CD4⁺CD25⁺ regulatory T cells in patients with Behçet’s disease

K. Hamzaoui¹, A. Hamzaoui¹,², H. Houman³

ABSTRACT

Objective. To investigate whether the CD4⁺CD25⁺ regulatory T cell (Treg) population, which plays important role in autoimmune diseases is related to the pathophysiology of Behçet’s disease (BD).

Methods. Forty-two patients with BD (20 patients in active disease) fulfilling the criteria of the International Study Group of BD. Twenty age-matched healthy controls were studied. We analyzed CD4⁺CD25⁺ T cells and the mRNA expression of Foxp3, cytotoxic T lymphocyte-associated antigen 4 (CTLA-4), and transforming growth factor-β (TGF-β) in BD. We have studied the ability of CD4⁺CD25⁺ (Treg) to regulate proliferation of CD4⁺CD25⁻ T cells during active BD stage.

Results. Active BD patients had significantly higher CD4⁺CD25⁺ T cells, as compared with BD in the remission stage, and healthy controls. There was no significant differences in the CD4⁺CD25⁺ T cells expression between healthy controls and remission BD. In active BD, mRNA for Forkhead box p3 (Foxp3) and cytotoxic T lymphocyte-associated antigen-4 (CTLA-4) were highly expressed when compared to remission BD and healthy controls. There was no differences in the mRNA expression for TGF-β in active BD, remission BD and healthy controls. Functionally, CD4⁺CD25⁺ T cells in active BD were impaired in their proliferative responses and could suppress the proliferation of their CD4⁺CD25⁻ counterparts.

Conclusion. These data demonstrate that CD4⁺CD25⁺ Treg cells, with the potential to regulate suppression of effector T cells, were increased in the peripheral circulation of active BD patients. The role of CD4⁺CD25⁺ T cells in the regulatory process of the inflammation in active BD, could be taken in account.

Introduction

Behçet’s disease (BD) is a multisystem inflammatory disease, characterized by recurrent attacks of uveitis, oral aphthous, genital ulcerations, skin and joint lesions of variable severity, complicated by central nervous manifestations (1, 2). BD could be in part considered as an autoimmune disease (3). The aetiology of BD remains unknown, but the most widely held hypothesis of disease pathogenesis is that a profound inflammatory response is triggered by an infectious agent in a genetically susceptible host. Investigation of the aetiology of BD has focused predominantly on herpes simplex virus immunopatology, streptococcal infection and autoimmunity to oral or cross-reactive microbial antigens (4, 5). The human 60-kD HSP are involved in the development of BD and suggested an activated innate immunity (6, 7). CD4⁺CD25⁺ T cells, which constitute 3% to 7% of peripheral CD4⁺ T cells in human subjects, maintain immunologic self-tolerance (8-10). Removal of this population from normal rodents leads to the spontaneous development of various autoimmune diseases. Recent reports have documented that CD4⁺CD25⁺ regulatory T (Treg) cells play critical roles not only in preventing autoimmunity but also in controlling various immune reactions against bacteria, viruses, fungi, and intracellular parasites (11-14). In the periphery, CD4⁺CD25⁺ regulatory T cells constitutively express a variety of phenotypic markers that has allowed a further characterization and subdivision of this cell population: glucocorticoid-induced TNFR (GITR) (15), OX40 (16), CD103 (17), and CTLA-4 (CD152) (18-19). However, their precise role in the predominantly contact-dependent activity of CD4⁺CD25⁺ regulatory T cells remains controversial and the topic of ongoing investigations. It is
Table I. Characteristics of 20 active patients with Behçet’s disease (BD). Overall burden of the disease manifestations in BD patients. Patients received colchicine, steroids/cyclosporine. Five patients with oral ulcer, genital ulcer, uveitis and vascular-symptoms were newly diagnosed.

