Soluble CD154 in Rheumatoid Arthritis: Elevated Plasma Levels in Cases with Vasculitis

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ABSTRACT. ***Objective.*** To determine the levels of soluble CD154 (sCD154) in the plasma of patients with rheumatoid arthritis (RA) and rheumatoid vasculitis (RV), and to examine the relationship between the levels of sCD154 in plasma and the clinical variables.

Methods. Levels of sCD154 were quantified in 39 plasma samples from patients with RA, including 9 patients who were also diagnosed with RV, and compared with those of 20 healthy subjects. An ELISA was established and specificity of the ELISA was tested by control ELISA using isotype-matched IgG and preabsorption assay. The titers of IgM and IgG rheumatoid factor (IgM-RF, IgG-RF) for each patient were determined simultaneously, and values of other laboratory variables were also determined.

Results. Levels of sCD154 in plasma were higher in patients with RA than in the healthy subjects ($p < 0.02$). Compared with RA patients without vasculitis, patients with RV had significantly higher levels of sCD154 in their plasma ($p < 0.001$). Control ELISA and absorption assay of sCD154 indicated that our ELISA system was capable of measuring plasma sCD154 in RA patients. Levels of sCD154 in RA plasma correlated significantly with both IgM-RF and IgG-RF titers ($r = 0.64$ and 0.61, respectively, both $p < 0.001$). The levels of sCD154 decreased after commencement of treatment for vasculitis in cases with RV.

Conclusion. We identified the presence of sCD154 in RA plasma, with especially high levels in cases with vasculitis. Correlation between sCD154 and RF titers indicates the CD154-CD40 pathway is likely related to pathogenic RF production. (J Rheumatol 2001;28:2583–90)

Key Indexing Terms:
RHEUMATOID ARTHRITIS RHEUMATOID FACTOR VASCULITIS

Rheumatoid arthritis (RA) is a systemic disease characterized by chronic synovitis of unknown etiology. One of the features of RA synovitis is persistent infiltration of mononuclear cells, including monocyte and CD4+ T cells, in a chronically inflamed synovium[1]. The infiltrating cells in the synovial fluid (SF) and tissues induce the production of proinflammatory cytokines such as interleukin 1 (IL-1), IL-6, tumor necrosis factor-α (TNF-α), and granulocyte-colony stimulating factor, which are implicated in the pathogenesis of RA[2,3]. A subset of patients develops rheumatoid vasculitis (RV), which is complicated by an extraarticular inflammation that involves small and medium size blood vessels. Cutaneous ulceration, neuropathy, and disorders of visceral organs may occur in patients with RV, and the development of RV is associated with increased levels of rheumatoid factor (RF)[4].

CD154 (CD40 ligand), a 39 kDa type II membrane glycoprotein belonging to the TNF family, is predominantly expressed on activated CD4+ T cells[5,6]. Signaling via CD40 induces antigen presenting cells such as B cells, monocytes, and dendritic cells to express various immune accessory molecules that are important in cognate cell-cell interaction[7-18]. Activated T cells expressing CD154 can also engage CD40 on endothelial cells to induce production of inflammatory cytokines and augmentation of cell adhesion molecule expression, such as CD54 and CD106[19-24]. The rapid and transient expression of CD154 allows activated T cells to stimulate only particular CD40-bearing cells to participate in an antigen-specific immune response.

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Supported by grants from the Ministry of Health and Welfare on Research Committee of Intractable Vasculitis Syndrome, Surveys and Research on Specific Diseases and the Ministry of Education, Science, Sports, Culture and Technology of Japan (No.12770237). Dr. Kipps was supported by grant AR44850-01A1 from the National Institutes of Health, Bethesda, MD, USA. Dr. Kato was supported by grants from the Ministry of Education, Science, Sports, Culture and Technology of Japan (No.12670447) and Japan Rheumatism Foundation.

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Submitted February 22, 2001; revision accepted June 18, 2001.

In patients with RA, the expression of functional CD40 on synovial monocytes, fibroblasts, and dendritic cells has been reported[25-27]. CD154 expression, which is capable of inducing B cell immunoglobulin (Ig) production and dendritic cell IL-12 expression[28], was also detected on RA peripheral and synovial lymphocytes. Moreover, blockade of CD154 has been shown to ameliorate collagen induced arthritis in a murine model of RA[29], and to abrogate RF production in mice transgenic for human RF[30]. These findings indicate that constitutive or excessive expression of CD154 would induce RF production and the perpetuation of inflammation in RA.

Similar to TNF-α, activated T cells not only express membrane-bound CD154 but also release a soluble form of CD154 (sCD154). Both native and recombinant sCD154 have proven to have biological function[31-37]. The production of sCD154 presumably occurs in the microsomes of T cells by stimulation-dependent proteolysis of full length CD154[32,34]. We detected functional sCD154 in the plasma of patients with systemic lupus erythematosus (SLE) and revealed that the levels of sCD154 significantly correlated with the disease activity, as well as the titers of anti-dsDNA[38]. Conceivably, elevated expression of sCD154 may contribute to the activation of the immune system and stimulate the autoantibody-producing B cells in patients with SLE. Since the importance of CD40 signaling delivered by CD154 has also been described in patients with RA, we determined the levels of sCD154 in plasma and evaluated if there exists a relationship with RF titers.

