

Th17 cells In Behçet's disease: a new immunoregulatory axis

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ABSTRACT

Accumulating evidence suggests that the abnormality of innate and adaptive immunity responses plays an important role in Behçet's disease (BD). T helper (Th) cells have a central role in modulating immune responses. Traditionally, BD is regarded as a Th1-mediated inflammatory disease. Recently, Th17 cells were identified as a new subset of Th cells unrelated to Th1 or Th2 cells, and several cytokines are involved in regulating their activation and differentiation. Naïve murine CD4⁺ Th can be induced to differentiate towards Th1, Th2, Th17 and Treg phenotypes according to the local cytokine milieu. The committed cells are characterised by expression of specific transcription factors, Tbet for Th1, GATA-3 for Th2, Foxp3 for Tregs and RORγt (RORγt/RORC) for Th17 cells. It has been demonstrated that the skewing of murine Th towards Th17 and Treg is mutually exclusive. Th17 cells regulate inflammation via production of distinct cytokines such as interleukin (IL)-17. There is growing evidence that Th17 cells are pathological in many human autoimmune and inflammatory diseases, leading to intense interest in defining their origins, functions and developing strategies to block their pathological effects. Evidence from human disease such as BD suggests that specialised antigen-presenting cells drive their in vivo development. Knowledge of how Th17 cells interact with other immune cells is limited, but recent data suggest that Th17 cells may not be subject to strict cellular regulation by T regulatory cells. Notably, Th17 cells and Treg cells appear to share common developmental pathways and both cell types retain significant plasticity. Herein, we will discuss the molecular and cellular regulation of Th17 cells with an emphasis on BD. The identification of Th17 cells helps us to explain some of

the anomalies seen in the Th1/Th2 axis and has broadened our understanding of the immunopathological effects of Th17 cells in the development of BD.

Introduction

Current evidence suggests that inflammation in BD was aroused from disruption of homeostasis in genetically susceptible individuals, resulting in altered innate and adaptive immunity responses, pathogenic T cell activation in the peripheral blood and in inflammatory sites (1-3). Among a variety of inflammatory cells, CD4⁺ T cells are thought to play a central role in both the induction and persistence of inflammation by producing pro-inflammatory cytokines. Studies have indicated that Th1-related cytokines as well as Th17-associated cytokines are markedly increased in BD, in the peripheral circulation (4-6) and in the inflammatory sites: the gastrointestinal tract (7), eye lesions (8), skin lesions (9-10) and central nervous system (CNS) (11-13). These pro-inflammatory cytokines are potent stimulators of effector T cells, adhesion molecule expression, chemokine and metalloproteases expression, and secretion of other pro-inflammatory cytokines.

The T helper cell subsets

Functionally distinct Th cells are induced when naive T cells are stimulated via T cell receptor engagement in conjunction with co-stimulatory molecules and cytokines produced by innate immune cells. Th1 cells are thought to regulate cellular immunity via production of IL-2 and IFN-γ, whereas Th2 cells regulate humoral immunity via production of IL-4, IL-5, IL-13 and IL-25 (14-17). Above the limits of the traditional paradigm explaining adaptive CD4 cell responses, other subsets of Th cells are investigated in inflammatory and autoimmune diseases. CD4⁺

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T cell-directed immune responses are mediated by Th1, Th2, Th17 and Treg cells (Fig. 1).

Th17 cells fill a gap between Th1 and Th2, contributing to immunity against certain extracellular bacteria and fungi, and are typically linked to the defence of mucosal surfaces (18). The Th profile-associated cytokines produced are regulated by the expression of subset-specific transcription factors. T-bet directs the Th1 cellular programme (19), while GATA3 is specific for Th2 cells (20). The human RORC mRNA transcript variant 2 was identified, and appears to be responsible for Th17 differentiation (21). The phenotype of CD4⁺CD25^{hi} Tregs cells (22) is directed by the transcription factor Foxp3, also frequently used as a specific marker of these cells (23). Tregs play an important role in peripheral tolerance through their ability to suppress effector CD4⁺ T cells. Recently, the cytokine IL-35, consisting of EB13 (Epstein-Barr virus induced gene 3) and p35 (IL-12a), was implicated as candidate for mediation of suppression and a downstream target of Foxp3 (24-27).

