A Role for TARC/CCL17, a CC Chemokine, in Systemic Lupus Erythematosus

HIROSHI OKAMOTO, KYOKO KOIZUMI, HISASHI YAMANAKA, TERUNOBU SAITO, and NAOYUKI KAMATANI

ABSTRACT. ***Objective.*** The Th2-type CC chemokine thymus and activation-regulated chemokine (TARC/CCL17) is one of the high affinity ligands for CCR4, a chemokine receptor predominantly expressed by Th2 cells. We examined serum and plasma concentrations of TARC/CCL17 in patients with systemic lupus erythematosus (SLE).

Methods. Serum and plasma levels of TARC/CCL17 and plasma levels of monocyte chemoattractant protein-1 (MCP-1/CCL2) and macrophage-derived chemokine (MDC/CCL22) in patients with SLE were determined by ELISA.

Results. There were significant differences in the plasma concentrations of TARC/CCL17 between the patients with untreated SLE and treated SLE ($p < 0.001$), rheumatoid arthritis (RA) ($p < 0.001$), and healthy controls ($p < 0.001$). In addition, the plasma levels of TARC/CCL17 correlated with the class of lupus nephritis (higher in class I or II than in class III or IV). There was close correlation between plasma levels of MDC/CCL22 and TARC/CCL17. There was no correlation between plasma levels of MCP-1/CCL2 and TARC/CCL17.

Conclusion. TARC/CCL17 may be a useful serological marker and may facilitate an assessment of the degree of disease activity in SLE. The development of SLE is closely related to the elevation of plasma TARC/CCL17 levels. (J Rheumatol 2003;30:2369–73)

Key Indexing Terms:

THYMUS AND ACTIVATION-REGULATED CHEMOKINE
SYSTEMIC LUPUS ERYTHEMATOSUS

Systemic lupus erythematosus (SLE) is an autoimmune disease characterized by autoantibody formation and the systemic deposition of immune complexes. Different forms of glomerulonephritis can occur in patients with SLE and these contribute to the associated morbidity and mortality of this disease. SLE has long been considered a disease in which the actions of peripheral T helper-type 2 (Th2) cells predominate over Th1 cells[1,2]. However, this notion was challenged by a recent study that reported a predominance of Th1 cells in SLE patients with World Health Organization (WHO) class IV lupus nephritis[3]. Therefore, the relative balance of Th1 versus Th2 cells in SLE patients remains controversial.

The Th2-type CC chemokine thymus and activation-regulated chemokine (TARC/CCL17) is one of the high affinity ligands for CCR4[4], a chemokine receptor predominantly expressed by Th2 cells[5]. Elevated circulating TARC/CCL17 concentrations have been reported in the plasma of patients with allergic diseases such as bronchial asthma and atopic dermatitis[6,7]. To assess the extent of Th2 predominance in patients with SLE, we measured the plasma concentration of TARC/CCL17 in various patient groups.

MATERIALS AND METHODS

Patients. Plasma and serum samples were obtained from 58 patients, as follows — SLE, n = 38, median age 29.6 years (range 20–41); and rheumatoid arthritis (RA), n = 20, median age 48 years (range 22–71); and 50 healthy controls (n = 50, median age 28 yrs, range 21–42). Blood samples were obtained on admission to our hospital. All the patients with SLE or RA fulfilled the American College of Rheumatology criteria for SLE and RA, respectively[8,9]. Informed consent was obtained from each individual in this study. Fourteen patients in the untreated SLE group underwent renal biopsy so that an assessment according to the WHO criteria for lupus nephritis could be performed. None of the 38 patients with SLE tested positive for antiphospholipid antibodies (aPL). Patients with allergic disorders such as bronchial asthma, atopic dermatitis, and allergic rhinitis were specifically excluded. Blood samples were centrifuged and the serum/plasma were frozen at –80°C until testing.