MATERIALS AND METHODS

Patient samples. Blood samples were drawn from 39 patients fulfilling criteria of the American College of Rheumatology (formerly, The American Rheumatism Association)[39] for the diagnosis of RA. There were 29 women and 10 men with a mean age ± standard deviation (SD) of 52.5 ± 12.4 years (range 27–76). All patients were treated with disease modifying antirheumatic drugs (DMARD): 17 with methotrexate, 10 with bucillamine, 9 with sulfasalazine, and 3 with D-penicillamine. Twenty-eight patients were also treated with steroid, 3 with 7.5 mg, 15 with 5.0 mg, and 10 with 2.5 mg/day of prednisolone. Patients were diagnosed as having RV if they had exhibited one or more of the following symptoms: mononeuritis multiplex, necrotizing glomerulonephritis, rapidly progressive interstitial lung disease/fibrosing alveolitis, necrotizing scleritis, or typical vasculitic skin lesions (petechiae, purpura). The RV cases in which biopsies unequivocally showed the presence of leukocytoclasis or fibrinoid necrosis of the vascular wall were also diagnosed as histologically established vasculitis[40,41]. Nine patients fulfilled these criteria. Blood samples from 20 healthy control subjects, 14 women and 6 men (age 47.8 yrs ± 18.6, range 31–69), were also examined. Further, plasma levels of sCD154 were determined in 10 patients with seronegative spondyloarthropathies (SpA) and 9 with primary vasculitis syndrome. There were 2 patients with psoriatic arthritis, 3 with reactive arthritis, and 5 with ankylosing spondylitis in the seronegative SpA group, and 4 patients with classic polyarteritis nodosa and 5 with antineutrophil cytoplasmic autoantibody positive microscopic polyangiitis in the primary vasculitis syndrome group. No patient or healthy subject had any known infectious diseases for at least 2 weeks before blood samples were drawn. Whole blood was collected into tubes containing heparin and cell-free plasma was separated immediately by centrifugation at 100 g. The plasma was isolated and stored at –80°C until analyzed.

Rheumatoid factors and other laboratory variables. IgM-RF was determined by latex turbidimetric immunoassay (TIA), and the normal range was less than 20 IU/ml. IgG-RF was measured by ELISA (Eitest IgG-RF, Eisai, Tokyo, Japan), and the value was expressed as an index (normal range < 2.0). Serum IgG and C-reactive protein (CRP) were determined by TIA (normal range 800–1800 mg/dl and < 0.3 mg/dl, respectively) and erythrocyte sedimentation rate (ESR) was determined in our central diagnostic laboratory.

Determination of sCD154 concentration by ELISA. The concentration of sCD154 in plasma was determined by sandwich ELISA using 2 non cross-blocking anti-human CD154 monoclonal antibodies (Mab)[38]. Briefly, a 96 well polystyrene ELISA plate (Corning Costar Corp., Cambridge, MA, USA) was coated with 5 μg/ml of anti-human CD154 Mab (TRAP-1; Pharmingen, San Diego, CA, USA) in phosphate buffered saline (PBS) in each well. After washing with PBS, the plates were treated with a blocking buffer consisting of 1% bovine serum albumin in PBS at room temperature for 2 h. The plates were washed 4 times with 0.05% Tween-20 in PBS (washing buffer) and incubated at 4°C overnight with the samples in the washing buffer. After washing, the plates were incubated with 2 μg/ml of biotinylated anti-human CD154 Mab (bio-M90; Genzyme, Cambridge, MA, USA) in the washing buffer. Then avidin and biotinylated horseradish peroxidase (Elite Vectastain™; Vector Laboratories, Burlingame, CA, USA) were added, incubated, and washed, followed by addition of 3,3′, 5,5′-tetramethylbenzidine peroxidase (Kirkegaard & Perry Laboratories Inc., Gaithersburg, MD, USA), which served as a substrate for detection. Absorbance at 450 nm was determined using a micro-ELISA reader. Serial dilutions of recombinant human sCD154 (Bender MedSystems, Vienna, Austria) were used to construct the standard curve. In the control ELISA, isotype-matched mouse IgG (MOPC-21; Sigma, St. Louis, MO, USA) was used as a coating antibody, and biotinylated mouse IgG1 (Ancell, Bayport, MN, USA) was used for detection.

To examine the absorption of sCD154, plasma samples incubated at room temperature for 1 h with anti-human CD154 Mab (5C8, purified from ascites of the hybridoma purchased from the American Type Culture Collection, Rockville, MD, USA) or isotype-matched IgG-conjugated sepharose were used for the ELISA. The absorption was also evaluated by incubating the samples with Ramos B cells (a Burkitt's lymphoma B cell line expressing CD40 on the surface, purchased from the American Type Culture Collection).

