Increase of Soluble FcγRIIIa Derived from Natural Killer Cells and Macrophages in Plasma from Patients with Rheumatoid Arthritis

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ABSTRACT. ***Objective.*** FcγRIII (CD16), one of the low affinity IgG Fc receptors, is found in 2 alternative forms, a transmembrane FcγRIIIa expressed on natural killer (NK) cells and macrophages, and a glycosylphosphatidylinositol-linked FcγRIIIb present on neutrophils. Both FcγRIII are released from the cell surface by proteolytic cleavage and these soluble forms (sFcγRIII) are present in plasma. Since NK cells and macrophages will be activated locally, leading to shedding of FcγRIIIa and its subsequent release into blood, we investigated whether sFcγRIIIa plasma concentrations would be a good marker for disease activity in patients with rheumatoid arthritis (RA).

Methods. We measured sFcγRIIIa with an immuno-PCR in plasma of NA(1+,2–) phenotyped donors. In this assay, we used CD16 GRM1, which recognizes NA2-FcγRIIIb and FcγRIIIa. We also analyzed precipitated sFcγRIIIa derived from plasma with immunoblotting with CD16 CLB-LM6.30.

Results. The concentration of sFcγRIIIa in patients with RA was about 3 times higher than in healthy controls. In controls, the sFcγRIIIa levels in plasma correlated with the number of NK cells in peripheral blood. In RA patients, sFcγRIIIa levels were increased directly proportionally to the concentrations of IgG, IgA, or IgM and to erythrocyte sedimentation rate or Lansbury Index. The electrophoretic mobility of plasma sFcγRIIIa corresponded with sFcγRIIIa derived from NK cells and/or macrophages. In general, plasma sFcγRIIIa originated from both cell types; however, the ratio of $sFc\gamma RIIIa^{NK}$ to $sFc\gamma RIIIa^{M\phi}$ varied in the RA patients.

Conclusion. Increased sFcγRIIIa levels in RA patients were found to be caused by NK cell and/or macrophage activation. Plasma sFcγRIIIa levels may serve as a marker for disease activity in RA. (J Rheumatol 2003;30:1911–7)

Key Indexing Terms:
MACROPHAGES NATURAL KILLER CELLS FC-RECEPTORS CD16 RHEUMATOID ARTHRITIS

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Supported in part by grants from the Ministry of Education, Grant-in-Aid for Scientific Research (C) (No. 07672505, 10672192 and 14572192), from the Charitable Trust Clinical Pathology Research Foundation of Japan, and from TERUMO Life Science Foundation (No. 99-319).

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Submitted June 24, 2002; revision accepted February 21, 2003.

FcγRIII (CD16), one of the low affinity receptors for the Fc region of IgG, exists in 2 alternative forms. FcγRIIIa is an integral membrane protein expressed on natural killer (NK) cells, on a subset of T lymphocytes, and on a subpopulation of monocytes and macrophages[1], and shows a cell type-specific glycosylation pattern[2,3]. FcγRIIIb is a glycosylphosphatidylinositol-linked protein expressed exclusively on neutrophils, and it can be induced on eosinophils[4]. The 2 allotypes of FcγRIIIb (NA1- and NA2-FcγRIIIb) differ by 4 amino acids in the membrane-distal Ig-like domain[5], resulting in different glycosylation patterns[6]. Several NA-specific monoclonal antibodies have been generated, namely CLBFcRgranII, CLB-LM1.7, and MG38 (which recognize NA1-FcγRIIIb) and GRM1 and PEN1 (which recognize NA2-FcγRIIIb and FcγRIIIa)[7].

Both FcγRIII are released from the cell surface. FcγRIIIa is released by the action of a metalloprotease upon *in vitro* activation of NK cells and macrophages[8,9]. FcγRIIIb is released upon activation and during apoptosis of neutrophils

by proteolytic activity rather than by a lipase[4,10,11]. The release of FcγRIIIb is inhibited by the serine protease inhibitors and metalloprotease inhibitors, depending on the stimulus used to activate the cells[12].

