ARTHRITIS & RHEUMATISM
Vol. 52, No. 12, December 2005, pp 3718–3730
DOI 10.1002/art.21476

Chemokine Receptor Expression in Rat Adjuvant-Induced Arthritis

Christian S. Haas,[1] Rita J. Martinez,[1] Naweah Attia,[1] G. Kenneth Haines, III,[2] Phillip L. Campbell,[1] and Alisa E. Koch[3]

Objective. **Chemokine receptors mediate leukocyte migration into inflamed rheumatoid arthritis (RA) synovial tissue (ST). Knowledge of their distribution is crucial for understanding the evolution of the inflammatory process. In this study, we used rat adjuvant-induced arthritis (AIA), a model for RA, to define the temporospatial expression of chemokine receptors.**

Methods. **ST from rats with AIA was immunostained, the percentage of cells expressing each receptor was determined, and findings were correlated with levels of inflammation. Chemokine receptor expression was evaluated on rat macrophages in vitro.**

Results. **CCR1, a receptor for macrophage inflammatory protein 1α (MIP-1α)/CCL3 and RANTES/CCL5, exhibited high constitutive expression on macrophages in AIA. CCR5, binding MIP-1α/CCL3 and RANTES/CCL5, was up-regulated on ST macrophages during the course of AIA, correlating with macrophage expression of CCR2, a receptor for monocyte chemoattractant protein 1/CCL2. Endothelial cell (EC) CCR2 was down-regulated as arthritis progressed, inversely correlating with inflammation. CCR3, another RANTES/CCL5 receptor, was constitutively high on macrophages in vivo and in vitro, with down-regulation during AIA. CXCR4, a receptor for stromal cell–derived factor 1/CXCL12), was prominently up-regulated on ECs, preceding the peak of inflammation.**

Conclusion. **These findings show that 1) constitutive expression of CCR1 on macrophages remains high during AIA; 2) CCR2 and CCR3 may play a role in initial recruitment of leukocytes to ST in AIA; 3) macrophage expression of CCR2 and CCR5 may be important for sustaining inflammatory changes; and 4) EC CXCR4 may be a harbinger of inflammatory changes. Our results may help guide chemokine receptor blockade–targeting treatment strategies in inflammatory arthritis.**

Rheumatoid arthritis (RA) is a chronic inflammatory disease characterized by infiltration of leukocytes into the joint, mediated in part by chemokines (1). Upon activation of the endothelium by proinflammatory cytokines, leukocytes adhere to endothelial cells (ECs) and subsequently transmigrate across the EC barrier into the synovium.

Chemokines form a large family of small, structurally and functionally related proteins that facilitate chemoattraction and migration of leukocytes from the circulation into sites of inflammation and promote angiogenesis, cell proliferation, and apoptosis (2,3). To date, more than 50 chemokines have been defined based on the number and spacing of cysteine residues in the amino-terminal region; they are termed C, CC, CXC, and CX_3C chemokines (4).

The biologic activity of chemokines is mediated via 7-transmembrane domain G protein–coupled receptors (5). Twenty chemokine receptors have been identified and are denoted as CCR1–11, CXCR1–7, XCR1, and CX_3CR1 (6). Certain chemokine receptors bind only a unique ligand and vice versa, while others are more promiscuous. There is evidence that chemokine

Supported by the NIH (grants AI-40987, HL-58694, and AR-48267), an American Heart Association postdoctoral fellowship (grant AHA0423758Z), the Frederick G. L. Huetwell and William D. Robinson, MD, Professorship in Rheumatology, and the Office of Research and Development, Medical Research Service, Department of Veterans Affairs.

[1]Christian S. Haas, MD, Rita J. Martinez, BS, Naweah Attia, BS, Phillip L. Campbell, BS: University of Michigan Medical School, Ann Arbor; [2]G. Kenneth Haines, III, MD: Northwestern University Feinberg Medical School, Chicago, Illinois; [3]Alisa E. Koch, MD: University of Michigan Medical School and VA Medical Service, Department of Veterans Affairs Medical Center, Ann Arbor, Michigan.

Address correspondence and reprint requests to Alisa E. Koch, MD, University of Michigan Medical School, Department of Medicine, Division of Rheumatology, 1150 West Medical Center Drive, Ann Arbor, MI 48109-0680. E-mail: aekoch@umich.edu.

Submitted for publication March 29, 2005; accepted in revised form September 7, 2005.

receptors are used sequentially, thereby serving a unique function in the migratory process (7). The putative complexity and redundancy emphasize the importance of both spatially and temporally well-defined secretion and presentation of chemokines and chemokine receptors in the synovial tissue (ST), as well as regulated expression of their counterparts on leukocytes during differentiation and activation (8).

To determine the specific role of chemokine receptors in the pathogenesis of RA and to design interventional approaches accordingly, it is important to understand the temporospatial expression of chemokine receptors in the development of inflammatory arthritis. The present study was undertaken to elucidate this.

MATERIALS AND METHODS

Induction of rat adjuvant-induced arthritis (AIA) and tissue sampling. Female Lewis rats (100 gm) were injected subcutaneously at the base of the tail with 300 μl (5 mg/ml) lyophilized *Mycobacterium butyricum* (Difco, Detroit, MI) in sterile mineral oil on day 0. Rats were killed at various times post–adjuvant injection, and joints harvested.

Immunohistochemistry. Immunohistochemistry studies were performed on ST cryosections (8 μm) from rats with AIA, for the chemokine receptors CCR5, CXCR4 (polyclonal goat antibodies cross-reacting with human, mouse, and rat; Santa Cruz Biotechnology, Santa Cruz, CA), CCR1, CCR2, CCR3, CCR4 (polyclonal rabbit antibodies cross-reacting with human, mouse, and rat; Santa Cruz Biotechnology), CXCR1, CXCR2, CXCR3, and CXCR5 (monoclonal mouse anti-human antibodies; R&D Systems, Minneapolis, MN). Antibody concentration was 2 μg/ml for CCR1–5 and CXCR4, and 10 μg/ml for CXCR1, CXCR2, and CXCR5. Isotype-matched nonspecific IgG was used as a negative control. Immunostaining was performed using Elite ABC kits (Vector, Burlingame, CA) and diaminobenzidine (Kirkegaard & Perry, Gaithersburg, MD) as a chromogen, followed by counterstaining with hematoxylin.

Microscopic analysis. ST elements examined included lining cells, macrophages, ECs, lymphocytes, and smooth muscle cells. Immunostaining was evaluated under blinded conditions and graded by a pathologist as described previously (9). Cell types were distinguished based on their characteristic morphology and/or immunohistochemical staining reaction (9–11). Macrophages were distinguished from fibroblasts based on morphology and immunoreactivity with monoclonal mouse anti-rat CD11b/c antibody (monocyte/macrophage marker; BD PharMingen, Franklin Lakes, NJ). Endothelium was verified using anti–von Willebrand factor (anti-vWF; Dako, Carpinteria, CA), synovial lining cells were determined based on morphologic features, and lymphocytes were identified by their characteristic nuclear morphology and chromatin pattern. Each ST component was graded for frequency of staining on a scale of 0–100%, where 0% indicates no staining and 100% indicates that all cells were immunoreactive. The percentage of reactivity was defined as the number of cells of a given type reacting with a specific antibody divided by the total number of cells of that given type. To confirm identification of macrophages, CD11b/c-positive cells coexpressing CCR1 and CXCR4 were determined using double staining. Lymphocytes were identified by CD3 staining. In addition, an inflammation score was obtained using the following scoring system: 1 = normal; 2 = increased number of inflammatory cells, arrayed as individual cells; 3 = increased number of inflammatory cells including distinct clusters (aggregates); and 4 = marked diffuse infiltrate of inflammatory cells. Score data were pooled, and the mean ± SEM was calculated in each data group.

Cell culture. NR8383 cells, a rat alveolar macrophage cell line (American Type Culture Collection, Manassas, VA), were maintained in Ham's F-12 medium (Invitrogen, Carlsbad, CA) containing 20% fetal bovine serum, 100 units/ml penicillin, 100 μg/ml streptomycin, and 2 m*M* glutamine at 37°C/5% CO_2. Cells (20,000/well) were cultured in 8-well Labtek chamber slides (BD PharMingen) until confluence was reached. The medium was changed to serum-free Ham's F-12 before stimulation for 3 hours with lipopolysaccharide (LPS; 10 μg/ml) (12).

Immunofluorescence. NR8383 cells were used for immunofluorescence staining after formalin fixation. Similarly, double immunofluorescence analysis was performed on ST from rats with AIA to determine colocalization of chemokine receptors with synovial cells, using the EC-specific rabbit anti-human vWF antibody, mouse anti-rat CD11b/c, or the T cell marker CD3. After blocking, tissues or cells were incubated with the primary antibodies for chemokine receptors, cell type markers, or control IgG, respectively. For detection, the following immunofluorescence dye–tagged secondary antibodies (1:200 in phosphate buffered saline) were used: Alexa Fluor 568–conjugated stained goat anti-rabbit antibody, Alexa Fluor 488–conjugated donkey anti-rabbit antibody, and Alexa Fluor 488–conjugated donkey anti-goat antibody (all from Molecular Probes, Eugene, OR). Nuclear staining was performed with 4′,6-diamidino-2-phenylindole-dihydrochloride (Molecular Probes). Immunofluorescence staining was detected using a BX51 fluorescence microscope system (Olympus, Melville, NY) with DP Manager imaging software. CD11b/c-positive cells in ST from rats with AIA, reflecting the influx of monocyte/macrophages, were assessed by counting the number of cells in 5 high-power fields (hpf; 400×) and calculating the average number of cells per hpf. CD11b/c-coexpressing CCR1 and CXCR4 were determined in the same way and presented as the percentage of chemokine receptor–positive cells per CD11b/c-expressing cell.