Statistical analysis. The mean concentrations of sCD154 in plasma were compared between groups using the Mann-Whitney nonpaired nonparametric test. For comparisons of sCD154 levels within the same individuals over time, the Wilcoxon matched pairs test was used. Relationships between the level of sCD154 and the values of the variables assessed were analyzed using the Spearman rank order correlation test.

RESULTS

Establishment of a specific ELISA for sCD154. We have developed a sandwich ELISA using 2 non-cross-blocking anti-CD154 Mab to determine the plasma concentration of sCD154[38]. Recombinant homotrimeric sCD154 (rsCD154) was used as a positive standard control for the ELISA (Figure 1A), and the minimum concentration of detectable sCD154 in this assay was about 40 pg/ml. To exclude the influence of plasma factors for the ELISA system, plasma samples from healthy controls were added to the rsCD154 at the concentration of 10%, which was the maximum concentration in this study. As shown in Figure 1B, there were no differences detected at rsCD154 concentrations of 0, 1.0, and 5.0 ng/ml with plasma from healthy subjects. Moreover, the specificity of this ELISA was confirmed by its inability to detect mouse sCD154 or human sCD27 (data not shown).

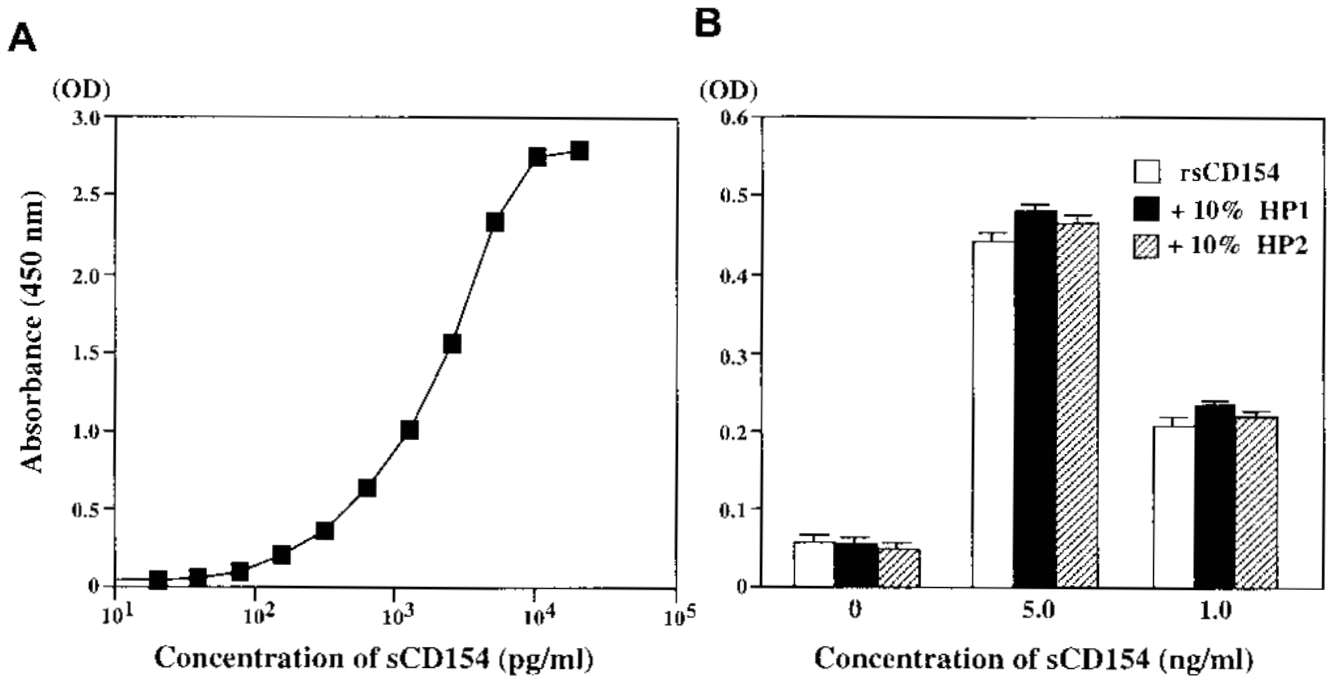

Figure 1. Detection of plasma concentration of sCD154 with ELISA. A. Serial dilutions of recombinant sCD154 were placed into separate wells of a 96 well microtiter plate precoated with anti-CD154 Mab (TRAP-1). A non-cross-blocking biotinylated anti-CD154 Mab (M90) was used to determine the bound recombinant sCD154. The mean absorbance of triplicate wells is plotted on the ordinate, and the logarithmic concentration of the recombinant sCD154 is indicated on the abscissa. B. The mean absorbance of triplicate wells of ELISA for rsCD154 at indicated concentrations was determined in the absence (white columns) or presence of 10% plasma sample from 2 healthy controls (HP1, black columns and HP2, shaded columns). The bars indicate SD of the mean.