<table>
<thead>
<tr>
<th>Major criteria</th>
<th>Number of patients (%)</th>
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<tbody>
<tr>
<td>Oral ulcer</td>
<td>20 (100)</td>
</tr>
<tr>
<td>Genital ulcer</td>
<td>20 (100)</td>
</tr>
<tr>
<td>Skin lesions (erythema nodosum, folliculitis)</td>
<td>15 (75)</td>
</tr>
<tr>
<td>Uveitis</td>
<td>20 (100)</td>
</tr>
<tr>
<td>Minor criteria</td>
<td></td>
</tr>
<tr>
<td>Arthritis</td>
<td>18 (90)</td>
</tr>
<tr>
<td>Vasculo-symptoms (+)</td>
<td>20 (100)</td>
</tr>
<tr>
<td>Intestinal symptoms</td>
<td>8 (40)</td>
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Also important to note that these markers, similar to expression of CD25, are differentially regulated upon activation of T cells and thus do not permit a categorical distinction between regulatory and recently activated T cells. Therefore, identification of Foxp3 as a signature transcription factor for development and function of CD4+CD25+ regulatory T cells in mice appeared to offer a solution for this dilemma (20-22). Yet, a recent report demonstrated that activation of human T cells, in contrast to mouse T cells, upregulates expression of Foxp3 and induces regulatory activity in CD4+CD25+ T cells (23). Although discrepancies between activation-induced Foxp3 expression in murine and human CD4+CD25 T cells, potentially the consequence of naive vs memory T cell populations investigated (23), remain to be studied in greater detail. The work by Walker et al. supports the contention that the particular phenotype of regulatory T cells may arise at least via two pathways, thymic selection, and peripheral T cell activation. The importance of latter pathway is further documented by the intriguing observation that TCR activation of murine CD4+CD25 T cells in the presence of transforming growth factor-β (TGF-β) induced CD25, CTLA-4 as well as Foxp3 expression and was associated with the acquisition of in vitro and in vivo regulatory activity by these cells (24).

We undertook this study to explore the presence and characteristics of CD4+CD25+ Treg cells in patients with BD. We show that the frequency of CD4+ T cells expressing CD25(high) is increased significantly in active BD compared to the remission BD stage. No significant difference was observed in the numbers of CD4+CD25(high) T cells in peripheral blood from remission BD patients and healthy controls. Phenotypically, CD4+CD25(high) T cells express high levels of mRNA expression of Foxp3 and CTLA-4. Importantly, isolated CD4+CD25(high) cells showed the classic functional characteristic of Treg cells, they suppressed the proliferation of CD4+CD25 T cells.

Materials and methods

Patients and control subjects

We studied 42 patients with BD (20 patients in active stage), and 20 healthy controls. The mean age of active BD patients (3 females and 17 men) was 32 years (range, 27-52 years) and the mean duration of disease was 74 months (range 10-168 months). The mean age of patients in remission BD (2 females and 20 men) was 42 years (range 35-47 years) and the mean duration of the disease was 128 months (range 22-176 months). All patients (5 females and 37 males) fulfilled the criteria defined by the International Study Group for diagnosis of Behçet’s disease (25). Disease activity was evaluated according to the published criteria (26). Clinical features of the patients with active and remission stages are given in Table I and Table II. Tables describe overall burden of the disease manifestations in BD patients. Active BD patients (15/20 patients) were always treated with steroids and colchicine. Five patients with active disease were newly diagnosed and received treatment before venipuncture. No patient was twice studied both during active and remission stages. Twenty healthy volunteers matched for age and sex (16 men and 3 women; age range 32-60 years, mean 42 years) were included as control subjects, none of them had evidence of acute infection or chronic disease (e.g., other autoimmune or atopic disorders). Venous blood samples (20 ml) were collected aseptically into tubes with anti-coagulant. The design of the study was approved by our National Ethics Committee.

Flow cytometry

Flow cytometry was performed by using an EPICS XL (Immunotech Coulter), as described previously (27). The forward and side light scatter gate was set to analyze viable cells and to exclude background artifacts. Multicolor staining was carried out with fluorescein isothiocyanate-, phycoerythrin-, or phycoerythrin-cyanin 5.1-conjugated monoclonal antibodies against CD25, CD3, and CD4 (R&D Systems Europe Ltd). Three-color flow cytometric analysis was performed on cells within the lymphocyte light scatter gate by using forward and side scatterers. The percentage of CD4+CD25+ T cells was determined by the ratio of CD4+CD25 T cells in lymphocytes. A flow cytometric enumeration and gating strategy of CD4+CD25+ T cells was represented in Figure 1A. The CD4+CD25(high) fraction was determined by fluorescence intensity > 10^2, with the same setting used in all BD patients and healthy controls. The gating strategy is shown for the analysis of CD4+CD25(low) (R1) and CD4+CD25(high) (R2) in one patient with active BD and in one patient in the remission stage.
The mRNA expression levels of the target and the housekeeping gene were defined by standard curves, which were drawn by the expression obtained from the GenBank database. Nucleotide sequences of PCR primers and TaqMan probes were as follows:

