Mod Rheumatol (2003) 13:114–120

ORIGINAL ARTICLE

Yumi Motoki · Kenji Tani · Teruki Shimizu
Hiroyuki Tamiya · Kayoko Hase · Yasukazu Ohmoto
Kouji Matsushima · Saburo Sone

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

Received: January 28, 2002 / Accepted: August 14, 2002

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

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

Y. Motoki · K. Tani · T. Shimizu · H. Tamiya · K. Hase · S. Sone (✉)
Third Department of Internal Medicine, School of Medicine, Tokushima University, 3-18-15 Kuramoto-cho, Tokushima 770-8503, Japan
Tel. +81-88-633-7127; Fax +81-88-633-2134
e-mail: ssone@clin.med.tokushima-u.ac.jp

Y. Ohmoto
Biological Research Institute, Otsuka Pharmaceutical Co., Ltd., Tokushima, Japan

K. Matsushima
Department of Molecular Preventive Medicine, School of Medicine, University of Tokyo, Tokyo, Japan

Introduction

CD4+ T lymphocytes can be subdivided into two distinct populations, T helper 1 (Th1) and Th2, defined by the spectrum of cytokines produced by these cells.[1] Th1 cells generate interleukin (IL)-2, interferon-γ, and tumor necrosis factor-β and promote cell-mediated immunity, whereas Th2 generate IL-4, IL-5, IL-6, and IL-10 and play a role in humoral immunity and allergic diseases.[2] There is evidence that a balance of Th1 and Th2 is crucial for an effective immune response and the outcome of infectious and autoimmune diseases.[3,4] Recently, chemokines have been suggested to have a role in effector and amplification mechanisms of polarized Th1- and Th2-mediated immune responses, and their receptors might serve as targets for selective modulation of T-cell-dependent immunity. Of these chemokine receptors, CXC chemokine receptor 3 (CXCR3), which is a receptor for interferon-γ-inducible protein 10 (IP-10; CXCL10) and monokine induced by interferon-γ (Mig; CXCL9), is predominantly expressed on Th1 cells.[5,6] In contrast, CC chemokine receptor 4 (CCR4), a receptor for thymus- and activation-regulated chemokine (TARC; CCL17) and macrophage-derived chemokine (MDC; CCL22), is selectively expressed on Th2 cells.[7–10] These observations suggest that the differential expression of chemokine receptor may be useful in determining T cells important to disease pathogenesis.

Rheumatoid arthritis (RA) is a chronic autoimmune disease characterized by persistent synovitis. Because chemotactic cytokines (chemokines) may play critical roles in the recruitment of leukocytes in RA, analysis for the expression of chemokines and their receptors should provide insight into events in synovial inflammation of RA. Chemokines have a role in joint inflammation, not only by inducing leukocyte chemotaxis but also by activating immune cells and angiogenesis.[11,12] Previous reports that showed the increased expression of Th1-related cytokines in cells of synovial fluid and synovial tissue speculated that Th1 cells may play an active role in the development of autoimmune responses in RA.[13–15] However, the data on the expression

of Th1- and Th2-type chemokine receptors in peripheral blood and inflamed joints of RA patients have been controversial.[16,17]

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

Materials and methods

Patients and controls

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

Sampling of peripheral blood

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

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

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

Values are mean ± SEM
RF, rheumatoid factor; ESR, erythrocyte sedimentation rate; CRP, C-reactive protein; WBC, white blood cells

FACS analysis

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

Assay for cytokines in serum

Enzyme immunoassays for IL-4 and IL-18 in serum were performed as described in detail previously.[22] The detection limit for cytokines was 20 pg/ml. IL-12, IL-13, and interferon-γ in serum were measured by performing assays with sandwich ELISA kits purchased from R&D Systems according to the manufacturer's instructions.

