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Immune regulation in rheumatoid arthritis, an insight into Treg cell heterogeneity, instability and plasticity

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Abstract

Rheumatoid arthritis (RA) is a chronic inflammatory autoimmune illness resulting from a fall in immunological self-tolerance, leading to irregular immune responses to autoantigens. Regulatory $CD4^+$ T-cells (Tregs) is considered one of the key mechanisms of self-tolerance and are a major concern for study in RA and other autoimmune illnesses. Understanding the complex mechanism of action of Tregs is important to produce a new and improved treatments to restore self-tolerance, and disease recovery. This review illustrates recent findings in the area of Tregs in RA, with specific attention to Treg cell heterogeneity, instability and plasticity.

Keywords: *Rheumatoid Arthritis, Treg cell, Heterogeneity, Instability, Plasticity, Th17 and FOXP3*

I. Introduction

Rheumatoid arthritis (RA) is a chronic inflammatory disorder resulting in joint damage unless efficient therapy intake. Widespread research has provided information to confirm the hypotheses that RA is an antigen-driven autoimmune disease [1]. In RA, it is known that an imbalance between pro- and anti-inflammatory cytokine actions supports the autoimmunity initiation, chronic inflammation and that way joint damage [2]. T cells obviously show a vital role in the initiation and maintenance of chronic inflammation established in RA. Two other subpopulations were subsequently identified in addition to the Th1 / Th2 cell populations: regulatory T cells (Treg), producing IL-10 and transforming growth factor (TGF)-β[3], and CD4 + Tcells (TH17) producing IL-17[4]. During tolerance and inflammatory responses these two subsets play a key role[5,6]. In recent years the role of TH17 cells in the development of autoimmune diseases has been largely established [6]. Such cell subsets can participate in the complex network of cell-cell interactions that control the development and chronicity of rheumatoid synovitis at various stages of RA disease, at different sites and with different intensities. Cells of T helper type 17 (TH17) that secrete interleukin (IL17) in humans[7], IL17 is a pleiotropic cytokine which induces

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the expression of proinflammatory cytokines and matrix metalloproteases[8], thus contributing to inflammation and destruction of tissues. The pathogenic effect of IL17[9] has been demonstrated in several experimental models of arthritis. Regulatory T (Treg) cells, on the other hand, are T cell subsets, whose special function is to inhibit autoreactive lymphocytes. We mediate immune tolerance, and seek to preserve homeostasis of the lymphocytes. The two separate actions of the T-cell subpopulations as well as their reciprocal plasticity illustrated the importance of TH17 / Treg cell disrupted equilibrium in RA pathogenesis. Consequently, a novel theory regarding the pathogenic mechanism of RA has been suggested that the TH17 / Treg imbalance, along with the TH1 / TH2 imbalance, could be responsible for the production and progression of RA [10-12]. In addition, the impact of existing RA therapeutic approaches have been studied on these lymphocyte subpopulations. A strong interest in the possible therapeutic targeting of these cells and their products has been developed to address the limitation of biological therapies currently being employed [13]. Blocking essential cytokines can result in a change from TH17 / TH1 phenotypes to Treg / TH2 in this polarization [14]. However, the findings are controversial [15]. In the meantime, differences in the response of patients to available anti-RA therapies corroborate the fact that RA is a heterogeneous and complex disease due to specific immune pathways. TH17 cell frequencies have been shown to increase in the peripheral mononuclear blood cells (PBMCs) of patients with RA relative to healthy controls [16]. There was an increased expression of IL-17 in rheumatoid synovium and synovial fluids in patients with early RA[17]. No studies were performed to determine the function of Treg cells in RA patients' blood. Conflicting findings regarding the proportion of Treg cells in RA peripheral blood have been published. The majority of studies highlight the existence of increased numbers of Treg cells in RA synovium [18, 19]. Treg cells isolated from RA patients' synovial fluid tend to be functionally inactive as regards their ability to suppress the development of pro-inflammatory cytokine [20]. IL-23 supports the growth and survival of TH17 cells. IL 23 promotes TH17 necrosis factor-α (TNF-α), IL-17, IL-6, IL-22 and GM-CSF cells that are associated with autoimmune inflammation induction [21].

