Differential expression of monocyte/macrophage markers between active and inactive stage of patients with Behçet’s disease

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Abstract

Although the exact etiology of Behçet’s disease (BD) remains unclear, a complex interaction between T cells and antigen-presenting cells is known to be involved in the immunopathogenesis of BD. This study aimed to identify differentially expressed cell surface markers of peripheral blood mononuclear cells (PBMCs) in active and inactive stage of BD patients. PBMCs were isolated from six healthy controls, eight inactive BD patients and five active BD patients. Different cell phenotypes were analyzed by flow cytometry, serum cytokine levels were detected by ELISA and the morphological structure of polymorphonuclear neutrophils (PMN) was revealed by transmission electron microscope (TEM). The CD11b monocyte marker was slightly decreased in active BD patients (91.5±10.9%) compared with healthy controls (97.5±1.3%), but was not different compared to inactive BD patients (88.8±12.2%). The CD14 monocyte marker was significantly increased in active BD patients (28.9±18.7%, p=0.05) and inactive BD patients (30.8±21.4%, p=0.08) compared to healthy controls (11.1±3.7%). However, CD16 (FcγRIIIA) was higher in inactive BD patients (93.9±2.4%) than active BD patients (85.3±14%), and CD32 (FcγRII) was down-regulated in active BD patients (26.6±18.1%) compared to inactive BD patients (46.6±30.3%) and healthy controls (71.7±17.4%; p=0.002). Most surprisingly, the mannose receptor marker CD206 was highly expressed with significance in active BD patients (49.7±35.2%) compared to healthy controls (7.4±0.8%) (p=0.02) and inactive BD patients (4.7±3.1%) (p=0.007). In spite of the up-regulation of CD206 in active BD patients, interleukin-10 was markedly increased in the inactive state after improving medication than in the active state. All these findings show that differential surface expression of PBMCs between the inactive and active state of BD patients may influence changes of the disease state following treatment.

Keywords: Behçet’s disease, monocyte/macrophage, active and inactive stage, surface markers

Introduction

Behçet’s disease (BD) is a rare chronic inflammatory disease characterized by recurrent oral and/or genital aphthous ulcersations, uveitis and skin lesions. Clinical presentation of this disorder is multifaceted with severe chronic inflammation accompanied by articular, central nervous system, gastrointestinal, renal, urogenital, pulmonary and cardiovascular manifestations, all of which are associated with systemic vasculitis, a pivotal pathophysiological feature of BD [1-4]. The exact pathogenesis of BD remains unclear, but autoimmune and autoinflammatory reactions are important [5]. Initially in BD, infiltrated types of cells include CD4+ and CD8+ T cells, macrophages and dendritic cells, followed by neutrophils [6]. Th1/Th2-type immune responses have been investigated in cell-mediated immunity and inflammation in BD [7]. T helper (Th) 1 and Th17 predominant response has been observed in many studies in patients with BD; the response involves the increased production of cytokines including interleukin (IL)-2, IL-6, IL-8, IL-17, IL-12, IL-18, tumor necrosis factor-alpha (TNF-α), and interferon-gamma (IFN-gamma) [8,9]. CD11b is expressed on neutrophils, monocytes, natural killer (NK) cells and a subset of lymphocytes. CD11b has been implicated as having a central role in the migration of leukocytes from peripheral blood to the sites of inflammation [10,11], and is also involved in adhesion, chemotaxis and diapedesis during the process of host defense [12]. A previous study involving the examination of cultured monocytes from BD patients reported the significantly elevated expression of the CD11a, CD11b and CD18 adhesion molecules compared to cells from healthy subjects [13].

