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Original Article

Selective Recruitment of CXCR3$^+$ and CCR5$^+$ CD4$^+$ T Cells into Synovial Tissue in Patients with Rheumatoid Arthritis

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The inflamed synovial tissue (ST) of rheumatoid arthritis (RA) is characterized by the selective accumulation of interferon γ-producing Th1-type CD4$^+$ T cells. In this study, we investigated whether the predominance of Th1-type CD4$^+$ cells in the ST lesion is mediated by their selective recruitment through Th1 cell-associated chemokine receptors CXCR3 and CCR5. The lymphocyte aggregates in the ST of RA contained a large number of CD4$^+$ T cells, which mostly expressed both CXCR3 and CCR5, but not CCR4. In contrast, the frequencies of CD4$^+$ and CD8$^+$ T cells expressing CXCR3 and CCR5 in the blood were significantly decreased in RA patients, compared with healthy controls (HC), although there was no difference in the frequencies of CCR4-expressing CD4$^+$ and CD8$^+$ T cells between RA and HC. CXCR3, CCR5, and CCR4 expression in blood CD4$^+$ T cells and CXCR3 expression in CD8$^+$ T cells were increased after interleukin-15 (IL-15) stimulation. Therefore, the distribution of Th1-type CD4$^+$ T cells into the ST from the blood in RA may be associated with the local expression of chemokines, both CXCR3 and CCR5 ligands, and IL-15 may play a role in enhancing these chemokine receptors on CD4$^+$ T cell infiltrates.

Key words: CXCR3, CCR5, CD4$^+$ T cells, interleukin-15, rheumatoid arthritis

The interaction of chemokines and their receptors play an important role in the migration of specific leukocyte subpopulations. The chemokine receptors CXCR3 and CCR5 are preferentially expressed in activated Th1 cells that can produce interferon-γ (IFN-γ), tumor necrosis factor β (TNF-β), and interleukin-2 (IL-2) [1, 2–7]. In contrast, receptors such as CCR4 are expressed on IL-4- and IL-5-producing Th2 cells [4, 8, 9]. In the early pathway of Th1 and Th2 cell differentiation, CXCR3$^+$CCR4$^-$ and CXCR3$^-$CCR4$^+$ T cells have been shown to express the Th1- and Th2-specific transcription factors T-bet and GATA-3, respectively [10]. The association between CXCR3 and CCR5 expression in Th1 cells remains obscure, but CXCR5 expression has been shown to be induced later during CXCR3$^+$ memory CD4$^+$ T cell differentiation [11]. Furthermore, recent evidence indicates that chemokine receptors are involved in T-cell costimulation [12]. The ligands for CXCR3 include monokine induced by IFN-γ (Mig), IFN-γ-inducible protein of 10 kDa (IP-10), and IFN-inducible T-cell

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α chemoattractant (I-TAC), and CCR5 ligands include regulated upon activation of normal T cell expressed and secreted (RANTES), macrophage inhibitory protein 1α (MIP-1α), and MIP-1β [2].

Rheumatoid arthritis (RA) is a chronic inflammatory disease that primarily affects multiple synovial joints. The inflamed synovial membrane is characterized by massive infiltration with lymphocytes and macrophages, and lymphocyte aggregates consists primarily of $CD4^+$ T cells that can produce the Th1 cytokines IFN-β and IL-17 [13–17]. Consistent with the Th1 predominance, studies of the expression of chemokines and chemokine receptors in synovial tissue (ST) samples from RA patients have demonstrated that both CXCR3 and CCR5 are strongly expressed in T-cell infiltrates [3, 18, 19], and that their ligands are abundantly produced by macrophages and synovial fibroblasts [20–22]. In the ST, where IL-2 is deficient, T-cell activation is thought to be mediated at least in part by IL-15, which shares the β and γ chains of the IL-2 receptor. IL-15 stimulates macrophage production of TNF- α through T-cell activation [23] as well as oligoclonal T-cell expansion [24].

In this study, we determined the expression of CXCR3, CCR5, and CCR4 in $CD4^+$ and $CD8^+$ T-cell infiltrates in the ST of RA, and then examined peripheral blood $CD4^+$ and $CD8^+$ T cells from RA patients for the frequencies of their chemokine receptor expression. In addition, we examined the effects of IL-15 stimulation on T-cell chemokine receptor expression, since IL-15 has been shown to induce T-cell expression of CCR4 and CCR5 [25].

Materials and Methods

Patients and samples. The total patient population consisted of 32 patients with RA (25 women and 7 men; mean $\pm$ SD age 59.1 ± 11.3 years and mean disease duration 8.9 ± 1.4 years), diagnosed according to the revised 1987 criteria of the American College of Rheumatology (formally, the American Rheumatism Association). Most patients were receiving nonsteroidal anti-inflammatory drugs (NSAIDs), prednisolone (≤ 10 mg/day), and disease-modifying anti-rheumatic drugs (DMARDs). Serum C-reactive protein (CRP) levels were 24.5 ± 29.9 mg/liter, and IgM class rheumatoid factor (RF) was positive in 24 patients (titer 76.7 ± 85.9 units/ml). Twenty-six healthy volunteers (10 women and 16 men) matched for age served as controls. ST samples were obtained from 3 patients with RA (2 women and 1 man; 51, 71, and 74 years old; and disease duration 8, 17, and 20 years) at the time of surgical treatment. These patients were all receiving NSAIDs, 5 mg/day of prednisolone, and DMARDs, showing CRP levels of 15 ± 4 mg/liter and positive RF (titer 69.4 ± 25.3 U/ml). All the patients gave informed consent.

