The expression of transmembrane and soluble CXCL16 and the relation with interferon-alpha secretion in patients with Behçet’s disease

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The aim of this study was to investigate the relation between the secretion of IFN-α and the expression of CXCL16 on surface of plasmacytoid dendritic cell from patients with Behçet’s disease, and compare it with patients with ankylosing spondylitis and healthy controls.

Methods. The study population consisted of 73 cases (35 with Behçet’s disease, 19 with ankylosing spondylitis and 19 controls). We investigated the expression of CXCL16 on surface of plasmacytoid dendritic cells by flow cytometry, and the serum levels of IFN-α and CXCL16 with ELISA.

Results. Serum levels of IFN-α in patients with Behçet’s disease were significantly higher than the controls (p=0.009), and than patients with ankylosing spondylitis, but not statistically significant (p=0.124). Serum levels of CXCL16 in patients with Behçet’s disease and patients with ankylosing spondylitis were significantly higher than controls (p=0.009, p=0.003, respectively). We found no difference in the percentage and MFI of plasmacytoid dendritic cells and CD123+CXCL16+ cells determined by flow cytometry among the study and control groups. In patients with Behçet’s disease, a positive correlation was found between the percentage of plasmacytoid dendritic cells and CD123+CXCL16+ cells (p<0.001). Furthermore, there was also a positive correlation between the percentage of plasmacytoid dendritic cells and serum levels of CXCL16 in patients with ankylosing spondylitis (p=0.001). In addition, there was a positive correlation between the percentage of CD123+CXCL16+ cells and serum levels of IFN-α in Behçet’s disease group (p=0.034). We could not find any significant difference in other comparisons.

Conclusion. We suggested that the expression of transmembrane CXCL16 on surface of plasmacytoid dendritic cell might contribute to high serum IFN-α levels seen in patients with BD.

Introduction

Behçet’s disease (BD) is a form of systemic vasculitis characterised by relapsing episodes of oral aphthous ulcers, genital ulcers, skin lesions and ocular lesions. Other systems including vascular, gastrointestinal and neurological systems can be affected, while much rare. It was first described in 1937, and since then, several pathogenic mechanisms have been proposed, although none of them is widely accepted (1).

One of the most postulated in the pathogenesis of BD has been the hypersensitivity of T cells to different antigens (2). Actually the cause of this hypersensitivity remains unclear. It has been proposed that this can be associated with abnormalities in antigen presenting cells (APCs) (3). In this regard, abnormalities in dendritic cells (DCs) have been suggested to have a major role in the pathogenesis of BD (4).

Dendritic cells are thought to be the most effective antigen presenting cells to activate T cells. There are two main subsets of DCs; myeloid and plasmacytoid DCs. Plasmacytoid dendritic cells (pDC) have important roles in innate immunity, and T cell activation. The
The study was approved by local ethics and controls signed informed consent. Non-steroidal anti-inflammatory agents were used to relieve any discomfort caused by the sampling, we excluded those who had active disease at the time of the sampling, showing at least 2 clinical manifestations of disease, including oral ulcer, genital ulcer, uveitis, uveitis, and vesicle involvement, which was the major mechanism for IFN-α secretion (6). Previously, we demonstrated pronounced IFN-α production from pDC in patients with Behçet’s disease (BD) following CpG D ODN stimulation (7). The aim of this study was to investigate the relation between the secretion of IFN-α and the expression of CXCL16 on surface of pDC from patients with BD, and compare with patients with ankylosing spondylitis (AS) and healthy controls (HC).

Patients and methods

Study population

A total of 73 patients, 35 with BD, 19 with AS and 19 HC were enrolled in the study (Table I). The patients with BD were clinically evaluated and only those who had active disease at the time of the sampling, showing at least 2 clinical manifestations of disease, including oral ulcer, genital ulcer, uveitis, arthritis, uveitis, and vesicle involvement, were enrolled. All of the patients with BD fulfilled the International Study Group criteria (8). Of those patients with BD, 22 had mucocutaneous, 6 had articular, 4 had ocular, and 3 had vascular involvement. All patients with AS fulfilled the modified New York classification criteria and active disease (Bath Ankylosing Spondylitis Disease Activity Index [BASDAI] score ≥4) (9). At the time of the sampling, we excluded all the patients in both groups who were using any drugs except colchicine and non-steroidal anti-inflammatory agents. The healthy control group consisted of volunteers who declared that they had no rheumatologic or chronic non-rheumatologic disorder. All patients and controls signed informed consent. The study was approved by local ethics committee.