The IL-17 cytokine family includes six members, IL-17A, IL-17B, IL-17C, IL-17D, IL-17E (or IL-25) and IL-17F. They act *in vitro* and *in vivo* as potent pro-inflammatory cytokines such as IL-6 and TNF, B-cell activating factor of the TNF family (BAFF), chemokines such as MCP-1 and MIP-2, and matrix metalloproteases, which mediate tissue infiltration and tissue destruction (28). IL-17 has pleiotropic activities, to promote immune response through the adaptive and innate immune systems. The number of Th17 cells is increased by the secretion of IL-1 and TNF- α , generating IL-6 and TGF- β (29, 30). Recent studies have highlighted further potential heterogeneity within Th17 cell populations by demonstrating that some may even secrete IL-10, a factor known to activate Treg cells and inhibit inflammation (30). Thus, it is possible that Th17 cells activity varies according to the local environment. Th17 cells may act as sentinels, which contribute to maintaining equilibrium functions. Th17 cells have to be analysed in the context of Treg cells.

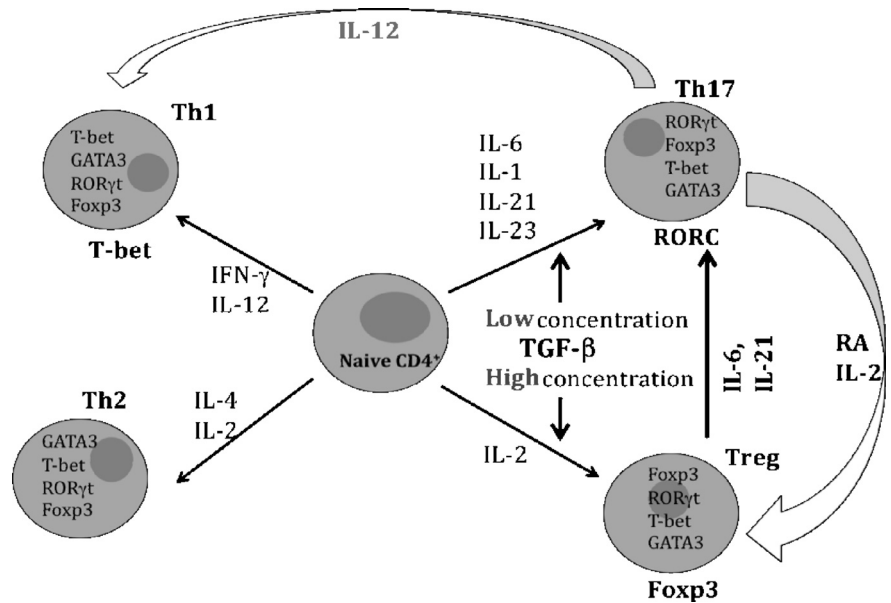


Fig. 1. Naïve CD4⁺ T cell differentiation and conversion. Upon encountering foreign antigens presented by antigen-presenting cells, naïve CD4⁺ T cells can differentiate into Th1, Th2, Th17 and Treg. These differentiation programs are controlled by cytokines produced by innate immune cells, such as IL-12 and IFN- γ , which are important for Th1 cell differentiation, and IL-4, which is crucial for Th2 cell differentiation. TGF- β together with IL-6 induces Th17 cell differentiation, whereas Treg differentiation is induced by TGF- β , retinoic acid (RA) and IL-2. The effector T cells had been thought to be terminally differentiated lineages, but it now appears that there is considerable plasticity allowing for conversion to other phenotypes. Each CD4⁺ T cell subset can adopt alternate cytokine profiles in response to cytokine environmental changes. Among four subsets of T cells, Treg cells and Th17 cells display the highest propensity to switch to other phenotypes. The permissive epigenetic marks at Foxp3 and ROR γ t gene loci, coexpression of Foxp3 and ROR γ t occurs in Treg cells but ROR γ t activity is inhibited by Foxp3 (16). Tregs can become IL-17-producing cells upon stimulation of IL-6 and IL-21. Th17 cells may also convert into IFN- γ -producing Th1 cells when stimulated by IL-12

