Up-regulation of Th17 and related cytokines in Behçet's disease corresponding to disease activity

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ABSTRACT

Objectives. The IL-23/IL-17 pathway is implicated in the development of certain inflammatory diseases. The aim of the present study was to investigate the expression of Th17 and related cytokines according to clinical activity in Behçet's disease (BD).

Methods. Peripheral blood mononuclear cells (PBMCs) from eleven patients with active BD, eleven patients with inactive BD, ten patients with recurrent aphthous ulcers, and ten healthy controls were cultured and stained with the appropriate fluorescent antibodies for analysis by flow cytometry. ELISA assays were utilised to determine the concentrations of IL-17, IFN- γ , IL-23, and IL-12/23p40 in serum and culture supernatants. IL-12p35, IL-12/23p40, and IL-23p19 transcript levels in PBMCs were measured by real-time PCR.

Results. Significantly higher frequencies of IL-17 and IFN- γ expressing CD4⁺ T cells were observed in patients with active BD compared with control groups. Similarly, levels of IL-17, IL-23, IL-12/23p40, and IFN- γ in serum and supernatants were significantly elevated in patients with BD despite the fact that IL-12p35 and IL-12/23p40 mRNA expression in PBMCs was upregulated in the inactive BD group. In the same patient, the frequency of IL-17 expressing cells decreased when the BD disease activity was stabilised.

Conclusion. The results of this study suggest that up-regulated IL-17 expression may be associated with clinical activity of BD.

Introduction

Behçet's disease (BD) is a multisystemic chronic inflammatory disease characterised by recurrent oral and genital ulcers, ocular lesions, skin lesions, and occasionally accompanied by articular, neurologic, vascular, and gastrointestinal manifestations (1). Although the exact aetiology of BD is not completely understood, it has been suggested that its pathogenesis is characterised by an exaggerated immune response in genetically susceptible individuals (2).

CD4⁺ T helper (Th) cells play an important role in the immune response by coordinating other cellular components of the immune system (3). Two decades ago, Th subsets were first classified as Th-1 and Th-2. Previous studies suggested that BD is prefaced by a Th-1 type immune response. Increased Th-1 related cytokines, mainly interferon (IFN)-y, IL-12, and tumour necrosis factor (TNF)- α , have been documented in BD patients, and this response is more significant in the active clinical stage (4, 5). However, treatment targeting TNF- α such as infliximab and etanercept, showed limited effects that prevented, but did not cure, the progression of BD (6). Therefore, it seems that other immunologic aberrations may be involved in BD pathogenesis.

Recently, a number of other T cell populations have been discovered, and IL-17- expressing Th17 cells have been recognised as a novel T cells group. Th17 cells are characterised by the lineage specific transcription factors RORyt and RORa, cell surface receptors IL-23R and CCR6, and by production of the pro-inflammatory cytokines IL-17A, IL-17F, IL-21, IL-22, and IL-26 (3). The differentiation and maintenance of Th17 cells have been linked not only to IL-1 β , IL-6, IL-21, and TGF- β , but also to IL-23, which is considered to be a key initiating cytokine in the autoimmune development (3). Th17 cells have been implicated in the tissue inflammation found in Crohn's disease and multiple sclerosis which were previously known for Th1-mediated autoimmune disease (7, 8). Furthermore, enhanced expression of IL-23/IL-17 axis has been established in certain inflammatory diseases, such as psoriasis, giving rise to the development of therapeutics that target these cytokines (9). Taken together, these results suggest that Th17 cells play a critical role in autoimmune inflammatory diseases.

In recent studies, Th1, Th17 related cytokines and signalling molecules have been shown to participate in the BD pathogenesis (10-12). Polymorphisms of Th17 related cytokine and receptors, such as IL-17F and IL-23R, have been associated to BD susceptibility (13, 14). The amelioration of intraocular inflammation in BD was accompanied by the suppression of IL-17 production after cyclosporine A administration (15). However, little is known concerning the role of IL-17 and related cytokines and their correlation with disease activity and different clinical disease symptoms. To further evaluate the immunopathological implications of Th17 cells and related cytokines in BD and their correlation to clinical activity, we investigated the intracellular expression of IL-17 and IFN-y on PBMCs and the concentrations of IL-17, IL-23, IL-12/23p40 and IFN-y from serum and culture supernatants. In addition, to clarify the mRNA expression levels of IL-23p19, IL-12p35, and IL-12/23p40, we performed real-time polymerase chain reaction (PCR).