CD14 is a co-receptor of innate immunity. BD patients display up-regulated CD14 expression on monocytes and neutrophils and elevated serum soluble CD14 levels [14,15]. Very early activation confirmed by CD69 and CD14 response to heat shock protein 60 (HSP60) on peripheral blood mononuclear cells (PBMCs) of BD patients might be associated with an HSP60-induced innate activation through antigen presenting cells (APCs) [16]. CD16 is a Fc receptor (Fc RIII) that has been directly associated with neutrophil activation. Normally, CD14 and CD16 are found together in secretory vesicles of neutrophils...
and, when neutrophils are stimulated, CD14 and CD16 co-
migrate to the plasma membrane [17]. The intensity of CD16
expression in patients with BD is equivocal [15,18]. In addition,
FcyRII, namely CD32, has been detected on T cells, mast cells,
monocytes, macrophages, and some epithelial and endothelial
cell lineages. The primary function of CD32 appears to be
antibody-mediated uptake of antigen and modulation of
cellular activation and maturation events [19,20].

Macrophage mannose receptor (MMR), also known as
CD206, is a scavenger receptor that is expressed primarily by
tissue macrophages and lymphatic and hepatic endothelia
in humans and mice [21,22]. MMR’s carbohydrate pattern
recognition, potent capacity of endocytosis, and role in
phagocytosis of microorganisms support a dual role in host
defense and homeostasis [23]. In addition, CD206 has been
identified in a variety of autoimmune and inflammatory
diseases, such as systemic lupus erythematosus, ulcerative
colitis, and Crohn’s disease [24,25]. However, the role of
mannose receptor with other cell surface marker on PBMCs
of active and inactive BD patients remain poorly understood
in the host defense. In the present study, we investigated
the pattern of cell-surface expression of CD11b, CD14, CD16,
CD32 and CD206 on PBMCs of active and inactive BD patients
before and after medications, respectively. We also attempted to characterize the serum IL-10 levels and
cell surface expression during transformation of active to
inactive form of BD.

Materials and methods

Patients and healthy controls
The patient population consisted of 13 patients with BD
who presented for the first time or were monitored at the
Department of Dermatology, Yonsei University Hospital,
Seoul, Korea. According to the International Study Group
for BD criteria, the presence of any two of the following
symptoms in addition to recurrent oral ulceration is diagnostic:
genital ulceration, skin lesions, joint involvement, and ocular
lesions. Presently, active BD patients had at least two of
the BD symptoms and inactive BD patients who received
anti-inflammatory medication were well controlled with no
symptomatic states. Two of eight inactive BD patients were
transferred from the active BD patient group. The control
group consisted of six healthy volunteers (three women
and three men; mean age, 29.6±3.5 years), the inactive BD
patients consisted of eight (six women and two men; mean
age, 48.4±15.0 years) and the active BD patients consisted
of five (four women and one man; mean age, 30.0±8.6 years).
Detailed clinical characteristics and therapeutic history of these
patients are presented in Tables 1 and 2. Written informed
consent was obtained from all participants prior to enrolling
them into this study in accordance with the guidelines of the
Declaration of Helsinki Principles.

Cell preparation
PBMCs were isolated from heparinized venous blood by ACK
lysing buffer. The cells were washed twice in phosphate-
buffered saline (PBS) and then resuspended in PBS. The cell
susensions were finally adjusted to a concentration of 1 x
10^6 cells/ml and were processed further for cellular staining
studies.

Flow cytometry
PBMCs were surface-stained with anti-human antibodies CD14
(phycoerythrin (PE)-cy7), CD11b (fluorescein isothiocyanate,
FITC), CD16 (PE), CD32 (Allophycocyanin, APC) (eBiosciences,
San Diego, CA, USA) and CD206 (Per-CP) (BD Biosciences

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Table 1. Clinical and laboratory characteristics of inactive and active BD patients.

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Pharmingen, San Diego, CA, USA) for 30 min at 4 °C in the dark. Isotype control antibodies were used to estimate the non-specific binding of target primary antibodies. Stained cells were analyzed by flow cytometry using a FACS Canto II (Becton Dickinson, San Jose, CA, USA) with ≥10,000 gated lymphocytes.