Two-color immunofluorescent staining. Cryostat sections (4 μm) from RA ST samples were fixed in acetone and blocked with 1.5% normal horse serum in phosphate-buffered saline (PBS). ST sections were incubated with 10 μg/ml of mouse anti-CXCR3 or mouse anti-CCR5 monoclonal antibody (mAb), followed by fluorescein isothiocyanate (FITC)-conjugated horse anti-mouse antibody (polyclonal Ab), FITC-conjugated mouse anti-CCR4 mAb, goat anti-CD4 (R&D systems, Minneapolis, MN, USA), or anti-CD8 (polycolonal Ab) (Santa Cruz, CA, USA), followed by incubation with rhodamin-conjugated rabbit anti-goat (polycolonal Ab), and isotype control mAb. The double immunofluorescence of sections was examined with an LSM510 inverted laser-scanning confocal microscope (Zeiss, Jena, Germany) and illuminated with 488 nm and 568 nm of light. Images decorated with FITC and rhodamine were recorded simultaneously through separate optical detectors with a 530-nm band-pass filter and a 590-nm long-pass filter, respectively. Pairs of images were superimposed for colocalization analysis, and chemokine receptor expression in $CD4^+$ and $CD8^+$ T cells was quantified using OPTIMAS software.

Flow cytometric analysis. Peripheral blood mononuclear cells (PBMCs) were prepared from heparinized blood samples by centrifugation over Ficoll-Hypaque density gradients (Pharmacia, Uppsala, Sweden). PBMCs in 1% heat-inactivated fetal calf serum (Life Technologies, Gaithersburg, MD)/PBS were incubated with FITC-conjugated anti-CXCR3 (Dako, Kyoto, Japan) or anti-CCR4 mAb (Kyowa Hakko, Tokyo, Japan), phycoerythrin (PE)-conjugated mouse anti-CCR5 mAb (Becton Dickinson, Franklin Lakes, NJ, USA), or Chlorophyll-Protein (Per-CP)-conjugated anti-CD4 or anti-CD8 (Becton

Dickinson, Franklin Lakes, NJ, USA), or isotype-matched control mAb. Flow cytometric analysis was performed on a FACScan flow cytometer (Becton Dickinson) by selective gating of lymphocytes based on parameters of forward and side light scatter.

T-cell stimulation with IL-15. PBMCs, at a density of 1×10^6 cells/ml in RPMI 1640 medium (Life Technologies, Gaithersburg, MD, USA) with 10% FCS, were incubated with 10 ng/ml of recombinant human IL-15 (Pepro Tech, Rocky Hill, NJ, USA) in 24-well plates (Corning, Corning, NY, USA) at 37℃ in a humidified atmosphere containing 5% CO_2. Cells were harvested 7 days later and measured for the frequencies of CXCR3, CCR5, and CCR4 expression in $CD4^+$ and $CD8^+$ T cells by flow cytometric analysis.

Colorimetric (MTT) assay for cell survival. PBMCs, at a density of 1×10^6 in 100 μl of RPMI 1640 with 10% FCS in 96-well plates (Corning, NY, USA) were incubated with or without 10 ng/ml of IL-15. After 7 days of incubation, the cell survival was determined by MTT assay, according to the manufacturer's instructions (Chemicon, Temecula, CA, USA). Cell survival with IL-15 was expressed by the relative index (the absorption of cultures with IL-15 minus that of the blank/the absorption of cultures without IL-15 minus that of the blank).

Statistical analysis. Data were expressed as the mean ± SEM of the number of samples evaluated. The statistical significance of deference between the 2 groups was determined by the Mann-Whitney U test or the Wilcoxon signed rank test. P values less than 0.05 were considered significant.