- For CD25, 5'-TGAGCCATTTGTTGCTGAT-3' (forward) and 5'-CAGCTGTACATTGGCTGGTTGTAC-3' (reverse) were used. The TaqMan probe, 5'-CAGCTGTACATTGGCTGGTTGTAC-3' with the reporter dye molecule FAM and the quencher dye molecule TAMRA, was used as an internal control. The final concentration of 1x Taqman universal master mix (PE Biosystems) was prepared at a 100x dilution, and TaqMan probe was labeled at the 5' end with the reporter dye molecule FAM and the 3' end with the quencher dye molecule TAMRA. The increase in fluorescence was proportional to the concentration of template in the PCR mixture, which varied from 1x to 10x.

- For TGF-β1, the primer set was 5'-CAGCTGTACATTGGCTGGTTGTAC-3' and 5'-CAGCTGTACATTGGCTGGTTGTAC-3'. The TaqMan probe, 5'-CAGCTGTACATTGGCTGGTTGTAC-3', was labeled with the reporter dye molecule FAM and the quencher dye molecule TAMRA. The TaqMan primer was labeled at the 5' end with the reporter dye molecule FAM and the 3' end with the quencher dye molecule TAMRA. The increase in fluorescence was proportional to the concentration of template in the PCR mixture, which varied from 1x to 10x.

- For CTLA4, the primer set was 5'-CAGCTGTACATTGGCTGGTTGTAC-3' and 5'-CAGCTGTACATTGGCTGGTTGTAC-3'. The TaqMan probe, 5'-CAGCTGTACATTGGCTGGTTGTAC-3', was labeled with the reporter dye molecule FAM and the quencher dye molecule TAMRA. The TaqMan primer was labeled at the 5' end with the reporter dye molecule FAM and the 3' end with the quencher dye molecule TAMRA. The increase in fluorescence was proportional to the concentration of template in the PCR mixture, which varied from 1x to 10x.

- For Foxp3, the primer set was 5'-CAGCTGTACATTGGCTGGTTGTAC-3' and 5'-CAGCTGTACATTGGCTGGTTGTAC-3'. The TaqMan probe, 5'-CAGCTGTACATTGGCTGGTTGTAC-3', was labeled with the reporter dye molecule FAM and the quencher dye molecule TAMRA. The TaqMan primer was labeled at the 5' end with the reporter dye molecule FAM and the 3' end with the quencher dye molecule TAMRA. The increase in fluorescence was proportional to the concentration of template in the PCR mixture, which varied from 1x to 10x.

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- For CTLA4, the primer set was 5'-CAGCTGTACATTGGCTGGTTGTAC-3' and 5'-CAGCTGTACATTGGCTGGTTGTAC-3'. The TaqMan probe, 5'-CAGCTGTACATTGGCTGGTTGTAC-3', was labeled with the reporter dye molecule FAM and the quencher dye molecule TAMRA. The TaqMan primer was labeled at the 5' end with the reporter dye molecule FAM and the 3' end with the quencher dye molecule TAMRA. The increase in fluorescence was proportional to the concentration of template in the PCR mixture, which varied from 1x to 10x.

- For Foxp3, the primer set was 5'-CAGCTGTACATTGGCTGGTTGTAC-3' and 5'-CAGCTGTACATTGGCTGGTTGTAC-3'. The TaqMan probe, 5'-CAGCTGTACATTGGCTGGTTGTAC-3', was labeled with the reporter dye molecule FAM and the quencher dye molecule TAMRA. The TaqMan primer was labeled at the 5' end with the reporter dye molecule FAM and the 3' end with the quencher dye molecule TAMRA. The increase in fluorescence was proportional to the concentration of template in the PCR mixture, which varied from 1x to 10x.
clear cells derived from theuffy coat. The value of each gene expression was divided by that of β-actin to calculate the relative amount of the gene in cells. All analyses were carried out in duplicate and were repeated for confirmation.