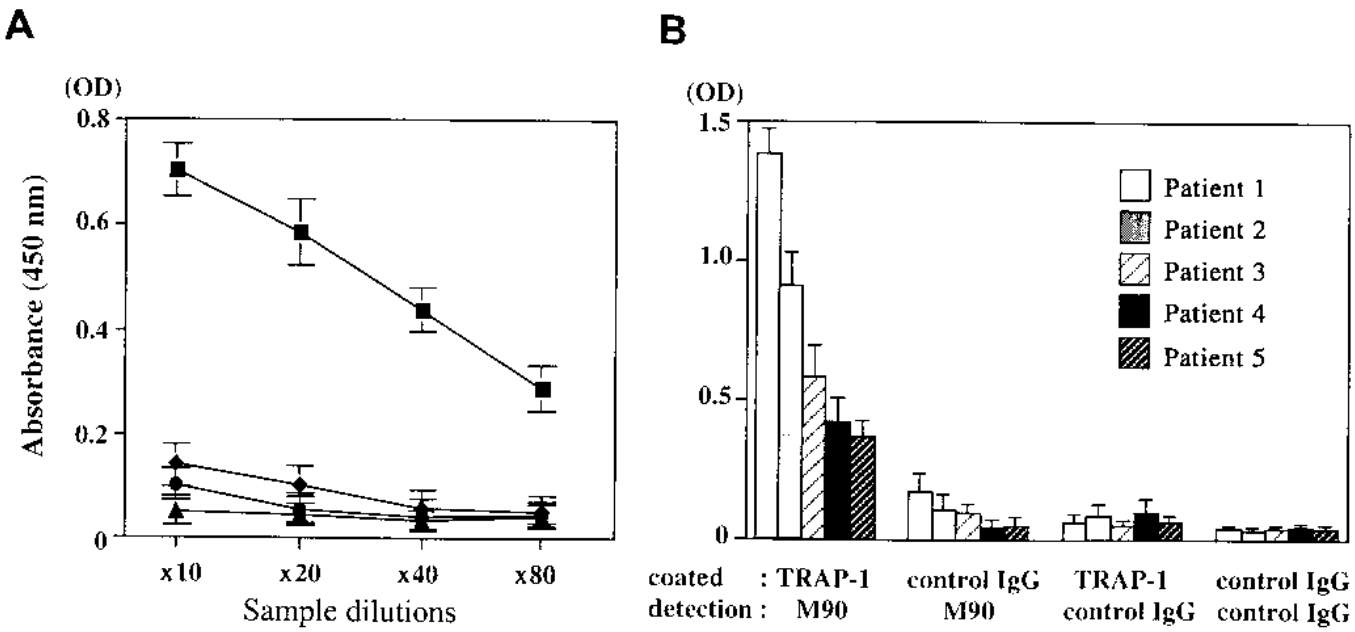

Figure 2. Specificity of sCD154 ELISA in RA plasma. A. Serial dilutions of plasma were determined in the sCD154 ELISA. The mean absorbance of triplicated wells is indicated with squares (■). To test the specificity of ELISA, isotype-matched mouse IgG or biotinylated mouse IgG was substituted for anti-CD154 Mab in the ELISA. The mean absorbance of triplicated wells in each ELISA is indicated as follows: substitution of control IgG for TRAP-1 (◆), bio-M90 (●), and both (▲). Bars indicate standard error of 3 independent experiments. B. Plasma from 5 patients with RA were diluted 20 times and determined with the same ELISA system. Combinations of antibody used for coating and detection are labeled at the bottom of columns. Columns and bars indicate absorbance and SE of 3 independent experiments, respectively.

Some of our results revealed high levels of sCD154 determined with the ELISA for RA patients evaluated. Therefore, to confirm the specificity of sCD154 in RA plasma samples, isotype-matched mouse IgG was substituted for the coated TRAP-1 and/or detection bio-M90 in the ELISA. As shown in Figure 2A, RA plasma in serial dilution revealed a linear dose-dependent value for sCD154 (squares). However, substitution with control mouse IgG for TRAP-1 or bio-M90 (diamonds and circles, respectively) clearly reduced the values of absorbance in each dilution. Further, significance was not established with sandwich ELISA in the 2 control IgG (triangles). The values of 5 RA plasma samples in 20 times dilution for each ELISA were compared in Figure 2B. In this dilution, values of sCD154 ELISA were significantly higher compared with those of the control IgG ELISA in all 5 samples that had various levels of sCD154 ELISA. Due to these results, plasma samples diluted 20 times were used to determine sCD154 levels in further experiments.

To confirm the presence of sCD154 in RA plasma, those with elevated levels of sCD154 were pretreated with 5C8 (anti-CD154 Mab) or isotype control IgG-conjugated sepharose. Preabsorption of the RA plasma with 5C8 resulted in about 90% reduction of sCD154 levels, whereas control IgG barely affected the values obtained (Figure 3). Further, plasma sCD154 was also absorbed by preincubation with a CD40-expressing B cell line (data not shown). Collectively, these data indicate that RA plasma contained sCD154.