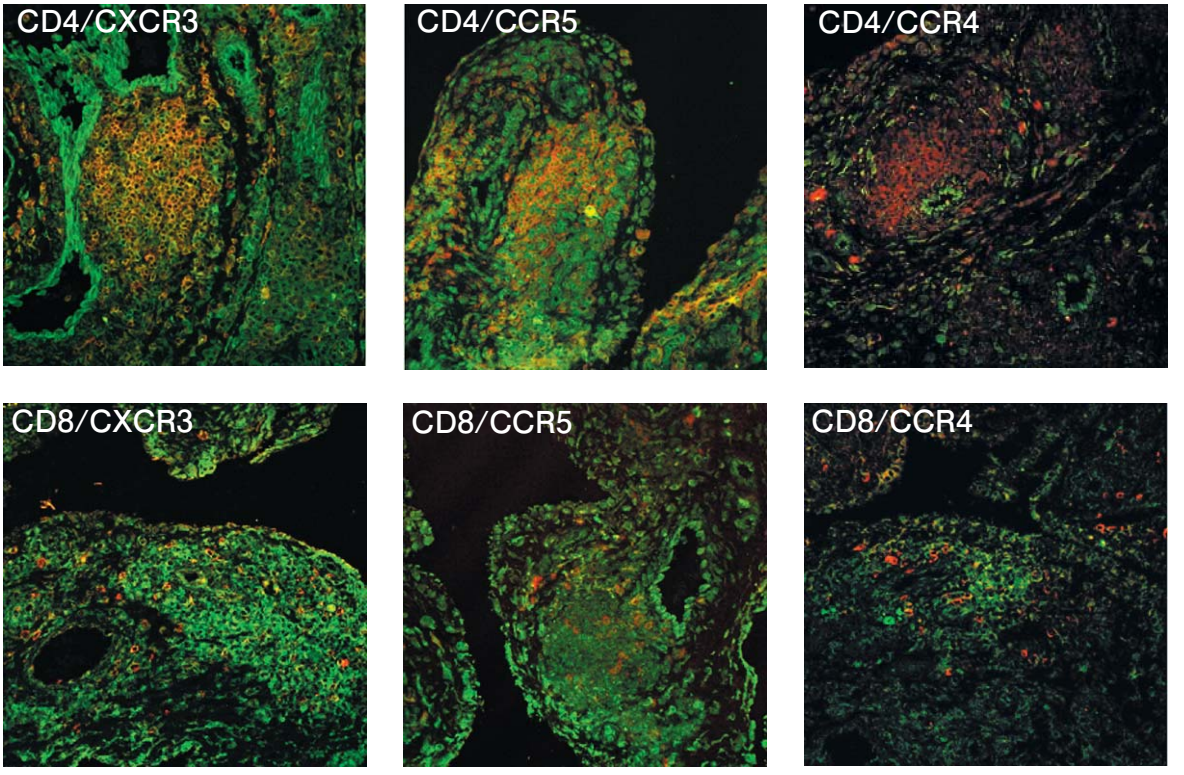

Fig. 1 Expression of CXCR3, CCR5, and CCR4 in $CD4^+$ and $CD8^+$ T cells in the synovial tissue (ST) of rheumatoid arthritis (RA). ST sections from RA patients were stained with mouse anti-CXCR3 or anti-CCR5 antibody (Ab), followed by incubation with FITC-conjugated anti-mouse IgG Ab, or FITC-conjugated anti-CCR4 Ab, and then with goat anti-CD4 or anti-CD8 Ab, followed by incubation with rhodamine-conjugated anti-goat IgG Ab. Two-color immunofluorescence confocal images were obtained for chemokine receptors and the CD4 or CD8 antigen (green and red staining, respectively). The 2 images were superimposed, and double-positive cells are shown in yellow. Representative staining patterns of chemokine receptors in $CD4^+$ and $CD8^+$ T cell infiltrates are shown. Similar staining patterns were obtained in additional analyses with 2 synovial tissue samples from different patients.

Results

CXCR3 and CCR5 expression in CD4$^+$ T-cell infiltrates in the ST of RA. To determine the significance of Th1 cell-associated chemokine receptors in CD4$^+$ and CD8$^+$ T-cell subpopulations in the ST, coexpression of the receptors CXCR3, CCR5, and CCR4, and the surface markers CD4 and CD8 in ST samples from 3 RA patients were analyzed by 2-color immunofluorescence labeling. Representative staining patterns of CXCR3, CCR5, and CCR4 expression in CD4$^+$ and CD8$^+$ T cells are shown in Fig. 1. CD4$^+$ T cells were densely localized in the sublining layer, forming the lymphoid aggregate surrounding blood vessels, whereas CD8$^+$ T cells were sparsely distributed in the periphery. The majority of CD4$^+$ T cells in the germinal center expressed both CXCR3 and CCR5. In contrast, CCR4 expression was rarely observed in the ST.

To quantitatively analyze CXCR3, CCR5 and CCR4 expression in the CD4$^+$ and CD8$^+$ T-cell infiltrates of RA, the fluorescence-stained area of chemokine receptors in the lymphoid aggregates was measured by OPTIMAS software. ST samples from the RA patients studied showed a predominance of CD4$^+$ T cells (mean $\pm$ SEM; $53.1 \pm 3.9\%$) over CD8$^+$ T cells ($7.8 \pm 2.1\%$). Both CD4$^+$ and CD8$^+$ T-cell subpopulations expressed high levels of CXCR3 ($89.3 \pm 1.7\%$ and $80.2 \pm 5.9\%$, respectively) and CCR5 ($58.6 \pm 4.2\%$ and $67.2 \pm 12.6\%$, respectively), but low levels of CCR4 ($1.4 \pm 0.4\%$ and $4.5 \pm 2.7\%$, respectively) (Fig. 2); CXCR3 expression was greater than that of CCR5. These results indicate that most CD4$^+$ and CD8$^+$ T cells in the ST of RA express Th1 cell-associated CXCR3 and CCR5, but not Th2 cell-associated CCR4.

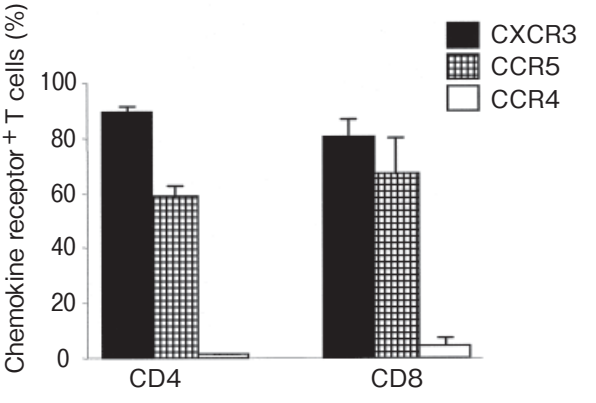

Fig. 2 Quantification of CXCR3, CCR5, and CCR4 expression in CD4$^+$ and CD8$^+$ cells in the lymphocyte aggregate of RA tissues. The proportion of chemokine receptor expression in the CD4$^+$ and CD8$^+$ cell areas of ST sections from 3 RA patients was measured by analyzing the superimposed images from 3 different sections of each patient using OPTIMAS software. The areas of lymphocyte aggregates in the sublining were identified by tracing the periphery of CD4$^+$ cell aggregates. The area of chemokine receptors, the CD4 or CD8 antigen, and coexpression was identified using the color threshold set for green, red, and yellow, respectively. The proportion of chemokine receptors in the CD4$^+$ and CD8$^+$ T-cell population was calculated as follows; chemokine receptor area (yellow)/CD4$^+$ or CD8$^+$ area (red plus yellow). Values are the mean $\pm$ SEM of a total of 9 tissue sections.