**Statistical analysis**
The differences in percentages of CD4+CD25-high cells and mRNA expression levels between each group were analyzed by using the Wilcoxon test. The Mann-Whitney U test was used to analyze the significance of the differences. Differences were considered to be significant when the P value was less than 0.05.

**Results**

**CD4+CD25+ T cells in BD**
We first analyzed the proportions of CD4+CD25-high cells (Rt cells) in 42 patients with BD (20 in active stage) by using flow cytometry. As shown (Fig. 1A, 1B) the proportions of CD4+CD25-high cells in active BD were significantly higher (median: 9; 25 percentile: 7; and 75 percentile: 12) than those in healthy controls (median: 4.5; 25 percentile: 3.575; and 75 percentile: 6) and BD patients in the remission phase (median: 3; 25 percentile: 2; and 75 percentile: 4.25). Patients in remission BD exhibited similar proportions of CD4+CD25+ than healthy controls. In patients with active BD, there was no difference between treated patients (colchicine, steroids, cyclosporine) and newly diagnosed active BD patients. A typical pattern of CD4+CD25-high T cell expression from one patient with active BD and one patient in remission stage are illustrated in Figure 1A.

**Foxp3 mRNA expression in BD**
To further characterize regulatory T (Treg) cells in patients with BD, the mRNA expression levels of Foxp3 was analyzed in purified CD4+CD25-high T cells in 10 active BD, in 12 remissions BD as well as in 10 healthy controls. A significant increase in Foxp3 mRNA expression was observed in freshly and unstimulated CD4+CD25-high T cells of patients with active BD (median: 7.5; 25 percentile: 6; and 75 percentile: 8.75) (Fig. 2) compared with remission BD (median: 1; 25 percentile: 0.25; and 75 percentile: 1) and healthy controls (median: 1.45; 25 percentile: 0.5; and 75 percentile: 2). No significant difference was observed between healthy controls and BD in remission stage. No difference was observed in the Foxp3 expression between treated and newly diagnosed active BD patients (P = 1).

**CTLA-4 and TGF-β mRNA expression in BD**
Relative CTLA-4 mRNA expression levels on purified CD4+CD25-high T cells were significantly higher in active BD patients (median: 4; 25 percentile: 3; and 75 percentile: 4.75; P < 0.01) than those in healthy controls (median: 1.25; 25 percentile: 0.7; and 75 percentile: 2) and recovered to the level of healthy controls at the remission phase (median: 2; 25 percentile: 1; and 75 percentile: 2) (Fig. 3A). No difference was observed in the CTLA-4 mRNA expression between treated and newly diagnosed active BD patients.

We also analyzed the mRNA expression of TGF-β, which is not specifically expressed on CD4+CD25-high regulatory T cells (28). Relative TGF-β mRNA expression levels at the active BD stage (median: 2; 25 percentile: 1.25; and 75 percentile: 3) and remission stage were not significantly different (median: 1.75; 25 percentile: 0.25; and 75 percentile: 3.5; P = 0.006) (Fig. 3B). No differences were observed between BD patients in the active and remission stages compared to healthy controls (median: 1.5; 25 percentile: 1; 75 percentile: 3).

**CD4+CD25-high T cells inhibit the proliferation of CD4+CD25 cells**
Hyporesponse to TCR-mediated stimu-
mRNA Foxp3 expression in Behçet’s disease

Fig. 2. Relative Foxp3 expression in Behçet’s disease (BD). Foxp3 expression was determined in cDNA samples by means of the real-time reverse transcriptase PCR method by using β-actin as an endogenous reference gene. Shown is the analysis of freshly and purified CD4+CD25+high cells from samples obtained from healthy control (n = 10), patients with active BD (n = 10), and remission BD (n = 12). Each bar represents a median with a range from the 25th percentile to the 75th percentile. NS: not significant.

Fig. 3. Relative CTLA-4 and TGF-β mRNA expression at the active and remission BD: CTLA4 (A), and TGF-β (B). mRNA expression levels of these genes were determined in cDNA samples by using a real-time reverse transcriptase PCR method, with β-actin as an endogenous reference gene. Shown is the analysis of freshly isolated CD4+CD25+high cells from samples from healthy control (n = 10), patients with active BD (n = 10), and remission BD (n = 12). Each bar represents a median with a range from the 25th percentile to the 75th percentile. NS: not significant.

