Duality and Plasticity of Th17 Cells in Behçet's Disease

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ABSTRACT

T helper type 17 (Th17) cells are active players in the establishment of inflammation in Behçet's disease (BD). How this process impacts the immune dysregulation observed in BD is far from fully understood. Plasticity within this subset is suggested by the existence of IL-17 secreting cells, which express either forkhead box p3 (Foxp3) or retinoic acid receptor-related orphan nuclear receptor (RORγt), signature transcription factors of Treg and Th17 respectively. Much more is unknown at the basic level, as IL-17 producing cells do not always contribute to inflammation, and may even acquire regulatory features depending upon environment modifications. This review aims to highlight Th17 plasticity and to discuss the role of Th17/IL-17-axis in BD inflammatory pathway.

Keywords: Behçet's disease; Th17; Inflammation; RORγt; Duality; Plasticity; BTLA

INTRODUCTION

CD4+ T cells' ability to exert their effector functions depends on the provision from antigen-presenting cells (dendritic cells: DC) of the immunological cues that prompt formation of Th subsets: Th1, Th2, Th9, Th17, Th22, and FoxP3+ regulatory T (Treg) cells (Figure 1) [1-3]. Th17 subset is so named due to its ability to secrete interleukin (IL)-17A, which has emerged as a major player in tissue-specific immune pathology, interacting with Th1 and Treg lymphocytes. The initial emphasis on the detrimental cytotoxic effects of Th17 is reflected by the plethora of early literature supporting such a role in both human and murine studies [2-4].

Th17 cells and their associated cytokines have been found to interact closely with other adaptive immune cells, raising interesting questions about how to select and design therapeutic strategies targeting this cell population [5]. New technologies such as transcriptome profiling, global epigenetic mapping and computerized simulation analysis [6-7] have captured an accurate picture of this T cell subset, revealing it to be more transient, complex and perhaps more reversible than previously imagined. Human Foxp3+ Treg cells can differentiate into IL-17 promoting cells *in vitro* [8]. Flexibility within this subset may allow Th17 cells to embrace either pro-inflammatory or protective roles in inflammatory and auto-inflammatory diseases by secreting a wide spectrum of cytokines (Figure 1).

