The chemoattraction of lymphocytes by rheumatoid arthritis – synovial fluid is not dependent on the chemokine receptor CCR5

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Objectives

The objective was to study the potential role of the chemokine receptor CCR5 in the chemoattraction of lymphocytes by rheumatoid arthritis synovial fluid (RA-SF).

Methods

The expression of the CCR5 receptor was studied by flow cytometry. Chemotaxis of peripheral blood lymphocytes in response to RA-SF was analyzed on transmigration chambers. Chemotaxis of immortalized lymphocytes from individuals homozygous for the Δ32 deletion of the CCR5 gene (CCR5−/−) was analyzed. The effect of a neutralizing anti-CCR5 antibody on the migration of CCR5+/+ cells was also studied.

Results

We confirmed an increase in the proportion of CCR5-expressing lymphocytes in RA-SF and a preferential migration of CCR5+ lymphocytes toward RA-SF in vitro. CCR5−/− lymphocytes showed decreased chemotactic responses to the chemokine MIP-1β but not to RA-SF. The chemotactic responses of CCR5+/+ lymphocytes to RA-SF were not modified by anti-CCR5 neutralizing antibody.

Conclusions

We confirm a preferential accumulation of CCR5-expressing lymphocytes into RA-SF. However, the chemotactic responses of lymphocytes to RA-SF were not dependent on a functional CCR5 receptor, suggesting that CCR5 is a marker of a lymphocyte subset rather than a specific mediator of chemotactic responses to chemokines in RA-SF.

Keywords

Rheumatoid arthritis · Chemokines · Chemotaxis · Lymphocytes · Synovial fluid · T cells

Introduction

The inflamed synovium of patients with rheumatoid arthritis (RA) is characterized by a dense infiltration of mononuclear cells, including CD4+ T lymphocytes [1]. The accumulated T cells in the RA synovium exhibit a specific phenotype characterized by the expression of memory and activation markers and cytokines with a Th1 profile that suggest a pathogenetic role [2, 3, 4]. Migration of these cells from the blood to the synovial tissue is a multistep process in which interactions of selectins and integrins with their ligands and a large variety of chemoattractant molecules are involved [5, 6].

Chemokines are an important family of mediators directing leukocyte trafficking under normal and pathologic conditions [7]. Chemokine receptors are a family of seven-transmembrane-domain proteins that signal through heterotrimeric guanosine triphosphate (GTP)-binding proteins and mediate chemotactic responses [8]. Most receptors recognize more than one chemokine, and several chemokines bind to more than one receptor, indicating that redundancy and versatility are characteristic for the chemokine system. Recent studies have demonstrated different patterns of chemokine receptor expression and responsiveness to different chemokines of the different T subsets. Memory and activated T cells displaying a Th1 phenotype preferentially express CCR5 and CXCR3 receptors [9, 10]. A selective accumulation of CCR5-positive lymphocytes has been demonstrated in the synovial membrane and SF of RA patients [11, 12]. Furthermore, the CCR5 ligands macrophage inflammatory protein (MIP)-1α, MIP-1β, and RANTES present in the RA synovial membrane and SF and seem to contribute partially to the strong chemoattractant activity of RA-SF [13, 14, 15]. However, the chemotactic activity of RA-SF is not induced by a single factor, and a large number of cytokines, chemokines, and other soluble factors are involved [15, 16, 17]. Therefore, whether the CCR5 receptor is simply a phenotypic marker for RA synovial T cells or is specifically
involved in the chemotactic attraction of these cells to the synovium is not known. We previously demonstrated a negative association between the homozygous Δ32 deletion of the CCR5 gene that leads to a nonfunctional CCR5 receptor and RA [18]. However, CCR5 null genotype does not provide complete protection for RA, since individuals carrying the homozygous Δ32 deletion and severe RA have been described [19].

Since chemokines and their receptors have been suggested as potential targets in the therapy of RA [20], a better characterization of the functional relevance of the CCR5 receptor on cell migration in response to synovial chemokines is warranted. To examine the participation of CCR5 receptor in the chemotactic responses to SF, we examined these responses in immortalized lymphocytes from healthy individuals genetically deficient in CCR5 or from individuals with a functional CCR5 receptor exposed to a neutralizing anti-CCR5 monoclonal antibody (mAb). Herpes virus saimiri (HVS) immortalized T cells permit their permanent growth without significantly modifying their functional responses [21].