Table I. Clinic and demographic features of study group.

<table>
<thead>
<tr>
<th>Case number</th>
<th>Behçet’s disease</th>
<th>Ankylosing spondylitis</th>
<th>Healthy controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>35</td>
<td>19</td>
<td>19</td>
<td></td>
</tr>
<tr>
<td>Age, years (mean ± SD)</td>
<td>32 ± 6.4</td>
<td>30 ± 7.1</td>
<td>29 ± 5.9</td>
</tr>
<tr>
<td>Disease duration, years (mean ± SD)</td>
<td>7.4 ± 3.2</td>
<td>5.4 ± 4.4</td>
<td></td>
</tr>
<tr>
<td>Sex, female/male</td>
<td>3/32</td>
<td>1/18</td>
<td>4/15</td>
</tr>
</tbody>
</table>

Flow cytometric analysis

Plasmacytoid dendritic cells with or without expressing CXCL16 in peripheral blood samples were analysed by six colour flow cytometry. Initially, whole blood samples (4–5 mL) were obtained by venipuncture into a sterile ACD blood collection tube. Two tubes containing 20 μL of an appropriate monoclonal antibody or isotype control were prepared for staining procedure. The directly conjugated monoclonal antibodies (CD303 BDCA-2 PE) (Miltenyi-Biotec, Bergisch Gladbach, Germany), CD123 PE-Cy 5 (Becton Dickinson, San Jose, CA, USA), CXCL16APC, (R&D Systems, UK), and Anti HLA DR FITC (Becton Dickinson, San Jose, CA, USA) were used for this purpose. Isotype control monoclonal antibodies were used to determine background fluorescence levels. After the staining procedure of whole blood (100 μL) samples, the cells were lysed and washed with appropriate solutions (lysing and phosphate buffered solutions). Thereafter, acquisition and analysis were performed using FACSCanto flow cytometer (Becton Dickinson, Immunocytometry Systems, San Jose, CA, USA) and FACSDiva software. The cells expressing CD303 and CD123 (CD303+CD123+) cells were considered to be pDCs (9).

Measurement of serum cytokines

Measurement of cytokine levels in sera obtained from all patients and controls was carried out at the same time. Analysis of IFN-α (Human Interferon Alpha Serum Sample ELISA kit [catalogue no.: 41110]), PBL Biomedical Laboratories, NJ, USA), and CXCL16 (Human CXCL 16 Immunoassay ELISA Kit, [catalogue no.: DCX160]), R&D Systems Inc Minneapolis, USA) were performed by ELISA according to the manufacturer’s instructions.

Statistical analysis

All of the statistical analyses were performed by using SPSS (SPSS 10.0 FW, SPSS Inc., Chicago, USA) statistical package. Descriptive statistics were presented as median (range) notation. For the tests of normality, Kolmogorov-Smirnov test was used. For multiple groups, we used one-way ANOVA test with Bonferroni’s correction. To investigate the relations among the variables, Spearman rank correlation test was used. P-values ≤0.05 were considered as statistically significant.

Results

Serum concentrations of IFN-α in patients with BD were found significantly higher than those of controls (p<0.009). These were also higher than those of patients with AS, but did not reach the statistically significant level (p=0.124) (Fig. 1). Although there was no significant difference between patients with BD and AS, serum levels of CXCL16 were significantly higher in both groups in comparison with those of healthy controls (p=0.009, p=0.003, respectively) (Fig. 2). There was no correlation between serum levels of IFN-α and CXCL16 in both patient and control groups.