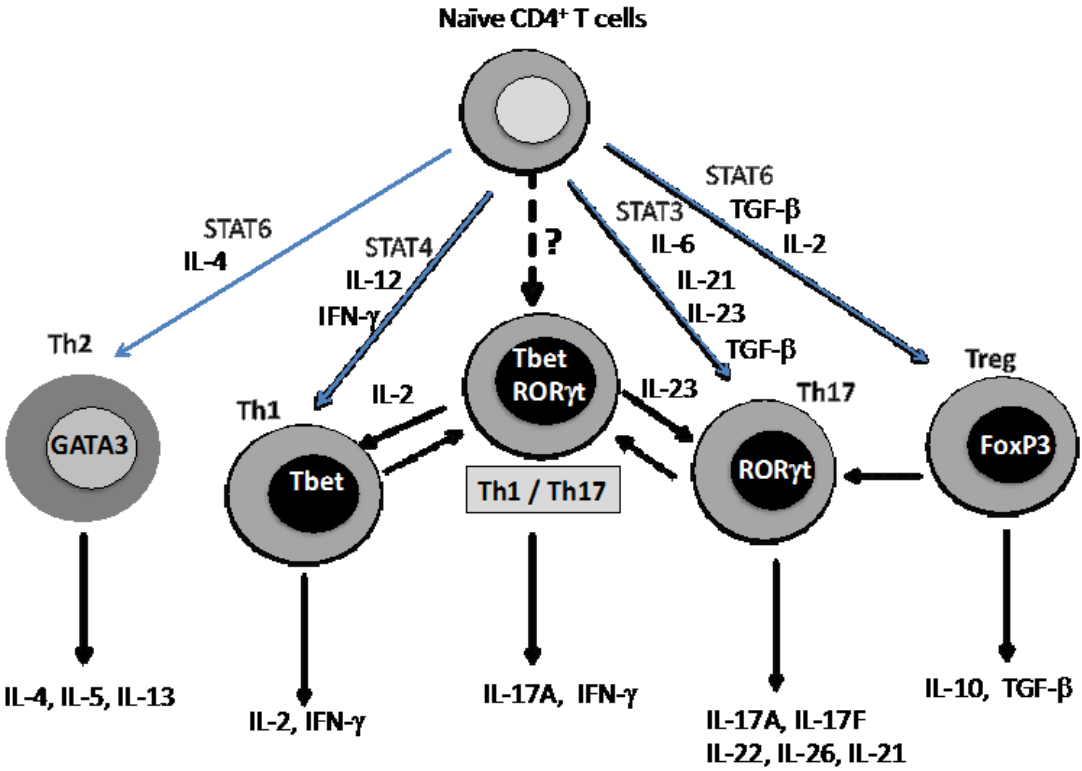

Figure 1: Cytokines and transcription factors required for helper (Th) differentiation.

In the presence of interleukin-6 (IL-6), IL-21, and transforming growth factor-beta (TGF-β), naïve CD4+ T cells differentiate into a Th17 cell phenotype, which is characterized by the expression of transcription factors retinoic acid receptor-related orphan receptor-γt (RORγt) and signal transducer and activator of transcription 3 (STAT3). IL-1β and IL-23 cytokines can promote and stabilize this phenotype during cell expansion. Once programmed, these cells secrete IL-17A, IL-17F, IL-21, and IL-22, which play a key role in enhancing autoimmunity and host defence. The differentiation of Th17 cells is promoted by activation of STAT3 and inhibited by activation of STAT1. Cytokines IL-12, IL-4, and TGF-β and transcription factors T-bet, GATA3, and FoxP3 have been shown to regulate Th1, Th2, and Treg cell development, respectively. These distinct subsets regulate immune response. (R): cytokine receptor.

In BD, an imbalance between Th17 cells and Treg cells may be generated quickly in order to establish immune homeostasis [9-11]. This ability to transition between functional states is defined as T cell plasticity [12]. Differentiated CD4+ T cell subpopulations display a high grade of plasticity. Their initial differentiation is not an endpoint of T cell development that allows a functional adaptation to various physiological situations during immune response. Plasticity of effector helper T cells offers the opportunity, for clinical immunologists, to interfere with the natural course of immune-mediated diseases. This could be possible either by blocking

environmental signals that drive the transition of T helper cells towards more aggressive phenotypes, or by promoting the differentiation towards less pathogenic phenotypes [13].

This review outlines the major features of Th17 plasticity including the Treg/Th17 paradigm and discusses this duality in Behçet's disease focusing on the maintenance of homeostasis.

TH17 LINEAGE: IL-17 AND IL-17 PRODUCING CELLS

Th17 cells are characterized by secretion of IL-17A, expression of chemokine receptor CCR6 and transcriptional factor RORγt [14]. Th17 pathogenicity is limited by Foxp3+ Treg and T regulatory type 1 (TR1) cells [14-15]. Treg cells are characterized by the transcription factor Foxp3, whereas TR1 cells secrete high levels of the anti-inflammatory IL-10 and express cell-surface markers CD49b [15-17]. Although Th17, Foxp3+ Treg and TR1 cells are functionally distinct subsets, they share several features. Their differentiation is promoted by transforming growth factor β (TGF-β) [18] and both Th17 and TR1 cells express CD49b and high levels of the transcription factor aryl hydrocarbon receptor 9 (AhR9). Moreover Th17 cells can transiently co-express RORγt with Foxp3 [19-20] and IL-17A with IL-10 [21-23] (Figure 2).