ELISA for chemokines. TARC/CCL17 concentrations were determined by ELISA using the Quantikine human TARC immunoassay (R&D Systems, Minneapolis, MN, USA). Monocyte chemoattractant protein-1 (MCP-1/CCL2) concentrations were determined by ELISA using the AN'ALYZA human MCP-1 immunoassay (Techne Corp., Minneapolis, MN, USA). Macrophage-derived chemokine (MDC/CCL22) concentrations were determined by ELISA using the AN'ALYZA human MDC immunoassay (Techne). Interferon-inducible protein-10 (IP-10/CXCL10) concentrations were determined by ELISA using the Quantikine human IP-10 immunoassay (R&D Systems).

Platelet aggregation test. Whole blood was obtained from a patient with a high circulating TARC level (serum concentration 521 pg/ml) and centrifuged at 500 rpm for 10 min. The supernatant was collected and

From the Institute of Rheumatology, Tokyo Women's Medical University, Tokyo, Japan.

Supported, in part, by grants-in-aid from the Ministry of Education, Culture, Sports, Science and Technology of Japan.

H. Okamoto, MD, PhD; K. Koizumi, MD; H. Yamanaka, MD, PhD; T. Saito, MD, PhD; N. Kamatani, MD, PhD.

Address reprint requests to Dr. H. Okamoto, Institute of Rheumatology, Tokyo Women's Medical University, 10-22 Kawada-cho, Shinjuku, Tokyo 162-0054, Japan. E-mail: hokamoto@ior.twmu.ac.jp

Submitted November 1, 2002; revision accepted April 11, 2003.

various concentrations of U-46619 (9,11-dideoxy-9α,11α-methanoepoxy prostaglandin F(2α)) were added and the sample incubated for 10 min at 37°C. The samples were then centrifuged at 3000 rpm for 20 min and the supernatant collected and stored at –20°C until the measurement of TARC/CCL17 and ß-thromboglobulin was performed.

Statistical analysis. The statistical analyses were by Mann-Whitney test and by determining Pearson correlation coefficients.

RESULTS

Plasma and serum samples were obtained from 58 patients [SLE, n = 38, median age 29.6 yrs (20–41); RA, n = 20, median age 48 yrs (22–71); and 50 controls, n = 50, median age 28 yrs (21–42)]. We found that the concentration of TARC/CCL17 was higher in serum than in plasma. In addition, there was a correlation between the absolute serum and plasma TARC/CCL17 concentrations (R = 0.4334) (Figure 1A, 1B). We speculated that platelet aggregation occurring during the separation of the serum might contribute to this difference in concentration. To verify this, we examined how increasing concentrations of the thromboxane analog U-46619 affected the concentration of TARC in whole blood obtained from a patient with an initial TARC/CCL17 serum concentration of 521 pg/ml. As shown in Figure 1C, the level of TARC/CCL17 tended to increase with an increase in U-46619 concentration, as did the concentration of ß-thromboglobulin, a protein known to be secreted from platelets. These results suggest that the higher TARC/CCL17 concentrations observed in serum compared to those in plasma were caused by the secretion of TARC/CCL17 from platelets following platelet aggregation in the course of the separation of serum. This is consistent with the recent report showing that platelets from patients with atopic dermatitis contain high levels of TARC/CCL17[10]. Therefore we decided to compare plasma and not serum levels of TARC/CCL17.

Plasma samples were obtained from 38 female patients with SLE who were divided into 2 groups: (1) untreated SLE patients, i.e., those who had not received treatment with corticosteroids or immunosuppressants (n = 18, median age 27.4 yrs, range 20–36) and (2) SLE patients who had received treatment with corticosteroids and/or immunosuppressants (n = 20, median age 32.4 yrs, range 26–41). We also evaluated plasma and serum levels of TARC/CCL17 in a group of 50 healthy age and sex matched controls, while 20 patients with RA served as disease controls. Plasma levels of TARC/CCL17 were significantly higher in untreated SLE patients than in healthy controls (mean ± SD 199.98 ± 58 pg/ml vs 23.56 ± 17.6 pg/ml, $p < 0.001$ by Mann-Whitney U test; Figure 2A). TARC/CCL17 levels in untreated SLE patients were significantly higher than in treated SLE patients (69.70 ± 36.82 pg/ml) and RA patients (45.69 ± 24.36) ($p < 0.001$), suggesting that Th2 cells contribute significantly to the pathogenesis of SLE.