Tregs are subdivided into many populations. Naturally occurring Tregs, expressing the forkhead transcription factor (FoxP3), are located in the thymus and in the peripheral blood where 5–10 percent of CD4 + T cells are present. FoxP3 is responsible for Tregs forming in the thymus. The inhibiting action of mature peripheral Tregs is also required and is a particular molecular marker for Tregs in the human peripheral blood. Decreased expression of FoxP3 allows the Tregs to be transformed into effector cells[22]. Therefore, the expression of FoxP3 is critical in recognizing Tregs carrying inhibitory activity [23]. TGF-b performs a complex and interconnected role in inflammation, involvement in cell lineage T, production of antibodies, immune suppression and tolerance. In the immune system, TGF-b can inhibit the production of Th17 effector cells and induce FoxP3 + Tregs, thereby preserving self-tolerance. TGF-b tends to be the mediator of Treg 's active immune suppression and cell differentiation regulation [24].

Three findings have altered the perception of what Treg cells are, how they act in peripheral lymphoid organs and non-immune tissues, how they respond to other immune and non-immune cells, and how their structure and role can be modulated, with strong implications for the creation of new therapeutic approaches for various autoimmune diseases. Those remarks are: 1) Instability of Treg cells and their acquisition of an effector phenotype after loss of Foxp3 expression under inflammatory conditions; 2) plasticity of the Treg cell phenotype with the acquisition of effector-like properties while preserving the Foxp3 expression; 3) Foxp3+ Treg heterogeneity. These

findings emerged at a time when the advancement of high-throughput genomic, epigenetic and proteomic technologies allows it possible to study uncommon cell populations at a single cell level, this would certainly enhance the visibility of basic Treg cell biology, human Treg cell biology and RA therapy in particular.

T_{reg} cell instability

Both Foxp3's expression and its stability have important roles in preserving Treg cell function [25]. Therefore, in mature Tregcells, the conditional removal of a Foxp3 allele results in effector T cells capable of causing inflammatory tissue lesions [22]. While the instability of Foxp3 in iTreg cells has been widely observed and is intrinsic to their developmental origin[25], tTreg cells have been investigated to establish how the instability of Foxp3 expression in particular tissue under basal or inflammatory conditions affects the production and recovery of autoimmunity[26,27,28]. Loss of expression of Foxp3 by tTreg cells was observed in vitro[29], in the adoption of cells to lymphopenic hosts[30], in infectious settings[31] and in graft-versus-host disease[32]. A fate-mapping mouse model in which a yellow fluorescent protein reporter marks all cells which were produced in both homeostatic conditions and autoimmune inflammatory conditions at any time expressing Foxp3. A small proportion of relatively healthy Treg cells lost the expression of Foxp3 in this model and acquired an effector-memory phenotype with varying rates of secretion of the pro-inflammatory cytokines IFN-γ and IL-17[28]. Based on their level of TSDR demethylation, these 'ex-Foxp3 cells' were able to elicit autoimmunity in an adoptive transfer model on the NOD context and composed of a combined population which would indicate that not all ex-Foxp3 cells in this sample were once de facto tTreg cells. Subsequent data indicated that this fate-mapping mouse model identified a proportion of Foxp3 + tTreg cells that either upregulated Foxp3 transiently, or were not completely committed to the tTreg cell lineage[33]. More evidence in support of the tTreg cell instability hypothesis showed that the expression of Foxp3 is lost in tTreg cells unique to an epitope of myelin oligodendrocyte glycoprotein (MOG; amino acids 38–49) Throughout the context of the development of experimental autoimmune encephalitis (EAE), with an increase in frequency of ex-Foxp3 cells in the central nervous system at preclinical and peak EAE stages declining throughout EAE resolution. These ex-Foxp3 Treg cells express IFN-γ and are capable of transmitting EAE [34]. If the decrease in ex-Foxp3 cells during disease recovery is due to the re-acquisition of the Foxp3 expression by ex-Foxp3 cells remains to be determined.

Another genetic fate-mapping mouse model has shown that, under homeostatic conditions, the majority of mature tTreg cells in spleen and lymph nodes are fairly stable[27]. This model , based on inducible labeling of Foxp3+cells after tamoxifen treatment, labels all those cells that expressed Foxp3 at the time of tamoxifen application, and, contrary to continuous labeling models[28], prohibits the identification of cells that express Foxp3 transiently. Even though the expression of Foxp3 is stable under homeostatic conditions, depriving growth factor cells or blocking the IL-2 receptor, which induces autoimmunity [35], The consequence is a large reduction in Foxp3 expression per cell in mature tTreg cells, and a small population loses Foxp3 expression altogether. We do not, however, generate pro-inflammatory cytokines [27], indicating some degree of instability under particular environmental settings. This apparent difference may arise from the different fate-mapping mouse models used and the form of Foxp3 cell labeling that could lead to the labeling of uncommitted Treg cells [28] or the lack of ex-Foxp3 cell labelling that occurred before tamoxifen administration [27]. The discordance in outcomes may also rely on the inflammatory stimuli used to test the stability of Foxp3. Nonetheless, there is a limited population of tTreg cells losing Foxp3 expression in either fate mapping models, and those Treg cells that remain 'stable' show