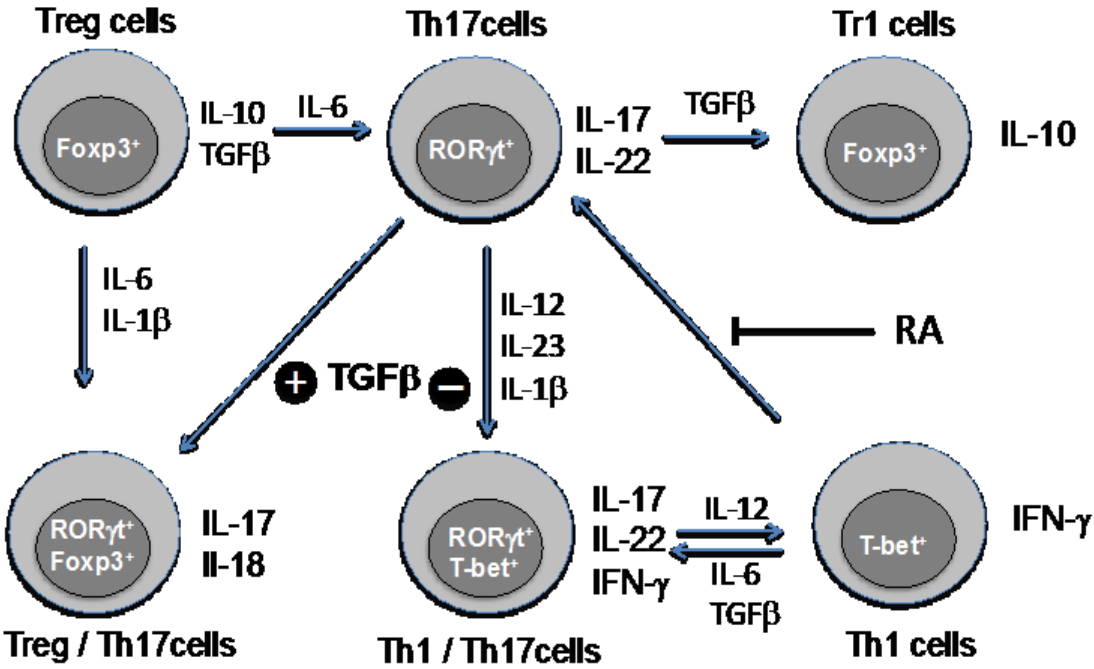

Figure 2: Duality of Th17 cells.

Th17 cells lose stability in the absence of TGFβ and presence of IL-12, IL-23, and IL-1β, favoring IFNγ expression and differentiation into Th1/Th17 cells that produce both Th1 (producing IFNγ) and Th17 (producing IL-17and IL-22) cytokines. Further augmentation of IL-12 can fully convert Th1/Th17 cells into Th1 cells, whereas this process can be reverted by either TGFβ and IL-6 or in the absence of retinoic acid (RA) in favor of Th1/Th17 or Th17 cells, respectively. Alternatively, the abundance of TGFβ in the absence of IL-6 drives Th17 cells toward regulatory phenotypes, such as either RORγt+Foxp3+ Treg/Th17 cells or Foxp3- Tr1 cells. If proinflammatory cytokines are present, including either IL-6 or IL-1β and IL-6, Foxp3+ Treg have the ability to transdifferentiate into either Th17 or Treg/Th17 cells, respectively.

TRANSDIFFERENTIATION OF TH17 CELLS

Inflammation is a beneficial host response to infection but can contribute to inflammatory disease if unregulated. Th17 cells have been involved in several human inflammatory diseases [24-25]. These cells exhibit both instability and plasticity upon *in vitro* re-stimulation. However, technical limitations have prevented the transcriptional profiling of pre- and post-conversion Th17 cells ex vivo during immune responses. Thus, it is unknown whether Th17 cell plasticity merely reflects change in cytokines expression, or if Th17 cells physiologically undergo global genetic reprogramming driving their conversion from one T helper cell type to another, a process known as transdifferentiation [4;26]. Furthermore, although Th17 cell instability/plasticity has been associated with pathogenicity [27], it is unknown whether this could represent a therapeutic

opportunity whereby formerly pathogenic Th17 cells could adopt an anti-inflammatory fate. The transdifferentiation of Th17 into Treg cells was illustrated by a change in their transcriptional profile signature and the acquisition of potent suppressive capacity. Comparisons of the transcriptional profiles of pre- and post-conversion Th17 cells also revealed a role for canonical TGF-β signalling and consequently for the AhR. Thus, Th17 cells transdifferentiate into Treg cells, and may contribute to the resolution of inflammation.