Materials and methods

Cells and synovial fluid

Peripheral blood mononuclear cells (PBMC) for chemotaxis studies were obtained by Ficoll-Hypaque density gradient centrifugation from selected healthy donors with either homozygous wild-type CCR5 genotype (CCR5+/+) or the homozygous Δ32 CCR5 mutation (CCR5–/–) identified by polymerase chain reaction (PCR) analysis as previously described [18]. These cells were resuspended at 5×10⁶ cells/ml in RPMI 1640 medium (Gibco, Grand Island, N.Y., USA) and immortalized by HVS infection [22]. Briefly, PBMC were resuspended in a mixture (1:1 proportion) of RPMI 1640 and cell growth (CG) medium (Vitromex, Vilshofen, Germany) supplemented with 10% of fetal calf serum (FCS), 2 mM L-glutamine, and 50 IU/ml of human rIL-2 (Hoffmann-La Roche, Nutley, N.J., USA). Finally, the cells were exposed to HVS supernatant and maintained in mixed medium containing human rIL-2.

Synovial fluids were obtained by arthrocentesis from 15 patients with RA diagnosed according to the American College of Rheumatology criteria [23]. Rheumatoid arthritic patients were heterogeneous regarding age, disease duration, and therapy and were selected by the presence of active knee arthritis. Synovial fluid cells were centrifuged at 800 G for 10 min and resuspended in phosphate buffered solution (PBS), and CCR5 expression was studied by flow cytometry. Supernatants were frozen at –80°C until used for chemotaxis assays.

Chemotaxis assays

Cell migration was evaluated in 3.0-μm pore size Transwell chemotactic microchambers (Costar, Corning, N.Y., USA). Synovial fluid from different RA patients were diluted (1:1) in fresh RPMI medium and placed into the lower wells of the chemotaxis chambers. The PBMC suspension (100 μl) at 5×10⁶ cells/ml was seeded in the upper chamber. As negative control, RPMI medium was added to the lower wells of the chemotaxis chambers. The chambers were incubated for 2 h at 37°C in a 5% CO₂ atmosphere, and migrated cells were harvested and immunolabeled with anti-CCR5, anti-CD4, and anti-CD45RO mAb (Becton Dickinson, San Jose, Calif., USA). The number of migrated lymphocytes and their expression of different molecules were analyzed by flow cytometry. The migration index (MI) was calculated as the x-fold increase in migration observed over the negative control.

To confirm the participation of the CCR5 receptor in the chemotactic response to a known chemokine, we used MIP-1β at 10 ng/ml (R&D Systems, Biesbaden-Nordenstadt, Germany) and HVS immortalized CCR5+/+ and CCR5–/– T cells. In some experiments, the CCR5 receptor was neutralized by preincubating cells with 5 μg/ml of human mAb anti-CCR5 (clone 2D7) or isotype-matched control murine IgG2a (Becton Dickinson) 30 min before the chemotaxis assay. At this concentration, clone 2D7 abrogates binding of chemokines to transmembrane CCR5 and chemotactic responses to CCR5 specific chemokines in human cells for periods up to 4 h [24].

Flow cytometric analysis

The PBMC from RA patients or healthy donors and migrated cells were three-color immunolabeled with monoclonal antibodies, anti-CCR5-FITC (clone 2D7), anti-CD45RO-phycoerythrin (clone UCLH1), and anti-CD4-PerCP (clone RPA-T4) (Becton Dickinson) and analyzed on an Epics Elite cytometer (Coulter, Brea, Calif., USA). Data are presented as the number of gated lymphocytes expressing these markers.

Results

To confirm the predominance of CCR5+ lymphocytes in the RA-SF used for the present studies, PBMC from 28 RA patients and SF from 15 RA patients were analyzed by CCR5 immunofluorescence and flow cytometry. The RA-SF samples contained a 3.5-fold higher proportion of CCR5+ lymphocytes (57 ± 26%, range 11.3–88.9%) than RA-PBMC samples (16 ± 9%, range 5.8–35.3%). The PBMC from RA patients (n = 28) or healthy controls (n = 22) contained a similar percentage of CCR5+ lymphocytes (Fig. 1). CCR5 expression by CD4+ or CD4+CD45RO+ lymphocyte subsets was also similar in PBMC from RA patients and controls (Fig. 1).

To analyze the chemotactic responses of genetically null CCR5–/– lymphocytes, we studied the response of CCR5–/– and CCR5+/+ immortalized T cells to