Enzyme-linked immunosorbant assay (ELISA)
Serum was obtained from patients and healthy controls and analyzed using commercial ELISA kits for the detection of IL-10 (R&D Systems, Minneapolis, MN, USA) according to the manufacturer’s instructions. The mean and standard deviation were calculated using ELISA values determined for each well. The ELISA reader was Bio-Rad 170-6850 microplate reader (Bio-Rad, Hercules, CA, USA) and samples were read at a wavelength of 450 nm.

Transmission electron microscopy (TEM)
PBMCs were isolated from whole blood of healthy control, inactive and active BD patients and the morphological changes were observed using EM 902A transmission electron microscope (Zeiss, Oberkochen, Germany). In brief, cells were fixed using Karnovsky’s fixative solution (2% paraformaldehyde, 2% glutaraldehyde, 0.5% calcium chloride in cacodylate buffer, pH 7.2) for 30 min, washed with cacodylate buffer, dehydrated in a series of graded ethanol and embedded in Epon mixture. After polymerization, ultrathin sections were cut using on Reichert Jung Ultracut S (Leica, Vienna, Austria), mounted on grids, stained with uranyl acetate and lead citrate and analyzed by TEM.

Statistical analysis
Statistical analysis was performed using SPSS 11.0 software (SPSS, Chicago, IL, USA) and analyzed by Kruskal-Wallis Test and Bonferroni correction. A value of p<0.05 was considered statistically significant.

Results
Clinical and laboratory features of BD patients
All five active BD patients had severe manifestations consisting of oral ulcers with genital ulcers and skin lesions during the course of the disease. One patient (patient b) did not show genital ulcers and skin lesions, but did have ocular lesions. In addition, two patients (patient c and patient d) had joint complications. However, gastrointestinal infection, neurological involvement and vasculitis were not observed during the study period (Table 1). The laboratory tests performed were pathergy test, HLA-B51 detection, erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP) level. Patients with active disease may generate an acute-phase response leading to a significantly raised ESR than inactive disease (37.8±21.39 mm/h and 11.5±6.76 mm/h, p=0.007; respectively). Serum level of CRP was varied from <0.01 mg/dL to 2.4 mg/dL in active BD patients, and in inactive BD patients was from <1.0 mg/dL to 1.54 mg/dL. Genetic factor HLA-B51 was positively detected in two active and two inactive BD patients. Finally, a pathergy test was performed; none of the active BD patients showed a positive result (Table 1). After blood sampling, active BD patients (Patient a and b) started treatment. Inactive BD patients were treated with colchicine, prednisolone, and azathioprine (n=1); colchicine, prednisolone, azathioprine, and cyclosporine (n=1); colchicine (n=3); colchicine and aspirin (n=2) and prednisolone (n=1). When the active BD patients were changed to inactive stage after medication (Table 2), blood was collected for laboratory analysis.

Differential surface expression on PBMCs of active and inactive BD patients
To identify differently expressed cell surface markers between active and inactive BD patients, PBMCs were isolated from healthy volunteers, active and inactive BD patients and labeled with antibodies and analyzed by flow cytometry. The CD11b monocyte marker was slightly decreased in active BD patients (91.5±10.9%) compared to healthy controls (97.5±1.3%), but was not different compared to inactive BD patients (88.8±12.2%). Monocyte marker CD14 was significantly increased in active BD patients (28.9±18.7%, p=0.05) and inactivate BD patients (30.8±21.4%, p=0.08) compared to healthy control (11.1±3.7%). Fc receptor contributes to the protective role of immune system by binding to pathogens.

Table 2. Therapeutic history of inactive BD patients.