Assay for cytokines in synovial fluid

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

Statistical analysis

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

Results

Expression of CXCR3 and CCR4 on peripheral blood CD4+ T lymphocytes

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

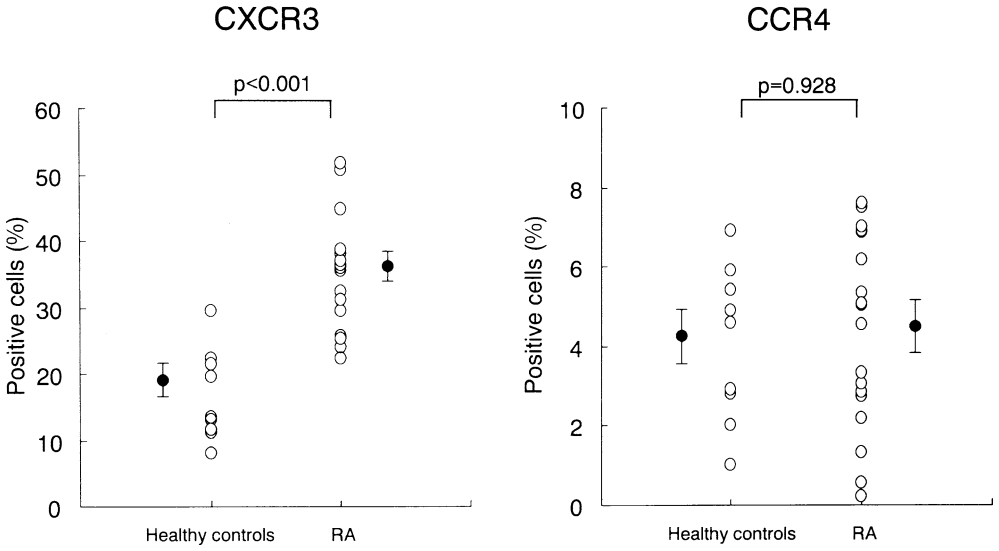

Fig. 1. CXC chemokine receptor 3 (CXCR3) and CC chemokine receptor 4 (CCR4) expression on CD4+ T lymphocytes from rheumatoid arthritis (RA) patients and healthy controls. Peripheral blood mononuclear cells (PBMC) were stained with fluorescein isothiocyanate (FITC)-conjugated anti-CXCR3 antibody or FITC-conjugated anti-CCR4 antibody and phycoerythrin (PE)-conjugated anti-CD4 antibody, and then analyzed by flow cytometry. Results are expressed as mean ± SEM

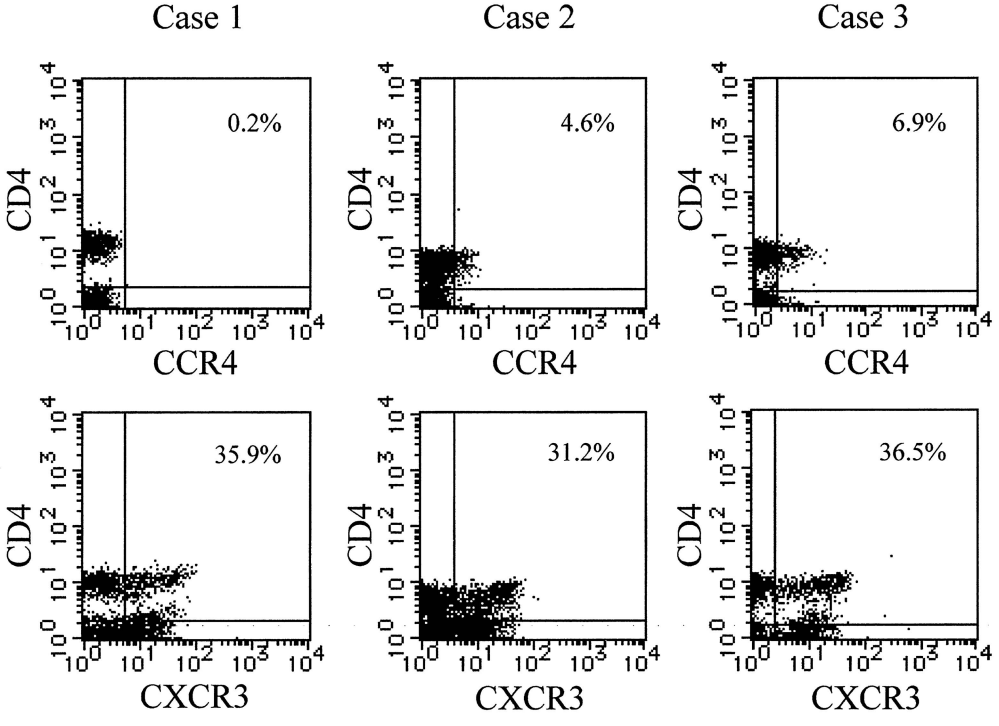

Fig. 2. CXCR3 and CCR4 expression on CD4+ T lymphocytes of RA patients. Data are from three cases of RA