Activation and mitogens is one characteristic of CD4+CD25− Treg cells (8). Therefore, we studied in 10 patients with active BD, the proliferation of sorted CD4+CD25+high and CD4+CD25− T cells in response to anti-CD3+anti-CD28 MoAbs. Significant, proliferative responses of CD4+CD25− cells were observed, whereas the proliferation of CD4+CD25+high T cells was almost undetectable in all samples studied (Fig. 4A) and reduced significantly in relation to that of CD4+CD25− cells in all the samples (P < 0.001). As one of the main functions of Treg cells is to inhibit the activation of T cells, we studied whether enriched CD4+CD25+high T cells are able to inhibit the proliferation of their CD4+CD25− counterparts. As shown in Figure 4B, the presence of CD4+CD25+high T cells clearly reduced the proliferative response of CD4+CD25− T cells to anti-CD3+anti-CD28 MoAbs in all samples tested. The degree of suppression was cell ratio-dependent, but difference was not significant (ratios CD25− : CD25+high, 2 : 1 and 4 : 1). These data confirm that CD4+CD25+high T cells in active BD show the in vitro functional activities of Treg cells.

Discussion

Behçet’s syndrome is a multisystemic inflammatory disorder with numbers of immunological alterations suggesting at least in part an autoimmune pathogenesis. Autoantibodies have been reported against mucosal cells (29), endothelial cells (30), and lymphocytes (31). In the hope to explore regulating pathways in BD, we focused on CD4+CD25+ T cells. Our study demonstrates increased CD4+CD25+high T cells in active BD compared to remission BD and healthy controls. CD4+CD25+high cells have been the object of numerous studies because their function appears critical in maintaining self tolerance. Baecher-Allan et al. (8) demonstrated that the regulatory CD4+ T cells in peripheral blood of healthy human subjects preferentially reside within the CD4+CD25+high T-cell population. In mice, the forkhead winged helix family protein Foxp3 has recently been shown to be expressed predominantly in Treg cells and to be critical for their generation and function (32). It is suggested that Foxp3 and CTLA-4 are associated with regulatory T cells (18, 19, 22). In this study we have demonstrated in active BD, that Foxp3, and CTLA-4 mRNA expression were increased in purified CD4+CD25+high T cells, compared to healthy controls and patients in remission BD. Taken together, our results indicated that CD4+CD25+ regulatory T cells were increased transitory during inflammatory manifestations (clinical exacerbation) and recovered normal values at the remission phase. The suppressive function of CD4+CD25+high T cells in active BD was confirmed by the observation that these cells were hyporesponsive and inhibited the proliferation of CD4+CD25− T cells after polyclonal stimulation.
There have been several reports demonstrating the relationship between CD4+CD25+ regulatory T cells and human inflammatory diseases, including noninfectious uveitis and rheumatoid arthritis (33-36). They showed increases of the CD4+CD25+ regulatory T-cell proportion in peripheral blood or its presence in target organs. In the opposite Kukreja et al. (37) have reported the depletion of CD4+CD25+ T cells in human immune-mediated diseases. Nevertheless, they did not analyze the CD4+CD25+ regulatory T cell-specific markers Foxp3. Therefore it is not clear whether the decreased CD4+CD25+ T cells represented the regulatory T-cell proportion. In the same way, Furino et al. (38) reported decreased CD4+CD25+ regulatory T cells in the acute phase of Kawasaki disease, correlated with a decrease in Foxp3. The precise role of Foxp3 in the development and/or function of Treg cells remains to be elucidated. Thorsten-son and Khorusts (39) have generated CD25+ T cells in the DO11.10 TCR transgenic mouse via oral i.v. administration of peptide Ag and demonstrated that the resulting CD25+ T cells are anergic and have Treg properties in vitro and in vivo (39). Upon adoptive transfer, the expression of CD25 in donor-derived cells is not stable, with CD4+CD25+ cells appearing in CD4+- CD25+ T cell-injected animals and vice versa. The maintenance of CD25 expression by CD4+CD25+ cells depends on IL-2 secreted by co-transferred CD4+CD25+ or Ag-stimulated T cells in peripheral lymphoid organs (40).