The WHO classification of lupus nephritis is based upon the renal biopsy findings. Class I exhibits a normal histological appearance on biopsy. Classes II and III exhibit mild to moderate mesangial immune deposits and mesangial proliferation, while Class IV is characterized by diffuse proliferative glomerulonephritis. To further analyze the Th1/Th2 balance in SLE patients with lupus nephritis, we studied the relationship between plasma TARC/CCL17 levels and the WHO lupus nephritis class in the 14 samples derived from untreated SLE patients who had undergone renal biopsy. Some reports show that serum levels of MCP-1/CCL2 are higher in SLE patients than in healthy controls[11]. To analyze the relationship between the levels of MCP-1 and TARC/CCL17, we measured levels of MCP-1/CCL2 in plasma from 32 SLE patients (median age 29 yrs, range 24–39) by ELISA. There was no correlation between

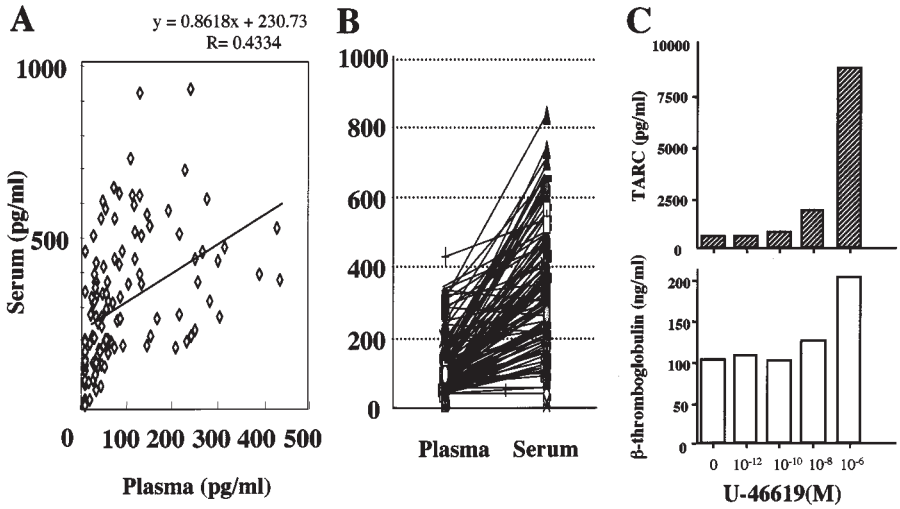

Figure 1. A. Comparison of TARC/CCL17 concentrations in plasma and serum. B. Correlation in TARC/CCL17 levels between plasma and serum. C. Contribution of platelets to the higher concentration in serum samples.