REGULATORY TH17 CELLS?

The relationship between Th17 cells and Treg cells is now the subject of intense research efforts in autoimmune and inflammatory diseases. During polarization, TGF-β and IL-6 polarize naive T cells toward a Th17 phenotype, whereas TGF-β alone induces Treg cells. Thus, it seems that these two 'lineages' share an intermediate stage in their development (Figure 2). Interestingly, Th17 cells that have been matured with TGF-β and IL-6 express strongly genes encoding RORγt, IL-17A, IL-17F and IL-21, produce IL-10 and are capable of regulatory functions. The discovery of human IL-17-producing RORγt+ Foxp3+T cells that retain their ability to suppress effector lymphocytes further, supports this 'dual-natured' hypothesis [28-29] (Figure 2).

INCREASED IL-17 LEVEL AND TH17 CELLS IN BD

Multiple independent studies have demonstrated that the level of IL-17A is higher in patients with activeBD [30- 34] compared to healthy controls ($P < 0.0001$ and $P < 0.005$). Moreover, the frequency of circulating Th17cells, IL-17A concentration in supernatant of lymphocytes culture, and IL-17A mRNA expression in activated peripheral blood mononuclear cells (PBMCs) are significantly increased ($P < 0.0001$) [9-11].

Most studies have shown that either the percentage of Th17 cells or the concentration of serum IL-17A correlate positively with BD activity [35-41].

Polarized BD Th17 cells expressed large amounts of transcription factor RORγt. In contrast, *in vitro*-treated infliximab Th17 cells expressed less RORγt. Stimulation of PBMCs with anti-CD3 and anti-CD28 antibodies resulted in the production of higher IL-17 levels in the cell culture supernatants of PBMCs from BD patients than those obtained from controls ($P < 0.001$) [36]. Addition of recombinant human interferon-α (rhIFN-α) to this cell culture model decreased significantly IL-17 production while increasing IL-10 production ("both $P < 0.001$) [36], inducing consequently TGF-β release and Treg cells differentiation. IFN-α activity was mediated via signal transducers and activators of transcription 2 (STAT2) phosphorylation. IFN-α modulated pro- and anti-inflammatory cytokines secreted by T cells increasing IL-10/IL-6 ratio in BD. IFN-α increased IL-10 secretion in each memory (m) subset mTh1, mTh2 and mTh17 [31]. The mechanism by which IFN-α exerts its inhibitory effect on IL-17 was partially mediated by IL-10 and was associated with an up-regulation of the level of p-STAT2.

Recent data demonstrated that increased activation of the Notch pathway is associated with an increased Th17 response in active BD patients [41]. Blocking the Notch pathway can preferentially attenuate the Th17 response by modulating STAT3 phosphorylation [41]. Additionally, the authors showed that decreased expression of miR-23b might contribute to activation of the Notch pathway and the expansion of Th17 cells in BD patients. These interesting results suggested that increased activation of the Notch pathway due to decreased expression of miR-23b might contribute to the pathogenesis of BD [41]. In BD, STAT3 expression was significantly elevated [42] and JAK1/STAT3 signalling pathway was activated, possibly through the activation of Th1/Th17-type cytokines such as IL-2, IFN-γ, IL-6, IL-17 and IL-23 [42].

Increased serum levels of IL-17A were observed in active BD and might serve as markers of disease activity [36; 40]. Moreover, cerebrospinal fluid (CSF) levels of IL-17A were demonstrated to be higher in BD patients with central nervous system involvement compared to NIND (non-inflammatory neurological disease) and HaBD (headache attributed to BD) patients ($P < 0.001$; $P = 0.0021$respectively) [9]. The relative expression of transcription factors of RORγt/FOXP3 ratio (Th17/Treg cells) in CSF was increased in neuro-BD (NBD) patients. These observations of increased expression of IL-17 in blood and in inflammatory sites (CSF) support a Th17-mediated pathogenesis [9]. This suggests the possibility that IL-17A somehow drives central nervous system inflammation in BD [9].