diminished single-cell Foxp3 expression[27]. As decreased levels of Foxp3 have been observed in Treg cells isolated from inflammatory sites in mouse models of autoimmunity[25,36] and in patients with autoimmune diseases[37,38,39], further research with these models is needed to establish the mechanisms and implications of long-term decreases in Treg cell expression Foxp3. More results supported the observation that most mature tTreg cells are stable in a recent fate-mapping mouse model in which Foxp3 lacks CNS1, but these cells become unstable when stimulated in vitro and in vivo in an EAE model, losing Foxp3 expression and gaining TH1 cell and TFH cell-like characteristics[33]. Although epigenetic changes such as CNS2 region re-methylation may account for the loss of Foxp3 expression in those settings[28,33], The molecular mechanisms responsible for the reduction of Foxp3 protein and the possible contribution of post-translational Foxp3 protein modifications to the development of ex-Foxp3 cells remain to be explored.

Ultimately, removing different partners from Foxp3 can also precipitate the presence of ex-Foxp3 cells. Treg cell – specific removal of the chaperone GP96 on the NOD basis , for example, leads to fatal autoimmunity due to deficient suppressive abilities of Treg cells in diabetes and colitis models. In this system, Treg cells are slowly losing Foxp3 expression and gaining IFN- γ secretion, while retaining their unique pattern of TSDR-demethylation [40]. Mice with Treg cell – severe deletion of the transcription factor Helios experience systemic autoimmune pathology characterized by increased germ-center development, lymphocytic infiltration into non-lymphoid organs and glomerulonephritis [41]. While Helios does not form protein complexes with Foxp3 or bind to the Foxp3 locus [41], helios-deficient Tregcells have increased IFN-α and IL-17 expression and are unstable, with decreased Foxp3 expression and a propensity to lose Foxp3 expression altogether [41,42]. Treg cells deficient in another transcription factor, Eos, exhibit increased IL-2 and IFN-ϒ expression along with reduced suppressive efficiency, although forced overexpression of Eos in Treg cells prevents Treg cell instability, even in inflammatory environments. EosloFoxp3 + tTreg cells are in vivo observable and have regulatory function, with the ability to acquire T cell-like effector characteristics while preserving Foxp3 expression; however, these cells display significant changes in their global DNA-methylation pattern [43].

T_{reg} cell plasticity

Plasticity is a feature inherent in most, if not all, immune cells, allowing them to modify their morphology and function to the evolving environment and to extracellular 'risk' signals. So it's no surprise that Treg cells have a degree of plasticity. Treg cells have the ability to obtain different features for the type of immune response they control, guided mainly by 'master' transcription factors, and controlled by environmental signals. Treg cells thus gain expression of the transcription factor T-bet to suppress inflammation of type 1 throughout infection [44,45], And they use the transcription factors IRF4 and STAT3 to inhibit the response of TH2 cells[46] and TH17 cells[47], respectively. Although this plasticity modality tends to be favorable to the host and beneficial to the outcome of the immune response, aberrant plasticity of Treg cells is also observed in many autoimmune disorders, with Treg cells expressing pro-inflammatory cytokines, developing T cell-like phenotypes and displaying reduced activity in most cases, but remaining Foxp3 expression [42,48,49,50]. Paradoxically, these T-cell helpers — like Treg cells — use the same transcription factors that Treg cells use to suppress different types of immune response. Thus, the secretion of IFN- γ by TH1-like Treg cells demands T-bet expression [49,50], while IL-6-driven TH17-like Treg cells need STAT3 to secrete IL-17 [32], and IL-4-driven TH2-like Treg cells upregulate IRF4 and Gata-3 [51,52]. In most cases, helper T cells — like Treg cells — have a demethylated TSDR in the Foxp3 locus even

though they share effector features, indicating that their phenotype could be reversible[48]. They show changes in their epigenetic signature characteristic of those of Treg cells [53]; That may be the fundamental mechanism allowing pro-inflammatory cytokine secretion. Current studies are focused on understanding the signaling mechanisms that cause this plasticity in different autoimmune diseases, harnessing this versatility to treat human disease and the role of Treg cell plasticity in tissues associated with autoimmune disease [50].