Functional link between Treg cells and of IL-17 producing Th1 cells

Early studies (31-33) suggested the existence of a functional antagonism between the Treg/Th17 subsets and a dichotomy, mutually exclusive, in their generation (Fig. 1). The relationship between the two subsets is complex, particularly during chronic inflammation. Recent results reported that murine Treg suppress both Th1 and Th2 cells; and they enhance IL-17 secretion by T cells, likely through production of TGF- β (34). Similarly, differentiation of conventional human CD4⁺ T cells into Th17 cells is enhanced in the presence of natural Treg (35). Memory Treg actually showed a more pronounced proficiency to give rise to Th17 cells than conventional memory CD4⁺ T cells, suggesting that they may be at least partially committed towards the Th17 differentiation pathway (34). Memory Treg populations contain high proportions of cells expressing CCR6, receptor of macrophage inflammatory

protein3 α (MIP-3 α /CCL20) that has been shown to characterise the Th17 lineage (36-37). Moreover, within memory conventional CD4⁺ T cells, expression of the Th17 lineage transcription factor ROR γ t has been shown to segregate with CCR6 expression (36) and it might be expected that Treg also express elevated levels of ROR γ t (Fig. 2). The proficiency of Treg cells to differentiate into Th17 cells was also apparent, but to a lesser extent. Induction of Th17 cells from Treg required activation of APC by microbial products through Toll-like receptors (TLRs) that are involved in innate immune functions and in autoimmune diseases as reported by Marshak-Rothstein *et al.* (38). IL-1 is a key regulator of inflammation. IL-1 β is able to mediate both the conversion of memory Treg into Th17 cells and the priming of Th17 cells from natural naïve Treg precursors. Ayyoub *et al.* (32) reported that murine Tregs cells can produce IL-17 while retaining Foxp3 expression and that Foxp3

Table I. Different properties of (T helper) Th1, Th2 and Th17 cells.

	Th1	Th2	Th17
Transcription factors	T-bet STAT4	GATA3 STAT6	ROR γ t STAT3
Differentiation cytokines	IL-12	IL-4	TGF- β IL-6
Secreted cytokines	IFN- γ LT- α	IL-4 IL-5 IL-13	IL-17A IL-17F IL-21 IL-22 IL-9 IL-6 TNF- α

IFN- γ : interferon-gamma; IL: interleukin; ROR: retinoid nuclear receptor; TGF: transforming growth factor; TNF: tumour necrosis factor.

inhibits IL-17 secretion by antagonising ROR γ t (16). IL-1 β and IL-6 in promoting Th17 cell differentiation from conventional CD4⁺ T cells (39-40), have also revealed their ability to subvert IL-2 mediated suppression of Th17 differentiation (40), providing further support for the IL-1 β /IL-2-mediated differentiation of Th17 cells from Treg. The finding that IL-1 β is critical for the conversion of Treg into Th17 cells further supports the use of this approach for treating inflammatory/autoimmune disease (41).

The differentiation program of Foxp3 Treg cells is not fixed (16), and Treg cells have the propensity to differentiate into Th17 cells in a pro-inflammatory cytokine environment (39). TCR-stimulated thymus-derived Foxp3 cells, which produce TGF- β , were shown to produce IL-17 after exposure to IL-6 (21, 31). As depicted in Figure 1, IL-6 produced by antigen-presenting cells as a result of TLR engagement, blocks CD4⁺ CD25⁺ Treg cell-suppressive activity (21, 26).