Rheumatoid arthritis (RA) is a chronic inflammatory disease affecting the joints and extraarticular tissues. Increased protein expression of a series of matrix metalloproteases (MMP), which are involved in the breakdown of extracellular matrix molecules, has been described in patients with RA[13]. Several reports[14-16] have described a reduced expression of FcγRIIIa on NK cells isolated from synovial fluid of affected joints of RA patients. It is possible that the activated metalloproteases cleave FcγRIIIa on NK cells. FcγRIIIa is detected in plasma from RA patients, and in very low amounts in plasma from healthy donors[3]. In addition to NK cells, mononuclear leukocytes, such as macrophages, lymphocytes, and plasma cells, are the most numerous cells in the synovium of the rheumatoid joint. Macrophage FcγRIIIa is distributed in restricted tissue[17], being expressed at high concentrations in synovial intimal tissue and other tissues, such as pericardium. FcγRIIIa is also expressed on a subset of T lymphocytes[1], which are characterized by a CD3+CD8+CD16^{dim} phenotype and are sometimes also CD56 positive. Especially in patients with RA, this subset may be increased.

Although soluble FcγRIII (sFcγRIII) has been detected in saliva, synovial and seminal fluid, serum, and plasma[3,6,10,18-20], none of the assays used discriminates sFcγRIIIa from sFcγRIIIb. Plasma sFcγRIII was shown to be mainly derived from neutrophils and to a lesser extent from NK cells[3,10]. sFcγRIIIa derived from macrophages has not yet been detected in plasma. Since NK cells and macrophages will be activated locally, leading to shedding of FcγRIIIa and subsequent release into the blood, we investigated whether the plasma concentration of sFcγRIIIa would be a good marker for the activity of RA. We measured sFcγRIIIa in plasma of NA(1+,2–) genotyped donors with CD16 GRM1[7], which recognizes NA2-FcγRIIIb and FcγRIIIa. The concentration of sFcγRIIIa, as well as the total level of sFcγRIII (sFcγRIIIb plus sFcγRIIIa), in RA patients was significantly higher than in healthy controls. In general, plasma sFcγRIIIa originates from both NK cells and macrophages. sFcγRIIIa may serve as a good marker for disease activity in RA.

MATERIALS AND METHODS

Patients. Patients were recruited from the Division of Rheumatology at our hospital, and were followed from April 1994 to April 1996. All patients met the American Rheumatism Association (ARA) criteria for RA[21]. Seventy-six patients with NA(1+,2–) phenotype were selected from 178 patients with RA. Four of these 76 patients were excluded for having nephritis and 4 for hepatic diseases, because sFcγRIII are probably catabolized by the liver, as well as excreted from the kidney[10,20]. As pathological controls, 8 patients with NA(1+,2–) phenotype were selected from 40 patients with osteoarthritis (OA). Laboratory findings in these patients are shown in Table 1. A Lansbury index was determined based on the duration of morning stiffness, erythrocyte sedimentation rate (ESR), grip strength, and joint score[22]. Informed consent was obtained from all patients, and the trial was approved by the ethical committee in our hospital.

Table 1. Laboratory findings in the patients and controls.

	Controls	RA Patients	OA Patients
n, F/M	28/12	54/14	7/1
Age, yrs	53.4 ± 13.4	56.1 ± 11.9	58.6 ± 7.4
IgG RF, U/ml	1.92 ± 1.63	12.58 ± 40.23	4.20 ± 4.68
IgM RF, IU/ml	< 10.1	131.6 ± 318.4	< 10.1
IgG, g/l	15.4 ± 2.2	21.5 ± 6.5**††	14.9 ± 2.7
IgA, g/l	3.1 ± 1.1	4.4 ± 1.9**	3.5 ± 1.6
IgM, g/l	1.8 ± 0.7	2.0 ± 1.0	2.0 ± 1.7
CRP, mg/l	< 1.0	34.2 ± 34.4†	7.1 ± 17.4
ESR, mm	—	48.1 ± 33.7	—
Lansbury Index	—	39.4 ± 18.9	—
WBC, /µl	5861 ± 1301	7348 ± 1990**	6857 ± 2525
Neutrophils, /µl	3684 ± 998	5316 ± 1877**	4500 ± 2552
Lymphocytes, /µl	1868 ± 514	1482 ± 468**†	1894 ± 458
NK cells, /µl	253 ± 112	263 ± 152	335 ± 146
Platelets, 10^4/µl	24.1 ± 6.0	31.2 ± 9.9**††	21.7 ± 5.4
Hb, g/l			
F	125 ± 9	110 ± 13**††	126 ± 9
M	143 ± 9	121 ± 19**	155

The data are mean ± SD. Significant differences vs healthy control, * $p < 0.05$, ** $p < 0.01$, or vs OA patients, † $p < 0.05$, †† $p < 0.01$. RF: rheumatoid factor; WBC: white blood cell count; Hb: hemoglobin.