Materials and methods

Patients

The patient population consisted of 22 BD patients (9 women and 13 men; mean±S.D. age, 43.0±6.1 years), who presented for the first time, or were monitored at the Department of Dermatology at Ajou University Hospital from April 2010 to September 2010. Diagnosis was made based on the criteria established by the international study group (16) and the Japanese group (17). BD patients were divided into two groups, the active group and the inactive group. Patients in the active group had at least one of the BD symptoms despite treatment and those in the inactive group were in well-controlled state by taking anti-inflammatory medication at the time of the visit. The disease control and healthy control (HC) group consisted of 10 age- and sex-matched recurrent aphthous ulcer (RAU) patients without any other evident disease and 10 healthy volunteers, respectively. Informed consent was obtained from patients prior to enrolling them into the study. This study was approved by the Institutional Review Board (IRB number: AJIRB-GN3-07-098).

Cell preparation

Peripheral blood mononuclear cells (PBMCs) were prepared from heparinised blood samples by Ficoll Hypaque density gradient centrifugation (Ficoll paque[™] plus; StemCell Technologies, Vancouver, BC, Cananda). Cells were washed with phosphate buffered saline (PBS; Sigma, St. Louis, MO, USA) with 2% heat-inactivated fetal bovine serum (FBS; Gibco-BRL, Grand Island, NY, USA)

Cell culture and stimulation of PBMCs To evaluate the changes of the expression of surface molecules and intracellular cytokines in PBMCs, PBMCs were cultured in medium (RPMI 1640 medium supplemented with 2mM Lglutamine, 100 U/ml penicillin and 100 µg/ml streptomycin) (RPMI 1640; Gibco-BRL, Grand Island, NY, USA) with 10% FBS at 37°C and 5% CO2 and stimulated. IFN- γ was detected in the cells stimulated with anti-CD3 (5 µg/ ml, clone OKT3) and anti-CD28 antibodies (1 µg/ml, clone CD28.6) (eBiosciences, San Diego, CA, USA) for 48 hours. IL-17 was detected in the cells stimulated with IL-1 β (10 ng/ml), IL-6 (20 ng/ml), IL-23 (100 ng/ml), TNF-α (10 ng/ml), and TGF-\beta (1 ng/ml) (R&D systems Inc., Minneapolis, MN, USA) in addition to anti-CD3 and anti-CD28 antibodies for 5 days. During the final 4 hours, 10 µg/ml of Brefeldin A (eBiosciences, San Diego, CA, USA) was added to the cultured PBMCs.

Flow cytometry

For examination of the cell expression of the indicated surface molecules and cytokines, cells were labelled with the following fluorescence conjugated antibodies: APC anti-CD4 (no. 555349), PE-Cy7 anti-CD8 (no. 557746), PE anti-CD45RO (no. 555493) (BD Biosciences Pharmingen, San Diego, CA, USA), PerCP-Cy5.5 anti-IL-17 (no. 45-7179), and FITC anti-IFN- γ (no. 11-7319) antibodies (eBiosciences, San Diego, CA, USA). Staining was carried out using an intracellular staining kit (eBioscience, San Diego, CA, USA) according to the manufacturer's instructions. Cell surface and intracellular expression of each molecule was then analysed by multi-colour flow cytometry (BD FACS ARIA III, San Jose, CA, USA).

ELISA

To measure the concentrations of IL-17, IL-23, IL-12/23p40 and IFN-γ in serum and in culture supernatants (48 hours, 5 days), ELISA was performed. ELISA kits (R&D systems Inc., Minneapolis, MN, USA) were used for measuring IL-17 (no. DY317), IL-23 (no. D2300B), IL-12/23p40 (no. DY1240) and IFN-γ (no. DY285) according to the manufacturer's protocol. All samples were processed in duplicates.

RNA preparation and real-time PCR

Total RNA was extracted using trizol (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions and reverse transcription of RNA was performed using dNTP's and an oligo(dT) primer (Invitrogen, Carlsbad, CA, USA). The samples were incubated for 5 min at 65°C. The cDNA was then amplified in a 20-µl final volume using the SuperscriptTM III kit (Invitrogen, Carlsbad, CA, USA), following the recommendations of the manufacturer. The obtained cDNA was analysed by real-time PCR with the ABI Prism 7000 Sequence Detection System (Applied Biosystems, Foster, CA, USA) according to the protocol provided by the manufacturer using the $2^{-\Delta\Delta Ct}$ method. Primers and internal probes for IL-23p19 (Hs00372324_m1), IL-12p35 (Hs01073447_m1), IL-12/23p40 (Hs01011518_m1), and GAPDH (Hs99999905_m1) were purchased as assays on demand primer-probe sets.

