



Mini Review

Unbalanced helper T cell function in Behcet's disease

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Behcet's disease (BD) is a multisystem inflammatory disease that is characterized by recurrent attacks of uveitis, oral aphthous ulcers, genital ulcers, and erythema nodosum. The etiology and pathogenesis of BD are largely unknown. We have presented evidence that supports a role of excessive Th1 cell activity in BD. Recently, it has been suggested that Th17 cells are associated with several autoimmune diseases, such as multiple sclerosis, rheumatoid arthritis, and inflammatory bowel disease, all of which are considered to be Th1 diseases. Therefore, it is interesting to study the role of Th17-related cytokines and Th17-associated signaling molecules in BD. Major Th17-related cytokines were not detected in peripheral blood mononuclear cells (PBMCs) and in skin lesions of BD patients. Expression of TGF-beta receptor and Smad2 mRNA was significantly higher in BD patients compared with the levels in normal controls. Interleukin (IL)-23 receptor, ROR-C, and Foxp3 are key transcription factors expressed by Th17 cells and regulatory T cells, respectively, and their expression was decreased in BD. These findings suggest that T cell function may be unbalanced in BD.

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Introduction

CD4⁺ T cells differentiate into several subsets in response to specific cytokine environments that lead to an effective response against various microorganisms. Recently, the Th1/Th2 paradigm was challenged by the discovery of several subsets of helper T (Th) cells, including follicular helper T (T_{FH}), Th17, Th9, and Th22 cells (Figure). However, we understand little about how these cells participate in the immune system and what circumstances lead to immune disturbance¹⁾.

Behcet's disease (BD) is a systemic inflammatory

condition that is presumably associated with autoimmunity. Many studies have revealed dominance of Th1 cells in BD, and recent studies have suggested involvement of Th17 cells in its pathogenesis^{2,3,4)}. Furthermore, several investigators have reported that regulatory T cells (Tregs) are paradoxically expanded in BD^{5,6)}. We have raised the possibility that immunological regulation of peripheral blood mononuclear cells (PBMCs) may be unbalanced in BD. Here we review recent advances in our understanding of the development of helper T cells and the proposed pathogenesis of BD in relation to polarization of T cell subsets.