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<th>Azathioprine</th>
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*, These two inactive patients were improved from active patients-a and b.
[26]. CD16 (FcyRIIIA) is expressed on NK cells, macrophages and neutrophils. Presently, CD16 was higher in inactive BD patients (93.9±2.4%) than active BD patients (85.3±14.9%). CD32 (FcγRII), which is important in regulating adaptive immunity [27], was down-regulated in active BD patients (26.6±18.1%) compared to inactive BD patients (46.6±30.3%; p=0.04) and healthy controls (71.7±17.4%, p=0.002). Most surprisingly, the mannose receptor marker CD206 was highly expressed with significance in active BD patients (49.7±35.2%) than in healthy controls (7.4±0.8%, p=0.02) and inactive BD patients (4.7±3.1%, p=0.007) (Figure 1).

**Characterization of CD11b+ subsets in active and inactive BD patients**

CD11b is present in neutrophils, NK cells and macrophages, and is overexpressed in chronic obstructive pulmonary disease (COPD) [28]. CD14, the receptor for lipopolysaccharide binding protein, is expressed to a higher degree in blood monocytes than in tissue macrophages [29]. To determine whether the frequencies of co-expressed markers with CD11b+, CD14+, CD16+ or CD32+ cells were analyzed in PBMCs from healthy controls, active and inactive BD patients by flow cytometry. The frequencies of the CD11b+CD14+ cells in inactive and active BD patients were almost similar (27.7±4.1% and 25.9±16.3%, respectively), but were higher than healthy controls (11.0±3.7%) (healthy vs inactive, p=0.03; healthy vs active, p=0.06). Although the frequencies of the CD11b+CD16+ cells were not significantly different among the groups (healthy 85.5±4.7%, inactive 85.7±9.4% and active 82.2±15.9%), and the frequencies of the CD11b+CD32+ cells were significantly down-regulated in active BD patients (24.8±18%) (p=0.002) and inactive BD patients (47.8±27.8%) (p=0.04), as compared to healthy controls (70.6±18.2%). In addition, active BD patients showed down-regulation of CD11b+CD32+ cells compared to inactive BD patients (Figure 2).

**Up-regulated expression of CD14+CD16+ subsets in active and inactive BD patients**

CD14+ cells were increased in both inactive and active groups as compared to healthy controls (Figure 1). Similarly, double positive CD14+CD16+ cells were also significantly increased in inactive BD patients (36.1±22.4%, p=0.008) and active BD patient (22.9±17.6%, p=0.02) compared to healthy control (1.7±0.6%). CD14+CD16+ cells were reduced in active BD patients compared to inactive BD patients. Although the difference was not found significant, a similar pattern of surface expression was observed after analysis of CD14+CD32+ cells in inactive BD patients (18.0±13.3%) and active BD patients (10.4±4.4%) and healthy controls (7.9±1.3%) (Figure 3).

**Increased expression of mannose receptor CD206 in active BD patients**

To investigate the expression of mannose receptor in association with monocyte/macrophage subsets, we analyzed...
CD11b⁺CD206⁻ and CD14⁺CD206⁻ cells in inactive and active BD patients. These populations were highly expressed in active BD patients with significance compared to healthy control (CD11b⁺CD206⁻ cells: 49.4±35.6% in active, 7.4±0.9% in healthy, p=0.02; and CD14⁺CD206⁻ cells: 14.6±9.6% in active, 5.6±1.4% in healthy, p=0.05) and inactive BD patients (CD11b⁺CD206⁻ cells: 49.4±35.6% in active, 4.4±3.1% in inactive, p=0.007; CD14⁺CD206⁻ cells: 14.6±9.6% in active, 4.4±2.9% in inactive, p=0.02) (Figure 4).

Change of PBMC surface markers after improvement in two BD patients
Among the five active BD patients, two patients (patient a and b) were followed after treatment with colchicine, prednisolone and azathioprine in the absence or presence of cyclosporine (Table 2). When the symptoms changed to the inactive state, the surface expression on PBMCs was analyzed by flow cytometry.