Statistical analyses

Statistical analysis was performed using SPSS 12.0 software (SPSS Inc., Chicago, IL, USA). Differences in the expression of cytokines in PBMCs, serum and culture supernatants between the groups were analysed by the Mann-Whitney U-test. A *p*-value of <0.05was considered statistically significant.

	Active BD (n=11)	Inactive BD (n=11)	RAU (n=10)	HC (n=10)
Age*	42.0 ± 5.1	43.9 ± 7.0	44.5 ± 8.6	39.5 ± 7.8
Gender				
Male (%)	5 (45.5%)	8 (72.7%)	4 (40.0%)	3 (30.0%)
Female (%)	6 (54.5%)	3 (27.3%)	6 (60.0%)	7 (70.0%)

Table I. Baseline demographics of active and inactive BD group, RAU group, and HC group.

Results

Participant characteristics

Baseline demographics and clinical characteristics of each subject group are summarised in Table I. Of the 22 BD patients, 11 (6 females, 5 males) were in the active state and 11 (3 females, 8 males) were in the inactive state. In the active BD group, six patients (54.5%) had oral aphthae, five (45.4%) had EN-like lesions, and one (9.1%) had vascular involvement at the time of sampling. One patient had both oral aphthae and EN-like lesions (Table II).

Total IL-17 expression in PBMCs

Total IL-17 expression on PBMCs of BD patients was investigated using flow cytometry. In the unstimulated state, there was no significant difference in the intracellular expression of IL-17 among the study groups (data not shown). After stimulation, increased frequency of IL-17 expressing T cells in PBMCs was observed in all study groups. The frequencies of IL-17 expressing T cells were significantly higher in the BD group compared with the HC and RAU

Table II. Clinical characteristics of active BD group (n=11) at the time of sampling.

Clinical characteristics	Cases, n.
Major symptoms	
Oral ulcer	6
Genital ulcer	0
Skin lesion	5
Ocular lesion	0
Minor symptoms	
Arthritis	0
Epididymitis	0
GI involvement	0
Vascular lesion	1
CNS involvement	
0 BD: Behçet's disease.	

groups. In addition, there was no significant difference between the active BD and inactive BD groups, IL-17 expressing T cells were significantly increased in the active BD group compared with the HC and RAU groups, but the frequencies of these cells were decreased in the inactive BD group. This resulted in an insignificant difference between the inactive BD group and the RAU group (Fig. 1A). The IL-17 expressing T cell frequency according to the clinical symptoms tended to increase in the patients with erythema nodosum-like lesions, although, due to sample size limitations, there was no significant difference among several clinical symptoms (Fig. 1B).

IL-17 and IFN-\gamma in CD4⁺ and CD8⁺ T cell subpopulations

In the unstimulated state, there was no significant difference in the intracellular expression of IL-17 and IFN-y in CD4⁺ and CD8⁺ T cells among the study groups (data not shown). At 5 days after stimulation, up-regulation of IL-17 expression in CD4+ and CD8+ T cells in response to anti-CD3, anti-CD28, IL-1 β , IL-6, IL-23, TNF- α , and TGF- β stimulation were observed in all study groups. The frequencies of IL-17 expressing CD4+ T cells were significantly increased in the active BD group compared with HC and the RAU groups, even though the inactive BD group exhibited a significant difference compared with the only HC group. In addition, the frequencies of IL-17 expressing CD8+T cells were significantly increased in active BD and inactive BD groups compared with the HC group (Fig. 1C-D). At 2 days after stimulation, up-regulation of IFN-y expression on CD4⁺ and CD8⁺ T cells in response to

anti-CD3 and anti-CD28 stimulation was observed in all study groups. The frequencies of IFN-γ expressing CD4+ T cells were significantly increased in the active BD group compared with HC and RAU groups, however, frequencies of these cells in the inactive BD group were significantly higher than those of HC group, but not the RAU group. The frequencies of IFN-γ expressing CD8+ T cells were significantly increased in the active BD and inactive BD groups compared with the HC and RAU groups (Fig. 2A-B). Our results suggest that expression of IL-17 and IFN- γ in BD patients is higher than those in the HC group.