Statistical analysis. Values are presented as the mean ± SEM. Statistical analysis was performed using Student's *t*-test. *P* values less than 0.05 were considered significant. Pearson correlation coefficients were assessed to describe the relationships of various parameters, using the mean from each time point during AIA.

RESULTS

Course of inflammation in rat AIA. Rats with AIA started showing clinical signs of inflammation on days 7–14 post–adjuvant injection. Histologic assess

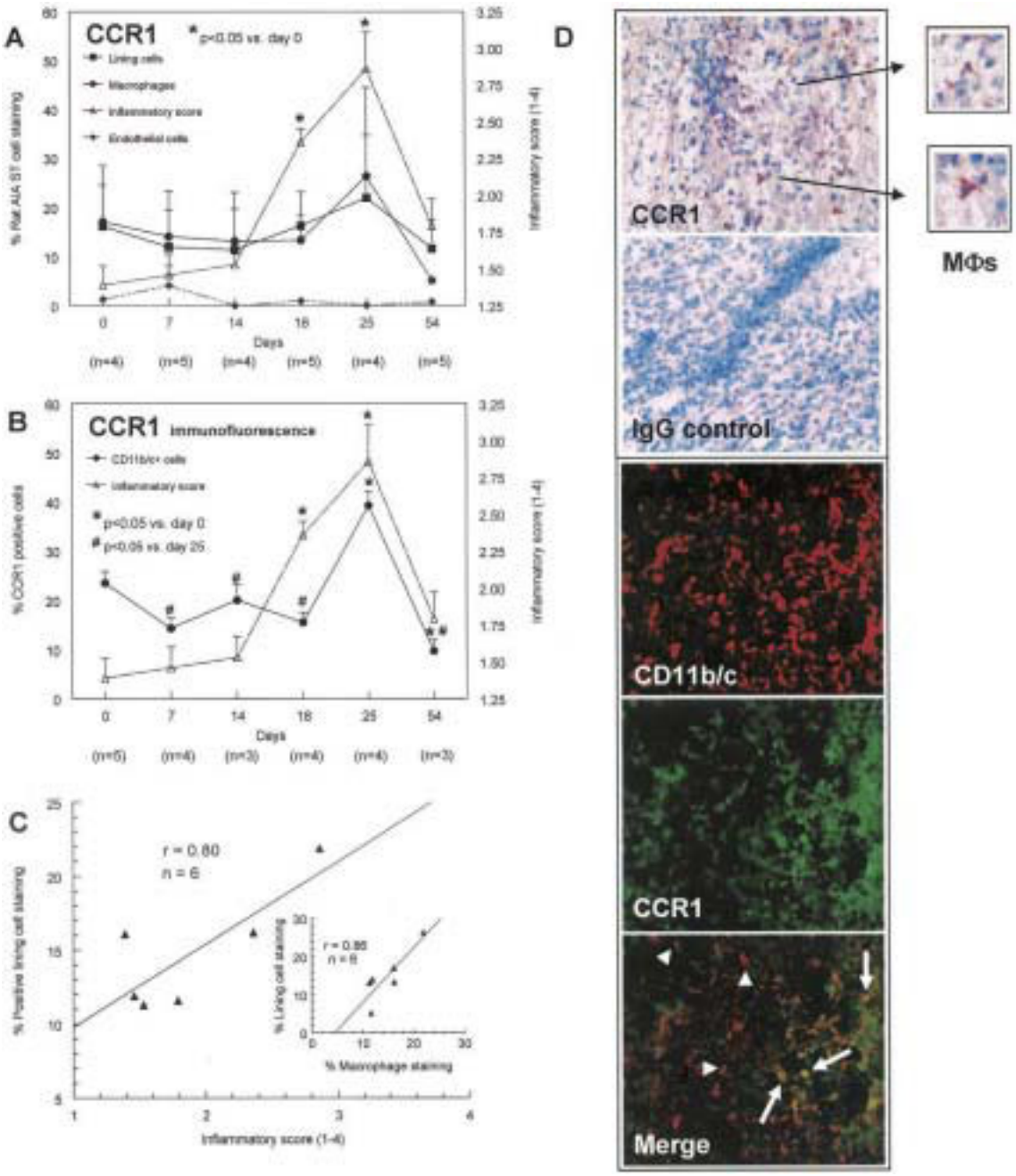

Figure 1. Kinetics of histologic inflammation and expression of CCR1 in synovial tissue (ST) from rats with adjuvant-induced arthritis (AIA). **A,** Inflammation score and expression of CCR1 by various cell types. Lining cells and macrophages (MΦs) showed constitutive expression of CCR1. The percentage of lining cells and macrophages that were immunopositive for CCR1 tended to be increased on day 25 (the time of maximum inflammation of the joints), declining thereafter. Values are the mean and SEM. **B,** Results of double staining in relation to inflammation score. Double staining with anti-CD11b/c using immunofluorescence confirmed the baseline expression of CCR1 in macrophages and revealed significant up-regulation on day 25 post–adjuvant injection, with subsequent down-regulation. Values are the mean and SEM. **C,** Correlations between CCR1 expression and inflammation score during the course of AIA. Expression of CCR1 on lining cells correlated with the histologic inflammation score over time and mirrored CCR1 staining on macrophages. n = number of time points. **D,** CCR1 expression by immunohistochemistry and immunofluorescence in ST from rats on day 0 (prior to adjuvant injection). There was intense staining for CCR1 on macrophages compared with staining with control IgG. Immunofluorescence costaining with anti-CD11b/c confirmed the high percentage of immunopositivity on macrophages, but suggested that CCR1 was expressed not only on the cell surface, but also on the cytoplasm (**arrows**). Merge = overlay of CD11b/c and CCR1 staining; **arrowheads** indicate CD11b/c-positive cells that were negative for CCR1 (original magnification × 100; the 2 smaller boxes show further magnifications of the indicated areas [original magnification × 250]).