Decreased frequencies of CXCR3- and CCR5-expressing CD4$^+$ T cells in the blood of RA. The frequencies of CXCR3-, CCR5-, and CCR4-expression in blood CD4$^+$ and CD8$^+$ T cells from 32 RA patients and 26 healthy controls (HC) were compared by flow cytometric analysis. Representative staining patterns of these chemokine receptors in the CD4$^+$ and CD8$^+$ subpopulations in the RA patient and HC groups are shown in Fig. 3A and B, respectively. The mean $\pm$ SEM frequencies of CXCR3-expressing blood CD4$^+$ T cells were significantly decreased in RA patients ($20.6 \pm 1.6\%$) compared with HC (32.8 ± 1.9 %, $p < 0.0001$). The frequencies of CCR5-expressing blood CD4$^+$ T cells were also significantly decreased in RA patients ($15.3 \pm 1.7\%$) compared with HC ($22.9 \pm 2.2\%$, $p < 0.005$). However, there was no difference in CCR4-expressing CD4$^+$ T cells between RA patients ($10.5 \pm 1.0\%$) and HC ($12.9 \pm 1.5\%$). Similarly, the frequency of CXCR3-expressing CD8$^+$ T cells in RA (39.2 ± 3.9) was decreased compared with HC ($57.0 \pm 3.7\%$, $p < 0.005$); however, no difference was noted in CCR5 expression in CD8$^+$ T cells between the RA patients (41.1 ± 3.2) and HC ($43.2 \pm 2.4\%$). CCR4 expression in CD8$^+$ T cells was extremely limited (RA vs. HC; 2.6 ± 0.5 vs.$1.5 + 0.4\%$), in agreement with previous observations [25] (Fig. 3C). CXCR3 and CCR5-coexpressing CD4$^+$ T cells were decreased in RA patients ($7.7\% \pm 2.3\%$) compared with HC (17.8 ± 3.2 %, $p < 0.0005$); however, coexpression of CCR4 and CCR5 was negligible. These results indicate that Th1-type CD4$^+$ T cells are decreased in the