Levels of sCD154 in plasma of patients with RA and RV. Using this sCD154-specific ELISA, we determined levels of sCD154 in plasma from healthy subjects and in patients with RA and RV (Figure 4). The average sCD154 concentration of RA patients (0.73 ± 1.11 ng/ml) was greater than that of healthy subjects (0.17 ± 0.19 ng/ml; $p < 0.02$). Moreover, levels of sCD154 (8.16 ± 10.12 ng/ml) were significantly higher in vasculitis cases (RV) than in RA without vasculitis ($p < 0.001$), and all 9 patients with RV had levels of sCD154 elevated above the mean + 3 SD of the healthy subjects. In all RA patients including RV cases, the average sCD154 concentration was 2.53 ± 5.74 ng/ml, and elevated sCD154 was detected in 19 of 39 patients (48.7%). There were no differences in the plasma levels of sCD154 among patients treated with different DMARD and between patients with and without prednisolone (data not shown). On the other hand, the plasma levels of sCD154 in patients with seronegative SpA (0.29 ± 0.28 ng/ml) were not elevated in comparison to the healthy subjects ($p = 0.21$). The average concentration of plasma sCD154 in patients with primary vasculitis syndrome (0.58 ± 0.72 ng/ml) was not greater than that of healthy subjects ($p = 0.13$) and was significantly lower than that of RV ($p < 0.001$).

Relationship between sCD154 levels and clinical variables. We previously reported that levels of sCD154 in plasma of

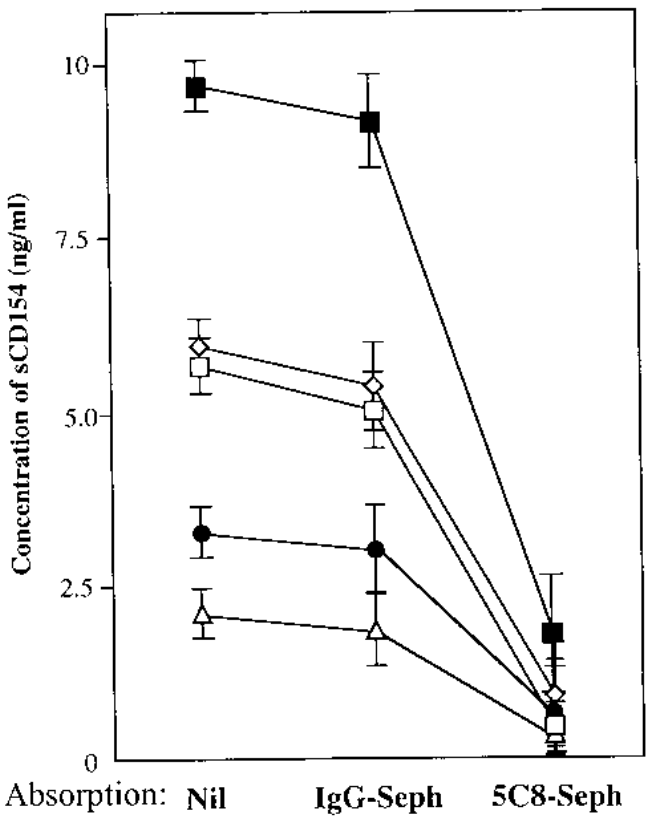

Figure 3. Absorption of sCD154 in RA plasma pretreated with anti-CD154 Mab. Plasma samples at various levels of sCD154 were preincubated with or without 5C8 (anti-CD154 Mab) or isotype-matched control IgG-conjugated sepharose (5C8-Seph and IgG-Seph), and the concentration of sCD154 was determined with ELISA. Mean absorbance of triplicate wells was compared with the standard curve constructed with serial dilutions of rsCD154. Bars indicate SE of 2 independent experiments. Each symbol represents samples from each patient.

patients with SLE correlated significantly with the SLE disease activity index and also with the titers of anti-dsDNA antibody. To assess whether plasma levels of sCD154 correlate with RF and other clinical variables of RA, we compared the values of IgM-RF and IgG-RF, ESR, CRP, and IgG of each patient with plasma concentration of sCD154. As shown in Figure 5, the values of both IgM-RF and IgG-RF correlated significantly with the levels of sCD154 ($r = 0.64$ and 0.61, respectively, both $p < 0.001$). However, the values of ESR and also CRP did not correlate with the sCD154 plasma levels ($r = 0.28$ and 0.29). Although a notable correlation was found between the sCD154 levels and the values of RF, the total plasma IgG levels did not significantly correlate with the sCD154 levels (data not shown).

sCD154 levels before and after commencement of treatment for vasculitis. Plasma levels of sCD154 were determined in 9 patients with RV (Table 1) after commencement of treatment for vasculitis (Figure 5). All patients received steroid