spectively. Based on two-color flow cytometry, CXCR3 was expressed on 16.7% ± 2.3% of CD4+ T lymphocytes of healthy controls, which is consistent with results in previous reports.[23,24] Significantly increased expression of CXCR3 was found on CD4+ T lymphocytes of patients with RA (35.9% ± 1.9%) (Figs. 1, 2). There was a significantly positive correlation ($r = 0.724$, $P < 0.001$) between the CXCR3 expression and age. Therefore, we next examined whether the expression of CXCR3 in RA patients is higher than that of healthy controls with age similar to RA patients (elder controls: $n = 9$; mean age, 51.9 years). Significantly higher CXCR3 expression was detected in the elder controls (28.4% ± 1.9%) than in the healthy controls. However, the CXCR3 expression was significantly ($P = 0.026$) higher in RA patients than the value of the elder controls. On the other hand, CCR4 expression was found in 4.0% ± 0.7% of CD4+ T lymphocytes of healthy controls, which is also consistent with results in previous reports.[23,24] The expression of CCR4 in RA patients (4.1% ± 0.6%) was similar to that in healthy controls. As shown in Figs. 1 and 2, however, the expression of CCR4 varied among patients with RA. There was no significant correlation between the expression of CCR4 and age (data not shown).

Expression of CXCR3 between RA patients with high-ESR and low-ESR groups

To determine the clinical significance of increased CXCR3 expression in RA patients, we examined the correlation of CXCR3 expression with various clinical parameters for RA.

When RA patients were separated into two groups, a low-ESR group (<50 mm/h) and a high-ESR group (≧50 mm/h), the low-ESR group consisted of 8 patients with a value of 26.4 ± 3.6 mm/h and the high-ESR group consisted of 11 patients with a value of 77.7 ± 7.3 mm/h. The level of CXCR3 expression in the high-ESR group (40.4% ± 2.2%) was significantly higher than that in the low-ESR group (29.6% ± 1.9%) (Fig. 3). There was no significant correlation between CXCR3 expression and the level of CRP or rheumatoid factor.

CXCR3 expression and disease duration of RA

We examined the difference of CXCR3 expression in disease duration of RA. The average disease duration of RA patients was 8.7 ± 2.3 years. RA patients were separated into two groups, a short-term group (<9 years, $n = 12$, 2.9 ± 0.8 years) and a long-term group (≧9 years, $n = 7$, 18.7 ± 3.6 years). The CXCR3 expression of the long-term group (40.8% ± 2.2%) was significantly higher than that of the short-term group (32.9% ± 2.5%) (Table 2). There was no significant difference in CXCR3 expression with sex, age, or pulmonary complication.

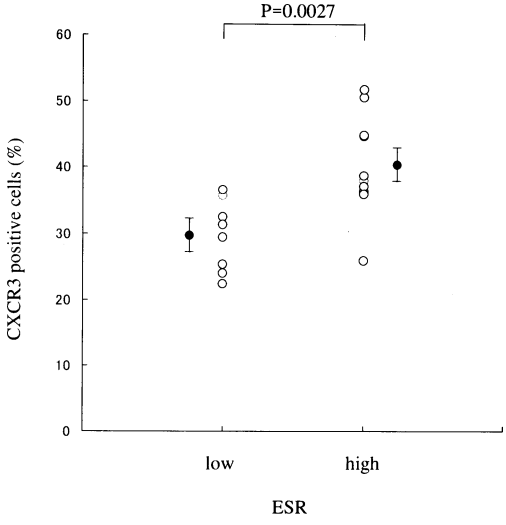

Fig. 3. Expression of CXCR3 between RA patients with high erythrocyte sedimentation rate (ESR) (≧50 mm/h) and low ESR (<50 mm/h) groups. PBMC were stained with FITC-conjugated anti-CXCR3 antibody and PE-conjugated anti-CD4 antibody and then analyzed by flow cytometry. Results are expressed as mean ± SEM

Serum cytokines

We measured the level of serum cytokines by ELISA. The serum level of IL-18 in RA patients (223.0 ± 44.6 pg/ml) was higher than that in healthy controls (120.3 ± 34.6 pg/ml) although a significant difference ($P = 0.171$) was not detected. The level of IL-12 in RA patients (56.7 ± 11.7 pg/ml) was similar to that in healthy controls (43.6 ± 6.0 pg/ml), and the level of IL-13 in RA patients (84.7 ± 7.3 pg/ml) was not significantly different from that in healthy controls (77.9 ± 1.0 pg/ml). Serum interferon-γ and IL-4 were under the detection limit in most RA patients or all healthy controls, except one RA patient had a high level of interferon-γ (150 pg/ml).