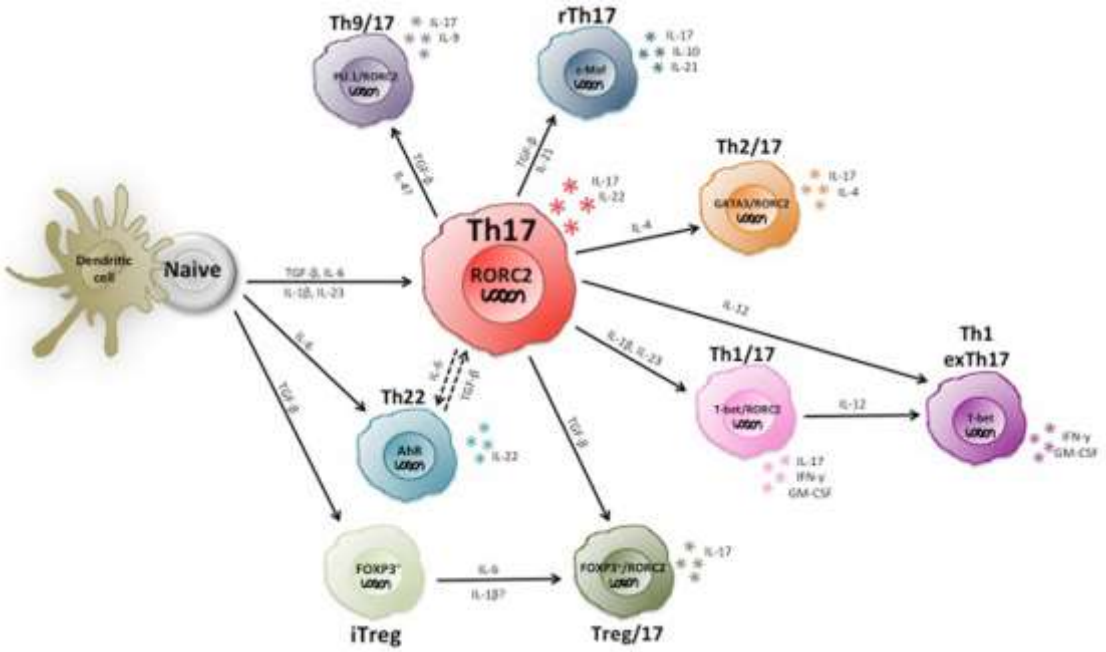

Figure (1): Heterogeneity and plasticity of human Th17 cells.

Th17 cells are extremely heterogeneous and, in addition to IL-17, contain various forms of other cytokines including the Th1 and Th2 marker cytokines IFN-ÿ and IL-4. Some IL-17 cells which produce T express Foxp3 and/or IL-10 and are suppressive. Moreover, after chronic inflammation, Th17 cells are partially unstable, and can become Th1 cells.

T_H17-like T_{reg} cells

A limited percentage of human peripheral Treg cells generate IL-17 in healthy individuals and upregulate the gene encoding the ex vivo transcription factor ROR γ t (RORC) (TH17-like Treg cells) while maintaining their inhibitory capacity [54]. It remains to be elucidated, given the well-established developmental relationship between Treg cells and TH17 cells, whether TH17-like Treg cells are a transitory stage in the de-differentiation of tTreg cells into TH17 cells, as suggested [55]. The conversion of Foxp3 + Treg cells into TH17 cells plays a key role in the pathogenesis of autoimmune arthritis in a collagen-induced mouse model of arthritis in support of that proposition. This conversion is powered by IL-1β[56] and IL-6, and Foxp3+IL-17 + Treg cells are present in the synovium of active rheumatoid arthritis subjects[57]. In addition, the transition of Treg cells to TH17 cells was also observed in the psoriasis model CD18hypo PL / J mouse [56], and TH17-like Treg cells were confirmed in psoriatic patient skin tissue [58].

The gastrointestinal tract is perhaps the tissue in which TH17-like Treg cells were best observed. In iTreg cells with high expression of the TH17 gene, the lamina propria tends to be enriched – defining the transcription factor ROR γ t. These TH17-like Treg cells tend to have a significant function, because their absence aggravates pathogenesis in many models of mucosal autoimmunity [59]. Studies have also proposed that glomerulonephritis is regulated by Foxp3+RORγt+ Treg cells [60], And the absence of TH17-like Treg cells results in higher mortality and systemic lupus erythematous organ pathology [61]. In addition to the role of IL-1β and IL-6 in influencing the synthesis of IL-17 by Treg cells[62], other environmental conditions have been shown to adjust the transition of TH17-like Treg cells either indirectly or directly, such as indoleamine 2,3-dioxygenase[63], ligation of the pattern-recognition receptor TLR2[64] and other infections[65].