After improvement, the frequencies of CD14⁺ cells were highly increased in both patients at the inactive stage compared to the active stage (patient a from 29.8% to 53.0%; patient b from 7.8% to 44.4%). The frequencies of CD11b⁺ cells were slightly decreased at the inactive stage (81.9%) compared to the active stage (90.2%) in patient a, and were not different in patient b (inactive 96.4% vs active 96.7%). The frequencies of CD14⁺CD11b⁺ cells were also higher at the inactive stage (patient a 37.7%, patient b 42.4%) compared to the active stage (patient a 25.9%, patient b 7.4%) (Figure 5A). The frequencies of CD16⁺ cells were slightly increased at the inactive stage (patient a 95.8%, patient b 97.6%) compared to the active stage (patient a 95.8%, patient b 97.6%) (Figure 5A). The frequencies of CD16⁺CD11b⁺ cells were lower at the inactive stage (78.7%) compared to the active stage (86.6%) in patient a, but was similar in patient b (inactive 94.6% vs active 93.0%). In addition, double positive CD16⁺CD14⁺ cells were up-regulated at the
Figure 4. Frequencies of CD206 subsets are highly up-regulated in active BD patients. PBMCs were isolated from healthy control (n=6), inactive (n=8) and BD (n=5) patients. The surface expression of CD11b+CD206+ and CD14+CD206+ was analyzed by flow cytometry.

inactive stage (patient a 53.1%, patient b 43.3%) compared to the active stage (patient a 25.5%, patient b 3.8%) (Figure 5A).

In patient a, the frequency of single CD32+ cells was decreased at the inactive stage (active 34.0% vs inactive 9.6%), but was increased in patient b (active 54.8% vs inactive 85.8%). The frequency of double positive CD32+CD11b+ cells in patient a was also decreased at the inactive stage (active 29.9% vs inactive 9.2%), but in patient b, the frequency of CD32+CD11b+ cells was increased (active 53.7% vs inactive 84.7%). In addition, the frequency of CD32+CD14+ cells was decreased at the inactive stage (active 17.8% vs inactive 9.6%) in patient a, but was increased in patient b (active 6.0% vs inactive 38.1%). The frequencies of CD32+, CD32+CD11b+, and CD32+CD14+ cells showed opposite pattern of result between patient a and b (Figure 5B).

The frequencies of CD206+ cells were highly up-regulated in the active stage (patient a 67%, patient b 68.1%) compared to the inactive stage (patient a 6%, patient b 2.5%). Furthermore, the frequencies of CD206+CD11b+ and CD206+CD14+ cells were also increased at the active stage (patient a 65.8% and 28.4%, respectively; patient b 67.9% and 7.3%, respectively) compared to the inactive stage (patient a 5.9% and 5.9%, respectively; patient b 2.4% and 2.5%, respectively) (Figure 5C).

Observation of intracellular morphology of polymorphonuclear neutrophils (PMNs) by TEM

PMNs contain two types of chemically distinct cytoplasmic granules, which appear at different stages of maturation. The larger and dense azurophilic granule (or primary granule) is formed during the promyelocyte stage and contains myeloperoxidase in addition to numerous lysosomal enzymes, neutral proteases, glycosaminoglycans, cationic bactericidal proteins and lysozyme. The specific granule (or secondary granule) is formed during the myelocyte stage. Mature PMNs contain both types of granules: 33% azurophilic and 67% specific granules [30]. To observe the intracellular changes occurred at different stages of BD, we isolated PBMCs from whole blood of healthy controls, and inactive and active BD patients, and observed with TEM. In this study, PBMCs had a normal structural appearance in the healthy control and inactive stage groups. In contrast, huge azurophilic granules were aggregated in the cytoplasm of the neutrophils in active BD patients (Figure 7).