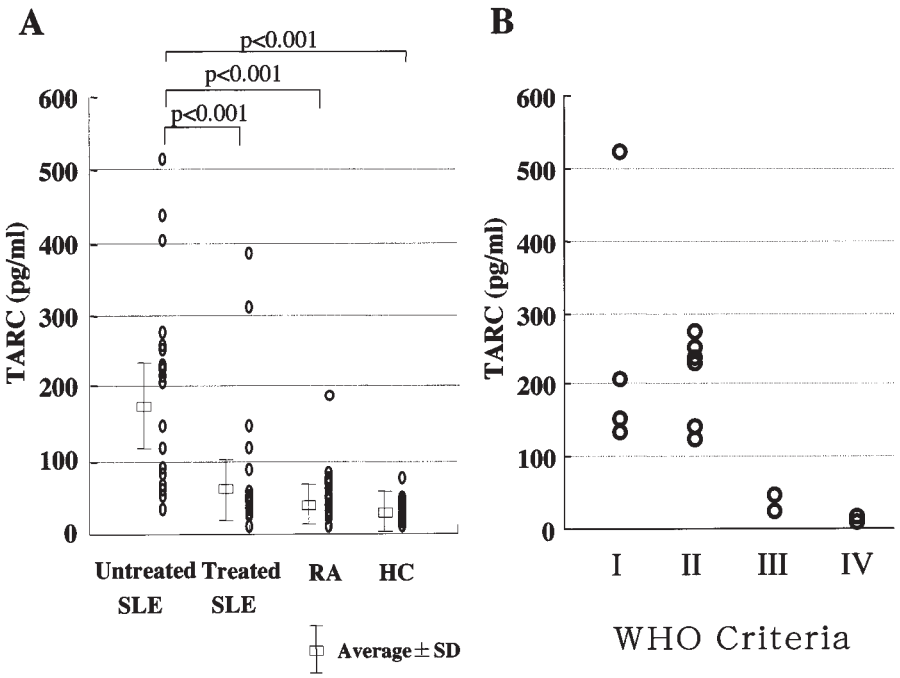

Figure 2. A. Plasma levels of TARC/CCL17 in patients with SLE. Untreated SLE: SLE patients who had never received corticosteroids or immunosuppressive agents (n = 18). Treated SLE: SLE patients who had been treated with corticosteroids and/or immunosuppressive agents (n = 20). RA: patients with rheumatoid arthritis (n = 20). HC: healthy controls (n = 50). The data are represented as mean ± standard deviation. B. Relationship between plasma TARC/CCL17 levels and WHO lupus nephritis class.

plasma levels of MCP-1/CCL2 and TARC/CCL17 (Figure 3A). To confirm the importance of the Th2-type CC chemokine, we next measured the levels of MDC/CCL22, another high affinity ligand for CCR4, in plasma from 32 SLE patients (median age 29 yrs, range 24–39) by ELISA[5]. There was a close correlation between the plasma levels of MDC/CCL22 and TARC/CCL17 (Figure 3B). Figure 4 illustrates the changes of plasma TARC/CCL17 levels during the course of 2 representative patients with SLE. Case A was a 34-year-old woman diagnosed with SLE after development of proteinuria (2.5 g/day) and immunological abnormalities such as a high titer of anti-dsDNA antibody and low levels of CH50. Her clinical and laboratory findings improved with steroid therapy. As the disease activity

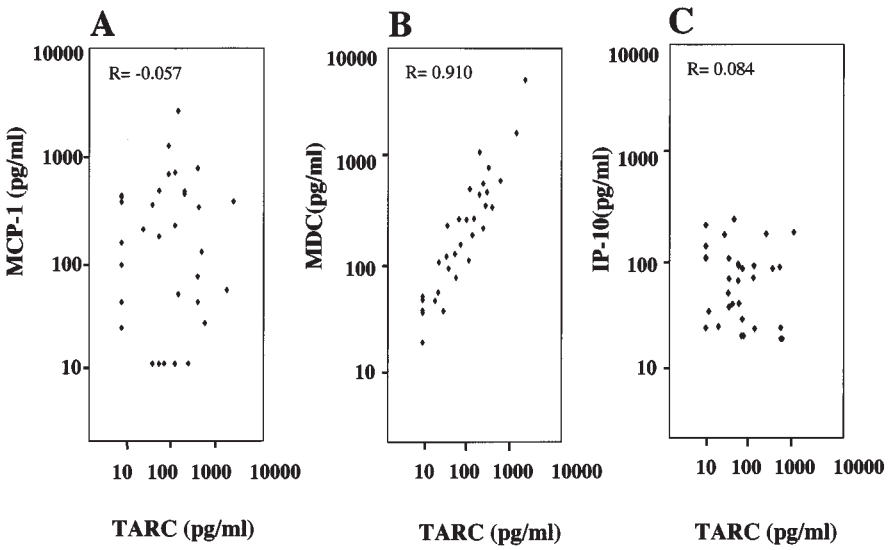

Figure 3. Relationship between plasma levels of other chemokines (MCP-1/CCL2, MDC/CCL22, or IP-10/CXCL10) and TARC/CCL17.

