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IL-8 producing cells in patients with Behçet's disease

C. Mantas[1], H. Direskeneli[2], D. Öz[1], S. Yavuz[2], T. Akoglu[1]

[1]*Division of Hematology and Immunology,* [2]*Division of Rheumatology, Department of Internal Medicine, Faculty of Medicine, Marmara University, Istanbul, Turkey*

C. Mantas, MD, PhD; H. Direskeneli, MD, Associate Professor; D. Öz, MsC; S. Yavuz, MD; T. Akoglu, MD, Professor.
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Please address correspondence to: H. Direskeneli, MD, Resit Pasa Sok 36/5, Kiziltoprak, Kadiköy, Istanbul, Turkey. E-mail: haner@marun.edu.tr

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ABSTRACT

Objective

IL-8 is thought to be the principal chemokine responsible for neutrophil activation and tissue infiltration in patients with Behçet's disease (BD). In various studies serum levels of IL-8 were reported to be increased.

Methods

IL-8 mRNA was purified from both peripheral whole blood samples and separated lymphocytes, granulocytes and monocytes of patients with BD and compared to that from healthy (HC) and disease controls. IL-8 sequences were revealed by PCR amplification using appropriate sequence-specific primers. mRNA levels were determined semi-quantitatively using an image analyser in comparison with β-actin.

Results

Although the differences did not reach statistical significance, BD patients tended to have higher IL-8 mRNA levels compared to HC in whole blood samples (2.0±1.4 vs 1.5±1.2) as well as in their lymphocytes (2.7±1.6 vs 1.5±0.9). No differences were observed between BD and HC in monocyte or granulocyte IL-8 mRNA levels.

Conclusion

Our results suggest that the cellular source of IL-8 is diverse in BD with a possible major contribution by lymphocytes.

Introduction

Behçet's disease (BD) is a chronic, inflammatory, multi-system disorder of unknown etiology. Clinically, it is characterized by recurrent oral and genital ulcerations, skin eruptions, uveitis, and vascular and neurological involvement. Histopathological studies have revealed a mixed cellular infiltration of different tissues, primarily composed of mononuclear cells and granulocytes (1). Although the etiopathogenesis of BD has not yet been clarified, different immunological abnormalities have been reported and increased levels of Th1 and pro-inflammatory cytokines and their receptors in patients' sera have been demonstrated. Serum levels of IFN-γ (2), sIL-2R (3), TNF-α, IL-1 (3, 4), and sTNFR-75 (5) were found to be increased in BD. Spontaneous secretion of TNF-α, IL-6 and IL-8 in monocyte cultures obtained from BD patients was also demonstrated to be high compared to healthy and disease controls (6).

Among the pro-inflammatory chemokines, IL-8 plays a role of particular importance in BD, as elevated serum levels have been reported by many investigators (7-10). IL-8 is produced mainly by monocyte/macrophages, T cells, granulocytes and endothelial cells. Other cells including fibroblasts, keratinocytes, hepatocytes and chondrocytes may also produce IL-8 in small amounts (11). Following inflammatory stimuli, especially by IL-1β and TNF-α, IL-8 is released within one hour and reaches maximum levels in about 4 hours (11). IL-8 is chemotactic for both granulocytes and T cells and is effective in the diversion of the inflammatory reaction from mononuclear cells to granulocyte infiltration. In order to further characterize IL-8 secretion in BD, we studied IL-8 mRNA levels in peripheral blood cells obtained from BD patients and investigated the cellular origin of IL-8 production.

Materials and methods

Patients and controls

Peripheral blood mononuclear cells obtained from 17 patients with Behçet's disease (10 male and 7 female, mean age 36.3±7.5, range 25-48), diagnosed according to the International Study Group criteria (12), who were being followed at the multi-disciplinary Behçet's disease out-patient clinic of Marmara University Hospital, were studied. At the time of the study, all patients had clinically active disease with at least one major clinical manifestation of the disease. As control groups, 11 healthy controls and 21 disease controls [11 patients with sepsis and 10 patients with rheumatoid arthritis (RA)] were also studied.

Cell separation

To study total IL-8 mRNA levels in the whole blood, erythrocytes were first lysed. The number of cells in each sample was then adjusted to 1.5 x 10^5/ml. For the analysis of IL-8 production by different cells, venous blood samples were collected from BD patients and controls into EDTA-containing tubes, and were diluted with phosphate buff-

ered saline (PBS) at a ratio of 2:1 (Blood: PBS). Cell suspensions were than layered onto lymphoprep (Nycomed Pharma AS Oslo, Norway) and centrifuged. The mononuclear cells were collected from the interphase and placed in siliconized test tubes and lysis buffer was added onto the cell pellet. After incubation in the dark, the tubes were centrifuged and the cell number adjusted to 1.5 x 10^5/ml.