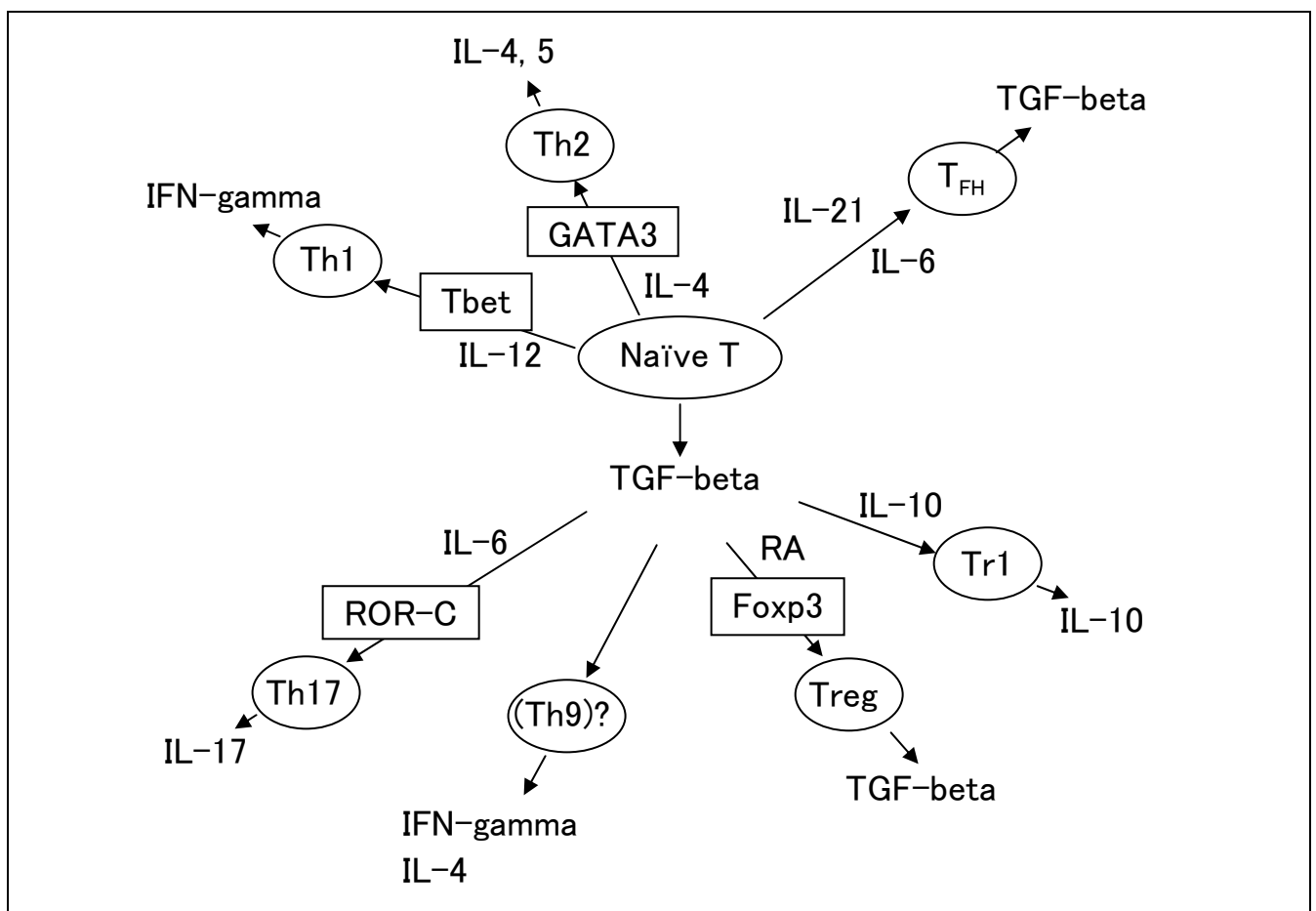


Figure. Primary polarization of diverse CD4⁺ T cell subsets¹⁾.

Various cytokines and transcription factors (listed inside rectangles) induce polarization (Ref. 1). For example, naïve T cells develop into Th17 cells through induction of ROR-C by stimulation with TGF-beta and IL-6, or into Tregs through induction of Foxp3 by stimulation with TGF-beta and retinoic acid (RA).

T cell hypersensitivity in BD

T lymphocytes are thought to play a central role in the pathogenesis of BD. In addition, immune hypersensitivity to certain antigens has been suggested to

trigger various manifestations of BD. Generally, there are two types of research that have suggested a role for immune hypersensitivity: the first is studies on the existence of hypersensitive T cells and the second is investigation of T cell polarization in BD,



evidence of which has recently been accumulating as mentioned below. Several antigens have been reported to stimulate a hypersensitive response from T cells. For instance, streptococcal or *E. coli* antigens can stimulate T cells to produce IFN-gamma in BD^{7,8}. We previously reported that heat shock protein increased the expression of IL-8 and TNF-alpha by PBMCs in patients with BD⁹, and also showed the involvement of innate immunity in hyperresponsiveness to microbial infections that leads to various manifestations of BD¹⁰.

Th1 cell dominance in BD

We previously reported that IFN-gamma-producing cells were detected in erythema nodosum skin lesions of patients with BD, while IL-4-producing cells were rarely detected¹¹. We have also observed heavy infiltration of CD4⁺ and CD8⁺ T cells into the intestinal lesions of BD patients, and have detected the expression of mRNAs for proinflammatory and Th1 cytokines/chemokines¹². Kobayashi et al. observed the accumulation of lymphocytes around the vasa vasorum in six patients with vascular BD, as well as expression of proinflammatory cytokines by these cells, including IL-1 alpha, TNF-alpha, and IFN-gamma¹³. Melikoglu et al. compared the time course of the pathergy reaction between ten BD patients and healthy controls, and found increased infiltration of mature dendritic cells, monocytes, and lymphocytes (including Tregs) in the patients. At the same time, enhanced expression of several proinflammatory cytokines (IFN-gamma, IL-12 p40, and IL-15), chemokines (MIP-3-alpha and IP-10), and adhesion molecules (ICAM-1 and VCAM-1) was observed after 48 hours in BD patients¹⁴. Ben Ahmed et al. studied skin biopsy specimens obtained from the cutaneous ulcers of BD patients, and noted a marked increase in the expression of mRNAs for IL-8, MCP-1, IFN-gamma, and IL-12¹⁵. Some re-

searchers have investigated intracellular cytokine production and have found predominant staining for INF-gamma in BD T cells^{16,17}. Th1 dominance is also observed in BD uveitis¹⁸ and stomatitis¹⁹. Moreover, Th1/Th2 imbalance has also been demonstrated by studies of serum/plasma and PBMCs^{20,21}, and these findings have established a role for Th1 dominance-related hypersensitivity due to loss of the Th1/Th2 balance in BD.

Th17 cells

Th17 cells produce a number of pro-inflammatory cytokines, such as IL-17A, IL-17F, IL-21, and IL-22, while TGF-beta and IL-6 are essential for their development. Transforming growth factor (TGF)-beta induces the expression of retinoic acid-related orphan receptor-gamma t (ROR-gamma t), which is the master transcription factor for Th17 cells. TGF-beta also induces Foxp3, while IL-6 reduces its expression. After stimulation by TGF-beta and IL-6, Th17 cells express IL-21, which expands these cells in an autocrine manner. IL-21 also induces IL-23 receptor expression, which allows Th17 cells to respond to stimulation by IL-23 and leads to their further expansion. Th17 cells account for the majority of intestinal helper T cells and have been shown to play a key role in maintaining peripheral immunity²². These cells have also been suggested to have a role in certain models of autoimmune disease, such as autoimmune brain inflammation²³, collagen-induced arthritis²⁴, and experimental autoimmune encephalomyelitis²⁵. Furthermore, an increase of IL-17 production has been detected in some human autoimmune diseases, including rheumatoid arthritis^{26,27}, inflammatory bowel disease²⁸, and multiple sclerosis²⁹. These findings suggest that dysregulation of Th17 cells may be involved in the pathogenesis of certain autoimmune diseases.

