The expression of chemokine receptor CXCR3: relevance to disease activity of rheumatoid arthritis

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Abstract CXC chemokine receptor 3 (CXCR3) is selectively expressed on T helper 1 (Th1) type T cells and has been shown to be responsible for Th1-dominant immune responses. In this study, we analyzed the expression of CXCR3 on peripheral blood T lymphocytes of patients with rheumatoid arthritis (RA) by FACS analysis using anti-human CXCR3 monoclonal antibody and determined the clinical relevance in this disease. Significantly higher expression of CXCR3 was found on peripheral blood CD4+ T lymphocytes of RA patients than healthy controls. The CXCR3 expression in RA patients with a high erythrocyte sedimentation rate was significantly higher than in those with a low erythrocyte sedimentation rate. Moreover, we found that the CXCR3 expression in RA patients with long-term disease duration was significantly higher than in those with short-term disease. On the other hand, CC chemokine receptor 4 (CCR4), which was shown to be selectively expressed on Th2-type T cells, was expressed at low levels in RA patients as well as in healthy controls. The serum level of interleukin (IL)-18 in RA patients was higher than that in healthy controls, although there was no statistically significant difference. This study suggests that the Th1 immune response is predominant in RA and that CXCR3 may have relevance in regard to the disease course in RA patients.

Key words CC chemokine receptor 4 (CCR4) · CD4+ T lymphocyte · CXC chemokine receptor 3 (CXCR3) · Rheumatoid arthritis (RA) · T helper 1 (Th1)

Introduction

CD4+ T lymphocytes can be subdivided into two distinct populations, T helper 1 (Th1) and Th2, defined by the spectrum of cytokines produced by these cells. Th1 cells generate interleukin (IL)-2, interferon-γ, and tumor necrosis factor-β and promote cell-mediated immunity, whereas Th2 generate IL-4, IL-5, IL-6, and IL-10 and play a role in humoral immunity and allergic diseases. There is evidence that a balance of Th1 and Th2 is crucial for an effective immune response and the outcome of infectious and autoimmune diseases. Recently, chemokines have been suggested to have a role in effector and amplification mechanisms of polarized Th1- and Th2-mediated immune responses, and their receptors might serve as targets for selective modulation of T-cell-dependent immunity. Of these chemokine receptors, CXC chemokine receptor 3 (CXCR3), which is a receptor for interferon-γ-inducible protein 10 (IP-10; CXCL10) and monokine induced by interferon-γ (Mig; CXCL9), is predominantly expressed on Th1 cells. In contrast, CC chemokine receptor 4 (CCR4), a receptor for thymus- and activation-regulated chemokine (TARC; CCL17) and macrophage-derived chemokine (MDC; CCL22), is selectively expressed on Th2 cells.

These observations suggest that the differential expression of chemokine receptor may be useful in determining T cells important to disease pathogenesis.

Rheumatoid arthritis (RA) is a chronic autoimmune disease characterized by persistent synovitis. Because chemotactic cytokines (chemokines) may play critical roles in the recruitment of leukocytes in RA, analysis for the expression of chemokines and their receptors should provide insight into events in synovial inflammation of RA. Chemokines have a role in joint inflammation, not only by inducing leukocyte chemotaxis but also by activating immune cells and angiogenesis. Previous reports that showed the increased expression of Th1-related cytokines in cells of synovial fluid and synovial tissue speculated that Th1 cells may play an active role in the development of autoimmune responses in RA. However, the data on the expression
of Th1- and Th2-type chemokine receptors in peripheral blood and inflamed joints of RA patients have been controversial.\textsuperscript{16,17}

In this study, we analyzed the expression of CXCR3 and CCR4 on peripheral blood CD4\textsuperscript{+} T lymphocytes of RA patients compared to that of healthy controls and demonstrated the predominance of Th1 in the pathogenesis of RA.

**Materials and methods**

**Patients and controls**

This study included 19 patients (5 men and 14 women) with RA (Table 1). Their mean age was 56.9 years (range, 25–75). The diagnosis of RA was based on the American College of Rheumatism (ACR) criteria for RA.\textsuperscript{18} Healthy controls (3 men and 6 women) (mean age, 26.2 years; range, 23–29) served as controls. None of the patients or controls showed any abnormalities on physical examination, chest radiography, or in lung function tests. Two RA patients had allergic diseases; 1 had bronchial asthma, and the other had allergic rhinitis. No allergic disease was observed in the healthy controls.