Mononuclear cells were spread on a plastic petri dish and incubated in 5% CO_2 at 37°C for 45 minutes. To obtain adherent cells (mainly monocytes), the petri dishes were washed twice with PBS, then tripsin-EDTA solution (0.25% trypsin; 0.02% EDTA in PBS, Biochrom KG Seromed) was added, and the dishes were incubated at 37°C for 10-20 min. Cells were aspirated and washed with 20% FCS to inactivate the trypsin. Then they were washed twice with the medium plus 5% FCS (RPMI-1640 containing L-Glutamine). The number of the cells was adjusted to 3 to 5 x 10^5/ml.

Purification of mRNA and cDNA synthesis

All cell samples were homogenized with GTC extraction solution containing guanidine thiocyanate and β mercaptoethanol (PolyATrack Series 9600 mRNA Isolation and cDNA Synthesis Systems, Promega-Madison, USA). Cell lysates were kept at -50° C until used.

PCR for IL-8

Previously reported PCR conditions and primer sets for IL-8 and β-actin were used (13, 14) with some modifications. The primers for IL-8 were: 5' ATT TCT GCA GCT CGT TGT GAA 3'; 5' TGA ATT CTC AGC CCT CTT CAA 3'; and for β-actin: 5' GTG GGG CGC CCC AGG CAC CA 3'; 5' GTC CTT AAT GTC ACG CAC GAT TTC 3'. The PCR mixtures for IL-8 and β-actin were similar, the contents being as follows: dNTP (0.2 mM/µl); Primary 1, 2 (10 pmol/µl); $MgCl_2$ (3 mM); cDNA (50-100 ng/µl); Taq Polimeraz (0.02 U/µl).

The following PCR conditions were used: IL-8: denaturation for 45 sec at 94°C, annealing for 45 sec at 50°C, and extension for 1 min at 72°C; 35 cycles were carried out; β-actin: denaturation for 1 min at 94°C, annealing for 1 min at 60°C, and extension for 1.5 min at 72°C. The PCR products were applied to 1.5% agarose gel and visualized by polaroid photography (Fig. 1). Bands were then analysed using the Collage Analysis program and a vision analysis device (CCD Camera Photodyn). β-actin was used as an internal control for the semi-quantitative analysis. The amount of IL-8 mRNA was calculated by dividing the value for IL-8 obtained from each patient by the value for β-actin obtained from the same patient.

Statistical evaluation

The ratios obtained were compared by Kruskal Wallis and one-way ANOVA tests using the SPSS statistics program for Windows.

Results

IL-8 mRNA levels in whole blood samples

The mean IL-8 mRNA levels in the whole blood white cell population was 2.0±1.4 in patients with BD, 1.4±0.9 in patients with sepsis, 1.8±1.0 in patients with RA and 1.5±1.2 in HC (Figs. 1 and 2). Although the mean IL-8 mRNA level in Behçet's patients was higher than that in the other groups, the difference did not reach statistical significance (BD vs HC: p=0.21; BD vs sepsis: p = 0.21; BD vs RA: p=0.76).

IL-8 mRNA levels in lymphocytes, monocytes and granulocytes in BD patients

The mean IL-8 mRNA levels in the peripheral blood cells obtained from BD patients (n = 9) was 2.7±1.6 in lymphocytes, 1.2 ±0.7 in monocytes and 1.3± 0.7 in granulocytes. In the healthy control group (n = 8), the mean IL-8 mRNA levels for the different cell subsets were: 1.5 ± 0.9 in lymphocytes, 1.2 ± 0.3 in monocytes and 1.8 ± 1.4 in granulocytes (Fig. 3). IL-8 mRNA levels in the lymphocyte population of BD patients was higher than in the healthy controls, but this difference did not reach statistical significance. Interestingly, IL-8 mRNA levels in the granulocyte subset were

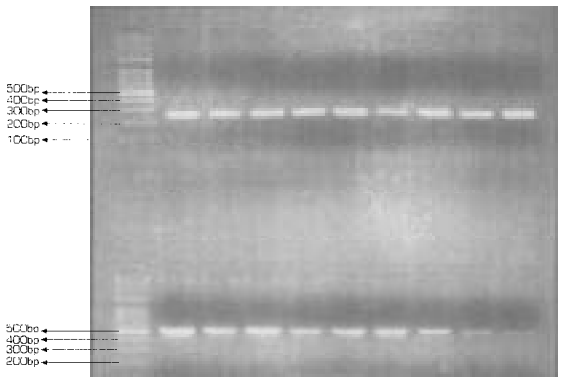

Fig. 1. Demonstration of IL-8 and β-actin mRNA levels in agarose gel: Upper bands (540 bp) represent β-actin and the lower bands (255 bp) IL-8 mRNA. Lines 1, 2, 3 represent the whole blood mRNA of Behçet's patients; line 4 represents lymphocytes alone, line 5 granulocytes, and line 6 monocytes of BD patients. Line 7 represents lymphocytes, line 8 granulocytes, and line 9 monocytes of healthy controls.