Discussion

The exact etiology and immunopathological features of BD are not clear yet. But, immunological properties may play a role in disease sequela. The frequencies of CD11b+ cells in neutrophils are higher in BD patients than in healthy controls [31]. CD8+CD11b+ cells are reportedly increased in BD patients compared to healthy controls [32], and thalidomide treatment down-regulates CD8+CD11b+ cells [18]. In another study, CD11b was significantly high in active compared to inactive BD patients or healthy control [33]. However, our data showed lower frequencies of CD11b in BD patients than in healthy controls, although the difference was not significantly different. The prior study reported on BD patients with ocular lesion,
Figure 5. The frequencies of surface expression were changed in after improvement of two BD patients. PBMCs were isolated from two BD patients (patient a and b) before and after treatment with colchicine, prednisolone and azathioprine with or without cyclosporine. A~C. The surface expression of CD11b, CD14, CD16, CD32, CD206 and their subsets was analyzed by flow cytometry.
but in our five patients, only one had ocular BD. Therefore, the difference of CD11b expression pattern to the study of Ahn et al., could be explained by different symptom composition.

Presently, the CD11b blood monocyte marker was lower in active BD patients than healthy controls, but the CD14 monocyte marker was highly expressed in inactive and active BD patients compared to healthy controls. In a previous study, Eksioglu-Demiralp et al., reported similar expression pattern of CD14 in BD [15]. Houman et al., reported no significant differences in the proportion of CD11b^+CD14^+ cells between the active and inactive stages, but CD11b^+CD14^+ cells were highly expressed compared to healthy controls [34]. Our data also demonstrated a similar expression pattern as reported by Houman. CD11b^+CD32^+ cells were significantly down-regulated in active and inactive BD patients compared to healthy controls, whereas no significant differences were evident in the proportion of CD11b^+CD16^+ cells between healthy controls and both active and inactive BD patients. These results indicate that among the CD11b^+ subsets, CD11b^+CD14^+ and CD11b^+CD32^+ cells are important in the induction of BD, but are unrelated with symptoms. The CD11b^+CD14^+ and CD11b^+CD32^+ subsets were not significantly different between active and inactive BD patients. The CD14^+ subsets and CD32^+ subsets in CD11b^+ population displayed a reciprocal negative regulation according to the inhibition of CD32^+ macrophage activation from CD14^+ monocytes. CD14 is a membrane-bound protein that is expressed in monocytes, macrophages, polymorphonuclear neutrophils [35] and dendritic cells [36]. CD32 is a marker of indication in monocyte activation [37] and one of the Fc-IgG receptors [38]. Fc-IgG receptors contribute to the pathogenesis of immune complex- and auto-antibody mediated diseases such as vasculitis, rheumatoid arthritis or autoimmune neutropenia [38,39]. CD32 also plays an essential role in the removal of antigen-antibody complexes from the circulation and cell-to-cell interactions mediating antibody-dependent cell-mediated cytotoxicity. CD14^+CD16^+ monocytes are reportedly increased in sepsis patients with severe infection [40] and efficiently produce the pro-inflammatory cytokine TNF-α, while they produce no or little of the anti-inflammatory cytokine IL-10 [41]. Our results also showed significantly up-regulated frequencies of CD14^+CD16^+ subsets in BD patients (Figure 3) and markedly elevated IL-10 levels in improved inactive BD patients compared to active BD patients (Figure 6). In BD patients, the frequencies of CD14^+CD16^+ cells were higher in the inactive stage than in the active stage, although the difference was not statistically significant. In addition, a similar pattern was also observed in CD14^+CD32^+ cells.