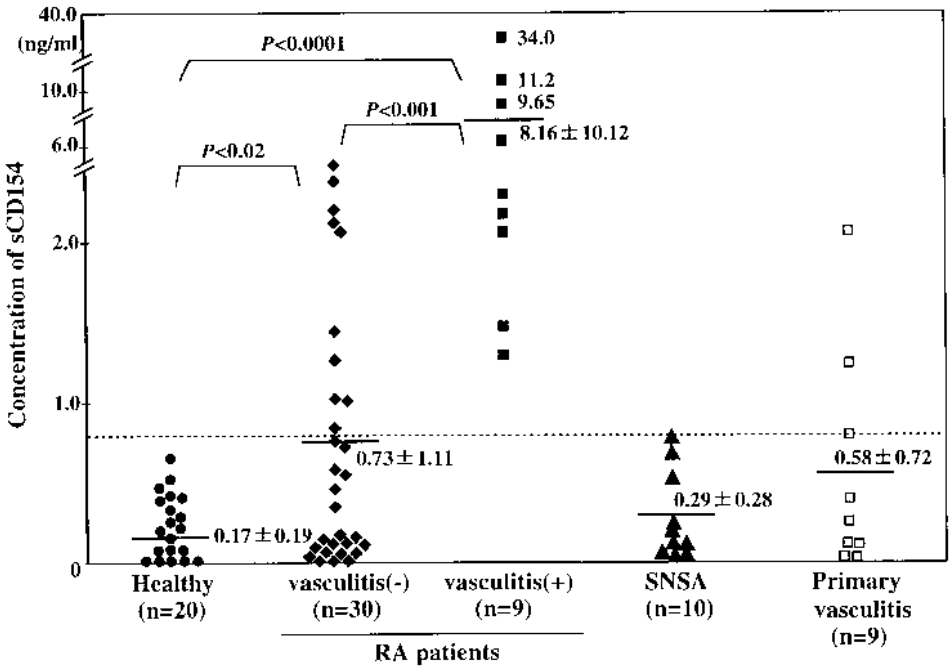

Figure 4. Concentration of sCD154 in plasma of RA and RV patients. The plasma concentrations of sCD154 (ng/ml) were determined by ELISA. Concentrations that were detected in the plasma of each of the 20 healthy subjects (●) or 30 RA patients (◆) are plotted. Nine RA patients with clinical and/or histological vasculitis (■) were separated as the RV group. Also illustrated are concentrations of plasma from patients with seronegative SpA (▲) and primary vasculitis syndrome (□). The plasma concentration of each symbol represents the concentration of sCD154 from a single subject. Bar indicates the mean concentration of each group. Numbers indicate the mean concentrations of each group ± SD of the mean. Broken line represents the mean + 3 SD of sCD154 of healthy subjects. P value is calculated for the difference between the means of the groups. The average sCD154 concentration of RA + RV was 2.53 ± 5.74 ng/ml.

therapy, at least 30 mg/day of prednisolone, for vasculitis, and 3 patients also received plasmapheresis. The mean level of sCD154 (8.16 ± 10.27 ng/ml) decreased to 2.67 ± 1.92 ng/ml ($p < 0.02$) 2 to 4 weeks after commencement of the treatment.

DISCUSSION

In our study we found that levels of sCD154 were elevated in plasma of patients with RA in comparison with healthy subjects ($p < 0.02$; Figure 2). Moreover, RA patients who had vasculitis revealed significantly higher plasma concentrations of sCD154 than those without vasculitis ($p < 0.001$).

To establish sCD154-specific ELISA, we tested several sets of anti-CD154 Mab for coating and detection, and selected a combination of Mab, TRAP-1, and biotinylated-M90 that gave minimal nonspecific interference. Further, to test whether nonspecific binding of RF to anti-CD154 Mab affects the levels of sCD154, values of RA samples in the control ELISA were substituted with isotype-matched mouse IgG for anti-CD154 Mab and evaluated. In the sCD154 ELISA, levels of sCD154 in serial dilution of RA plasma responded in a linear dose-dependent manner, while the values of absorbance were obviously reduced in the control ELISA substituted with isotype-matched mouse IgG for TRAP-1 and/or bio-M90 (Figure 2). This indicated that the influence of RF on sCD154 levels was unlikely in the sCD154 ELISA. Moreover, absorption of sCD154 with anti-CD154 Mab, but not with isotype-matched control IgG (Figure 3), confirms the presence of sCD154 in the plasma of patients with RA, indicating that there is no considerable interference of RF in the ELISA. In addition, the plasma sCD154 was also absorbed by incubation of the plasma with the CD40-expressing B cell line (data not shown), indicating that the plasma sCD154 is capable of binding to CD40.

CD154 exists ordinarily as a 39 kDa membrane-anchored type II glycoprotein expressed predominantly on CD4+ T cells[5,6]. The brief expression of CD154 is capable of allowing activated T cells to stimulate only particular CD40-bearing cells that participate in antigen-specific immune response. CD154 has also been identified as an 18–20 kDA soluble protein[31-36], suggesting that full length CD154 undergoes a stimulation-dependent proteolytic process to generate[32,34]. In patients with RA, the expression of functional membrane-bound CD154, which is capable of inducing Ig production by B cells and IL-12 production by dendritic cells, has been detected on synovial and peripheral blood T cells[28]. Most of the CD4+ T cells in RA synovium are postulated to be memory cells that have been repeatedly stimulated by an antigen or antigens[42]. These antigen-stimulated T cells are capable of expressing CD154 and also

Table 1. Characteristics of cases with rheumatoid vasculitis. Values in parentheses were determined after beginning treatment for vasculitis.