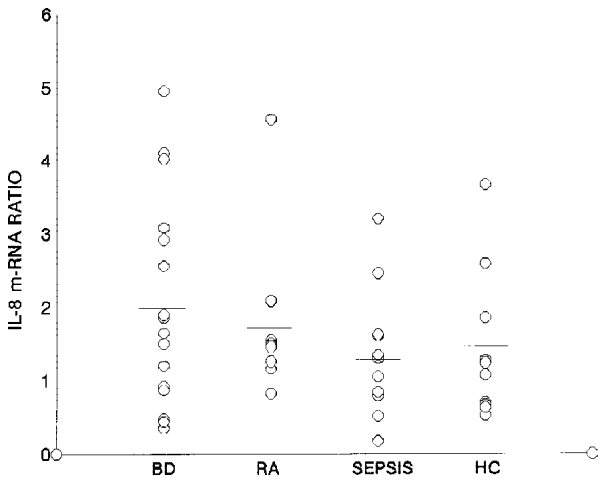

Fig. 2. Whole blood m-RNA ratios in patients with Behçet's disease (BD), rheumatoid arthritis (RA), sepsis, and in healthy controls (HC).

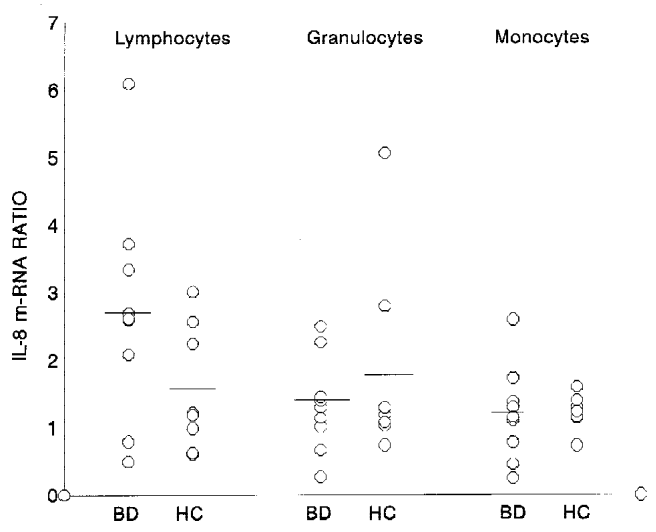

Fig. 3. Lymphocyte, granulocyte and monocyte m-RNA ratios in patients with Behçet's disease (BD) and in healthy controls (HC).

surprisingly lower than in the control groups, but again the difference was not statistically significant. No correlation was found between the absolute number of leucocyte subsets and the m-RNA ratios (data not shown).

Discussion

Behçet's disease is characterized by systemic inflammation, and a number of pro-inflammatory cytokines and chemokines such as IL-6, IL-8 and TNF-α have been demonstrated to be high in the patients' sera and in *in vitro* culture supernatants. Among these, IL-8 has particular importance as it exerts a specific upregulatory effect on granulocytes, one of the major cell subsets in Behçet's lesions. IL-8 is produced *in vitro* by many different cells, especially lymphocytes, monocytes, granulocytes and endothelial cells.

In our study we have tried to demonstrate the source of production of IL-8 in Behçet's patients, apart from the endothelial cells, which present difficulties in terms of specimen collection. Although the differences did not reach statistical significance due to the small sample size, BD patients tended to show higher IL-8 mRNA levels compared to healthy controls in both whole blood white cells and the lymphocyte subpopulations. The dominance of lymphocyte participation in IL-8 production is in accordance with various studies in which T cells were found to be activated in BD (15). IL-8 secretion was also observed after the stimulation of T cells by possible triggering antigens such as KTH-1 and the 65 kD heat shock protein peptide 336-51 in BD patients (16).

Interestingly, IL-8 production by granulocytes was lower than in healthy individuals, although the differences did not reach statistical significance. Continuous stimulation of granulocytes by different chemokines, including the IL-8 produced by mononuclear cells, might cause over-exhaustion and prevent granulocytes from continuing their normal activities and IL-8 production. Another possibility is a probable constitutional abnormality in granulocyte function, which has been suggested in HLA-B51 positive individuals and in HLA-B51 transgenic mice and which might be linked to the IL-8 expression of granulocytes (17). Pathergy positivity similar to the BD observed in chronic myeloid leukemia, a disease with increased granulocytes and granulocyte precursors, after interferon-α treatment also supports this observation (18).

In conclusion, we observed spontaneous IL-8 mRNA production mainly by lymphocytes in BD patients, which might participate in immune activation and further stimulate other pro-inflammatory cytokines causing inflammation and tissue damage.

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