Functional heterogeneity in human FoxP3+T Reg cells

Given FoxP3 maintaining an efficient immunosuppressive phenotype, the human FoxP3 + Treg cells have substantial functional heterogeneity. A single-cell strategy has been developed to investigate the phenotypical and functional heterogeneity of human CD4+CD25highCD127low Treg cells compared to the expression of FoxP3 from the blood of healthy people WAS[66]. While highly enriched in inhibitory FoxP3 + T cells, CD4+CD25high / bright Treg cells harbor a pool of bona fide FoxP3 + Treg cells with impaired suppressive function, while retaining the hallmark phenotypic, epigenetic and transcriptional features of Treg cells. These FoxP3 + Treg cells also develop proinflammation cytokines with compromised suppressive function such as IL-2, IL-17 and IFN-γ following polyclonal activation [66]. It remains to be decided if this heterogeneity refers to FoxP3 + Treg cell subsets which have acquired distinct effector functions, or if Treg cells lose their phenotype. These non-suppressive CD25highFoxP3 + cells in normal peripheral blood may be engaged in the creation of autoimmune or inflammatory conditions, and further study is needed to better understand their health and disease functions.

Identification of Helios, a transcription factor for the Ikaros family, to be expressed preferentially on suppressive Treg cells as opposed to non-suppressive Treg cells in human blood during immune quiescence and disease[67]. Although Helios was originally suggested as a marker for identifying among both tTreg and iTreg cells, no evidence supporting this definition exists in humans. Co-expression of the surface receptors T cell immunoreceptor with immunoglobulin (Ig) and ITIM domains (TIGIT) and Fc receptor-like 3 (FcRL3) detected most peripheral Helios+ Treg cells and were unavailable from Teff cells at a steady state and after TCR stimulation[67]. Treg cell heterogeneity also lies in TCR specificities which drive their ontogenic growth, peripheral homeostasis, and effector functions. As we all know, the cells of tTreg and pTreg vary in their ontogenies. TTreg cells therefore have a broad, self-restricted TCR range that is distinguished from Tconv cells, whereas pTreg cells preserve the antigen specificities of their naïve Tconv precursors. In-vitro data proves that the TTreg cell suppressive role is TCR-dependent on activation, and while non-specific antigen inhibition has been defined, antigen-specific signals are largely considered important for optimal peripheral tTreg cell suppressive functions [68]. Expressing elevated amounts of the prosurvival molecule Bcl-2 and depending on peripheral IL-7 signaling, naïve tTreg cells are rapidly proliferative and readily distinguish by their cognate self-antigen into powerful suppressive memory tTreg cells upon TCR involvement [70]. Also involved in taking a role in autoimmunity and cancer were antigen-specific Treg cells. TCR-sequencing of synovial CD14−CD4+CD25highCD127− Treg cells in rheumatoid arthritis (RA) yielded some extended clonotypes[71].

Such Treg cells were enriched in suppressive and activated (HLA-DR+) Treg cell subsets, thus illustrating functional significance in RA for the specificity of Treg cells in antigen.

II. Summary

Regulatory cells T (Treg) form an essential component of peripheral tolerance. Because of their strong immunosuppressive roles supervised by the lineage-defining transcription factor (FoxP3), these cells' clinical modulation of autoimmunity is a promising therapeutic objective. Recent evidence suggests that Treg cells represent a heterogeneic population, both phenotypically and functionally. In addition there are both suppressive and non-suppressive Treg cells in human blood that are otherwise distinct from each other using classic Treg cell markers such as CD25 and FoxP3. In addition, Treg cells show a range of plasticity by which they obtain the required trafficking paths to home tissues containing T (Teff) cells. However, this plasticity can also contribute to instability of the Treg cell lineage and to the acquisition of proinflammatory Teff cell functions. Accordingly, these dysfunctional CD4+FoxP3 + T cells that fail to keep peripheral tolerance and help immunopathology instead. SO the stability of the FoxP3 + Treg cell lineage phenotype and functional heterogeneity of human Treg cells, and how abolition of these mechanisms can give rise to lineage instability and Treg cell dysfunction in rheumatoid arthritis.

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