Synovial fluid cytokines

We measured IL-12, IL-18, and interferon-γ in synovial fluid of other RA patients and osteoarthritis (OA) patients. The level of IL-12, IL-18, and interferon-γ in synovial fluid of RA patients was higher than that in OA patients, and a statistically significant difference was observed in the level of IL-18 (Table 3).

Discussion

In this study, we found increased expression of CXCR3 on peripheral blood CD4+ T lymphocytes of RA patients. This article demonstrates that CXCR3 is a useful marker in Th1 predominance and the disease activity of RA and may have relevance in regard to the course of the disease.

The predominance of either Th1 or Th2 immune response may be of great importance for various autoimmune

Table 2. CXCR3 expression on CD4+ T lymphocytes of RA patients

	Number of cases	CXCR3-positive cells (%)	*P*
Sex			
Male	5	32.0 ± 3.9	NS
Female	14	37.7 ± 2.3	
Age (years)			
<57	9	35.9 ± 3.6	NS
≧57	10	35.8 ± 2.1	
Disease duration (years)			
<9	12	32.9 ± 2.5	<0.05
≧9	7	40.8 ± 2.2	
Pulmonary fibrosis			
Positive	5	39.1 ± 4.7	NS
Negative	14	34.7 ± 2.1	

Values are mean ± SEM
CXCR, CXC chemokine receptor

Table 3. Cytokine levels in RA and osteoarthritis (OA) synovial fluids

Cytokine	RA ($n = 32$)	OA ($n = 11$)	P
IFN-γ	19.6 ± 6.5	<10	NS
IL-12	24.6 ± 8.4	2.1 ± 0.0	NS
IL-18	30412.6 ± 4177.1	3008.3 ± 660.5	<0.0005

Data are expressed as pg/ml; values are mean ± SEM
Detection limit of interferon-γ (TFN-γ) was 10 pg/ml
Detection limit of interleuken-18 (IL-18) was 20 pg/ml

diseases including RA. Previous reports have suggested that RA might be a disease characterized by Th1 because the Th1-related cytokines IL-2 and interferon-γ are increased in synovial fluid of RA patients and increased numbers of IL-2- and interferon-γ-producing cells predominate in T lymphocytes from synovial fluids and synovial tissues of RA patients.[25–27] Recently, great emphasis has been placed on the possibility of distinguishing Th1 and Th2 cells on the basis of the differential expression of chemokine receptors because chemokines and their receptors have been shown to play a role in effector and amplification mechanisms of polarized Th1- and Th2-mediated immune responses in T-cell-dependent immunity.[5] Of the chemokine receptors, CXCR3 and CCR5 are selectively expressed on Th1-type T cells and have been shown to be responsible for Th1-dominant immune responses.[13,24] Yamamoto et al.[23] recently showed that CXCR3 is a more useful marker for Th1 predominance than CCR5. On the other hand, CCR4, CCR3, and CCR8 expression is associated with Th2 cell differentiation.[28,29] CCR4 is a new Th2 marker and has been shown to have important roles in Th2-related diseases such as allergic dermatitis.[30,31] We recently reported increased expression of CCR4 on the peripheral blood CD4+ T cells of SLE patients that corresponds to the disease activity.[10] These results suggest that CCR4 is the most critical marker to determine Th2 predominance. Therefore, in this study we examined the expression of CXCR3 and CCR4 to determine Th1 or Th2 predominance in the pathogenesis of RA.

Previous reports have shown increased expression of CCR5 on T cells in synovial fluid and synovial tissue from patients with RA, suggesting Th1 predominance in RA,[14,15,32] but there is only one paper that reported increased CXCR3-expressing T cells in synovial fluid and synovial tissue of RA.[13] On the other hand, some reports have suggested that the Th2-type response may be important as well as the Th1 response in RA. Al-Janadi et al.[33] reported that serum levels of IL-6 in RA patients were elevated, and Thompson et al.[34] showed accumulations of both CCR4+ and CCR5+ T cells in synovial fluid of juvenile rheumatoid arthritis, suggesting that both Th1- and Th2-type responses are involved in the immune response of RA. However, the results of this study, examining the expression of chemokine receptors CXCR3 and CCR4 showed that the expression of CXCR3 but not CCR4 was increased on CD4+ T lymphocytes from RA patients, indicating that the Th1 response may have a critical role in the pathogenesis of RA. However, because the expression of CCR4 on CD4+ T cells varied among individuals, further studies are necessary to determine the role of the Th2 response in the pathogenesis of RA.