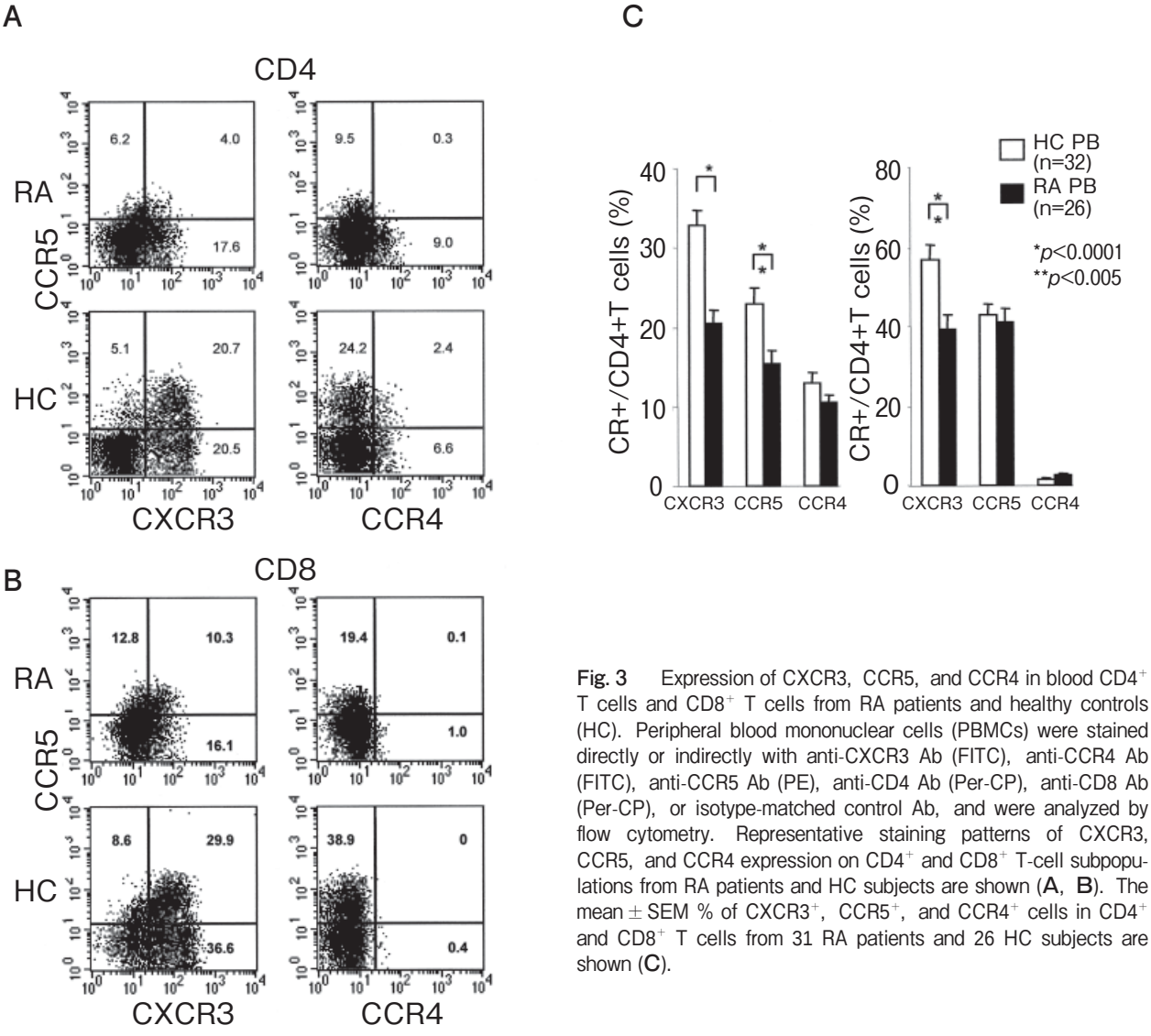

Fig. 3 Expression of CXCR3, CCR5, and CCR4 in blood $CD4^+$ T cells and $CD8^+$ T cells from RA patients and healthy controls (HC). Peripheral blood mononuclear cells (PBMCs) were stained directly or indirectly with anti-CXCR3 Ab (FITC), anti-CCR4 Ab (FITC), anti-CCR5 Ab (PE), anti-CD4 Ab (Per-CP), anti-CD8 Ab (Per-CP), or isotype-matched control Ab, and were analyzed by flow cytometry. Representative staining patterns of CXCR3, CCR5, and CCR4 expression on $CD4^+$ and $CD8^+$ T-cell subpopulations from RA patients and HC subjects are shown (**A**, **B**). The mean ± SEM % of $CXCR3^+$, $CCR5^+$, and $CCR4^+$ cells in $CD4^+$ and $CD8^+$ T cells from 31 RA patients and 26 HC subjects are shown (**C**).

peripheral blood of RA in terms of chemokine receptor expression.

Enhancement of CXCR3 and CCR5 expression in blood $CD4^+$ T cells by IL-15 stimulation. To determine the local regulation of CXCR3 and CCR5 expression in ST, $CD4^+$, and $CD8^+$ T cells from HC and RA patients after being stimulated with 10 ng/ml of IL-15 for 7 days, we measured the surface expression of chemokine receptors by flow-cytometric analysis. The frequencies of CXCR3-, CCR5-, and CCR4-expressing $CD4^+$ T cells in both RA patients and HC were significantly increased after IL-15 stimulation (Fig. 4). CXCR3- and CCR5-expressing $CD8^+$ T cells were also increased, but the effects of IL-15 stimulation were less prominent in $CD8^+$ T cells than in $CD4^+$ T cells (Fig. 4). These results suggest that IL-15 may enhance T-cell expression of CXCR3 and CCR5 in the ST of RA.

We found that the number of cells was maintained after 7 days of IL-15 stimulation (with and without IL-15, 1.1×10^6/ml and 0.8×10^6/ml, respectively). Consistent with this finding, the survival cell index of the cell culture with IL-15 relative to that with no

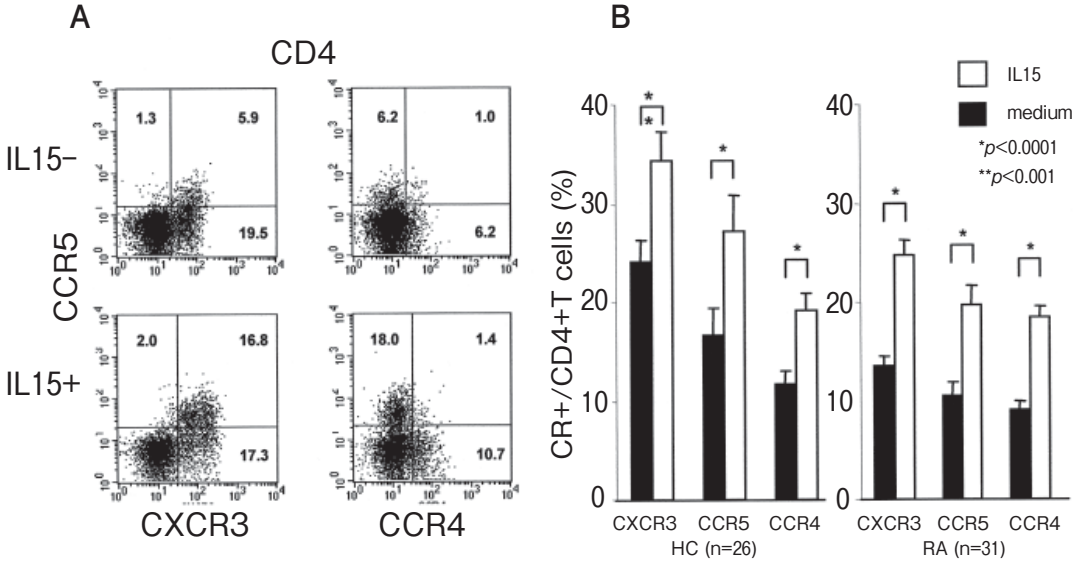

Fig. 4 Enhancement of CXCR3, CCR5, and CCR4 expression in blood $CD4^+$ T cells from HC and RA patients after IL-15 stimulation. PBMCs from HC and RA patients (1×10^6/ml in RPMI 1640 culture medium with 10% FCS) were incubated for 7 days with or without IL-15 (10 ng/ml), and $CXCR3^+$, $CCR5^+$, and $CCR4^+$ $CD4^+$ T cells were determined by flow cytometric analysis. Representative staining patterns of CXCR3, CCR5, and CCR4 expression on $CD4^+$ T cell cells with or without IL-15 stimulation are shown (**A**). Mean ± SEM percentages of $CXCR3^+$, $CCR5^+$, and $CCR4^+$ $CD4^+$ T cells from 31 RA patients and 26 HC subjects with or without IL-15 stimulation are shown (**B**).