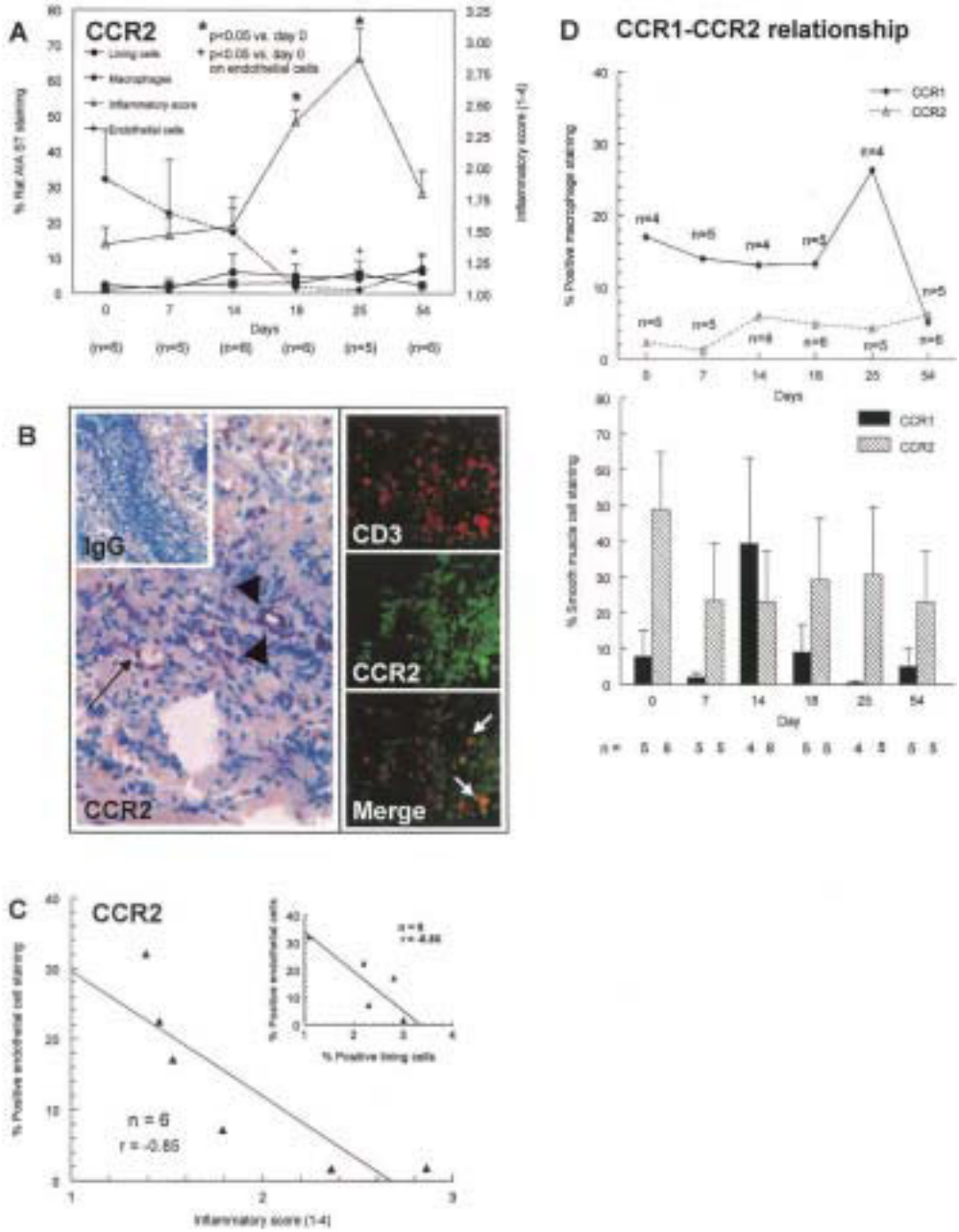

Figure 2. Kinetics of histologic inflammation and expression of CCR2 in ST and comparison with CCR2 expression in macrophages and smooth muscle cells in rats with AIA. **A,** Inflammation score and expression of CCR2 by various cell types. Endothelial cells (ECs) constitutively expressed CCR2. ST EC expression of CCR2 remained high in early AIA, but decreased dramatically by day 18, with the decrease persisting to day 25 (the time of maximum inflammation of the joints) and an increase tending to occur thereafter. Macrophages showed only slight expression of CCR2, but with a trend toward an increase on day 14. Values are the mean and SEM. **B,** CCR2 expression determined by immunohistochemistry in ST of a rat with AIA compared with an IgG-treated control (**inset**) on day 14, showing occasional macrophage staining (**black arrow**) and intense EC staining in the ST microvasculature (**arrowheads**). CD3 staining (as a marker for lymphocytes) on day 18 showed only occasional colocalization with CCR2 in inflammatory ST (**white arrows**). Merge = overlay of CD11b/c and CCR1 staining (original magnification × 200 in left image [including inset]; original magnification × 100 in right images). **C,** Course of EC expression of CCR2 in rat AIA, showing a negative correlation with the degree of inflammation and with CCR2 expression on lining cells. n = number of time points. **D,** Percentages of macrophages stained for CCR1 and CCR2 during the course of rat AIA, indicating inverse expression of the 2 chemokine receptors over time. Smooth muscle cells that were immunopositive for CCR1 and CCR2 in normal rats (day 0) are shown for comparison. CCR2 showed abundant constitutive expression (49 ± 6%), and tended to decrease on day 14 of AIA (23 ± 14%), followed by a rebound on day 25. In contrast, CCR1 expression on normal ST smooth muscle cells was low, but increased slightly on day 14 with a subsequent decline to baseline values. Values are the mean and SEM. See Figure 1 for other definitions. Color figure can be viewed in the online issue, which is available at http://www.arthritisrheum.org.

ment, based on the appearance of infiltrating inflammatory cells, demonstrated significant deterioration on day 18 ($P < 0.05$), with peak inflammation on day 25 (Figure 1A). Immunofluorescence data on CD11b/c-positive macrophages paralleled the inflammation score, showing significant cell influx ($P < 0.05$) starting on day 14 (mean ± SEM 16 ± 2 cells/hpf, compared with 8 ± 1 cells/hpf in nonarthritic controls [day 0]), with further increases on day 18 (20 ± 4 cells/hpf) and day 25 (38 ± 6 cells/hpf) and a subsequent decline on day 54 (12 ± 2 cells/hpf).

Up-regulation of constitutive CCR1 expression in rat AIA. Among the important receptors in RA is CCR1, with its ligands RANTES/CCL5 and macrophage inhibitory protein 1α (MIP-1α)/CCL3. CCR1 expression in ST macrophages was constitutive and showed an increase on day 25 post–adjuvant injection (which was the time of maximum joint inflammation), with a subsequent decline to baseline expression on day 54, as determined by both immunohistochemistry and immunofluorescence (Figures 1A and B). Surprisingly, lining cells also exhibited major constitutive expression of CCR1, which tended to increase at the peak of inflammation with a subsequent decline, the number mirroring CCR1 expression on macrophages (r = 0.86) (Figures 1A and C). Expression of CCR1 on lining cells also correlated with the histologic inflammation score (r = 0.80) (Figure 1C), while ECs and lymphocytes were nonreactive for CCR1 (data not shown).

Role of CCR2 in the initial recruitment of peripheral blood monocytes to ST in rats with AIA. Macrophages in normal rat ST were only occasionally immunopositive for CCR2, a receptor for monocyte chemoattractant protein 1 (MCP-1)/CCL2, but in macrophages from rats with AIA, CCR2 levels tended to be increased on day 14 post–adjuvant injection and thereafter (Figure 2A). In contrast, ST ECs showed constitutive expression of CCR2 that remained high in early AIA (Figures 2A and B), but decreased dramatically by day 18 (with the decrease persisting to day 25 [at the peak of inflammation] [$P < 0.05$]) and tended to increase thereafter. These data suggest a role for CCR2 on ECs in early leukocyte recruitment and transendothelial migration. Moreover, EC expression of CCR2 was inversely correlated with both the degree of inflammation (r = −0.85) and CCR2 expression on lining cells (r = −0.85) (Figure 2C). CD3+ lymphocytes expressed CCR2 only occasionally (Figure 2B).

Inverse correlation of CCR1 and CCR2 expression on ST in AIA. Figure 2D shows the interrelation of CCR1 and CCR2 expression on macrophages and in smooth muscle cells in rat AIA. Interestingly, the pattern of expression of CCR2 on those cell types was almost inverse to that of CCR1 expression on macrophages. This observation was corroborated by observations of CCR1 and CCR2 expression on smooth muscle cells in ST of rats with AIA at different time points compared with day 0. CCR2 was amply constitutively expressed in smooth muscle cells but showed a trend toward decreased immunoreactivity on days 7 and 14 of AIA, followed by a slight rebound. In contrast, CCR1 expression on normal ST smooth muscle cells was low, but increased on day 14 with a subsequent decline to baseline values, underscoring the different time and nature of action of the 2 receptors in leukocyte recruitment and retention in rat AIA.

Role of CCR3 in early AIA in rats. CCR3, a receptor for RANTES/CCL5 and eotaxin/CCL11, was present on macrophages in rats with early AIA (mean ± SEM 42 ± 11%), but expression tended to decrease during the course of AIA (23 ± 14% on day 18) (Figures 3A and B), supporting the notion of their initial involvement in leukocyte recruitment to the joint. CCR3 expression on lining cells in ST of rats with AIA exhibited a similar time course (Figure 3A), emphasizing the role of the receptor in this context. Conversely, ECs barely expressed CCR3, and lymphocytes did not show significant immunopositivity (data not shown), as confirmed by immunofluorescence costaining with CD3.

CCR4 expression on synovial endothelium promotes leukocyte recruitment in rats with early AIA. CCR4, a receptor for thymus and activation–regulated chemokine (TARC)/CCL17 and macrophage-derived chemokine (MDC)/CCL22, may be particularly important for CD3+,CD4+ memory T cell migration to synovium in RA (13,14). As shown in Figure 3C, constitutive CCR4 EC expression was down-regulated as inflammation progressed, supporting the idea that CCR4 has a specific role in leukocyte recruitment in the early phase of AIA. In contrast, CCR4 expression on macrophages was weak and unaltered, coinciding with the finding that ST lining cells and lymphocytes did not express CCR4 in AIA (data not shown).

Importance of CCR5 expression on macrophages for sustaining the inflammatory changes in rat AIA. Investigators at our laboratory have shown previously that CCR5, the receptor for RANTES/CCL5, MIP-1α/CCL3, and MIP-1β/CCL4, is up-regulated at the protein and messenger RNA (mRNA) levels on various ST cells during peak inflammation in rat AIA (11). In the present study we expanded those data by defining CCR5 expression at the protein level at different time points during the course of AIA in the context of the expression patterns of other chemokine receptors.

Rat ST macrophages exhibited weak constitutive expression of CCR5 that tended to be up-regulated in

AIA starting on day 7, with a significant increase on day 54 ($P < 0.05$) (Figures 4A and B), suggesting a distinct role for CCR5 and its ligands particularly in maintaining the inflammatory process. CCR5 expression on macrophages correlated well with CCR5 immunoreactivity on lining cells (r = 0.90) and tended to have a course similar to that of macrophage staining for CCR2 (r = 0.76), suggesting a close pathophysiologic relationship of the 2 chemokine receptors (Figure 4C). Immunofluorescence analysis for CCR5 and CD11b/c on day 18 confirmed colocalization of CCR5 in rat macrophages, revealing both cytoplasmic and cell surface expression (Figure 4D). ECs showed only weak CCR5 expression, with a moderate, but not statistically significant, increase in early AIA (Figure 4A), while lymphocytes were essentially immunonegative for the receptor (data not shown).