On the other hand, we found no difference in the percentages of CD123+CD303 and CD123+CXCL16+ pDCs determined by flow cytometry between the both study and control groups. There was also no significant difference in the mean fluorescence intensities of CXCL16 on pDCs among the both study and control groups. However, there was a positive correlation between the percentages of CD123+CD303+ and CD123+CXCL16+ pDCs (p<0.001) in patients with BD. Serum levels of CXCL16 was neither related to the percentages of CD123+CD303+ pDCs nor to the percentages of CD123+CXCL16.
While a positive correlation was detected in patients with AS between the percentage of CD123+CD303+ pDCs and serum levels of CXCL16 \((p=0.001)\), there was also a positive correlation between the percentage of CD123+CXCL16+ pDCs and serum levels of IFN-α in BD group \((p=0.034)\) (Fig. 3). Apart from these, we could not find any significant difference in other comparisons.

**Discussion**

In the present study we found that serum IFN-α levels were significantly higher than the healthy controls, which is consistent with our previous study results (7). While not statistically significant, these levels were also higher than the patients with AS, and it can be suggested that IFN-α itself or factors that increasing its level might have a role which seems more important in BD in comparison with AS. Actually, in a previous study we had shown a significant difference between patients with BD and AS regarding with IFN-α levels.

CXCL16 is a relatively a new discovered chemokine; its role has been studied in a variety of diseases including inflammatory and malignant disorders. Especially there are increasing body of evidence regarding its contribution of joint inflammation in rheumatoid arthritis (11, 12). Similarly CXCL16 was found to be highly elevated in both plasma and synovial fluid which suggesting a significant role in gouty arthritis (13, 14). Its role in psoriasis, but not in psoriatic arthritis has recently been investigated and authors concluded that CXCL16 contribute to the pathogenesis of psoriasis (15). The present study is the first report that investigating the role of CXCL16 in BD and indirectly in AS patients, and we found higher levels of CXCL16 in both groups in comparison to the healthy controls. The relation between IFN-α and BD has been investigated by several groups of investigators. It has been demonstrated that serum IFN-α levels were increased in Behçet patients with ocular involvement (16). A similar association has been shown by a report of our group in which there were increased levels of IFN-α in a group of patients with BD (4). As
Behçet’s disease is a form of systemic vasculitides that can involve both arteries and veins in any diameter. Although our study is the first study that explores the relation of the CXCL16 levels with BD, a recent study was evaluated the importance of this chemokine in another vasculitis, Henoch Schönlein purpura (23). The authors demonstrated that serum levels of CXCL16 are higher in the acute phase of the disease and found to be more elevated in patients with internal organ involvement. In our study, we have shown that CXCL16 levels were increased in patients with BD, whether it correlates with the stages of disease or organ involvement merits further studies. In conclusion, we demonstrated that the increased expression of trans-membrane CXCL16, which found on the surface of pDC, might contribute to high serum IFN-α levels seen in patients with BD. The ultimate result of the increased IFN-α is Th1 type immune response, which is considered the key process in the pathogenesis of the disease. Its potential as a surrogate marker that predicts the extent or severity of disease should be evaluated prospectively in larger studies.

References
20. MINAMI M, KUME N, SHIMAOKA T et al.: Expression of SR-PSOX, a novel cell-surface scavenger receptor for phosphatidylserine and oxidised lipoprotein (SR-PSOX) was found to be identical to CXCL16 (19). In addition to this, it has been detected in the samples obtained from carotid endarterectomy (20). These findings suggest that CXCL16 might therefore have a role in the atherosclerosis process. In BD, there are conflicting data regarding the risk of the development of atherosclerosis. Since there are adaverse reports, a study has demonstrated that carotid intima-media thickness, which is considered as an indirect finding of atherosclerosis, was higher in patients with BD in comparison to healthy controls (21). Furthermore, perhaps as a casually related to this, an atherogenic lipid profile with a higher LDL-cholesterol/HDL-cholesterol ratio was found in BD patients in comparison to healthy controls (22). Increased levels of CXCL16 found in our study, might be underlying mechanism for both atherogenic lipid profile and accelerated atherosclerosis in BD.