Three hundred forty-two healthy volunteers were randomly recruited from the hospital staff. One hundred individuals were selected for NA(1+,2–) phenotype to contribute the pooled plasma. Forty-one age matched individuals were selected as healthy controls. No control individual had any evidence of renal, hepatic, infectious, or inflammatory disease or diabetes mellitus, and none was taking any medication.

FcγRIIIB-NA(1/2) genotyping assays. Genomic DNA (gDNA) was extracted from leukocytes by standard techniques. Genotyping for the FcγRIIIB-NA1/2 polymorphism was performed as described[23]. In brief, 2 sets of primers specifically annealing to either an NA1-FcγRIIIB or an NA2-FcγRIIIB fragment were used. NA1-FcγRIIIB- and NA2-FcγRIIIB-specific fragments were separately amplified from gDNA in a Perkin Elmer GeneAmp PCR System 9600 (Perkin Elmer, Foster City, CA, USA) in a total volume of 20 µl.

ELISA for total sFcγRIII. The total sFcγRIII (sFcγRIIIa + sFcγRIIIb) concentrations were measured by ELISA as described[20]. Briefly, an ELISA plate with 96 wells (Nunc Immunoplate Maxisorp, Roskilde, Denmark) was coated with CD16 CLBFcRgranI. After unbound sites had been blocked with 2% milk in phosphate buffered saline (PBS), diluted EDTA plasma in high performance ELISA buffer (HPE buffer; CLB, Amsterdam, The Netherlands) was incubated in the wells for 1 h at room temperature. After washing with PBS containing 0.05% (v/v) Tween-20, the plates were incubated with a biotin labeled rabbit anti-FcγRIII antibody. After incubation with horseradish-peroxidase labeled streptavidin, the amount of sFcγRIII was detected with tetramethylbenzidine and H_2O_2. A calibration curve was constructed with pooled plasma from 100 healthy NA(1+,2–) phenotyped donors. The concentration of total sFcγRIII in this pool was set at 100 arbitrary units (AU).

Immuno-polymerase chain reaction (PCR) method for sFcγRIIIa. The sFcγRIIIa concentrations were measured by immuno-PCR according to Furuya, *et al*[24] with some modifications. Briefly, thin-wall 96-well polypropylene plates fit for thermocycling (Bio Medical Equipment, Tokyo, Japan) were coated with CD16 GRM1. After unbound sites had been blocked with 1 g/l salmon sperm DNA, 1% fetal calf serum, 5% milk, and 1% gelatin in PBS, diluted EDTA-plasma in HPE buffer was incubated

in the wells overnight at 4°C. After washing with PBS containing 0.05% (v/v) Tween-20, the plates were incubated with biotin labeled CD16 CLBFcRgranI. After incubation with NeutrAvidin (ImmunoPure grade; Pierce Chemical Co., Rockford, IL, USA) (1 mg/l in HPE buffer), the plates were incubated with the biotinylated DNA (5 nM in HPE buffer containing 1 g/l salmon sperm DNA). Biotinylated DNA was produced from plasmid Bluescript by PCR amplification with biotinylated M13 primer and nonbiotinylated Rev primer, as described[24]. The amount of sFcγRIII was detected by real-time PCR in an ABI Prism® 7700 Sequence Detection System (Applied Biosystems, Foster City, CA, USA). A calibration curve was constructed with the plasma pool, and the concentration of sFcγRIIIa in the pooled plasma was set at 100 AU. CD16 GRM1 was a generous gift from Dr. F. Garrido, Hospital des Nieves, Granada, Spain.

Recovery of sFcγRIIIa. sFcγRIII concentrations are presented as the percentage of sFcγRIII compared with the amount of sFcγRIII in a standard plasma pool. To determine the relative concentrations of FcγRIIIa and FcγRIIIb in plasma, we added purified sFcγRIIIa to pooled plasma, measured sFcγRIIIa and total sFcγRIII, and then calculated how many AU had been added. sFcγRIIIa was prepared from culture supernatant of monocytes by affinity chromatography with Lentil Lectin-Sepharose and CD16 CLBFcRgranI-Sepharose. Monocytes were purified and cultured 4 days as described[25] (see below). Contaminating monoclonal antibody (Mab) was removed by filtration with a 100 kDa cutoff membrane (Omega™ disc; Pall Filtron Co., Northborough, MA, USA), followed by concentration of sFcγRIIIa (OD 280: 0.457). Purified sFcγRIIIa or PBS (1/10 volume) was supplemented to pooled plasma from healthy NA(1+,2–) phenotyped donors and then sFcγRIIIa and total sFcγRIII were measured 10 times each.