Although Th17 cells are named after their ability to produce IL-17 and represent a main source of IL-17 production, other cells can secrete IL-17. TCRγδ T cells are an important source of IL-17 during infections [43]. Mice that lack TCRγδ T cells produce less IL-17. TCRγδ T cells are activated in BD producing high levels of cytokines [44].

Neutrophils, when stimulated with IL-15, are also able to secrete IL17. Studies have shown that certain memory CD8+ T cells, after stimulation with PMA and ionomycin, can produce IL-17 [45]. BD as systemic inflammatory disorder is characterized by recurrent episodes of acute inflammation consisting mainly of neutrophil infiltration around blood vessels in affected tissues.

Stimulated Natural killer T cells (NKT) IL-23R+ cells, when cultured with IL-23 and anti-CD3, also produce IL-17 [46]. NKT cells that are possibly down regulated by type-I interferon, were also reported in BD [47].

Taken together, these data suggest that IL-17 is not produced solely by a specific set of T cells but a more generic cytokine that is secreted by several cell populations such as TCRγδ cells, neutrophils and NKT cells in an environment of pro-inflammatory cytokines, leading in turn to propagation of the inflammatory response. In patients with BD, IL-17A promotes the inflammatory process by inducing local production of cytokines and chemokines from multiple cell types including epithelial cells [23; 47- 55]. Geri provided the first evidence of the critical role of IL-21 in driving inflammatory lesions in BD by promoting Th17 effectors and suppressing Treg cells [52].

In BD as reported in autoimmune and inflammatory diseases, Th17 cell duality/plasticity has been associated with pathogenicity and it is unknown whether this could represent a therapeutic opportunity, whereby formerly pathogenic Th17 cells could adopt an anti-inflammatory fate.

ASSOCIATIONS OF GENETIC VARIATIONS OF IL17 WITH BEHÇET'S DISEASE

Behçet's disease is generally considered to be a multifactorial disease with important genetic and environmental components. Genetic polymorphisms of inflammatory cytokines have been associated with BD susceptibility. Recent data suggested that Th17 cell-related genes could act as susceptibility genes for BD in Korean population [53;56], particularly in BD patients with uveitis. Uveitis is a sight-threatening intraocular inflammatory disease that is estimated to account for ~10% of blindness. Based on its etiology, uveitis can be classified into two categories: uveitis related to infection and uveitis that is not. Noninfectious uveitis is frequently associated with autoimmune diseases, including BD, Vogt-Koyanagi-Harada (VKH) syndrome, systemic lupus erythematosus, sarcoidosis, autoimmune hepatitis, and multiple sclerosis [57]. Hou et al. reported that IL17F gene expression was increased in male BD patients [58]. Moreover, this study showed that high copies of IL17F were positively related with the expression of IL17F and may enhance peripheral blood cells proliferation. Such findings were consistent with data shown in SLE patients [59]. IL17 secretion was elevated in uveitis BD and VKH patients [60] supporting the important role of Th17 cells in the pathogenesis of intraocular inflammation.

Considering the role of Th17 cells in experimental autoimmune uveo-retinitis [61], the results suggested that IL17F copy number variant (CNV) might be involved in uveitis via the upregulated expression of IL17F [58]. However, Shu, et al. found no association between BD disease and two single nucleotide polymorphisms (SNPs) of IL-17A and IL-17F whereas a positively association was observed with VKH syndrome [62]. Recent clinical trials of secukinumab, a fully human anti–IL-17A monoclonal antibody, demonstrated its efficacy and safety for the treatment of chronic and active noninfectious uveitis requiring corticosteroid-sparing immunosuppressive therapy [63].