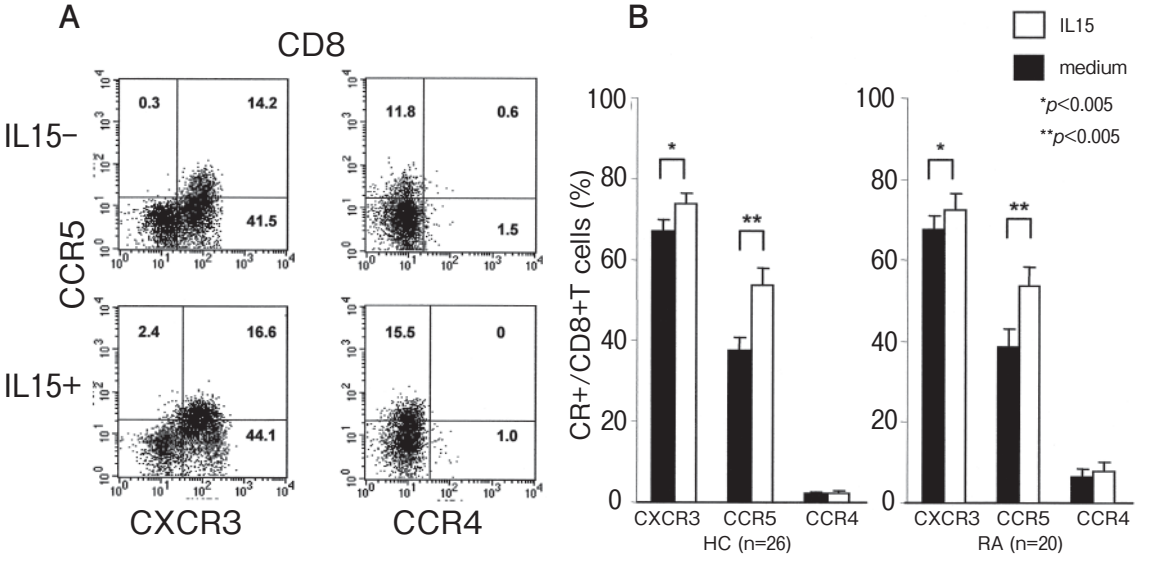

Fig. 5 Enhancement of CXCR3, CCR5, and CCR4 expression in blood $CD8^+$ T cells from HC and RA patients after IL-15 stimulation. $CXCR3^+$, $CCR5^+$, and $CCR4^+$ $CD8^+$ T cells were determined by flow cytometric analysis. Representative staining patterns of CXCR3, CCR5, and CCR4 expression on $CD8^+$ T cell cells with or without IL-15 stimulation are shown (**A**). Mean ± SEM percentages of $CXCR3^+$, $CCR5^+$, and $CCR4^+$ $CD8^+$ T cells from 31 RA patients and 26 HC subjects with or without IL-15 stimulation are shown (**B**).

stimulation, as determined by MTT assay, was 1.50 ± 0.14 ($n = 5$). Thus, IL-15 appears to enhance T-cell survival.

Discussion

In the inflamed ST of RA, perivascular lymphocyte aggregates are frequently found, with the Th1 cytokine IFN-γ being predominantly expressed [27]. We confirmed by 2-color immunofluorescence staining that high levels of CXCR3 and CCR5, but low levels of CCR4, were expressed in $CD4^+$ T cells localized to the lymphocyte aggregates of RA tissues. In contrast, CXCR3 and CCR5 expression in peripheral blood $CD4^+$ T cells from RA patients was significantly decreased when compared with HC $CD4^+$ T cells, despite there being no difference in $CD4^+$ T cell expression of CCR4. On the other hand, $CD8^+$ T cells were sparsely found in the periphery of $CD4^+$ T cell aggregates, which also express CXCR3 and CCR5.

The accumulation of Th1-type $CD4^+$ T cells in ST lesions is thought to be induced by the clonal expansion and selective infiltration of Th1 cells. In the ST of RA, IL-12 and IL-18, known to be crucial in Th1 cell activation, are substantially expressed [28], indicative of the local expansion of Th1 cells. On the other hand, our study and others have shown that T cells strongly express CCR5 and CXCR3 [18, 20, 29, 30]. In addition, high concentrations of ligands for these receptors such as MIP-1α [31], MIP-1β [29], RANTES [32], IP-10[33, 34], Mig [33, 35], and ITAC [21] have been detected in the synovial fluid of active RA, and these chemokines have been shown to be produced by macrophages and synovial fibroblasts. These findings suggest that the Th1 predominance in the ST may also be mediated by selective lymphocyte trafficking due to the preferential expression of CXCR3 and CCR5 on Th1 cells and the local abundance of their agonistic ligands.

CXCR3 and CCR5 expression on blood T cells in RA patients remain controversial. Some studies have demonstrated increased frequencies of CXCR3- and CCR5-expressing T cells [18], which indicates the systemic deviation of T-cell responses toward the Th1 type in RA. However, others have found decreased frequencies of such T cells [20, 29, 36]. The reason for the differences in these observations is unknown, but our flow cytometric analysis indicated the selective depletion of CXCR3- and CC5-expressing T cells in the blood compartment. It is of interest that the accumulation of $CXCR3^+$ T cells in the blood was found in RA patients after TNF-α inhibition with infliximab and etanercept [26]. These results indicate that CCR5- and CXCR3-expressing T cells are actively recruited into inflammatory sites and that treatment with TNF-α inhibition prevents Th1-cell infiltration into the ST by suppressing the expression of chemokines and adhesion molecules specific to Th1 cell migration. We therefore believe that blood $CXCR3^+$ and $CCR5^+$ Th1 cells aggressively migrate into the ST when joint inflammation is severe, resulting in a depletion of such Th1 cells in the blood compartment.