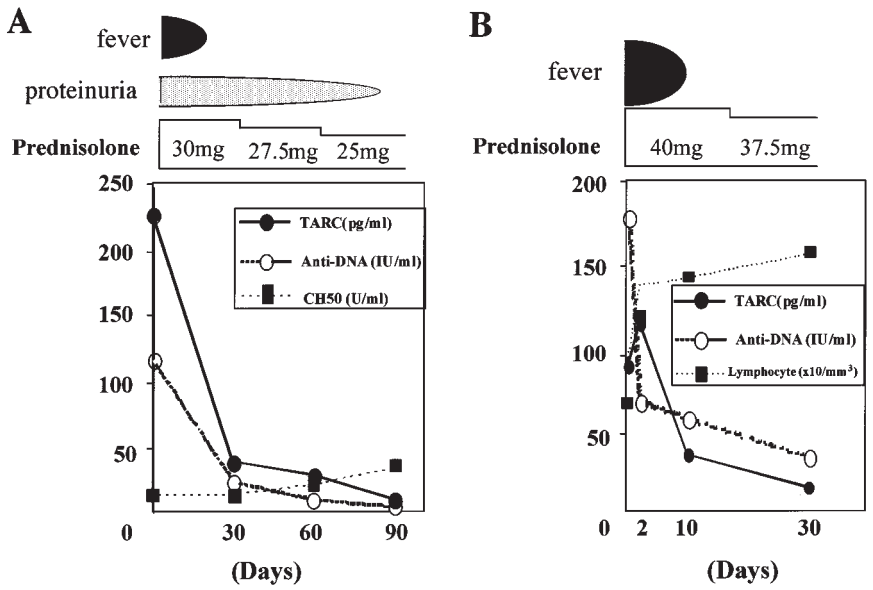

Figure 4. Clinical course and plasma levels of TARC/CCL17 in 2 representative cases. Case A was a 34-year-old woman diagnosed as having SLE by the findings of proteinuria and immunological abnormalities. Her clinical and laboratory findings improved with steroid therapy. As disease activity declined, her plasma TARC/CCL17 level decreased, and this correlated with serum anti-DNA levels. Case B was a 21-year-old woman whose chief complaint was high fever. She was diagnosed as having SLE from a combination of lymphopenia and immunological abnormalities. Her clinical and laboratory findings improved with steroid therapy.

declined, her plasma TARC/CCL17 level decreased, and this correlated with the serum anti-DNA antibody levels (Figure 4A). Case B was a 21-year-old woman with a high fever, diagnosed as having SLE by the findings of lymphopenia (760/mm^3) and immunological abnormalities such as a high titer of anti-dsDNA-antibody and low levels of CH50. Her clinical and laboratory findings improved with steroid therapy. As the disease activity declined, her plasma TARC/CCL17 level decreased. Of note, 2 days after the initial steroid therapy, the plasma level of TARC/CCL17 increased temporarily and decreased in the course of the treatment, indicating that a change in the coagulation state after the initiation of steroid therapy might be involved in the temporal increase of TARC/CCL17 in plasma (Figure 4B).