Genome-wide association (GWAS) and replication studies identified in BD a susceptibility locus around STAT4 which expression could regulate IL-17 production [64]. Increased expression of STAT4 was observed in individuals carrying the rs897200 risk genotype AA together with increased IL17 messenger RNA and protein levels. Furthermore, Kim et al [65] demonstrated that interactions of particular IL17A, IL23R, and STAT4 SNPs modulate susceptibility to intestinal BD in the Korean population.

Additional studies are needed to ascertain whether the results presented here can be extrapolated to other ethnic groups in the world.

B AND T LYMPHOCYTE ATTENUATOR IN BD MAY TRIGGER ABNORMAL TH17 IMMUNE RESPONSES

B and T-lymphocyte attenuator (BTLA, also known as CD272) is a member of the B7/CD28 superfamily and was first identified as an inhibitory receptor on T cells on the basis of the enhanced T cell responses that were observed in Btla-knockout mice [66]. Its ligand herpes virus entry mediator (HVEM, also known as TNFRSR14) is a member of TNF/TNFR superfamily. BTLA is broadly expressed on B cells, T cells, DCs, macrophages, and NK cells [67].

Recent studies have investigated whether BTLA activation could be exploited to inhibit the development of abnormal immune responses in BD patients [68]. BTLA mRNA and protein expression were found significantly decreased in BD patients with active ocular inflammation compared to the normal controls ($P < 0.001$). Decreased percentages of $BTLA^{high}$ cells and $CD4+BTLA^{high}$ cells were also observed in peripheral blood of these patients ($P = 0.011$; $P = 0.010$) while IL-17-positive cells were significantly increased in the $CD4+BTLA^{lo}$ cells population ($P < 0.01$, $P < 0.001$)[68]. In BD patients and controls, the agonistic anti-BTLA antibody reduced the frequency of IL-17- and IFN-γ-producing CD4+ T cells ($P< 0.05$, $P < 0.05$) and inhibited the production IL-17 cytokine ($P < 0.05$) ($P < 0.05$). BTLA-mediated inhibition of T cell activation occurred during both primary CD4+ T cell responses and secondary CD4+ and CD8+ T cell responses, suggesting that BTLA ligation sends a constitutive ''off'' signal to T cells and thus might play an important role in the maintenance of T cell tolerance [69].

FACTORS SUPPRESSING TH17 PRODUCTION IN BD

Vitamin D (Vit D) deficiency was observed in BD patients and correlated with inflammatory status [70] while its supplementation had favourable effects on endothelial function [71]. Single nucleotide polymorphisms (SNPs) of Vit D family genes increased the susceptibility risk for BD [72-73]. Recent data showed that vitamin D was an important promoter of T cell regulation *in vivo* in BD patients and modulated inflammatory mediators production. Its deficiency was associated with increased levels of Th17 cell cytokines (IL-17 and IL-21) [70]. IL-17+cells were negatively correlated with vitamin D levels ($r = - 0.462$; $P = 0.0403$).The inhibitory effect of Vit D on the Th17 and Th1 responses was mediated via both T cells and DCs while IFN regulatory factor 8 (IRF-8) pathway was involved in inhibition of Th17 cell differentiation [74].

Certain mediators exert broad inhibitory properties on the innate inflammatory and acquired immune responses. Studies were set up to investigate the expression of IL-27 and IL-37 in Behçet disease (BD) and to explore their possible regulatory role during inflammation, particularly on the suppression ofTh17 cells activity.

IL-27, a heterodimeric cytokine composed of two subunits: p28 (IL-27p28) and the Epstein-Barr virus–induced gene 3 (EBI3), is produced mainly by activated antigen-presenting cells (APCs) [75]. The IL-27 receptor is widely expressed on naïve T cells, natural killer cells, mast cells, monocytes, keratinocytes, vascular endothelium, activated B cells, DCs and Langerhans cells. Low

IL-27 expression was reported in BD patients compared to inactive BD patients (P = 0.009) and to healthy controls (P < 0.001) [76]. Recombinant IL-27 was able to inhibit Th17 differentiation in both BD patients and healthy controls. Results from Wang [76] provided evidence that the negative regulatory effect of IL-27 on Th1 and Th17 cells was mediated via DCs and suggested the involvement of the IRF-8 pathway in this suppressive effect. A decreased expression of IL-27 was associated with active intraocular inflammation in BD [76].