Levels of IL-17, IFN-γ, IL-23, and IL-12/23p40

In order to determine the quantities of Th17 and Th1 related cytokine in patients with BD, levels of IL-17, IL-23, IL-12/23p40, and IFN-y in serum and culture supernatants were measured. IL-17 levels in the serum were significantly up-regulated in the active BD group compared with the HC and RAU groups. The expression of IL-17 in the supernatants of cultured PBMCs 5 days after stimulation was significantly higher in the active and inactive BD groups compared with the HC and RAU groups (Fig. 3A). The expression of IL-23 in culture supernatants 2 days after stimulation were significantly higher in the active BD and inactive BD groups compared with the HC and RAU groups although IL-23 levels in the serum and culture supernatants at 5 days after stimulation showed no significant difference among the study groups (Fig. 3B). The expression of IL-12/23p40 in the serum and culture supernatants at 5 days after stimulation were significantly higher in the active BD and inactive BD groups compared with the HC and RAU groups, although IL-12/23p40 level in the culture supernatants at 2 days after stimulation showed no significant difference among the study groups. The expression of IL-12/23p40 in serum was significantly higher in the active BD group compared with the inactive BD group (Fig. 3C). IFN-y level was undetectable in the serum of all patients and control groups (data not



Fig. 1. Expression of IL-17 in PBMCs. Isolated PBMCs were stimulated with anti-CD3, anti-CD28, IL-1 β , IL-6, IL-23, TNF- α , and TGF- β for 5 days. The cells were stained for surface marker expression with the appropriate conjugated antibodies and analysed by flow cytometry. Frequencies of total IL-17 expressing T cells in PBMCs according to study groups (**A**) and active BD symptoms (**B**) and dot plots of IL-17 expression in CD4⁺ and CD8⁺ T cells (**C**) and frequencies of IL-17 expressing CD4⁺ and CD8⁺ T cells (**D**) were shown (*p < 0.05). BD: Behçet's disease; RAU: recurrent aphthous ulcer; HC: healthy control; OU: oral ulcer; EN: erythema nodosum-like lesions; VESSE: vascular involvement.

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Fig. 2. Intracellular expression of IFN- γ in CD4⁺ and CD8⁺ T cells. FACS analysis of IFN- γ expression in CD4⁺ and CD8⁺ T cells response to anti-CD3 and anti-CD28 stimulation was performed. Dot plots of IFN- γ expression in CD4⁺ and CD8⁺ T cells (**A**) and frequencies of IFN- γ expressing CD4⁺ and CD8⁺ T cells (**B**) were shown (**p*<0.05). BD: Behçet's disease; RAU: recurrent aphthous ulcer; HC: healthy control.

shown). The expression of IFN- γ in the supernatants of cultured PBMCs 2 days after stimulation was significantly higher in the active BD group compared with the HC and RAU groups. However, frequencies of these cells in the inactive BD group were significantly higher than those of the HC group, but not the RAU group. IFN- γ expression of in the supernatant of cultured PBMCs 5 days after stimulation was significantly higher in the active and inactive BD groups compared with the HC and RAU groups (Fig. 5D). Taken together, the related secretory proteins of Th17 and Th1 were significantly up-regulated in BD patients compared with control groups. Moreover, these cytokines have a tendency to increase in the active BD group compared with the inactive BD group, although a significant difference in IL-12/23p40 was found only in the serum.

mRNA levels of IL-23p19,

IL-12p35, and IL-12/23p40

To support further differences in the Th17 and Th1 response between the BD and control groups, the mRNA level of IL-23p19, IL-12p35, and

IL-12/23p40 in PBMCs was quantified. IL-23p19 mRNA expression was significantly higher in the active BD group compared with the HC group at 2 days after stimulation. In contrast, there were no significant differences between the study groups in the serum and culture supernatants at 5 days after stimulation (Fig. 4A). Expression of IL-12p35 mRNA was significantly higher in the inactive BD group compared with the HC and the RAU groups in the resting state, and IL-12p35 mRNA was significantly higher in the inactive BD group compared with ac-

Supernatant 5 Days



Fig. 3. Level of IL-17 (**A**), IL-23 (**B**), IL-12/23p40 (**C**) and IFN- γ (**D**) in sera and culture supernatants at 2 and 5 days. The production of cytokines in the serum and supernatants, which were stimulated PBMCs in the presence of anti-CD3 and anti-CD28 for 2 days and in the presence of anti-CD3, anti-CD28, IL-1β, IL-6, IL-23, TNF- α , and TGF- β for 5 days, were shown (**p*<0.05). BD: Behçet's disease; RAU: recurrent aphthous ulcer; HC: healthy control.