CXCR4 expression on ECs precedes the peak of inflammation in rat AIA. CXCR4, the receptor for stromal cell–derived factor 1 (SDF-1)/CXCL12 (which plays a critical role in monocyte localization to inflamed RA synovium [15], as well as in promoting neovascularization in RA ST [16]), showed prominent expression on ECs starting on day 14 ($P < 0.05$) (Figure 5A). CXCR4 expression preceded the peak of inflammation and was followed by a slow decline until day 54, emphasizing its potential role in retention of inflammatory effector cells in the joint (Figure 5A). Double immunofluorescence studies for vWF and CXCR4 confirmed the endothelial expression during the course of rat AIA (Figure 5C). Normal macrophages (i.e., day 0) expressed minimal CXCR4, but expression of the receptor on macrophages was increased starting on day 18 of AIA ($P < 0.05$) (Figures 5A and B). In addition, immunofluorescence staining revealed CXCR4 expression predominantly on the cell surface (Figure 5D). In contrast, ST lining cells expressed CXCR4 constitutively (mean ± SEM 22 ± 9%), followed by significant CXCR4 down-regulation in the early phase of AIA ($P < 0.05$) (Figure 5A). Again, lymphocytes in ST from rats with AIA only occasionally expressed CXCR4, and costaining with the T cell marker CD3 corroborated these findings (data not shown).

Chemokine receptor expression on stimulated NR8383 cells reflects the in vivo situation in early AIA in rats. To investigate whether expression of chemokine receptors on rat macrophages in vitro reflects in vivo conditions and if expression changes in response to a proinflammatory stimulus, we used the rat macrophage cell line NR8383 to determine baseline expression and intracellular distribution before and after stimulation with LPS (Figure 6A). NR8383 cells showed intense constitutive, predominantly cytoplasmic, expression of CCR1 which was not affected by LPS stimulation, corroborating the in vivo data. NR8383 cells demon-

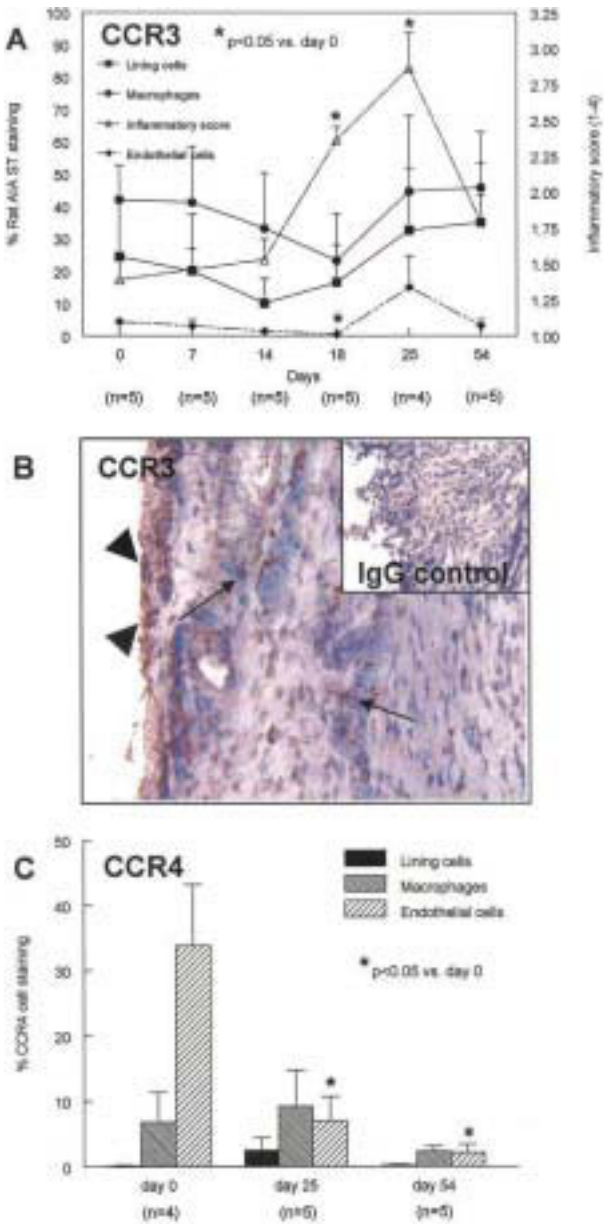

Figure 3. Kinetics of histologic inflammation and expression of CCR3 and CCR4. **A,** Inflammation score and expression of CCR3 by various cell types. CCR3 was constitutively expressed on rat macrophages (42 ± 11%) and lining cells (25 ± 18%), but its expression tended to decrease during rat AIA, with a rebound on days 25–54. Endothelial cells barely expressed CCR3, except later in the course of AIA. Values are the mean and SEM. **B,** CCR3 expression determined by immunohistochemistry in ST of a rat with AIA compared with an IgG-treated control (**inset**) on day 14, showing intense staining for CCR3 on lining cells (**arrowheads**) and macrophages (**arrows**) (original magnification × 200). **C,** Percentages of various cell types stained for CCR4 on day 25 and day 54 in rats with AIA, compared with findings in normal rats (day 0). While constitutive endothelial cell CCR4 expression was significantly down-regulated during inflammation, no major changes were observed regarding CCR4 expression on macrophages and lining cells. Values are the mean and SEM. See Figure 1 for definitions. Color figure can be viewed in the online issue, which is available at http://www.arthritisrheum.org.

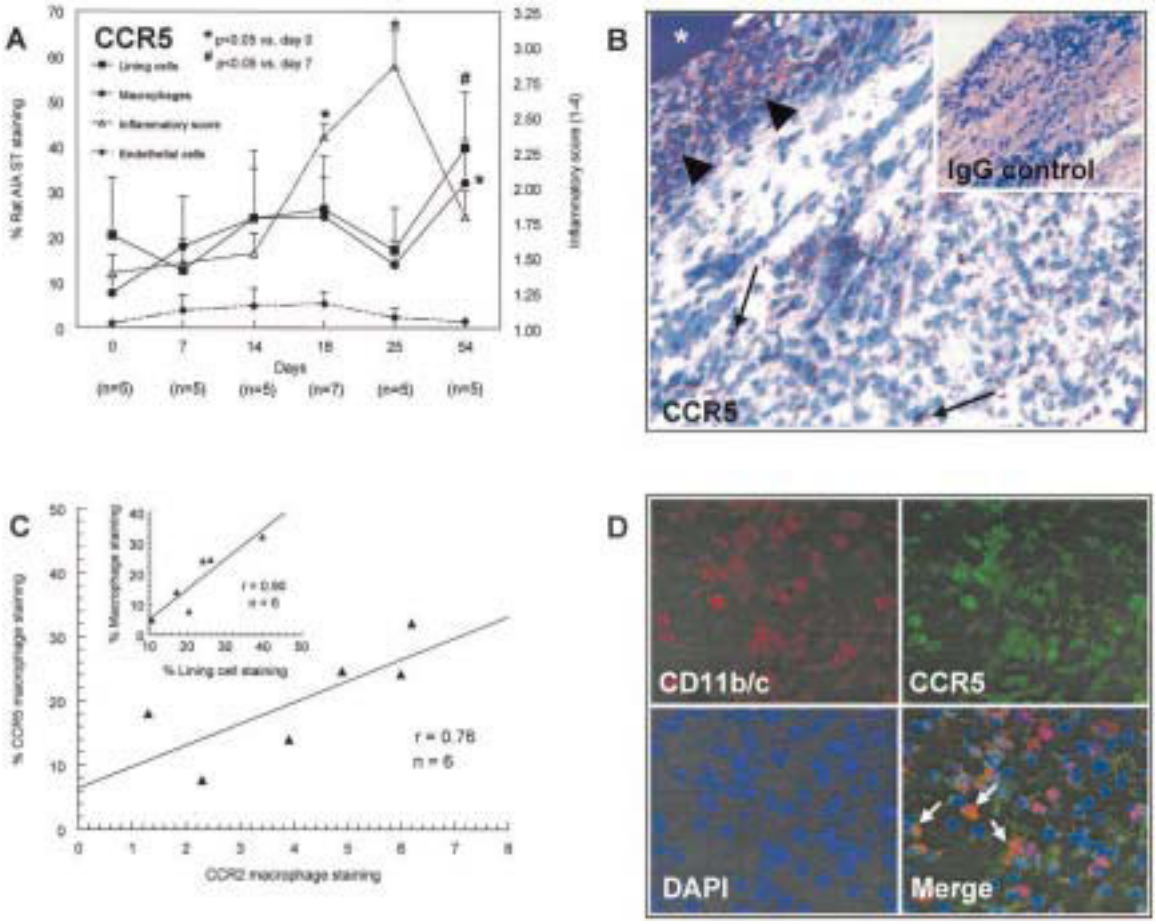

Figure 4. Kinetics of histologic inflammation and expression of CCR5 in ST from rats with AIA. **A,** Inflammation score and expression of CCR5 by various cell types. Low constitutive CCR5 expression on macrophages showed a trend toward up-regulation during AIA, and was significantly increased on day 54. A similar pattern was observed for CCR5 on ST lining cells. Endothelial cells showed weak CCR5 expression, with a moderate increase in early AIA that was not statistically significant. Values are the mean and SEM. **B,** CCR5 expression determined by immunohistochemistry in ST of a rat with AIA compared with an IgG-treated control (**inset**) on day 18, showing macrophage staining (**arrows**), as well as CCR5-positive lining cells (**arrowheads**); **asterisk** indicates adjacent bone (original magnification × 200). **C,** Correlations of CCR5 expression on macrophages with CCR2 expression on macrophages and with CCR5 staining on lining cells during the course of AIA. Macrophage CCR5 staining clearly mirrored lining cell CCR5 staining and correlated with macrophage CCR2 staining. n = number of time points. **D,** Double immunofluorescence staining for the macrophage marker CD11b/c and CCR5, showing colocalization after merging (**arrows**). Counterstaining was performed with 4′,6-diamidino-2-phenylindole-dihydrochloride (DAPI) (original magnification × 200). See Figure 1 for other definitions. Color figure can be viewed in the online issue, which is available at http://www.arthritisrheum.org.