IL-37 that belongs to the members of the IL-1 family has been described as an anti-inflammatory cytokine in several autoimmune/Inflammatory diseases. IL-37 can be induced in various types of cells such as PBMCs, epithelial cells, DCs, monocytes and keratinocytes [77]. IL-37 was significantly decreased in active BD patients compared to healthy controls (P < 0.0001), and could effectively decrease the productions of pro-inflammatory cytokines TNF-α, IL-6, IL-1β and IL-17. Furthermore, recombinant IL-37 (rIL-37) exerted a more suppressive effect on IL-17 production in active BD patients than in healthy controls [78]. Ye et al. reported that rIL-37-treated DCs in BD notably inhibited Th17 and Th1 cell responses as compared to control DCs [79]. These findings suggest that IL-37 could play a potent immunosuppressive role in the pathogenesis of BD via the down regulation of IL-17.

In recent trials, anti-IL-17 and anti-IL-17 receptor antibodies induced a rapid and robust clinical improvement in several autoimmune diseases such as rheumatoid arthritis, ankylosing spondylitis, psoriasis [80-82]. These antibodies were not tried in BD. Interferon alpha (IFN-α) is an effective treatment for patients with active Behçet disease (BD). Besides its antiviral property, IFN-α can generate an anti-inflammatory environment or inhibit specific inflammatory T cells such as Th1 and Th17 cells [34].

Anti-TNF-α therapy inhibited *in vivo* local IL-17 production in ocular fluids from BD patients with uveitis. Moreover, *in vitro* infliximab reduced RORγt expression and IL-17 production from BD TH17 cells [38].

DISTURBED BALANCE BETWEEN TREG AND TH17 CELLS IN BD

The pathologic hallmarks of BD are based on a dysregulated immune response, and include subsequent inflammatory tissue injury. It is widely acknowledged that the level of IL-17A and the percentage of Th17 cells are increased in BD animal models and BD patients. Regulatory T cells as well as Th17 cells have been shown to possess a certain degree of plasticity (Figure 3). It has been described that mouse Foxp3+ Treg cells are able to transdifferentiate into Th17-like cells due to the action of IL-6 in the absence of TGFβ [83-84]. In humans, Treg cells are able to adapt a Th17-like phenotype, which is accompanied with the production of IL-17 [8]. It appears that Treg cells that produce IL-17 can retain their suppressive function until they are triggered by IL-6 and IL-1β [19]. Dynamic changes in the cytokine milieu may transiently disturb the balance between Th17 cells and Treg cells, thereby driving flares of active BD disease [52,85,86].