DISCUSSION

We studied plasma levels of thymus and activation-regulated chemokine (TARC/CCL17) in patients with SLE and compared levels with those in patients with RA or in healthy controls. There were significant differences between the patients with untreated SLE and treated SLE ($p < 0.001$) and RA ($p < 0.001$) and healthy controls ($p < 0.001$) in the plasma concentrations of TARC/CCL17 (Figure 2A). In addition, plasma levels of TARC/CCL17 were dependent upon the class of lupus nephritis, being higher in class I or II than class III or IV disease. In some patients, a decrease of TARC/CCL17 was observed in proportion to disease activity (Figure 4).

Chemokines are small secreted polypeptides that play an important role in a wide range of inflammatory and immunological processes by recruiting selected subsets of leukocytes[12,13]. TARC/CCL17 is a recently identified lymphocyte-directed CC chemokine that is one of the high affinity ligands for CCR4 and specifically chemoattracts Th2 cells.

Th2 predominance has been investigated by analyzing the cytokine production of lymphocytes derived from SLE patients. However, to our knowledge, TARC/CCL17 levels have not been measured in patients with SLE to date. As TARC/CCL17 can be measured in plasma samples, we were able to evaluate the Th1/Th2 balance at different stages of this disease. We observed decreased plasma levels of TARC/CCL17 in SLE patients treated with immunosuppressive drugs. Decreased TARC/CCL17 levels were observed in proportion to the disease activity in patients who presented with a fresh diagnosis of SLE. Thus, TARC/CCL17 may be a useful serological marker and may facilitate assessment of the degree of disease activity in SLE. In addition, plasma levels of TARC/CCL17 are dependent upon the class of lupus nephritis. The TARC plasma concentrations were higher in patients with class I or II lupus nephritis than in patients with class III or IV lupus nephritis (Figure 2B). This suggests that Th2 cells contribute to lupus nephritis with immune deposits and cellular invasion, with TARC acting to attract and recruit Th2 cells to the renal tissue, where they may be involved in

the development of lupus nephritis. Recently, Yamada, *et al* showed that CCR4+ CD4+ T cells accumulate in the kidneys of patients with lupus nephritis, whereas these cells were less prevalent in the peripheral blood of these patients, suggesting that chemokines actively recruit CCR4+ cells to inflamed renal tissue[14]. Our results are consistent with this observation, since patients with lupus nephritis exhibited elevated circulating TARC/CCL17 concentrations, which may facilitate the recruitment of CCR4+ CD4+ T cells to renal tissues. TARC/CCL17 levels may be lower in patients with severe lupus nephritis, such as class IV disease, as there may be severe damage to the cells responsible for the production of TARC/CCL17 or loss of proteins including TARC/CCL17 due to increased proteinuria. Indeed, in patients with type III or IV nephritis, more proteinuria was observed than in patients with type I or II nephritis (mean proteinuria 2.43 vs 0.53 g/day, type III, IV nephritis vs type I, II nephritis, respectively). However further studies are needed to identify the source of TARC/CCL17 in lupus nephritis. Some reports show that serum levels of MCP-1/CCL2 are higher in SLE patients than healthy controls[11]. However, we were unable to observe any correlation between plasma levels of MCP-1/CCL2 and TARC/CCL17, as shown in Figure 3A. MCP-1/CCL2 is one of the ligands for CCR2, a chemokine receptor that is crucial for the function of Th1 cells[15,16]. Thus MCP-1/CCL2 might play an important role in cellular immunity in the later stages of the pathogenesis of SLE than the stage in which TARC/CCL17 contributes. We also measured serum/plasma levels of interferon-inducible protein-10 (IP-10/CXCL10) in some patients, but there was only a tenuous relationship between the 2 markers (Figure 3C).

Our data suggest that the development of SLE is closely related to the elevation of plasma TARC/CCL17 concentrations. To date, there is no difference between untreated freshly diagnosed SLE patients with high TARC levels and patients with low levels in terms of clinical phenotype. Further studies should be performed to analyze the relationship between clinical presentation and circulating TARC/CCL17 levels. These studies may assist the development of new therapeutic strategies to suppress SLE disease activity.

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