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tive BD group. At 2 and 5 days after stimulation, there were no significant difference of IL-12p35 mRNA levels among the study groups (Fig. 4B). Expression of IL-12/23p40 mRNA was significantly higher in the inactive BD group compared with the HC and RAU groups at 5 days after stimulation (Fig. 4C). IL-12/23p40 mRNA was significantly higher in the inactive BD group compared with the active BD group compared with the active BD group. Taken together, increased mRNA expression of IL-23p19, IL- 12p35, and IL-12/23p40 were found in BD patients compared with the HC group although this difference was not significant in the active BD group but was significant in the inactive BD group for IL-12p35 and IL-12/23p40.

Frequency of IL-17 expressing T cells after stabilisation of clinical symptoms of Behçet's disease

Among patients showing stabilisation of their disease, the PBMCs from three patients were estimated using FACS to evaluate the frequency of IL-17 expressing cells between the two clinical states. At the time of initial blood collection, they suffered from BD-related symptoms such as oral ulcers, ery-thema nodosum-like lesions and deep vein thrombosis, respectively, which disappeared in the follow-up period after about 2 years. Corresponding to disease activity of BD, all of the three patients exhibited decreased IL-17 expression at the stable state as compared to their active state (Fig. 5).

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Fig. 4. Relative mRNA expression of IL-23p19 (**A**), IL-12p35 (**B**), IL-12/23p40 (**C**) in the PBMCs. PBMCs were cultured in the presence of anti-CD3 and anti-CD28 for 2 days. They were also cultured in the presence of anti-CD3, anti-CD28, IL-1 β , IL-6, IL-23, TNF- α , and TGF- β for 5 days. Total RNAs were analysed by real-time PCR using specific probes for human IL-23p19, IL-12p35, and IL-12/23p40 cDNA sequences, respectively. GAPDH mRNA was used as an internal control (*p<0.05). BD: Behçet's disease; RAU: recurrent aphthous ulcer; HC: healthy control.



Fig. 5. Frequency of IL-17 expression cells according to clinical disease activity of Behçet's disease (n=3). FACS analysis of IL-17 expression in T cells response to anti-CD28, IL-1 β , IL-6, IL-23, TNF- α , and TGF- β stimulation was performed. In the same patient, PBMCs were taken at the active disease state and the stable state of Behçet's disease.

Discussion

Previous studies published conflicting results regarding Th17 related cytokines in patients with BD. The level of IL-23 and IL-17 in the sera showed a significant increase in BD patients with active uveitis compared to BD patients without active uveitis and healthy controls (18). Furthermore, increased expression of IL-23p19 mRNA was found in the EN-like lesion of BD (19). The expression of RORC, known for Th17 related transcription factor, was increased in the cerebrospinal fluid from patients with neuro-Behcet's disease, which indicate that Th17 cells may be involved in BD pathogenesis (20). In contrast, Ferrante et al. (21) found that mRNA and serum level of IL-17 and IL-23 in BD patients, who have gastrointestinal involvement, were not significantly different from healthy controls.

In the current study, IL-17 expressing T cells were significantly increased in patients with BD compared with the RAU and HC groups. This is especially the case for the active BD group, which showed a marked increase of IL-17 expression compared to both control groups (Fig. 1A). Furthermore, the concentrations of IL-17 in serum and culture supernatant were significantly up-regulated in the active BD group as compared with the HC and RAU groups (Fig. 3A). Our results support previous studies that suggest that IL-17 may be involved in the pathogenesis of BD (22, 23) and that the production of IL-17 has a tendency to increase in the active BD group compared to the inactive BD group although this was not significant. We also evaluated the expression of IL-17 in PBMCs according to BD patient symptoms (Fig. 1B).

However, since the sample size of each symptom group was small, it is difficult to evaluate the tendency of IL-17 expression as it relates to BD symptoms. Future studies with a larger sample size are needed to identify the exact level of IL-17 and the correlation of IL-17 and each BD symptoms.