Suzuki et al.[14] reported decreased expression of CCR5, another Th1-related chemokine receptor, in PBMCs of active RA patients. This discrepancy may be due to the difference in specificity to Th1 cells between CXCR3 and CCR5, because a recent report[35] showed that most Th1 cells (on average, 90%) express CXCR3 but only 50% of Th1 cells express CCR5, suggesting that CXCR3 is a more specific marker for Th1 cells than CCR5. On the other hand, Th0 and Th2 cells were shown to partially express CXCR3,[35] but these cells may have less participation in the CXCR3-expressing T-cell population because there are fewer of these cells in peripheral blood.[35.] Therefore, CXCR3 may be the most useful chemokine receptor to determine Th1 predominance at present.

In this study, to assess the Th1 predominance of RA, various serum cytokines were measured. The serum level of IL-18 was higher in RA patients than healthy controls, although there was no significant difference. Significantly increased IL-18 was found in synovial fluid from RA patients when compared with that from OA patients. IL-18 was shown to induce the development of Th1-related responses through interferon-γ production.[36] IL-18 is expressed in synovial tissue of RA patients and has been shown to have an important role in the pathogenesis of joint inflammation of RA.[37,38] These results suggest that IL-18 detected in serum of RA patients is derived from Th1-related inflammation of RA joints.

Examining the expression of CXCR3 on peripheral blood lymphocytes is suggested to be more clinically useful than that on synovial lymphocytes for the following two reasons. First, cells of peripheral blood are easier to obtain than those of synovial fluid and synovial tissue. Second, samples from inflamed joints such as synovial fluid and synovial tissue are useful to examine the pathogenesis of rheumatoid joint inflammation but sometimes fail to reflect the total activity of joint inflammation in the body because most inflamed joints are not accompanied by synovial fluids.

This study showed that a significantly higher expression of CXCR3 was found in the high-ESR group than in the low-ESR group, and the expression was higher in RA patients with long-term disease duration that those with short-term disease. These results suggest that the expression of CXCR3 may correspond to the disease activity and disease course of RA. However, the expression of CXCR3 did not correspond to the level of CRP, the number of tender joints, or the number of swollen joints (data not shown). Further clinical assessments are necessary to clarify the significance of CXCR3 expression in the clinical course of RA.

The role of CXCR3 in the pathogenesis of RA remains unclear. CXCR3 is a receptor for IP-10/CXCL10 and MIG/CXCL9, which are produced mainly by macrophages and fibroblasts.[39] Previous reports have shown that IP-10/CXCL10 and MIG/CXCL9 are preferentially expressed in inflamed joints of RA patient,[32] suggesting that these

chemokines may participate in the selective recruitment of T cells. IP-10/CXCL10 and MIG/CXCL9 were produced in inflamed joints. These chemokines, in Th1-dominant conditions, induce chemotactic migration of circulating CXCR3-expressing Th1 cells. Recruited Th1 cells produce cytokines such as IL-2 and interferon-γ, which further activate synovial macrophages. Thus, production of IP-10/CXCL10 and MIG/CXCL9 by macrophages and fibroblasts and selective expression of CXCR3 on Th1 cells may represent an important biological amplification mechanism to promote local Th1-type responses.

In conclusion, we have shown that CXCR3-expressing Th1 cells are increased in RA patients and might play an active role in the development of the autoimmune disorder in RA. Our recent report showed that peripheral blood CD4+ T cells from patients with active systemic lupus erythematosus (SLE) express increased CCR4, indicating that SLE is a Th2-dominant disease.[10] Thus, determination of Th1 and Th2 preference may lead us to new insight for the pathogenesis of collagen vascular diseases. Although neutralization of CXCR3 may have any therapeutic effect in vivo, further study is necessary to elucidate the critical role of CXCR3 in the pathogenesis of RA.