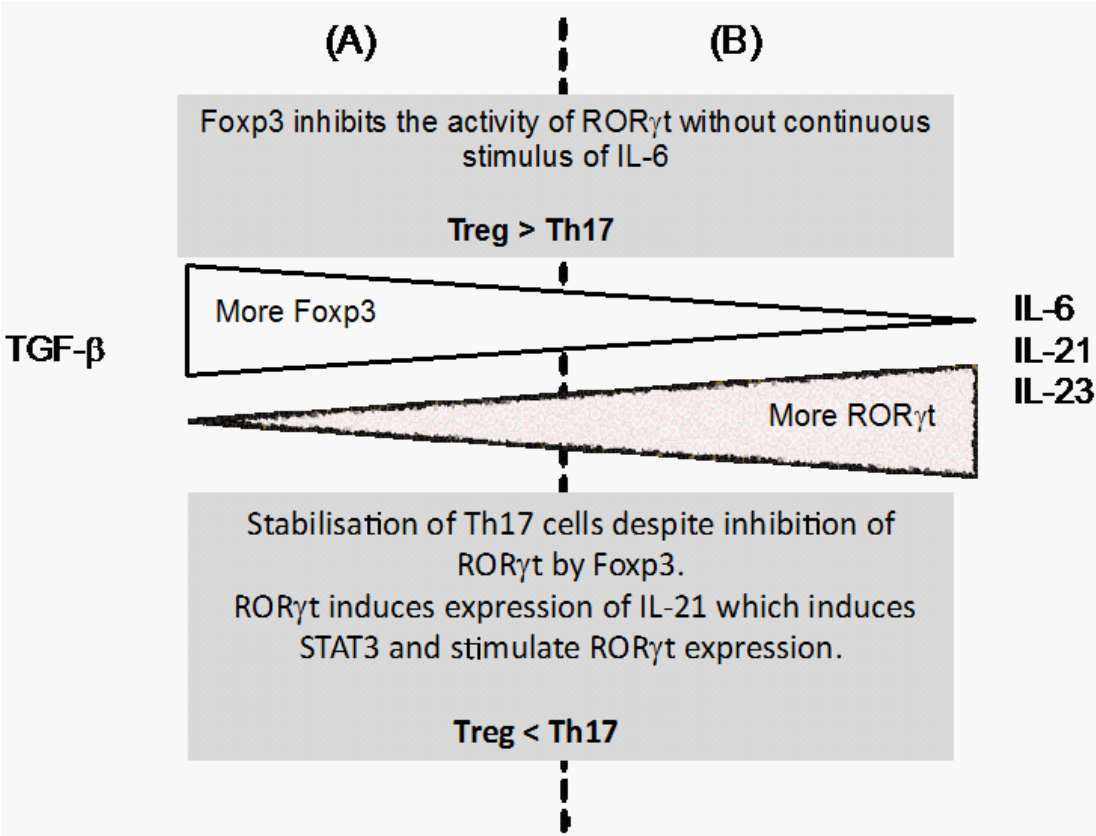

Figure 3: Hypothetical Plasticity between Treg and Th17 cells in Behçet disease.

(A): Possible transcription factor interactions regulating intermediate CD4+ T cell transitions. Foxp3 inhibits the activity of RORγt, without a reciprocal inhibition or feedback from RORγt. This circuit might favor Treg over Th17 development, making the Treg state more stable. Without continuous stimulus by IL-6 to induce RORγt, Foxp3 might tend to repress Th17 development. (B) Second possibility, Th17 state is stabilized despite inhibition of RORγt by Foxp3 owing to a known feedback loop, in which RORγt induces expression of IL-21, which acts in an autocrine manner to further induce STAT3 and stimulate RORγt expression. This feedback stabilizes the Th17 state relative to the Treg state.

CONCLUDING REMARKS

The discovery of Th17 cells has led to a plethora of studies targeting their role in BD inflammatory pathways. A number of basic findings have also lightened the autoimmune / inflammatory field through characterization of Th17 cells as a distinct subset that builds on theTh1/Th2 paradigm. However, as discussed herein, the role of the expanded Th17subpopulation in BD immunity remains ambiguous and appears to be dependent upon several factors such as co-stimulatory molecules, signal transducers and activators of transcription (STATs).

Plasticity between Treg and Th17 likely occurs in the context of dynamic changes in the inflammatory environment. Thus, pro-inflammatory stimuli may promote conversion of immune-suppressive regulatory T cells into pro-inflammatory Th17 cells, while resolution of inflammation may trigger or even require the alternate shift from Th17 to Treg. This concept is just becoming appreciated and requires further study to correlate both causes and outcomes.

A number of crucial questions remain to be answered. How might Th17/Treg imbalance be induced and lead to disease exacerbation, inducing immune pathogenesis of uveitis, central nervous system involvement and lung manifestations? Further understanding of the mechanisms of Th17/Treg-mediated inflammatory immune responses, in tilting the balance between destructive inflammation and homeostasis, may open new lines of investigation for BD treatment in the future.

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