Effects of IgG complexes in RA samples. A γ-globulin fraction was prepared from pooled RA patient serum by precipitation with 30% saturated ammonium sulfate. After dialysis against PBS, the fraction was passed through a column of CD16 CLBFcRgranI-bound Sepharose CL-4B beads (Amersham Pharmacia Biotech AB, Uppsala, Sweden) to remove sFcγRIII. The concentrated γ-globulin fraction with or without sFcγRIIIa was added to the pooled plasma or to an RA plasma (R56), and subsequently the amounts of the 2 types of sFcγRIII were measured.

Anti-FcγRIII immunoblot analysis. Patient plasma was diluted 2 times with 1% Nonidet P-40 (Sigma, St. Louis, MO, USA) in 110 mM NaCl and 50 mM Tris (pH 7.5), precleared 3 times with BSA bound to Sepharose CL-4B beads, and incubated at 4°C for 1 h with purified CD16 GRM1 coupled to Protein G-Sepharose 4 Fast-Flow beads (Amersham).

Neutrophils were isolated from blood of healthy NA(1+,2–) phenotyped donors by Percoll density (1.077 g/cm^3) centrifugation and subsequent lysis of erythrocytes with ammonium chloride[25]. Monocytes and large granular lymphocytes (LGL) were isolated from a buffy coat prepared from citrated blood of healthy donors by Percoll density centrifugation and subsequent counterflow centrifugal elutriation of the mononuclear leukocytes, as described[25]. The purified NA1-neutrophils, LGL, or monocytes were cultured at 2×10^7 cells/ml for 6 h, 16 h, or 4 days, respectively, in Iscove's modified Dulbecco's medium supplemented with 10% fetal calf serum. Culture supernatants of NA1-neutrophils, LGL, or monocytes were also precleared 3 times with BSA bound to Sepharose CL-4B beads and incubated at 4°C for 1 h with CD16 CLBFcRgranI or GRM1 coupled to Protein G-Sepharose 4 Fast-Flow beads.

Anti-FcγRIII immunoprecipitates were analyzed by one-dimensional sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) under nonreducing conditions and transferred to nitrocellulose. After blotting with CD16 CLB-LM6.30[7] and incubation with a peroxidase conjugated goat-antimouse IgG antibody (Jackson Immuno-Research, West Grove, PA, USA), the FcγRIIIa and NA1-FcγRIIIb were detected by enhanced chemiluminescence (Boehringer Mannheim GmbH, Germany).

Statistical analysis. Differences in sFcγRIII concentrations or laboratory data among the groups were tested by analysis of variances (ANOVA) with Fisher's PLSD. Correlations were tested by Bartlett's test.

RESULTS

In RA patients, the concentrations of sFcγRIIIa and of total sFcγRIII were significantly higher than in healthy controls (Figure 1). The sFcγRIII concentrations were also measured in OA patients as pathological arthritis controls. Neither sFcγRIIIa nor total sFcγRIII levels in OA patients were different from those in healthy controls, and they were significantly lower than levels in patients with RA.

As shown in Table 2, the sFcγRIIIa levels in plasma correlated with the number of NK cells in peripheral blood, and total sFcγRIII levels correlated with the number of neutrophils in peripheral blood in healthy controls, but not in patients with RA. Both sFcγRIII levels correlated with each other in RA patients, but not in healthy controls. In RA patients, there was a statistically significant correlation between both sFcγRIII levels and the concentrations of IgG, IgA, and IgM. Both sFcγRIII levels correlated with the rise in C-reactive protein (CRP), ESR, and Lansbury Index (Figure 2).