References

1. Mosmann TR, Coffman RL. Th1 and Th2 cells: different patterns of lymphokine secretion lead to different functional properties. Annu Rev Immunol 1989;7:145–73.
2. Romagnani S. Lymphokine production by human T cells in disease states. Annu Rev Immunol 1994;12:227–57.
3. Surcel HM, Troye-Blomberg M, Paulie S, Andersson G, Moreno C, Pasvol G, et al. Th1/Th2 profiles in tuberculosis, based on the proliferation and cytokine response of blood lymphocytes to mycobacterial antigens. Immunology 1994;81:171–6.
4. Tani K, Murphy WJ, Chertov O, Salcedo R, Koh CY, Utsunomiya I, et al. Defensins act as potent adjuvants that promote cellular and humoral immune responses in mice to a lymphoma idiotype and carrier antigens. Int Immunol 2000;12:691–700.
5. Bonecchi R, Bianchi G, Bordignon PP, D'Ambrosio D, Lang R, Borsatti A, et al. Differential expression of chemokine receptors and chemotactic responsiveness of type 1 T helper cells (Th1s) and Th2s. J Exp Med 1998;187:129–34.
6. Sallusto F, Lenig D, Mackay CR, Lanzavecchia A. Flexible programs of chemokine receptor expression on human polarized T helper 1 and 2 lymphocytes. J Exp Med 1998;187:875–83.
7. Imai T, Baba M, Nishimura M, Kakizaki M, Takagi S, Yoshie O. The T cell-directed CC chemokine TARC is a highly specific biological ligand for a CC chemokine receptor 4. J Biol Chem 1997;272:15036–42.
8. Imai T, Chantry D, Raport CJ, Wood CL, Nishimura M, Godiska R, et al. Macrophage-derived chemokine is a functional ligand for the CC chemokine receptor 4. J Biol Chem 1998;273:1764–8.
9. Imai T, Nagira M, Takagi S, Kakizaki M, Nishimura M, Wang J, et al. Selective recruitment of CCR4-bearing Th2 cells toward antigen-presenting cells by the CC chemokines thymus and activation-regulated chemokine and macrophage-derived chemokine. Int Immunol 1999;11:81–8.
10. Hase K, Tani K, Shimizu T, Ohmoto Y, Matsushima K, Sone S. Increased CCR4 expression in active systemic lupus erythematosus. J Leukoc Biol 2001;70:749–55.
11. Burmester GR, Stuhlmüller B, Keyszer G, Kinne RW. Mononuclear phagocytes and rheumatoid synovitis. Mastermind or workhorse in arthritis? Arthritis Rheum 1997;40:5–18.
12. Szekanecz Z, Strieter RM, Kunkel SL, Koch AE. Chemokines in rheumatoid arthritis. Springer Semin Immunopathol 1998;20:115–32.
13. Qin S, Rottman JB, Myers P, Kassam N, Weinblatt M, Loetscher M, et al. The chemokine receptors CXCR3 and CCR5 mark subsets of T cells associated with certain inflammatory reactions. J Clin Invest 1998;101:746–54.
14. Suzuki N, Nakajima A, Yoshino S, Matsushima K, Yagita H, Okumura K. Selective accumulation of CCR5+ T lymphocytes into inflamed joints of rheumatoid arthritis. Int Immunol 1999;11:553–9.
15. Mack M, Bruhl H, Gruber R, Jaeger C, Cihak J, Eiter V, et al. Predominance of mononuclear cells expressing the chemokine receptor CCR5 in synovial effusions of patients with different forms of arthritis. Arthritis Rheum 1999;42:981–8.
16. Katschke KJ, 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.
17. Nanki T, Lipsky PE. Lack of correlation between chemokine receptor and Th1/Th2 cytokine expression by individual memory T cells. Int Immunol 2000;12:1659–67.
18. Arnett FC, Edworthy SM, Bloch DA, McShane DJ, Fries JF, Cooper NS, et al. The American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis. Arthritis Rheum 1988;31:315–24.
19. Yano S, Yanagawa H, Nishioka Y, Mukaida N, Matsushima K, Sone S. T helper 2 cytokines differently regulate monocyte chemoattractant protein-1 production by human peripheral blood monocytes and alveolar macrophages. J Immunol 1996;157:2660–5.
20. Yano S, Nishioka Y, Izumi K, Tsuruo T, Tanaka T, Miyasaka M, et al. Novel metastasis model of human lung cancer in scid mice depleted of NK cells. Int J Cancer 1996;67:211–7.
21. Yano S, Nishioka Y, Nokihara H, Sone S. Macrophage colony-stimulating factor gene transduction into human lung cancer cells differentially regulates metastasis formations in various organ microenvironments of natural killer cell-depleted scid mice. Cancer Res 1997;57:784–90.
22. Sone S, Yanagawa H, Nishioka Y, Orino E, Bhaskaran G, Nii A, et al. Interleukin-4 as a potent down-regulator for human alveolar macrophages capable of producing tumor necrosis factor-alpha and interleukin-1. Eur Respir J 1992;5:174–81.
23. Yamamoto J, Adachi Y, Onoue Y, Adachi YS, Okabe Y, Itazawa T, et al. Differential expression of the chemokine receptors by the Th1- and Th2-type effector populations within circulating CD4+ T cells. J Leukoc Biol 2000;68:568–74.
24. Loetscher P, Uguccioni M, Bordoli L, Baggiolini M, Moser B, Chizzolini C, et al. CCR5 is characteristic of Th1 lymphocytes. Nature (Lond) 1998;391:344–5.
25. Nanki T, Lipsky PE. Cytokine, activation marker, and chemokine receptor expression by individual CD4(+) memory T cells in rheumatoid arthritis synovium. Arthritis Res 2000;2:415–23.
26. Berner B, Akca D, Jung T, Muller GA, Reuss-Borst MA. Analysis of Th1 and Th2 cytokines expressing CD4+ and CD8+ T cells in rheumatoid arthritis by flow cytometry. J Rheumatol 2000;27:1128–35.
27. Bourg V, Portaies P, Fiorito S, Combe B, Jorgensen C, Sany J, et al. Intracytoplasmic Th1 and Th2 cytokines in rheumatoid arthritis blood and synovial tissue. J Autoimmun 1999;13:415–22.
28. D'Ambrosio D, Iellem A, Bonecchi R, Mazzeo D, Sozzani S, Mantovani A, et al. Selective up-regulation of chemokine receptors CCR4 and CCR8 upon activation of polarized human type 2 Th cells. J Immunol 1998;161:5111–5.
29. Sallusto F, Mackay CR, Lanzavecchia A. Selective expression of the eotaxin receptor CCR3 by human T helper 2 cells. Science 1997;277:2005–7.
30. Wakugawa M, Nakamura K, Kakinuma T, Onai N, Matsushima K, Tamaki K. CC chemokine receptor 4 expression on peripheral blood CD4+ T cells reflects disease activity of atopic dermatitis. J Invest Dermatol 2001;117:188–96.
31. Nakatani T, Kaburagi Y, Shimada Y, Inaoki M, Takehara K, Mukaida N, et al. CCR4 memory CD4+ T lymphocytes are increased in peripheral blood and lesional skin from patients with atopic dermatitis. J Allergy Clin Immunol 2001;107:353–8.