Redifferentiation of Treg cells into effector T helper cells has also been suggested. A fraction of purified Treg cells can express IFN- γ and T-bet and maintain Foxp3 expression after culture in Th1 cell polarising conditions as reported by Wei *et al.* (42). Under homeostatic or inflammatory conditions, IL-17⁺ IFN- γ ⁺ double producer cells are easily detected, suggesting that there may be some intricate relationship between the Th1 and Th17 cell differentiation program (Fig. 1).

Innate lymphoid cells expressing the nuclear hormone receptor RORC have emerged as important players in human mucosal immunity as reported by Cornelissen *et al.* (43). These cells combine innate modes of activation such as Toll-like receptor signalling with secretion of adaptive effector molecules including IL-2, BAFF and the Th17 cytokines. This endows these cells with the ability to rapidly respond to changes in cytokine milieu as well as changes in microbial composition and to affect both intestinal homeostasis and activation of adaptive immune cells. In the same line, Foxp3⁺ Treg cells as well as Th17 cells are abundant at mucosal barriers and may participate in early responses to infection by attracting mediators of innate immunity (21). Specific commensal microbacteria are required for differentiation or migration of Th17 cells to gut connective tissue (16).

Pathogenic role of Th17 cells in Behçet's disease

Etiopathogenesis of BD remains largely unclear. Immune dysregulation involving T and B cells with hyperreactive neutrophils, supposedly triggered by infectious agents, contribute to disease pathogenesis in addition to genetic predisposition. BD is classified as an auto-inflammatory disease, as exacerbations are not linked to a definite external agent. However, some characteristics of autoimmunity were reported (44). Enhanced inflammatory reaction is the hallmark of pathological findings in BD, which strongly suggests innate im-

mune system activation. TLRs (TLR-6 and TLR-2) pathways are likely to contribute to the pathogenesis of BD as reported Yavuz *et al.* (45).

The importance of excessive Th1 (IL-1, TNF- α , IL-6 ...) was highly depicted in BD (46-47). BD patients exhibited high levels of TNF- α , IFN- γ and IL-1 in peripheral circulation (47-48) and inflammatory sites such as cerebrospinal fluid (CSF) and bronchoalveolar lavage (BAL) (48-49). BD is a Th1 driven disease and immunogenetic studies sustain that IL-1 and TNF- α polymorphisms have a functional effect in BD patients (50, 47).

However, the difficulty in predicting the consequences of interactions between different cytokine networks has increased with the expansion of the Th cell universe and the discovery of numerous B lymphocyte-derived cytokines. Consequently, it is now difficult to conceptualise a straightforward view of the contribution of these disturbances to the pathogenesis of BD. Th1 cells, which produce IFN- γ , IL-2 and IL-6, and Th17 cells, which release IL-17 and TNF- α , have been cast in the leading roles of the play. Experimental studies always are helpful to understand the cytokines interplay in BD. Herpes simplex virus (HSV1) type 1 inoculation of the earlobes of ICR mice resulted in the development of BD-like symptoms (51). To find out whether downregulation of IL-6 would affect the symptoms of BD, IL-6 small interfering RNA (siRNA) was administered. IL-6 siRNA injection downregulated serum IL-6 level and the severity score were decreased. Foxp3, ROR γ t, IL-17A, IL-17F and TNF- α were also influenced in IL-6 siRNA-injected BD mice compared with scramble-injected BD mice. Downregulation of IL-6 improved the inflammatory symptoms in BD mice through upregulation of regulatory T cells and inhibition of Th17 cells (51). In the same way, to inhibit the expression of TNF- α , Choi *et al.* (52) used siRNA to reduce over expression of TNF- α *in vitro* in cell cultures and in an *in vivo* Behçet's disease-like (BD) mouse model, contributing to inflammation inhibition (52).