The present study shows that patients with active BD, remission BD and healthy subjects expressed similar levels of TGFβ mRNA expression. Treg activity generated by CD25+ cells did not require TGF-β (23, 41). Whatever, the engagement of CTLA-4 was further found to be linked to the production of TGF-β (42). The role of TGF-β in the context of Treg cells has been recently described to be in the induction phase of regulatory process and not necessarily in the suppression itself (41). CD4+CD25+ peripheral blood T cell compartment does not exclusively include TGF-β producing Th1 subtype of regulatory T cells, which is suggested to be antigen-induced regulatory T cells at the tissue site of inflammation (43-45). CD4+ T cells that primarily produce TGF-β appear to be a unique T-cell subset that includes mucosal helper T-cell function and downregulated properties for Th1 and other immune cells and that have been termed Th2 cells (44). Th2-type regulatory cells that secrete TGF-β have been described in human conditions (44). As outlined by Prud’homme GJ and Piccirillo CA (43), TGF-β is produced at high levels by CNS including neurons, and that circulating or locally produced TGF-β can bind to a wide variety of tissue matrix proteins, which creates a tissue reservoir of this cytokine (46). The intensity of CTLA-4 expression has been correlated to the suppressive capacity of Treg cells (47). CD4+ CD25+ Treg cells constitutively express CTLA-4 (47). Signalling through CTLA-4 has been suggested to be essential for the suppressive function of Treg cells.

Recent evidence indicates that CD4+ CD25+ regulatory T cells can also modulate immune responses against pathogenic microbes. Hori et al. (48) have demonstrated that transfer of T cells depleted of CD4+CD25+ regulatory T cells elicited severe pneumonitis in mice with severe combined immunodeficiency (SCID) that were chronically infected with Pneumocystis carinii, whereas transfer of T cells not depleted for regulatory cells did not. Similarly, inflammatory bowel disease in mice with SCID was induced by the transfer of T-cell suspensions depleted of CD4+CD25+ T cells (49), although transfer of nondepleted T-cell suspensions produced no inflammatory bowel disease. Furthermore, mice with SCID raised in germ-free conditions did not have the disease. These findings suggest that CD4+CD25+ regulatory T cells act to suppress hyperimmune responsiveness against microbes.

BD was associated with autoimmune and/or viral aetiology (4). The aetiology of BD remains unknowns with major contributors to inflammatory conditions (IL-6, TNF-α, IFN-γ, …) and CD4+ CD25+ Treg cells implication should be performed with caution. Investigation of the aetiology of BD has focused on HSV (5, 50) and microbial antigens (4, 51). It was shown that microbial induction of Toll pathway in dendritic cells blocked immunosuppression by CD4+CD25+ Tregs, which was in part dependent on IL-6 (52). These “danger” signals could therefore override immnosuppression by Tregs, thus allowing immunity to infection to occur. Such disturbance in
BD process could contribute to the development of inflammation.

The ability of CD4⁺CD25⁺ regulatory T cells to down-regulate immune responses to pathogens argues against their function being inhibited by inflammatory signals (53). On the contrary, local CD4⁺CD25⁺ regulatory T cells homeostasis and function may even be enhanced following infection reactivation, hyperstimulation by HSP, as found in active BD. More investigations are needed in BD to discuss such hypothesis.

In active BD, CD4⁺CD25⁺ T cells may directly or indirectly inhibit the expansion of rival T cells by secretion of inhibitory cytokines. Interleukin-10 (IL-10) have been implicated in T-cell regulation (54, 55). Active BD was characterized by a higher increase of IL-10 compared to remission BD (56, 57) inducing the equilibrium of the immune system and the avoidance of immune pathology that the exuberant expansion of effector T cells is kept in check. In this study we have demonstrated the presence of CD4⁺CD25⁺ regulatory T cells in active BD. Defining the position of CD4⁺CD25⁺ regulatory T cells in BD is a challenge. Regulatory role of CD4⁺CD25⁺-high T cells in TH1 (IFN-γ) production and correlation of these cells with HSP production is under investigation. Further studies are needed to reveal the exact role of Treg cells in BD and to identify the factors regulating their function and accumulation in the peripheral circulation. BD is a multisystemic disease, where blood could reflect certain immune dysregulations. The characterization of the CD4⁺CD25⁺high T cells appears central to defining aberrancies in autoimmunity, transplantation, cancer and chronic inflammation, continued exploration of this population will uncover potential regulatory pathways in the treatment of multiple immunopathological conditions as suspected in BD.

References