We found that IL-15, the cytokine known to be responsible for T-cell activation in RA joints, enhances the expression of all CXCR3, CCR5, and CCR4 on $CD4^+$ and $CD8^+$ T cells, as well as their cell survival. These results correspond with those of a previous study demonstrating induction of CCR4 and CCR5 expression in IL-15-activated T cells [25]. Thus, IL-15 appears to activate T cells to express both Th1 cell- and Th2 cell-associated chemokine receptors. In the ST of RA, where Th1 cells are already enriched due to their selective recruitment, IL-15 may play a role in enhancing or maintaining CXCR3 and CCR5 expression in $CD4^+$ T-cell infiltrates. This upregulation of chemokine receptors may be crucial to the organized distribution of Th1-type $CD4^+$ T cells in the ST, which depends on chemokine expression. IL-15 has recently been shown to enhance CD40L and CCR5 expression in RA T cells and to promote the secretion of the chemokine MAP-1α. The key roles of IL-15 in RA pathogenesis have been well-supported [37, 38], and AMG 714, a human anti-IL-15 antibody, appears to ameliorate disease activity in RA patients [39]. The reduction in Th1 responses in response to IL-15 blockade may be one of the mechanisms responsible for its efficacy.

In summary, CXCR3- and CCR5-expressing $CD4^+$ T cells predominate in the lymphoid aggregates of RA tissues, and such $CD4^+$ T cells are decreased

in the blood compartment, presumably due to active, selective recruitment of Th1 cells into the ST by the local abundance of CXCR3 and CCR5 agonistic chemokines. IL-15 may play a role in the organization of $CD4^+$ T cells through chemokine receptor enhancement after migration.

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References

1. Laing KJ and Secombes CJ: Chemokines. Dev Comp Immunol (2004) 28: 443–460.
2. Koch AE: Chemokines and their receptors in rheumatoid arthritis: future targets? Arthritis Rheum (2005) 52: 710–721.
3. Campbell JD and HayGlass KT: T cell chemokine receptor expression in human Th1- and Th2-associated diseases. Arch Immunol Ther Exp (Warsz) (2000) 48: 451–456.
4. Mantovani A: The chemokine system: redundancy for robust outputs. Immunol Today (1999) 20: 254–257.
5. Loetscher P, Uguccioni M, Bordoli L, Baggiolini M, Moser B, Chizzolini C and Dayer JM: CCR5 is characteristic of Th1 lymphocytes. Nature (1998) 391: 344–345.
6. Qin S, Rottman JB, Myers P, Kassam N, Weinblatt M, Loetscher M, Koch AE, Moser B and Mackay CR: The chemokine receptors CXCR3 and CCR5 mark subsets of T cells associated with certain inflammatory reactions. J Clin Invest (1998) 101: 746–754.
7. D'Ambrosio D, Iellem A, Colantonio L, Clissi B, Pardi R and Sinigaglia F: Localization of Th-cell subsets in inflammation: differential thresholds for extravasation of Th1 and Th2 cells. Immunol Today (2000) 21: 183–186.
8. Bonecchi R, Bianchi G, Bordignon PP, D'Ambrosio D, Lang R, Borsatti A, Sozzani S, Allavena P, Gray PA, Mantovani A and Sinigaglia F: Differential expression of chemokine receptors and chemotactic responsiveness of type 1 T helper cells (Th1s) and Th2s. J Exp Med (1998) 187: 129–134.
9. Moser B, Wolf M, Walz A and Loetscher P: Chemokines: multipl e levels of leukocyte migration control. Trends Immunol (2004) 25: 75–84.
10. Song K, Rabin RL, Hill BJ, De Rosa SC, Perfetto SP, Zhang HH, Foley JF, Reiner JS, Liu J, Mattapallil JJ, Douek DC, Roederer M and Farber JM: Characterization of subsets of CD4+ memory T cells reveals early branched pathways of T cell differentiation in humans. Proc Natl Acad Sci USA (2005) 102: 7916–7921.
11. Rabin RL, Alston MA, Sircus JC, Knollmann-Ritschel B, Moratz C, Ngo D and Farber JM: CXCR3 is induced early on the pathway of CD4+ T cell differentiation and bridges central and peripheral functions. J Immunol (2003) 171: 2812–2824.
12. Molon B, Gri G, Bettella M, Gomez-Mouton C, Lanzavecchia A, Martinez AC, Manes S and Viola A: T cell costimulation by chemokine receptors. Nat Immunol (2005) 6: 465–471.
13. Brennan F and Foey A: Cytokine regulation in RA synovial tissue: role of T cell/macrophage contact-dependent interactions. Arthritis Res (2002) 4 Suppl 3: S177–182.
14. Morita Y, Yamamura M, Kawashima M, Harada S, Tsuji K, Shibuya K, Maruyama K and Makino H: Flow cytometric single-cell analysis of cytokine production by CD4+ T cells in synovial tissue and peripheral blood from patients with rheumatoid arthritis. Arthritis Rheum (1998) 41: 1669–1676.
15. Chabaud M, Durand JM, Buchs N, Fossiez F, Page G, Frappart L, and Miossec P: Human interleukin-17: A T cell-derived proinflammatory cytokine produced by the rheumatoid synovium. Arthritis Rheum (1999) 42: 963–970.
16. Hessian PA, Highton J, Kean A, Sun CK and Chin M: Cytokine profile of the rheumatoid nodule suggests that it is a Th1 granuloma. Arthritis Rheum (2003) 48: 334–338.
17. Loetscher P and Moser B: Homing chemokines in rheumatoid arthritis. Arthritis Res (2002) 4: 233–236.
18. Ruth JH, Rottman JB, Katschke KJ, Jr., Qin S, Wu L, LaRosa G, Ponath P, Pope RM and Koch AE: Selective lymphocyte chemokine receptor expression in the rheumatoid joint. Arthritis Rheum (2001) 44: 2750–2760.
19. Nanki T and Lipsky PE: Cytokine, activation marker, and chemokine receptor expression by individual CD4(+) memory T cells in rheumatoid arthritis synovium. Arthritis Res (2000) 2: 415–423.
20. Suzuki N, Nakajima A, Yoshino S, Matsushima K, Yagita H and Okumura K: Selective accumulation of CCR5+ T lymphocytes into inflamed joints of rheumatoid arthritis. Int Immunol (1999) 11: 553–559.
21. Ueno A, Yamamura M, Iwahashi M, Okamoto A, Aita T, Ogawa N and Makino H: The production of CXCR3-agonistic chemokines by synovial fibroblasts from patients with rheumatoid arthritis. Rheumatol Int (2005) 25: 361–367.
22. Shadidi KR, Aarvak T, Henriksen JE, Natvig JB and Thompson KM: The chemokines CCL5, CCL2 and CXCL12 play significant roles in the migration of Th1 cells into rheumatoid synovial tissue. Scand J Immunol (2003) 57: 192–198.
23. McInnes IB, Leung BP, Sturrock RD, Field M and Liew FY: Interleukin-15 mediates T cell-dependent regulation of tumor necrosis factor-alpha production in rheumatoid arthritis. Nat Med (1997) 3: 189–195.
24. Masuko-Hongo K, Kurokawa M, Kobata T, Nishioka K and Kato T: Effect of IL-15 on T cell clonality in vitro and in the synovial fluid of patients with rheumatoid arthritis. Ann Rheum Dis (2000) 59: 688–694.
25. Perera LP, Goldman CK and Waldmann TA: IL-15 induces the expression of chemokines and their receptors in T lymphocytes. J Immunol (1999) 162: 2606–2612.
26. Aeberli D, Seitz M, Juni P and Villiger PM: Increase of peripheral CXCR3 positive T lymphocytes upon treatment of RA patients with TNF-alpha inhibitors. Rheumatology (Oxford) (2005) 44: 172–175.
27. Sweeney SE and Firestein GS: Rheumatoid arthritis: regulation of synovial inflammation. Int J Biochem Cell Biol (2004) 36: 372–378.
28. Aita T, Yamamura M, Kawashima M, Okamoto A, Iwahashi M, Yamana J and Makino H: Expression of interleukin 12 receptor (IL-12R) and IL-18R on CD4+ T cells from patients with rheumatoid arthritis. J Rheumatol (2004) 31: 448–456.
29. Wang CR and Liu MF: Regulation of CCR5 expression and MIP-1alpha production in CD4+ T cells from patients with rheumatoid arthritis. Clin Exp Immunol (2003) 132: 371–378.
30. Garcia-Vicuna R, Gomez-Gaviro MV, Dominguez-Luis MJ, Pec MK, Gonzalez-Alvaro I, Alvaro-Gracia JM and Diaz-Gonzalez F: CC and CXC chemokine receptors mediate migration, prolifera-