Recent results from Kim *et al.* (5)

confirm that Th17 cells are instrumental in Behçet's inflammatory sites. They reported that peripheral blood Th17/Th1 ratio was significantly higher in patients with active BD compared to healthy controls. This increase was more prominent in BD patients with uveitis or folliculitis. In contrast, Ferrante *et al.* (7) evaluating the IL-17/IL-23 axis in parallel with Th1 response reported that inflammatory local reactions seen in the intestine of BD patients were Th1 and not Th17 responses. The mechanisms by which IL-17 induces the expression of pro-inflammatory mediators may be cell type-dependent involving gene transcription and modulation of mRNA processing (53). ROR γ t expression, the specific transcription factor of Th17 cell lineage (54), has also been associated with autoimmune diseases. A functional role for Th17 cells in inflammatory/autoimmune diseases was proposed based on the demonstration that the level of IL-17A was elevated, which induced elevated expression of messenger RNA (mRNA) for adhesion molecules in human umbilical vein endothelial cells (HUVECs) and elicited T cell adherence to HUVEC (16). IL-17A-induced signalling of adhesion molecules might play a key role by eliciting T cell adhesion. Our recent results (55) indicated that IL-17 contributes to the active pro-inflammatory pattern that is characteristic of inflammatory diseases and patients with active BD. The percentage of circulating Th17 cells and the ability to produce IL-17A were increased in patients with active BD. Patients with BD in remission stage expressed low Th17 levels compared to active BD patients. The decreased Th17 level could be explained by a probable conversion of Th17 into Treg cells. Foxp3 has been suggested to inhibit Th17 differentiation by antagonising ROR γ t function (55). Our results confirmed several recent studies indicating the existence of a close interplay between Treg and Th17 cells in regulating some autoimmune conditions (56). Regardless of whether it is a cause or a consequence, the expansion of Th17 cells is related to distinct cytokine environment in active BD that influence the extent inflamma-

