugs tested in this series, included, methotrexate, abatacept, tocilizumab, and adalimumab. These drugs had previously been shown to be clinically effective in RA patients, although a percentage of RA patients have an inadequate clinical response to them [30].

The association between pro-inflammatory cytokines and T-cell co-stimulatory molecules such as CTLA-4 as it pertains to Treg cell function is exemplified by the effect of the anti-IL-6R antibody, tocilizumab and the anti-CTLA-4 antibody, abatacept [31-33]. Thus, tocilizumab was shown to induce Treg cells as well as inhibiting Th17 and/or the differentiation of Th1 cells [31] whereas the anti-CTLA-4 antibody, abatacept increased the Treg cell to effector T-cell ratio [32]. In addition, IL-6 was identified as one of the cytokines, along with IL-17, and other molecules, including, TGF-β1, STAT3, RORγt and interferon regulatory factor-4 in the differentiation of Tc17 cells expressing CD161, a subset of $CD8^+$ T-cells [34,35]. Importantly, Khan and Ghazanfar [36] pointed out that failure to regulate several checkpoints governing T-cell expansion could result in the expansion of self-reactive T-cells that is a prominent feature of autoimmune diseases such as RA. Moreover, several of the therapeutic targets, as indicated, above involve receptor-mediated inhibition of individual cytokines, namely, IL-6 and IL-17.

However, newer drugs, exemplified by tregalizumab have been developed to specifically target and restore Treg cell function. Thus, tregalizumab was shown to selectively induce Treg cell activation [37,38]. However, Treg cell activation in response to tregalizumab occurred without altering typical Treg cell activation biomarkers, namely, CD25, CD39, cytotoxic T-lymphocyte-associated protein-4, glycoprotein-A repetitions predominant (Lrrc32), lymphocyte-activation gene 3, or HLA-DR.

Of note, a clinical trial employing tregalizumab in RA patients who showed an inadequate clinical response to methotrexate concluded that the drug failed to demonstrate clinical efficacy although the study did confirm its putative effect by modulating CD4 (presumably Treg cell) function [39]. Importantly, it has also been reported the extent to which high levels of thioredoxin-1 in RA patients might have blunted the response to tregalizumab by reducing the binding of the drug to its intended CD4 target [40]. This drug interaction may be responsible for the lack of published clinical trials data with tregalizumab since 2018 [41].

It is reasonable for us to conclude from the aforementioned commentary and the latest published results on the subject that Treg cells in RA, in fact, are relatively dysfunctional [42]. This assertion also suggested that molecular manipulation of Treg cells either *in vitro* and/or *ex vivo* could potentially be employed to correct Treg cell dysfunction(s) which can then be used to treat RA and other autoimmune diseases. In addition, we now know that dysfunctional Treg cells displaying the "memory phenotype" accumulate in the synovial fluid of patients with active RA [43]. Thus, synovial fluid could constitute the appropriate reservoir for capturing these dysfunctional Treg cells. Moreover, the Treg cells in RA synovial fluid were shown to actively proliferate, so one could expect to find no deficit of Treg cell numbers in RA synovial fluid [43].

Of note, the active proliferation of Treg cells in RA synovial fluid was traced to the expression of high levels of Bcl-2 and miR-21 when compared to other T cell populations or to Treg cells in the peripheral circulation [44]. This novel finding is critical in order to develop methods to correct Treg cell dysfunction recovered from RA synovial fluid. In that regard, Dong et al. [45] showed that miR-21 was a novel regulator of Treg cells in RA and that expression of miR-21 defined the level of imbalance between Th17 cells and Treg cells, towards Th17 cells.

So, where do Treg cell correction studies stand in 2019? Earlier results reported by Beavis et al. [46] showed that *ex vivo* forced (i.e. ectopic) FoxP3 expression in activated T cells captured from RA synovial fluid upregulated the key molecules associated with Treg cell function and diminished their responsiveness to cytokines that were also associated with inhibition of NF-κB activity. A few years later, Nie et al. [47] proved that phosphorylation of FoxP3 was the key step controlling Treg cell function(s) and that phosphorylation of FoxP3 was inhibited by TNF-α. This result solidified the connection between the elevated levels of TNF-α in RA synovial fluid and it's correlation with diminished Treg cell functions, the increased number of pathologic Th17 cells, and interferon-γ-expressing $CD4^+$ cells. Thus, regulating the increased level of TNF-α in RA synovial fluid via TNF-blockade would be expected to restore Treg function(s) in RA and should be associated with overcoming the negative effect of TNF-α on dephosphorylation of FoxP3 by the protein phosphatase, PP1. More recently, the combination of IL-2 and calcineurin inhibitors (e.g. tacrolimus; cyclosporine) was shown to modulate Treg cell function(s) while also regulating the negative effects of cytopathic T cells [48]. In that regard, it might be feasible to induce those changes in Treg cells *ex vivo* prior to returning them to the patient with the goal of overcoming the lack of immunosuppression by dysfunctional Treg cells isolated from those patients. Most recently, Goldberg et al. [49] showed that Treg cells isolated from patients diagnosed with Crohn's disease, which like RA, exhibit an imbalance of effector and Treg cells that express a lower level of expression of integrin α4β7 than Treg cells from a control group. In this study [49], *ex vivo* expansion of Treg cells with the mechanistic target of rapamycin (mTOR) molecule rapamycin together with retinoic acid receptor-α induced higher expression of integrin α4β7 that improved the suppressive effect of these Treg cells not only in *in vitro* but also in murine intestinal xenografts *in vivo*. Similar strategies are likely to be employed for future studies of Treg cells isolated from RA patients.

Conclusions and Future Perspective

Recent advances provide the impetus for treating RA and other autoimmune diseases, such as IBD, psoriatic arthritis and others by restoring the major function(s) of Treg cells through *in vitro* and *ex vivo* manipulation followed by returning the functionally corrected Treg cells to the patient. Thus, it appears to be only a matter of time before such a novel therapy will be affirmed, first in well-validated animal models of inflammatory arthritis and other autoimmune diseases and, afterward having achieved positive effectiveness in clinical trials moving forward.

Conflict of Interest Statement

The author declares no conflict of interest.

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