Patient	Age, Sex	Duration of RA, yrs	Extraarticular Symptoms and Signs	sCD154, ng/ml	ESR, mm/h	CRP, mg/dl	RF, IU/ml	IgG-RF, Index	DMARD	Major Treatment for Vasculitis
1	50M	5	Mononeuropathy multiplex, pleuritis, nodules	11.2 (2.87)	54 (32)	2.3 (0.7)	921 (410)	8.2 (5.3)	MTX 7.5 mg/wk	PSL 40 mg/day
2	46F	14	Episcleritis, skin ulcers, nodules	9.65(4.32)	56 (44)	3.9 (2.8)	210 (118)	4.6 (3.0)	Buc 200 mg/day	PSL 30 mg/day
3	51F	20	Gangrene of toes, pleuritis, pericarditis pancreatitis, acute myocardial infarction	5.38 (1.67)	126 (42)	27.0 (3.6)	819 (322)	7.8 (2.8)	MTX 7.5 mg/wk	mPSL 500 mg/day for 3 days, PP, PSL 40 mg/day
4	64M	6	Skin ulcers, interstitial pnemonia, nodules, episcleritis	4.76 (2.25)	66 (54)	2.5 (2.8)	53 (58)	1.6 (1.2)	SASP 1.0 g/day	PSL 60 mg/day
5	54F	16	Digital ulcers, episcleritis, nodules, pleuritis	2.21 (1.04)	78 (37)	5.5 (1.0)	203 (144)	5.1 (ND)	Buc 100 mg/day	PSL 30 mg/day
6	51F	12	Skin ulcers, interstitial pneumonia	2.19 (0.68)	52 (35)	6.6 (4.7)	290 (77)	5.4 (2.1)	PC 100 mg/day	PSL 40 mg/ day
7	67F	9	Episcleritis, nodules, mononeuropathy multiplex	1.98 (1.50)	67 (62)	4.9 (1.5)	570 (446)	2.6 (ND)	MTX 7.5 mg/wk	PSL 50 mg/day
8	55M	12	Interstitial pneumonia, gangrene, nodules, necrotizing glomerulonephritis	34.0 (6.87)	118 (43)	18.0 (3.8)	951 (398)	8.3 (ND)	SASP 1.0 g/day	mPSL 500 mg/day for 3 days, PP, PSL 40 mg/day
9	65M	10	Episcleritis, skin ulcer, nodules	2.09 (2.87)	52 (30)	4.2 (2.9)	71 (56)	8.9 (7.2)	MTX 10 mg/week	PSL 30 mg/day

MTX: methotrexate: Buc: bucillamine; SASP: sulfasalazine; PSL: prednisolone; mPSL: methylprednisolone; PP: plasmapheresis; ND: not determined.

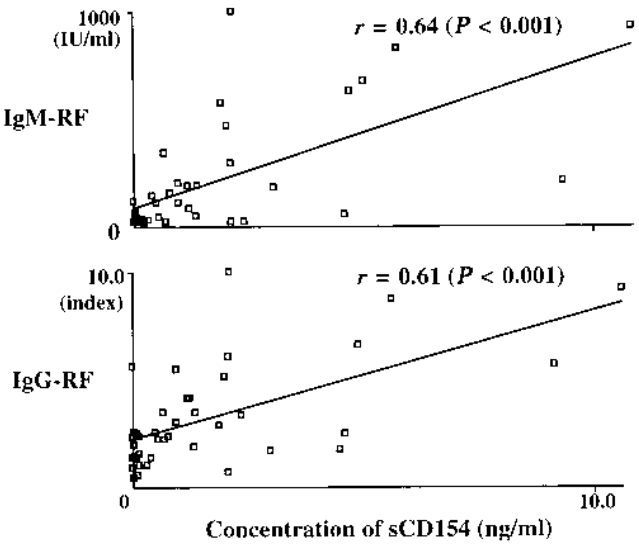

Figure 5. Relationship between sCD154 plasma concentration and RF. Relationships were evaluated between the determined concentrations of sCD154 in plasma (indicated on the abscissa in ng/ml) and values of IgM-RF or IgG-RF in each of 38 RA patients as indicated on the ordinate (the patient who had the maximum concentration of sCD154 was omitted from this figure; however, no p value changed even when the patient was included). The line represents the ultimate correlation between the plasma concentration of sCD154 and values of IgM-RF or IgG-RF (Spearman r = 0.64 and 0.61, respectively, both p < 0.001).

releasing sCD154 by cleavage. On the other hand, recent studies have revealed transient expression of CD154 on human activated platelets[43,44]. We cannot completely exclude the possibility that the activated platelets also release sCD154 since thrombocytosis is frequently recognized and platelets have a shortened half-life in RA. However, secondary activation of platelets *ex vivo* is unlikely in this study because the plasma was isolated immediately after phlebotomy and the sCD154 levels were not elevated in any of the samples from the healthy subjects (Figure 4).