32. Patel DD, Zachariah JP, Whichard LP. CXCR3 and CCR5 ligands in rheumatoid arthritis synovium. Clin Immunol 2001;98:39–45.
33. Al-Janadi M, al-Balla S, al-Dalaan A, Raziuddin S. Cytokine profile in systemic lupus erythematosus, rheumatoid arthritis, and other rheumatic diseases. J Clin Immunol 1993;13:58–67.
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. Kim CH, Rott L, Kunkel EJ, Gnovese MC, Andrew DP, Wu L, et al. Rules of chemokine receptor association with T cell polarization in vivo. J Clin Invest 2001;108:1331–9.
36. Okamura H, Tsutsui H, Komatsu T, Yutsudo M, Hakura A, Tanimoto T, et al. Cloning of a new cytokine that induces IFN-γ production by T cells. Nature (Lond) 1995;378:88–91.
37. Yamamura M, Kawashima M, Taniai M, Yamauchi H, Tanimoto T, Kurimoto M, et al. Interferon-γ-inducing activity of interleukin-18 in the joint with rheumatoid arthritis. Arthritis Rheum 2001;44: 275–85.
38. Gracie JA, Forsey RJ, Chan WL, Gilmour A, Leung BP, Greer MR, et al. A proinflammatory role for IL-18 in rheumatoid arthritis. J Clin Invest 1999;104:1393–401.
39. Proost P, Schutyser E, Menten P, Struyf S, Wuyts A, Opdenakker G, et al. Amino-terminal truncation of CXCR3 agonists impairs receptor signaling and lymphocyte chemotaxis, while preserving antiangiogenic properties. Blood 2001;98:3554–61.