Table. mRNAs Expressions of cytokines and signaling molecules in BD patients PBMC under lectin stimulations

PHA stimulation		
increase		Smad2*, IL-17, IL-23, TGF-beta receptor 1*, 2*, 3, SOCS1*
decrease		IL-23 receptor, ROR-C
PWM stimulation		
increase		Smad2, IL-23, TGF-beta receptor 1, 2, 3
decrease		IL-17F, IL-21, IL-23 receptor, STAT1, SOCS3, Foxp3, ROR-C*

*: p<0.05



Th17 cells in BD

There have been several reports about Th17 cells in BD. Chi et al. mentioned that the production of IL-23 and IL-17 by PBMCs was upregulated in patients with BD²⁾. On the contrary, Zhao et al. reported that PBMCs of BD patients did not produce much IL-17 after stimulation with S-antigen³⁾. Cañete et al. revealed that CD3 T cells were significantly increased in BD synovitis compared with psoriatic arthritis. Investigation of the lymphocyte population showed no clear shift of the Th1/Th2/Th17 profile⁴⁾.

We attempted to study the expression of Th17-related cytokines and signaling proteins in BD. As a result, we did not detect mRNAs for Th17-related cytokines (IL-17A, IL-17F, IL-21, and IL-22) or IL-23 in PBMCs or in skin lesions, whereas IFN-gamma mRNA was four-fold higher than in normal controls. With regard to mRNAs for signaling proteins, TGF-beta receptor and Smad2 showed significantly higher expression, while IL-23 receptor, ROR-C, and Foxp3 showed lower levels of expression (Table). These results showed complicated and unbalanced T cell signaling in patients with active BD. Up-regulation of INF-gamma and TGF-beta/Smad signaling along with down-regulation of Th17 cells seems to indicate a discrepancy with respect to our understanding about the role of Th1 dominance in BD³⁰⁾, and may suggest the possibility that dysfunction of T cell regulatory mechanisms contributes to this disease.

Regulatory T cells

Kuniyasu et al. found that the adoptive transfer of CD25⁺CD4⁺ T cells induced several organ-specific autoimmune diseases in athymic nude mice³¹⁾. Thereafter, they identified a small population of CD25⁺CD4⁺ T cells that maintain self-tolerance, suppress autoimmune responses, and control inflammatory reactions. They called these cells regulatory T cells (Tregs) and demonstrated that peripheral immune tolerance is controlled by such cells.

There are two types of Tregs. One is known as natural regulatory T (nTreg) cells, which develop in the thymus and are stable cells of the true CD4⁺ T cell lineage. nTreg cells express the transcription factor Foxp3, which is a reliable marker of this cell type and directs their development. The other type is known as induced regulatory T (iTreg) cells, which express Foxp3 and are induced in the periphery as a

result of stimulation by TGF-beta in the presence of retinoic acid (Figure). These cells produce various anti-inflammatory cytokines (such as TGF-beta, IL-10, and IL-35) to suppress the immune response, but sometimes undergo transformation to pathogenic effector T cells^{32,33)}. However, the contribution of Tregs to the pathogenesis of human autoimmune diseases is considered to be limited.

Regulatory T cells in BD

Recently, Hamazaoui et al. reported that CD25⁺CD4⁺ Treg cells were increased in both the peripheral blood and the cerebrospinal fluid of BD patients only in the active phase, as compared with BD in remission and healthy controls, and proposed a local immunosuppressive effect of these cells from both immunopathogenetic and therapeutic aspects^{5,6)}. Nanke et al. investigated the percentage of CD25⁺CD4⁺ T cells among helper T cells before and after ocular attacks in BD patients, suggesting that the level was significantly lower before ocular attack than afterward³⁴⁾. They speculated that the decreased percentage of Treg cells in the peripheral blood before ocular attacks reflected trafficking to the eyes. These findings may explain our observations with respect to regulatory dysfunction of T cells in BD and suggest that it is very important to consider status of each individual for proper our evaluation of T cell function. As far as we could determine, there have been no reports about the prevalence or functional characteristics of T_{FH}, Tr1, and Th9 cells in BD. Further studies of the relationship between T cell polarization and the pathogenic immune response in BD are needed. In conclusion, we reviewed the data about T cell polarization in BD and the findings suggest the possibility of a new approach to the pathogenesis of this disease.

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