strated less CCR2 expression both with and without LPS stimulation, also paralleling the results obtained for CCR2 staining on normal and AIA ST. Likewise, CCR4 could not be detected on NR8383 cells in vitro (results not shown), while high constitutive cell surface expression of CCR3 showed a slight decrease in response to LPS. Surprisingly, stimulation of quiescent NR8383 cells resulted in clear up-regulation of CCR5. In contrast, a faint but distinct cell surface pattern for CXCR4 essentially did not change after LPS treatment.

CXCR1 expression on smooth muscle cells in rat ST. Immunohistochemistry analysis for CXCR1 in normal rat ST (Figure 6B) showed constitutive expression on smooth muscle cells (mean ± SEM 9 ± 7%), which tended to be decreased on day 18 of rat AIA (4 ± 3%) and thereafter (2 ± 1% on day 54). Conversely, in lining cells and macrophages, CXCR1 expression was increased during rat AIA compared with expression in normal rats ($P < 0.05$ for macrophages on day 18).

Role of CXCR2, CXCR3, and CXCR5 in rat synovium. Evaluation of additional CXC chemokine receptors in normal and arthritic rat ST demonstrated expression only on lining cells and macrophages (Figure 6C), but virtually no staining on ECs or lymphocytes (data not shown). CXCR2, a receptor for the human chemokines growth-related oncogene α/CXCL1 and

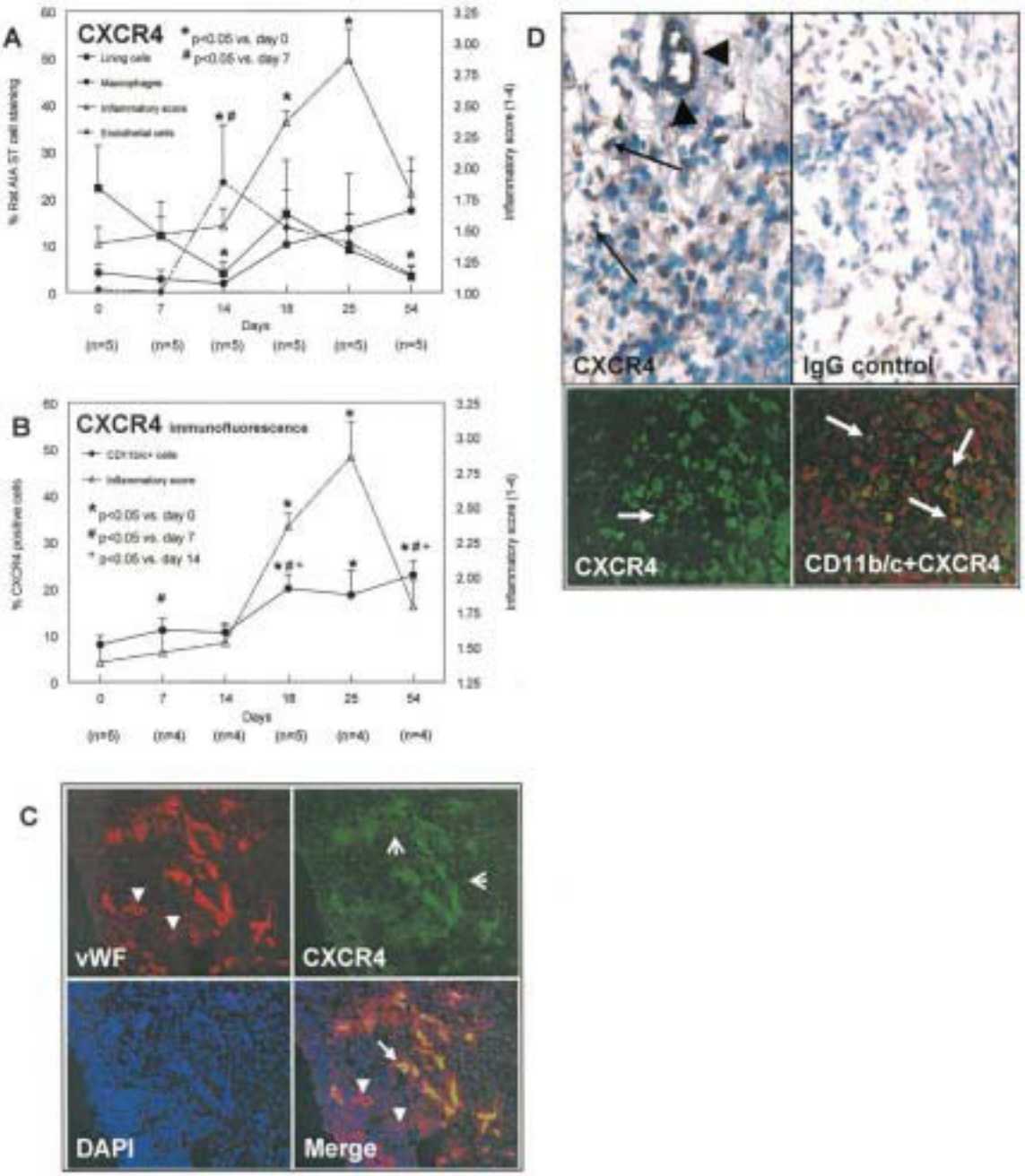

Figure 5. Kinetics of histologic inflammation and expression of CXCR4 in ST from rats with AIA. **A,** Inflammation score and expression of CXCR4 by various cell types. Compared with nonarthritic controls (day 0), endothelial cells (ECs) showed prominent CXCR4 expression on day 14, preceding the peak of inflammation and declining thereafter. ST lining cells, which constitutively expressed CXCR4, exhibited decreased expression of this receptor in early AIA, while CXCR4 expression on macrophages tended to increase in late AIA. Values are the mean and SEM. **B,** Results of double staining in relation to inflammation score. Double staining with anti-CD11b/c using immunofluorescence confirmed the observation of low baseline CXCR4 expression on macrophages with a subsequent significant increase starting on day 18. Values are the mean and SEM. **C,** Double immunofluorescence for the EC marker von Willebrand factor (vWF) and CXCR4. Merging of the two revealed colocalization (**arrow** in lower right image). **Arrows** in upper right image indicate CXCR4 staining not associated with ECs; **arrowheads** in upper left and lower right images indicate ECs not positive for CXCR4. Counterstaining was performed with 4′,6-diamidino-2-phenylindole-dihydrochloride (DAPI) (original magnification × 100). **D,** CXCR4 expression by immunohistochemistry in ST of a rat with AIA compared with an IgG-treated control on day 18, showing macrophage staining (**arrows** in upper left and lower left images) and positive staining on ECs (**arrowheads**). Double immunofluorescence staining with anti-CD11b/c revealed CXCR4 expression on macrophages predominantly on the cell surface (**arrows** in lower right image) (original magnification × 200). See Figure 1 for other definitions. Color figure can be viewed in the online issue, which is available at http://www.arthritisrheum.org.