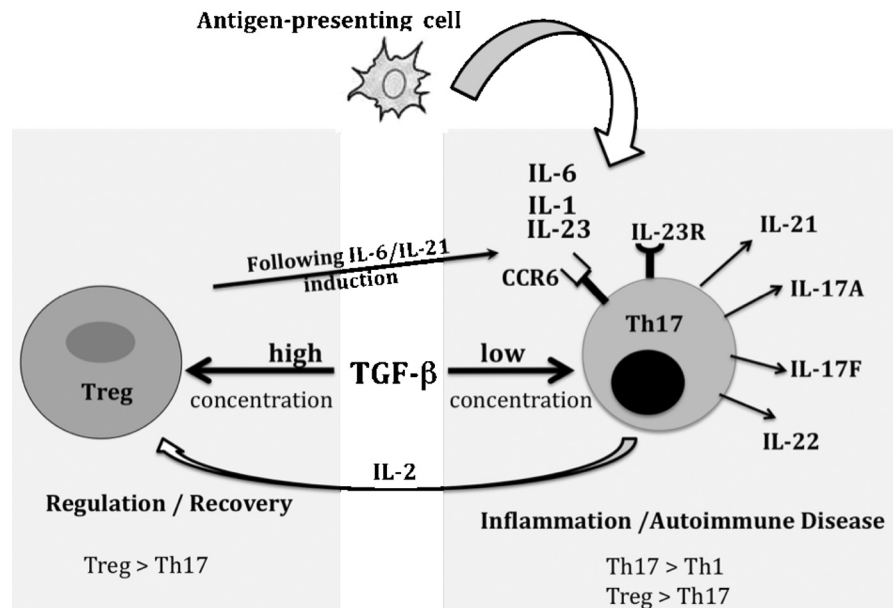


Fig. 2. Developmental pathways for the generation of pathogenic effector T Th17 cells and regulatory T cells (Treg). At the steady-state level TGF- β produced in the immune system will suppress the generation of effector T cells and induced Foxp3⁺ Treg cells, and then maintain homeostasis. However, on infection by unknown antigen (viral/bacterial antigens (HSV-1, *S. Sanguis*, *Mykobacteries*); super-antigens (*Staphilococcus*); autoantigens (HSP60, $\alpha\beta$ -crystallin, Retinal S, α -enolase))(6; 44) inducing inflammation, IL-6 produced by the activated innate immune system suppress the generation of TGF- β -induced Treg cells and induces pro-inflammatory Th17 cells. IL-1 or IL-23 drives differentiation of Th17 cells that express IL23R and CCR6. The effect of IL-1 may be enhanced by IL-23 and/or IL-6. The changes in the Treg/Th17 and Th17/Th1 ratios show the plasticity between these T cell subpopulations.

tion within tissues. The expression of adhesion molecule mRNA and adherence of T cells to HUVEC, underscores the role of Th17 cells in vascular inflammation in active BD (51). Inflammatory cytokines (IL-6, TNF- α , and IFN- γ) have been investigated in the CSF of patients with neuro-Beçet's disease (NBD) as reported by Saruhan-Direskeneli *et al.* (57), and the theory of simple Th1/Th2 polarisation in BD may be an oversimplification of complex interactions as observed in the peripheral circulation. The CD4⁺ population in NBD was then evaluated in the context of T cell-related transcription factor in cerebrospinal fluid (CSF) (13). Expression of TBX21, RORC and Foxp3 were found increased in NBD patients compared to headache attributed to BD (HaBD) and to non-inflammatory neurological disease (NIND) patients. Analysis of transcription factor ratios, revealed an increase in the RORC/FOXP3 and TBX21/GATA3 ratios in NBD patients. Our findings indicate that both Th1 and Th17 mRNA expressions involved a possible impair-

ment of Treg cells. The RORC/FOXP3 ratios dysregulation in NBD are consistent with those reported in other inflammatory diseases and underline the plasticity existing between Th1, Th17 and Treg cells during inflammation (13). Relative Treg deficiencies in suppression of Th-mediated pathogenic mechanisms probably exist in NBD (12-13). The increase in Th17/Th1 and Th17/Treg ratios result is correlated with the extent of inflammation. Recent evidence on T-cell subset reciprocal regulation and counterbalance between Th1, Th17 and Tregs has greatly influenced our understanding of immunoregulation in inflammatory diseases, particularly in BD (Fig. 2). T cells are exposed to a complex cytokine milieu (IL-1, TNF- α , IL-6, IL-23) and display considerable plasticity, traduced in our reports by Treg/Th17 and Th17/Th1 ratios. A switch exists between T cell subpopulations with variable Treg/Th17 and Th17/Th1 ratios according to recovery or inflammatory situations under the control of cytokines concentration (Fig. 2). The possible synergistic or

antagonistic interactions between these subsets in BD need to be more fully clarified.

Conclusion

Our results show the plasticity existing between Th1, Th17 and Treg cells during inflammation in the peripheral circulation and in inflammatory sites. They are not specific to BD, but could be generalised to most of the inflammatory diseases. Through the potential role of Th17 cells in BD animal models as well as in human BD, target therapy directed against the Th17/IL-17 axis may have a therapeutic role to stop the inflammation. However, the precise mechanisms of the Th17/Treg axis homeostasis should be further elucidated in animal models and human Behçet's disease. The best way to target the Th17 axis in BD is far from obvious. It will probably require many years of careful clinical and immunological studies to determine this. Such studies are likely to offer further insights into the pathogenesis of BD and the role of the Th17 pathway. A cogent description of the individual contributions to disease by Th17 cells, Th1 cells and Treg cells should be a goal of future research.

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