The CD4+CD45RO⁺ (memory) T cells have been demonstrated to be a major resource in the production of IL-17 in humans (24). In the present study, we observed that IL-17-expressing CD4⁺ memory T cells were significantly increased in patients with BD compared to control groups (Fig. 1D), in consistence with Chi *et al.* (18). Interestingly, increased expression of IL-17 was also seen in CD8⁺ T cells in this study, which had rarely been reported in BD pathogenesis previously. It has been demonstrated that CD8⁺ T cells in inflammatory skin disease are significant source of Th17 related cytokines including IL-17 and IL-22(25). Huber *et al.* (26) also reported that Th17 accumulation and development of autoimmunity required IL-17 producing CD8⁺ T cells (Tc17), indicating that Tc17 cells are essential to promote CD4⁺ T cell-mediated induction of autoimmunity. Taken together, we suggest that both Th17 and Tc17 cells play a critical role in the development and maintenance of BD and their amount may correlate with the active inflammation exhibited by BD patients.

Recently, it has been established that inflammatory cytokines such as IL-21, IL-23, IL-27 are required in induction of Th17 cells (27-29). In particular, there is growing evidence that IL-23 is linked to the pathogenesis of autoimmune inflammation (9, 30, 31). Therefore, we investigated the role of IL-23 in BD pathogenesis. From our results, IL-23 levels in culture supernatants and IL-23p19 mRNA in active BD patients were significantly elevated compared with those in the HC group after the stimulation (Fig. 3B, 4A). In a similar manner, Habibagahi et al. (32) found higher IL-23 in sera of patients with BD, and other investigators revealed that expression of IL-23p19 mRNA was up-regulated in BD patients with active uveitis and EN-like lesions, respectively (18, 19). Moreover, it has recently been proven that IL-17 mRNA expression was induced only in the presence of IL-23 (28) and recombinant IL-23 augments the production of IL-17 in CD4⁺ T cells in BD patients (33). We have also shown that IL-12/23p40 cytokine levels and mRNA expression, which is a common subunit of IL-12 and IL-23, were increased in BD patients compared with control groups (Fig. 3C, 4C). IL-12 and IL-23 are the critical cytokines to promote the differentiation to Th1 and Th17, respectively. Along with the above-mentioned data, we demonstrated increased frequencies of IFN-y expressing T cells and increased serum levels of IFN-y in active BD patients compared with control groups (Fig. 2, 3D). This finding implies that BD is associated with a mixture of Th1/Th17 cytokines. Similarly, increased memory CD4⁺ T cells producing IL-17 and IFN- γ simultaneously were observed in BD patients (28). All these data suggest that IL-23 has a possible role in the Th1 and Th17 induction in the pathogenesis of BD. Interestingly, mRNA expression of IL-12/23p40 was significantly increased in the inactive BD group compared to the active BD group, which did not correlate with serum levels. This result suggests that other regulatory mechanism may be involved in the IL-12/23 production found in BD.

We also investigated the frequency of IL-17 expressing cells corresponding to disease activity of BD in PBMCs from the same patient. The mean duration turning the active to the inactive state was 23 months. All Three patients in the active state showed an increased tendency of IL-17 expression than those in the stable state (Fig. 5). Similarly, several investigators demonstrated elevated levels of IL-17 in active BD patients compared to BD patients in remission after treatment (15, 22, 34). From these results, it is plausible to suggest that IL-17 may be involved in acute inflammatory attack of BD. In the present study, RAU patients were enrolled as a disease control, which is different from other studies. Unlike BD patients, RAU patients have only recurrent oral ulcers without any systemic manifestations and our results revealed that expression of IL-17 was significantly different between active BD and RAU groups, indicating that BD may be a distinct entity from RAU. We also cultured PBMCs with several cytokines, which induce Th17 differentiation. Although up-regulation of IL-17 expression was observed in all study groups compared with resting state, this increment was significantly higher in the active BD group compared to control groups. Similarly, production of IL-17 was significantly enhanced in active BD compared with healthy controls in response to phorbol myristate acetate and ionomycin, or interphotoreceptor retinoid binding protein stimulation (22, 23). These results imply that Th17 immune response by certain stimulus may be more sensitive in BD patients and associated with BD pathogenesis.

Conclusion

In conclusion, expression of IL-17 and IL-23 was significantly increased in patients with active BD group compared with control groups. Corresponding to the clinical activity, expression of IL-17 after disease stabilisation was decreased compared to the active state from the same patient. These results suggest that Th17 cells may be associated with the active inflammation of BD.

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