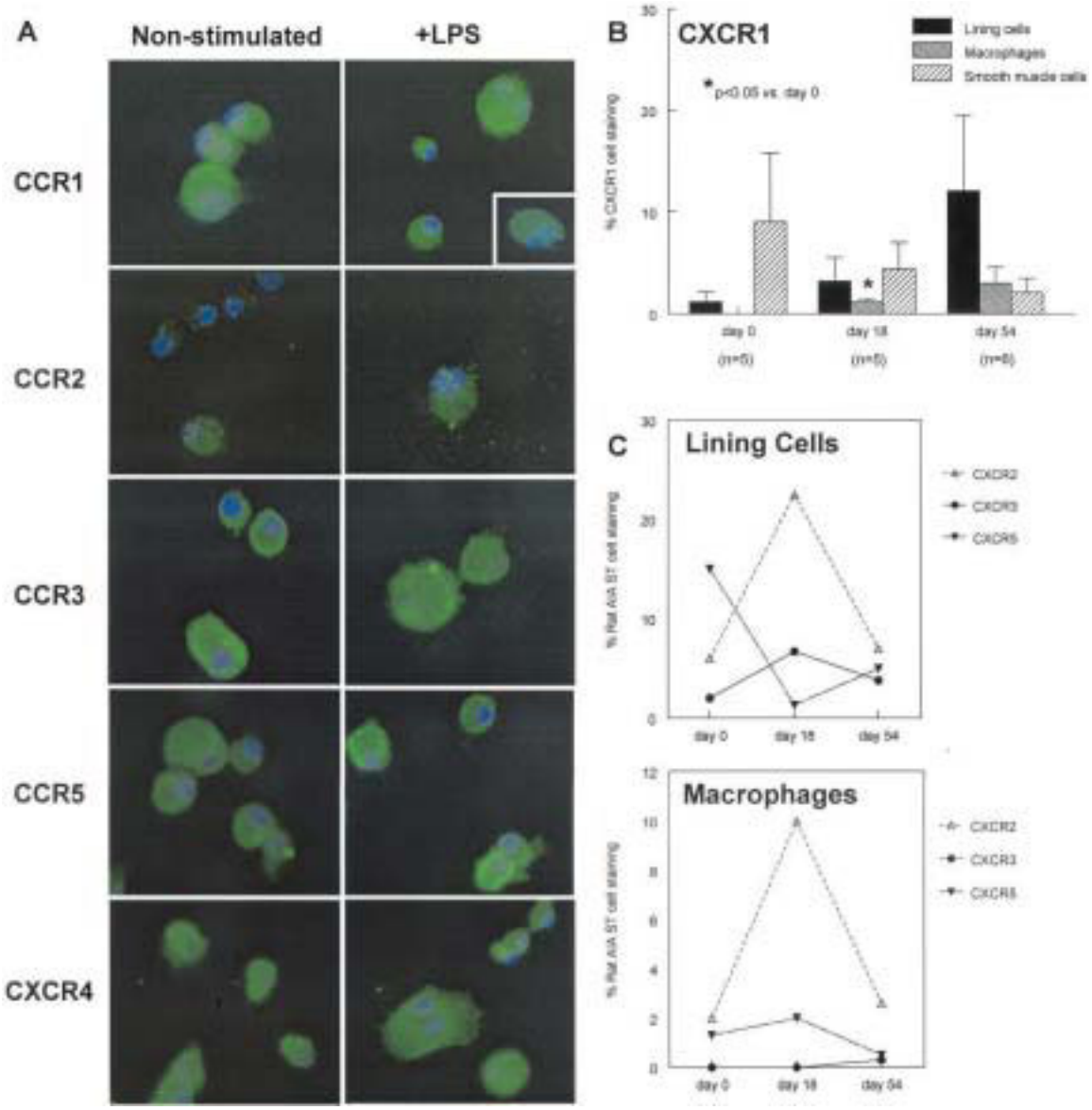

Figure 6. Chemokine receptor expression in the rat macrophage cell line NR8383 and expression of CXCR on ST from rats with AIA. **A,** Immunofluorescence staining for chemokine receptors on NR8383 cells, without stimulation and after stimulation with lipopolysaccharide (LPS; 10 μg/ml), showing intense constitutive cytoplasmic expression for CCR1, very low baseline expression for CCR2, and distinct cell surface expression for CXCR4, none of which was significantly altered by LPS. IgG isotype was used as a control (**inset**). High baseline expression for CCR3, showing immunopositivity predominantly on the cell surface, was only slightly affected by LPS stimulation. Stimulation of quiescent NR8383 cells resulted in a dramatic up-regulation of CCR5, with a clear cell surface pattern (original magnification $\times$ 1,000). **B,** Percentages of various cell types stained for CXCR1 on day 18 and day 54 in rats with AIA, compared with findings in normal rats (day 0). CXCR1 was constitutively expressed on smooth muscle cells, followed by a decline during rat AIA. Conversely, lining cells showed a trend toward increased CXCR1 expression over time, and macrophages showed de novo expression of CXCR1 on day 18 of rat AIA, which was significant compared with findings in normal rat ST macrophages. Values are the mean and SEM. **C,** Trends in CXCR2, CXCR3, and CXCR5 expression on lining cells and macrophages in ST from rats with AIA on day 18 and day 54 post–adjuvant administration, compared with day 0. Values are the mean. See Figure 1 for other definitions. Color figure can be viewed in the online issue, which is available at http://www.arthritisrheum.org.

epithelial neutrophil–activating protein 78/CXCL5 or the rat homologs cytokine-induced neutrophil chemoattractant 1/CXCL1 and LPS-induced CXC chemokine/CXCL5, tended to increase during the inflammatory phase of rat AIA, on day 18. CXCR3, a receptor for interferon-γ–inducible 10-kd protein (IP-10)/CXCL10 and monokine induced by interferon-γ (Mig)/CXCL9 showed a similar trend on lining cells, while CXCR5, a

receptor for B cell–attracting chemokine 1/CXCL13, showed baseline expression on lining cells with a trend toward a decrease during the course of AIA.

DISCUSSION

Chemokines and their receptors have increasingly been considered to be interesting potential targets for therapy in RA (17). The prevailing paradigm is that leukocyte influx into the joint is orchestrated through a sophisticated network of expressed chemokine receptors and their ligands. Thus, knowledge of defined expression patterns is needed in order to develop means of blocking chemokine interactions with their receptors, thus modifying the inflammatory process.

In human RA, CCR1-positive cells, mainly macrophages, are scattered throughout the synovium (14). Studies of CCR1 disruption in mice have yielded conflicting results regarding inflammatory responses, with both abrogation (18) and enhancement (19) being observed. However, blocking of CCR1 ligands resulted in impaired monocyte migration in vitro in response to RA SF and in reduced synovial inflammation and joint destruction in rodent models of RA (20,21). Moreover, a double-blind, placebo-controlled, phase Ib clinical trial using an oral CCR1 antagonist showed improved histologic features in ST of patients with RA (17).

In our study, constitutive CCR1 expression on ST lining cells tended to increase at the time of maximum inflammation in AIA. ST macrophages also showed constitutive expression of CCR1, with a significant increase at the time of peak inflammation on day 25, supporting findings in human RA (14). Surprisingly, we observed cytoplasmic and cell surface expression of CCR1 both in vivo and in vitro, potentially indicating a yet-unknown function of this receptor. These results suggest that CCR1 has a distinct role in recruiting and retaining leukocytes in rat AIA, and underscore our recent finding that blocking of CCR1 in rat AIA significantly reduced joint inflammation and monocyte/macrophage influx (22). These data also parallel findings on the fractalkine receptor CX_3CR1 in rats, i.e., lack of EC staining but high constitutive macrophage staining, maintained during AIA (10).

CCR2 is expressed on T cells and monocytes infiltrating human RA ST (23). In studies of blocking of the CCR2 ligand MCP-1/CCL2, there was partial improvement of murine arthritis (24). Surprisingly, in a collagen-induced arthritis (CIA) model, the disease was worse in CCR2 gene–deficient mice compared with wild-type controls (25); this suggested that CCR2 can serve as a negative regulator of arthritis onset and severity, conflicting with the current paradigm and implicating a more variable role of CCR2 than initially anticipated. In the present study, CCR2 protein expression was occasionally observed in macrophages from normal rat ST and tended to increase slightly on day 14 of AIA and thereafter, while expression of CCR2 mRNA in the whole joint has been shown to be clearly up-regulated at the time of maximum inflammation (11).

Interestingly, immunoreactivity for CCR2 on ECs was high constitutively as well as in early AIA, but had decreased dramatically by day 18 post–adjuvant administration, correlating negatively with the degree of inflammation. This distinctive expression of a chemokine receptor on endothelium, varying over time, may result in temporospatially specific leukocyte adhesion and transmigration, thus controlling the inflammatory process (26). Recently, Dzenko et al showed that CCR2 expression by ECs is critical for macrophage transendothelial migration in the brain (27). Our findings suggest that CCR2 on ECs mediates leukocyte recruitment in early AIA by facilitating transendothelial migration of mononuclear cells, but may function in a different way at a later time point. This notion of a dual role is supported by the results of a study in which blockade of CCR2 during the early phase of CIA resulted in improved clinical signs of arthritis and histologic scores, whereas arthritis was aggravated following CCR2 blockade in late stages of CIA (28).

Of note, on macrophages and smooth muscle cells, CCR2 expression and CCR1 expression displayed inverse patterns over time, thereby indicating essentially different roles of the 2 chemokine receptors in recruiting and retaining leukocytes in rat AIA. Both CCR1 and CCR2 have previously been reported to be expressed by human vascular smooth muscle cells in vitro (29), which could explain the ability of smooth muscle cells to respond to certain chemokines, thereby modifying proliferation and migration. The significance of this observation in the context of RA, however, needs further evaluation.

CCR3 was initially thought to be specific for eosinophils but was subsequently identified to mediate T cell recruitment (30). CCR3 has been demonstrated to be moderately expressed on CD14+ peripheral blood monocytes in patients with RA, while normal control subjects showed minimal expression (14), thereby indicating a potential role of CCR3 in monocyte recruitment. In the present study, CCR3 was detected on ST macrophages and lining cells in early AIA with a subsequent trend toward declining expression, supporting the idea of initial involvement in leukocyte trafficking to the synovial joint. High constitutive expression of CCR3 on rat ST macrophages was confirmed in experiments with resting rat macrophages in vitro, with only a slight

decrease after LPS stimulation, reflecting the in vivo situation.