tion, and matrix metalloproteinase production by fibroblast-like synoviocytes from rheumatoid arthritis patients. Arthritis Rheum (2004) 50: 3866–3877.
31. Koch AE, Kunkel SL, Harlow LA, Mazarakis DD, Haines GK, Burdick MD, Pope RM and Strieter RM: Macrophage inflammatory protein-1 alpha. A novel chemotactic cytokine for macrophages in rheumatoid arthritis. J Clin Invest (1994) 93: 921–928.
32. Rathanaswami P, Hachicha M, Sadick M, Schall TJ and McColl SR: Expression of the cytokine RANTES in human rheumatoid synovial fibroblasts. Differential regulation of RANTES and interleukin-8 genes by inflammatory cytokines. J Biol Chem (1993) 268: 5834–5839.
33. Patel DD, Zachariah JP and Whichard LP: CXCR3 and CCR5 ligands in rheumatoid arthritis synovium. Clin Immunol (2001) 98: 39–45.
34. Hanaoka R, Kasama T, Muramatsu M, Yajima N, Shiozawa F, Miwa Y, Negishi M, Ide H, Miyaoka H, Uchida H and Adachi M: A novel mechanism for the regulation of IFN-gamma inducible protein-10 expression in rheumatoid arthritis. Arthritis Res Ther (2003) 5: R74–81.
35. Konig A, Krenn V, Toksoy A, Gerhard N and Gillitzer R: Mig, GRO alpha and RANTES messenger RNA expression in lining layer, infiltrates and different leucocyte populations of synovial tissue from patients with rheumatoid arthritis, psoriatic arthritis and osteoarthritis. Virchows Arch (2000) 436: 449–458.
36. Haringman JJ, Smeets TJ, Reinders-Blankert P and Tak PP: Chemokine and chemokine receptor expression in paired peripheral blood mononuclear cells and synovial tissue of rheumatoid arthritis, osteoarthritis and reactive arthritis patients. Ann Rheum Dis (2006) 65: 294–300.
37. McInnes IB and Gracie JA: Interleukin-15: a new cytokine target for the treatment of inflammatory diseases. Curr Opin Pharmacol (2004) 4: 392–397.
38. Perera LP: Interleukin 15: its role in inflammation and immunity. Arch Immunol Ther Exp (Warsz) (2000) 48: 457–464.
39. Pollard L and Choy E: Rheumatoid arthritis: non-tumor necrosis factor targets. Curr Opin Rheumatol (2005) 17: 242–246.