**Sampling of peripheral blood**

Blood samples were collected in sterile tubes containing 100 U/ml heparin. Peripheral blood mononuclear cells (PBMC) were isolated from peripheral blood on lymphocyte separation medium (ICN Biomedicals, Aurora, OH, USA) by the density gradient separation method.\textsuperscript{19} The purity of PBMC, which was determined by cell differentiation after cytocentrifugation and staining with May–Giemsa stain, was 96%. More than 98% of the cells were viable, as judged by the trypan blue dye exclusion test. PBMC were washed twice with phosphate-buffered saline (PBS) containing 1% bovine serum albumin, resuspended in PBS, and used in fluorescence-activated cell sorter (FACS) analysis as described next. Serum was separated from freshly drawn blood and stored at \(-20^\circ\text{C}\) until cytokine analysis.

**FACS analysis**

The expression of CXCR3 and CCR4 was determined by FACS analysis as previously described.\textsuperscript{20,21} The generation of a monoclonal antibody (mAb) against CCR4 (KM2160, mouse IgG1) was described previously.\textsuperscript{9} The anti-CXCR3 (mouse IgG1) mAb was obtained from R&D Systems (Minneapolis, MN, USA). PBMC were counted and adjusted to \(1 \times 10^6\) cells/mL. The cells were simultaneously stained directly with the optimal dilution of fluorescein isothiocyanate (FITC)-labeled anti-human CCR4 mouse mAb or FITC-labeled anti-human CXCR3 mouse mAb and phycoerythrin (PE)-labeled anti-human CD4 mouse mAb (PharMingen, San Diego, CA, USA). All incubations were performed for 20 min followed by two washes. The stained cells were analyzed using a FACSscan flow cytometer (Becton Dickinson, San Jose, CA, USA). Appropriate FITC and PE control antibodies (PharMingen) were also included to monitor nonspecific antibody binding.

**Assay for cytokines in serum**

Enzyme immunoassays for IL-4 and IL-18 in serum were performed as described in detail previously.\textsuperscript{22} The detection limit for cytokines was 20 pg/mL. IL-12, IL-13, and interferon-\(\gamma\) in serum were measured by performing assays with sandwich ELISA kits purchased from R&D Systems according to the manufacturer’s instructions.

**Assay for cytokines in synovial fluid**

Studies were made on another 32 patients with RA (6 males; mean age, 59.8 years) and 11 patients with OA (7 males; mean age, 70.4 years). The levels of C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), and rheumatoid factor in serum of RA patients were 3.0 ± 0.4 mg/dl, 62.0 ± 6.6 mm/h, and 127 ± 34 U/ml, respectively. Enzyme immunoassays for IL-12, IL-18 and interferon-\(\gamma\) were performed as described earlier.

**Statistical analysis**

All results are expressed as mean ± SEM. Statistical analysis was performed using the Student’s two-tailed unpaired \(t\) test for comparisons between two groups. Correlations between two parameters were evaluated using Pearson’s test. Differences were considered significant if \(P\) values were 0.05 or less. Data were analyzed on a Windows computer using Statview software.

**Results**

Expression of CXCR3 and CCR4 on peripheral blood CD4\textsuperscript{+} T lymphocytes

CXCR3 and CCR4 expression was analyzed by flow cytometry using anti-human CXCR3 and CCR4 mAb, re-

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**Table 1. Clinical data of rheumatoid arthritis (RA) patients**

<table>
<thead>
<tr>
<th>Number</th>
<th>19</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>56.9 ± 3.4</td>
</tr>
<tr>
<td>Sex (% female)</td>
<td>73.7</td>
</tr>
<tr>
<td>Disease duration (years)</td>
<td>8.3 ± 2.2</td>
</tr>
<tr>
<td>RF (U/ml)</td>
<td>102 ± 36</td>
</tr>
<tr>
<td>ESR (mm/h)</td>
<td>56 ± 7</td>
</tr>
<tr>
<td>CRP (mg/dl)</td>
<td>2.59 ± 0.57</td>
</tr>
<tr>
<td>WBC (per µl)</td>
<td>6758 ± 504</td>
</tr>
<tr>
<td>Pulmonary fibrosis (% positive)</td>
<td>26.3</td>
</tr>
</tbody>
</table>

Values are mean ± SEM

RF, rheumatoid factor; ESR, erythrocyte sedimentation rate; CRP, C-reactive protein; WBC, white blood cells