CCR4, initially thought to be restricted to T cells and basophils, has subsequently been detected on peripheral blood monocytes of RA patients (14). In human RA ST, however, CCR4 was not detected on macrophages (14). Findings of other studies support the idea that CCR4 may be crucial to the pathogenesis of RA (13,31), although only limited data on the role of the CCR4 ligands TARC/CCL17 and MDC/CCL22 are available (32,33). The weak expression of CCR4 on synovial macrophages as well as its virtual absence on lining cells in rat AIA are supportive of the hypothesis that this receptor does not have an essential proinflammatory role, despite the observation that total CCR4 mRNA expression in joints increased during rat AIA (11). In some studies, CCR4 has even been considered to have an antiinflammatory role (14,34). In contrast, constitutive CCR4 expression in ECs was significantly down-regulated during inflammation, suggesting a possible role of CCR4 in leukocyte recruitment and transmigration in the early phase of AIA. Interestingly, CCR4-deficient mice are resistant to LPS-induced endotoxic shock, indicating a defect in macrophage function (35). It would be very informative to observe the effect of CCR4 deletion in an arthritis model.

Of particular interest regarding therapeutic intervention in RA is CCR5, although in a recent study the course of CIA was not significantly modified in CCR5-deficient mice (25). However, in a nonhuman primate model of RA, treatment with a CCR5 antagonist resulted in clinical improvement of the disease (36), supporting the notion that this chemokine receptor has a pathogenic role in arthritis and providing strong evidence for the potential value of therapy targeting this receptor.

In the present study, CCR5 expression on synovial macrophages showed a trend toward up-regulation starting on day 7, with significantly increased levels of CCR5-immunopositive macrophages on day 54, confirming observations in ST of humans with RA (14). CCR5 expression on lining cells showed a similar pattern, with protein expression following up-regulation of CCR5 in the joint at the mRNA level (11). These data suggest a distinct role for CCR5 and its ligands, particularly in maintaining the inflammatory process. However, CCR5 up-regulation on rat macrophages in response to LPS, together with increased CCR5 expression in vivo, may indicate an important pathophysiologic role over time in arthritis. An array-based analysis of not-yet-inflamed joints in mice with autoimmune arthritis identified CCR5 as one of the most highly up-regulated genes (37). In addition, mice that were deficient for the CCR5 ligand MIP-1α/CCL3 exhibited milder arthritis (38). We also recently showed that blocking of CCR5 in rat AIA resulted in reduced joint destruction (22). Taken together, these findings indicate that CCR5 targeting is an interesting potential therapeutic approach, possibly in later rather than earlier stages of inflammatory arthritis.

SDF-1/CXCL12 has been demonstrated to be highly expressed in RA ST, and expression of its receptor, CXCR4, was observed on CD4+ T cells following their entry into the synovium (39,40). Surprisingly, we did not detect substantial CXCR4 expression on lymphocytes in ST from rats with AIA, suggesting that murine lymphocytes may play a different role than in human RA. Similarly, we demonstrated in an earlier study that CX_3CR1 was virtually absent on lymphocytes during rat AIA (10), although it was clearly expressed on human T cells in the arthritic joint (13). In contrast, CXCR4 was prominently expressed on ST ECs in rat AIA, starting on day 14 and preceding the inflammatory peak. Likewise, Burman and coworkers found high expression of the ligand SDF-1/CXCL12 on vascular endothelium in inflamed RA ST (41). The expression of both receptor and ligand on ECs indicates a potential self-perpetuating pro-angiogenic mechanism, possibly via autocrine expression of vascular endothelial growth factor (42). Also, these data support the notion of a potential role of CXCR4 in retention of inflammatory effector cells and transendothelial migration in the joint, thereby driving the inflammatory process (43). EC expression of different chemokines may result in directional migration of leukocytes (26), with CXCR4 being a crucial part of this intercellular communication.

Macrophages showed increased expression of CXCR4 starting on day 18 after AIA induction. Similarly, CXCR4 has been implicated in monocyte localization to inflamed RA ST (15). In our study, ST lining cells expressed CXCR4 constitutively, followed by down-regulation of CXCR4 in the early phase of AIA, suggesting a self-limiting process.

Interestingly, immunohistochemistry analysis revealed a cytoplasmic staining pattern for CXCR4, while immunofluorescence clearly identified the receptor on the cell surface. This is consistent with previous observations, reflecting the limitations of the detection method used (10,11). Additional in vitro experiments using the rat macrophage cell line NR8383 confirmed the predominant cell surface expression not only of CXCR4 but also of other receptors. Expression in response to LPS essentially paralleled the in vivo situation in early rat AIA. Thus, NR8383 cells may be a useful in vitro tool to study the inflammatory response in

rat AIA, as previously shown for other inflammatory diseases (12,44).

Human interleukin-8 (IL-8)/CXCL8 is known to mediate inflammation in RA, and there is some evidence for a role for its receptor, CXCR1, in RA (45). Although CXCR1 is present in rats, a rodent homolog of IL-8/CXCL8 has not been identified. In our study, CXCR1 was constitutively expressed on smooth muscle cells, with a subsequent decrease during AIA. As with CCR1 and CCR2, the role of the CXCR1 that is substantially constitutively expressed on synovial smooth muscle cells remains unclear. Conversely, lining cells and macrophages showed increased CXCR1 expression during the course of arthritis, indicating a possible role in maintaining the inflammatory process.

Results of staining for CXCR2 on both lining cells and macrophages suggested a potential role in the inflammatory phase, consistent with data regarding mediation of inflammation in RA by its ligands, likely via promotion of angiogenesis (46). The chemokines IP-10/CXCL10 and Mig/CXCL9 have been detected in human RA (47), with their receptor CXCR3 demonstrated on ECs in inflamed tissue (48). However, in the present study, staining for CXCR3 failed to reveal substantial expression on macrophages or ECs in rat ST, implying that this receptor may be part of leukocyte ingress in inflamed synovium only if expressed on the recruited cells.

A recent study demonstrated reduced severity of CIA in mice in response to inhibition of the CXCR5 ligand CXCL13 (49), which indicated a potential role for CXCR5 in inflammatory arthritis. CXCR5 was also found to be up-regulated in human RA synovium (50). Indeed, in our study, CXCR5 was also present on lining cells in normal and arthritic rat ST and showed minor expression on macrophages.

In summary, this study provides information on cell type–specific protein expression of CC and CXC chemokine receptors during the course of rat AIA. This knowledge of the temporospatial expression of chemokine receptors may help to optimize target-oriented blocking of chemokine receptors at different phases of inflammatory arthritis.

REFERENCES

1. Firestein GS. Evolving concepts of rheumatoid arthritis. Nature 2003;423:356–61.
2. Gerard C, Rollins BJ. Chemokines and disease. Nat Immunol 2001;2:108–15.
3. Rossi D, Zlotnik A. The biology of chemokines and their receptors. Annu Rev Immunol 2000;18:217–42.
4. Zlotnik A, Yoshie O. Chemokines: a new classification system and their role in immunity. Immunity 2000;12:121–7.
5. Luster AD. Chemokines: chemotactic cytokines that mediate inflammation. N Engl J Med 1998;338:436–45.
6. D'Ambrosio D, Panina-Bordignon P, Sinigaglia F. Chemokine receptors in inflammation: an overview. J Immunol Methods 2003;273:3–13.
7. Rabin RL, Park MK, Liao F, Swofford R, Stephany D, Farber JM. Chemokine receptor responses on T cells are achieved through regulation of both receptor expression and signaling. J Immunol 1999;162:3840–50.
8. Heydtmann M, Adams DH. Understanding selective trafficking of lymphocyte subsets. Gut 2002;50:150–2.
9. Volin MV, Szekanecz Z, Halloran MM, Woods JM, Magua J, Damergis JA Jr, et al. PECAM-1 and leukosialin (CD43) expression correlate with heightened inflammation in rat adjuvant-induced arthritis. Exp Mol Pathol 1999;66:211–9.
10. Ruth JH, Volin MV, Haines GK III, Woodruff DC, Katschke KJ Jr, Woods JM, et al. Fractalkine, a novel chemokine in rheumatoid arthritis and in rat adjuvant-induced arthritis. Arthritis Rheum 2001;44:1568–81.
11. Shahrara S, Amin MA, Woods JM, Haines GK, Koch AE. Chemokine receptor expression and in vivo signaling pathways in the joints of rats with adjuvant-induced arthritis. Arthritis Rheum 2003;48:3568–83.
12. Wong LY, Cheung BM, Li YY, Tang F. Adrenomedullin is both proinflammatory and antiinflammatory: its effects on gene expression and secretion of cytokines and macrophage migration inhibitory factor in NR8383 macrophage cell line. Endocrinology 2005;146:1321–7.
13. Ruth JH, Rottman JB, Katschke KJ Jr, Qin S, Wu L, LaRosa G, et al. Selective lymphocyte chemokine receptor expression in the rheumatoid joint. Arthritis Rheum 2001;44:2750–60.
14. Katschke KJ Jr, Rottman JB, Ruth JH, Qin S, Wu L, LaRosa G, et al. Differential expression of chemokine receptors on peripheral blood, synovial fluid, and synovial tissue monocytes/macrophages in rheumatoid arthritis. Arthritis Rheum 2001;44:1022–32.
15. Blades MC, Manzo A, Ingegnoli F, Taylor PR, Panayi GS, Irjala H, et al. Stromal cell-derived factor 1 (CXCL12) induces human cell migration into human lymph nodes transplanted into SCID mice. J Immunol 2002;168:4308–17.
16. Koch AE. Angiogenesis as a target in rheumatoid arthritis. Ann Rheum Dis 2003;62 Suppl 2:ii60–7.
17. Haringman JJ, Kraan MC, Smeets TJ, Zwinderman KH, Tak PP. Chemokine blockade and chronic inflammatory disease: proof of concept in patients with rheumatoid arthritis. Ann Rheum Dis 2003;62:715–21.
18. Gerard C, Frossard JL, Bhatia M, Saluja A, Gerard NP, Lu B, Steer M. Targeted disruption of the β-chemokine receptor CCR1 protects against pancreatitis-associated lung injury. J Clin Invest 1997;100:2022–7.
19. Topham PS, Csizmadia V, Soler D, Hines D, Gerard CJ, Salant DJ, et al. Lack of chemokine receptor CCR1 enhances Th1 responses and glomerular injury during nephrotoxic nephritis. J Clin Invest 1999;104:1549–57.
20. Volin MV, Shah MR, Tokuhira M, Haines GK, Woods JM, Koch AE. RANTES expression and contribution to monocyte chemotaxis in arthritis. Clin Immunol Immunopathol 1998;89:44–53.
21. Barnes DA, Tse J, Kaufhold M, Owen M, Hesselgesser J, Strieter R, et al. Polyclonal antibody directed against human RANTES ameliorates disease in the Lewis rat adjuvant-induced arthritis model. J Clin Invest 1998;101:2910–9.
22. Shahrara S, Proudfoot AE, Woods JM, Ruth JH, Amin MA, Park CC, et al. Amelioration of rat adjuvant-induced arthritis by Met-RANTES. Arthritis Rheum 2005;52:1907–19.
23. Bruhl H, Wagner K, Kellner H, Schattenkirchner M, Schlondorff D, Mack M. Surface expression of CC- and CXC-chemokine receptors on leucocyte subsets in inflammatory joint diseases. Clin Exp Immunol 2001;126:551–9.
24. Gong JH, Ratkay LG, Waterfield JD, Clark-Lewis I. An antagonist of monocyte chemoattractant protein 1 (MCP-1) inhibits arthritis in the MRL-lpr mouse model. J Exp Med 1997;186:131–7.