A recent study reported that anti-TNF-α monoclonal antibodies (Infliximab and Adulimumumab) are effective in treating patients with Crohn’s disease (CD), which might contribute to the resolution of inflammation [42,43]. In addition, antibody against TNF-α induced the formation of a new population of macrophages in a Fc region-dependent
manner; these macrophages had an immunosuppressive phenotype because they inhibit the proliferation of activated T cells, produce anti-inflammatory cytokines, and express the macrophage marker CD206 [44]. However, in this study, a clear and significant increase was observed in the frequencies of CD206+CD11b+CD206+ cells and CD14+CD206+ cells in active BD patients compared to the inactive stage. Significant down-regulation was also observed in each individual inactive BD patient, which was recovered after receiving a combined therapy. Although patients with inflammatory bowel disease responding to infliximab displayed increased numbers of CD206+ cells [45], our data show that combination therapy with colchinic, prednisilone and azathioprine with or without cyclosporine improved BD symptoms with the reduction of CD206 macrophages. Although there have been several reports on mannose binding lectin polymorphism in BD [46-48], until now there was no report on mannose receptor related one. Here, we report that mannose receptor CD206 is related to BD, and specifically the presence of symptoms in active BD patients.

Although no differences were evident in the frequencies of CD11b+CD14+ cells between the active and inactive stages (Figure 2), after improvement with the combination therapy the frequencies of CD11b+CD14+ cells were markedly increased in the improved stage in both patient groups.

In a previous study, the frequencies of CD14+CD16+ monocytes were lower in rheumatoid arthritis patients compared to normal subjects [49]. In this study, the frequencies of CD14+CD16+ were higher in BD patients compared to normal subjects (Figure 3), and treatment up-regulated CD14+CD16+ cells in both patients. No effect of cyclosporine related to the frequencies of CD16+CD14+ cells after improvement was evident (Figure 5A). An opposite pattern of expression was observed in CD32+ subsets between both patients after improvement, in which the frequencies of CD32+CD11b+ and CD32+CD14+ cells were lower in the inactive stage of patient a, but was higher in patient b. These data show that a complicated immune response regulated the improvement of BD patients, which may vary with their choice of drugs or may be related to the ocular involvement.

PMNs are the most abundant white blood cells in the peripheral blood of humans, and are associated with the host defense. They are known as the “first line defense”, particularly against bacterial infections [50]. Because of their cytotoxic and proteolytic potential, PMNs can also attack and damage the surrounding tissue, and thus can contribute to destructive inflammatory processes [51]. There is increasing evidence that PMNs are not only effector cells of the acute inflammatory reaction, but that they also participate in chronic inflammatory diseases, such as rheumatoid arthritis, primary vasculitis and inflammatory bowel disease [52,53]. In this study, we found a distinct morphological difference in PMNs of active BD patients, in which huge azurophilic granules were aggregated in the cytoplasm of the neutrophil in active BD patients. In contrast, in inactive BD patients, the granules had disappeared or were decreased. Koga et al., also reported that the quantity of intracytoplasmic granules in blood monocytes and macrophages were correlated with disease severity in Kawasaki disease [54]. These intracytoplasmic granules store inflammatory mediators and are considered structural markers of inflammation [55], therefore, having a role in pathology of inflammatory diseases.

In conclusion, a comparative analysis of different expression of monocyte/ macrophage markers revealed a considerable variation in phenotypes between active and inactive stage of BD patients. The CD14 monocyte marker was highly expressed in active and inactive BD patients compared to healthy controls, but another monocyte marker, CD11b, was decreased in active BD patients compared to healthy control. Moreover, their subsets were also expressed differently in active BD patients compared to healthy control. Mannose receptor (CD206) and its subset were consistently highly expressed in active BD patients compared to inactive BD patients. Furthermore, the recovery state of the BD patients showed down-regulated frequencies of CD206 in both patients with or without ocular symptoms after treatment with colchicine, prednisilone and azathioprine with or without cyclosporine. In accordance with CD206, anti-inflammatory cytokine IL-10 was highly up-regulated in improved state with combined drugs with or without cyclosporine. Taken together, the data reveal that the peripheral inflammatory environment during active stage of BD might be dominated by monocytes, which depend on the expression of surface markers representing polarized phenotypes. In the future, we shall perform the large scale study to overcome this limited cases.

Competing interests
The authors declare that they have no competing interests.

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References


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