The elevated levels of plasma sCD154 appear to be associated with autoantibody production. Plasma levels of sCD154 significantly correlated with RF titers in patients with RA (Figure 5). On the other hand, the plasma samples from patients with seronegative SpA did not have elevated levels of sCD154 (Figure 4). Further, patients with bacterial pneumonia also had no or extremely minute detectable levels of plasma sCD154 (data not shown). The interaction between CD154 and CD40 provides one of the most essential signals for B cell proliferation, Ig production, and class switching[5,6,10,11]. Moreover, the importance of the interaction between CD154 and CD40 on antigen-presenting cells for cell-mediated immune response has also been reported[7,8,12-14]. Native or recombinant sCD154 has been shown to alter membrane-bound CD154 to induce some of these signals *in vitro*[13,19,32-37]. We and others recently reported that the plasma of patients with active SLE

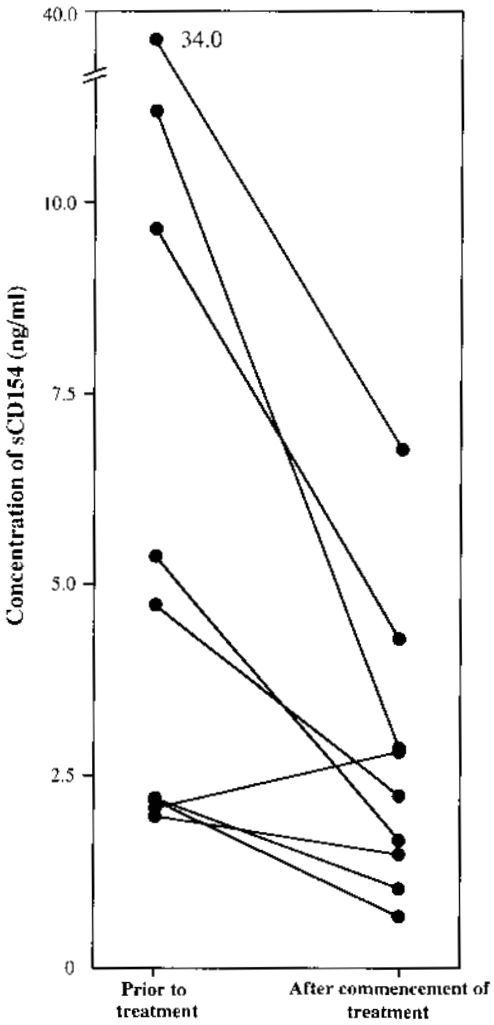

Figure 6. Levels of plasma sCD154 in patients with RV before and after commencement of treatment for vasculitis. Levels of plasma sCD154 were determined in 9 cases of RV before and 2 to 4 weeks after commencement of treatment for vasculitis. All patients received steroid, at least 30 mg/day prednisolone, and 3 patients were simultaneously treated with plasmapheresis. Mean levels of sCD154 before and after commencement of treatment were 8.16 ± 10.27 and 2.67 ± 1.92 ng/ml, respectively ($p < 0.02$).

contained sCD154 that had biological activity, and the levels of sCD154 in the plasma significantly correlated with the titers of anti-dsDNA antibody[38,45]. In the collagen-induced murine arthritis model, the administration of neutralizing anti-CD154 Mab ameliorated the arthritis with reduced titers of anti-type II collagen antibody[30]. Further, production of high affinity pathogenic RF was abrogated by anti-CD154 Mab treatment in mice transgenic for a human IgM-RF[29]. These findings suggest that the interaction between CD154 and CD40 plays a critical role in the development of RA, and collectively, the positive correlation of plasma sCD154 with RF titers supports the assumption that sCD154 also contributes to the stimulation of RF-producing B cells.

In our study, the levels of sCD154 in the plasma of patients with RV were significantly higher than those of RA patients without vasculitis (Figure 4). All RV patients had elevated levels of plasma sCD154, while only 27% of the RA patients without vasculitis had levels above the mean + 3 SD range of healthy subjects. In contrast, patients with primary vasculitis syndrome had lower concentrations of plasma sCD154 than patients with RV (Figure 4; $p < 0.001$), although 3 patients with microscopic polyangiitis had positive values for sCD154. This may be explained by correlation of sCD154 and RF titers, since vasculitis ordinarily occurs in RA patients who have high titers of RF, and these are associated with a severe course of the disease[4,46-48]. Further, identification of several autoantibodies, such as antibodies to endothelial cells, the collagen-like region of C1q, and lactoferin[49-51], suggests that activation of autoantibody-producing B cells is more common in RV. The levels of sCD154 decreased after the commencement of steroid therapy in patients with vasculitis (Figure 6). These findings indicate that sCD154 is a marker of pathogenic B cell activation in RA, which may often occur in cases with vasculitis.

It is noteworthy that endothelial cells express CD40 on their surface, and marked upregulation of CD40 expression was detected on dermal endothelial cells of patients with inflammatory skin disease[19]. CD154-expressing T cells or recombinant sCD154 is capable of inducing endothelial cells to secrete proinflammatory cytokines, to express adhesion molecules[19-23], and to upregulate procoagulant activity[24]. Further longitudinal studies are necessary to establish that sCD154 in plasma contributes to the development of vasculitis in patients with RA. If this finding is confirmed, anti-CD154 Mab therapy may be beneficial in the treatment of rheumatoid vasculitis.

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