25. Quinones MP, Ahuja SK, Jimenez F, Schaefer J, Garavito E, Rao A, et al. Experimental arthritis in CC chemokine receptor 2-null mice closely mimics severe human rheumatoid arthritis. J Clin Invest 2004;113:856–66.
26. Hillyer P, Mordelet E, Flynn G, Male D. Chemokines, chemokine receptors and adhesion molecules on different human endothelia: discriminating the tissue-specific functions that affect leucocyte migration. Clin Exp Immunol 2003;134:431–41.
27. Dzenko KA, Song L, Ge S, Kuziel WA, Pachter JS. CCR2 expression by brain microvascular endothelial cells is critical for macrophage transendothelial migration in response to CCL2. Microvasc Res. 2005;70:53–64.
28. Bruhl H, Cihak J, Schneider MA, Plachy J, Rupp T, Wenzel I, et al. Dual role of CCR2 during initiation and progression of collagen-induced arthritis: evidence for regulatory activity of CCR2+ T cells. J Immunol 2004;172:890–8.
29. Hayes IM, Jordan NJ, Towers S, Smith G, Paterson JR, Earnshaw JJ, et al. Human vascular smooth muscle cells express receptors for CC chemokines. Arterioscler Thromb Vasc Biol 1998;18:397–403.
30. Ochi H, Hirani WM, Yuan Q, Friend DS, Austen KF, Boyce JA. T helper cell type 2 cytokine-mediated comitogenic responses and CCR3 expression during differentiation of human mast cells in vitro. J Exp Med 1999;190:267–80.
31. Yang PT, Kasai H, Zhao LJ, Xiao WG, Tanabe F, Ito M. Increased CCR4 expression on circulating CD4(+) T cells in ankylosing spondylitis, rheumatoid arthritis and systemic lupus erythematosus. Clin Exp Immunol 2004;138:342–7.
32. Ho CY, Wong CK, Li EK, Tam LS, Lam CW. Suppressive effect of combination treatment of leflunomide and methotrexate on chemokine expression in patients with rheumatoid arthritis. Clin Exp Immunol 2003;133:132–8.
33. Radstake TR, van der Voort R, ten Brummelhuis M, de Waal Malefijt M, Looman M, Figdor CG, et al. Increased expression of CCL18, CCL19, and CCL17 by dendritic cells from patients with rheumatoid arthritis, and regulation by Fcγ receptors. Ann Rheum Dis 2005;64:359–67.
34. Thompson SD, Luyrink LK, Graham TB, Tsoras M, Ryan M, Passo MH, et al. Chemokine receptor CCR4 on CD4+ T cells in juvenile rheumatoid arthritis synovial fluid defines a subset of cells with increased IL-4:IFN-γ mRNA ratios. J Immunol 2001;166: 6899–906.
35. Power CA. Knock out models to dissect chemokine receptor function in vivo. J Immunol Methods 2003;273:73–82.
36. Vierboom MP, Zavodny PJ, Chou CC, Tagat JR, Pugliese-Sivo C, Strizki J, et al. Inhibition of the development of collagen-induced arthritis in rhesus monkeys by a small molecular weight antagonist of CCR5. Arthritis Rheum 2005;52:627–36.
37. Adarichev VA, Vermes C, Hanyecz A, Mikecz K, Bremer EG, Glant TT. Gene expression profiling in murine autoimmune arthritis during the initiation and progression of joint inflammation. Arthritis Res Ther 2005;7:R196–207.
38. Chintalacharuvu SR, Wang JX, Giaconia JM, Venkataraman C. An essential role for CCL3 in the development of collagen antibody-induced arthritis. Immunol Lett 2005;100:202–4.
39. Nanki T, Hayashida K, El-Gabalawy HS, Suson S, Shi K, Girschick HJ, et al. Stromal cell-derived factor-1-CXC chemokine receptor 4 interactions play a central role in CD4+ T cell accumulation in rheumatoid arthritis synovium. J Immunol 2000;165:6590–8.
40. Buckley CD, Amft N, Bradfield PF, Pilling D, Ross E, Arenzana-Seisdedos F, et al. Persistent induction of the chemokine receptor CXCR4 by TGFβ1 on synovial T cells contributes to their accumulation within the rheumatoid synovium. J Immunol 2000; 165:3423–9.
41. Burman A, Haworth O, Hardie DL, Amft EN, Siewert C, Jackson DG, et al. A chemokine-dependent stromal induction mechanism for aberrant lymphocyte accumulation and compromised lymphatic return in rheumatoid arthritis. J Immunol 2005;174: 1693–700.
42. Pablos JL, Santiago B, Galindo M, Torres C, Brehmer MT, Blanco FJ, et al. Synoviocyte-derived CXCL12 is displayed on endothelium and induces angiogenesis in rheumatoid arthritis. J Immunol 2003;170:2147–52.
43. Van Buul JD, Hordijk PL. Signaling in leukocyte transendothelial migration. Arterioscler Thromb Vasc Biol 2004;24:824–33.
44. Ikezumi Y, Atkins RC, Nikolic-Paterson DJ. Interferon-γ augments acute macrophage-mediated renal injury via a glucocorticoid-sensitive mechanism. J Am Soc Nephrol 2003;14:888–98.
45. Erdem H, Pay S, Serdar M, Simsek I, Dinc A, Musabak U, et al. Different ELR (+) angiogenic CXC chemokine profiles in synovial fluid of patients with Behcet's disease, familial Mediterranean fever, rheumatoid arthritis, and osteoarthritis. Rheumatol Int 2004. E-pub ahead of print.
46. Szekanecz Z, Kim J, Koch AE. Chemokines and chemokine receptors in rheumatoid arthritis. Semin Immunol 2003;15:15–21.
47. Patel DD, Zachariah JP, Whichard LP. CXCR3 and CCR5 ligands in rheumatoid arthritis synovium. Clin Immunol 2001;98:39–45.
48. Garcia-Lopez MA, Sanchez-Madrid F, Rodriguez-Frade JM, Mellado M, Acevedo A, Garcia MI, et al. CXCR3 chemokine receptor distribution in normal and inflamed tissues: expression on activated lymphocytes, endothelial cells, and dendritic cells. Lab Invest 2001;81:409–18.
49. Zheng B, Ozen Z, Zhang X, De Silva S, Marinova E, Guo L, et al. CXCL13 neutralization reduces the severity of collagen-induced arthritis. Arthritis Rheum 2005;52:620–6.
50. Schmutz C, Hulme A, Burman A, Salmon M, Ashton B, Buckley C, et al. Chemokine receptors in the rheumatoid synovium: upregulation of CXCR5. Arthritis Res Ther 2005;7:R217–29.