We measured the sFcγRIII concentrations with the CD16 CLBFcRgranI, which recognizes the IgG binding site of FcγRIII. Because sFcγRIII has retained its IgG-binding capacity[11,18], the presence of immunocomplex may influence the sFcγRIII measurement. To detect the effect of IgG complexes in RA plasma samples, we mimicked RA samples by adding to pooled normal plasma sFcγRIIIa prepared from culture supernatant of monocytes and the γ-globulin fraction prepared from RA patient serum. As expected, the levels of sFcγRIII decreased with increasing amounts of supplemented RA γ-globulin fraction. High levels of IgG complexes apparently decreased the sFcγRIII levels by 10–15% in our assay.

Reports have shown that plasma sFcγRIII is mainly derived from neutrophils and to a lesser extent from NK cells[3,10]. To determine the relative levels of FcγRIIIa and FcγRIIIb in plasma, we added purified sFcγRIIIa to pooled plasma, measured sFcγRIIIa and total sFcγRIII, and then calculated how many AU had been added. The supplemented sFcγRIIIa were found to be 95.78 ± 7.38 AU in the sFcγRIIIa assay and 1.93 ± 0.93 AU in the total sFcγRIII assay.

FcγRIIIa is expressed on NK cells and macrophages[1] with a cell type-specific pattern of glycosylation[2,3] that causes differences in the electrophoretic mobility. Using this phenomenon, we analyzed the origin of the sFcγRIIIa in plasma. As shown in Figure 3, the electrophoretic mobility of plasma sFcγRIIIa corresponded to those of sFcγRIIIa from NK cells (lower spots) and from macrophages (upper spots), but not NA1-sFcγRIIIb. NA1-sFcγRIIIb could not be precipitated with CD16 GRM1. The dim spot at about 50 kDa in this line is protein G that was used in the immunoprecipitation step and recognized by conjugated antimouse Ig antibody. In general, plasma sFcγRIIIa originates from both cell types, in healthy controls and also in patients with

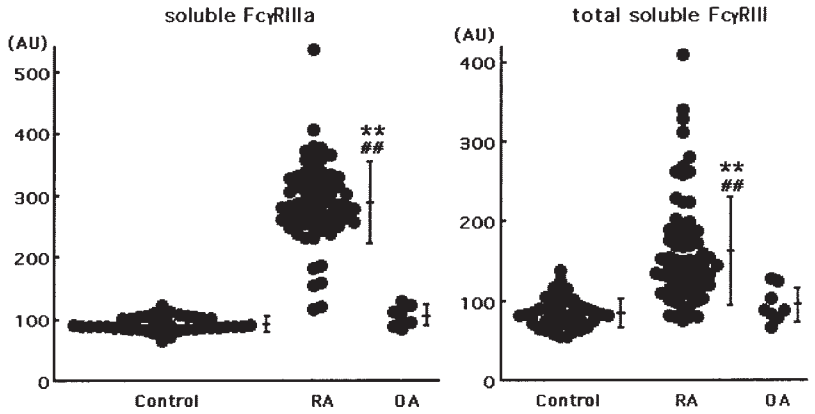

Figure 1. Concentration of sFcγRIIIa and total sFcγRIII in plasma from RA patients. The sFcγRIIIa (left) or total sFcγRIII (right) concentrations were measured by immuno-PCR or ELISA, respectively, and are presented as the percentage of sFcγRIII compared to the amount of sFcγRIII in the pooled plasma. Significant differences vs healthy controls, *p < 0.05, **p < 0.01, or vs OA patients, #p < 0.05, ##p < 0.01.

Table 2. Correlation between sFcγRIII concentrations and laboratory findings in RA patients.

	Correlation Coefficient			
	Control		RA	
	sFcγRIIIa	Total sFcγRIII	sFcγRIIIa	Total sFcγRIII
Age	–0.097	–0.013	–0.008	–0.161
IgG RF	–0.080	0.290	0.097	0.225
IgM RF	—	—	0.127	0.152
IgG	–0.179	0.029	0.326**	0.382**
IgA	0.167	–0.002	0.268*	0.254*
IgM	0.109	0.101	0.253*	0.432**
CRP	—	—	0.419**	0.153
ESR	—	—	0.318**	0.251*
Lansbury Index	—	—	0.447**	0.312**
WBC	0.022	0.062	–0.012	0.060
Neutrophils	–0.195	0.351*	0.035	0.056
Lymphocytes	0.298	–0.026	–0.091	–0.008
NK cells	0.701**	0.189	0.011	–0.017
Platelets	0.093	–0.213	0.230*	0.123
Hb	–0.108	–0.118	0.012	0.043
Total sFcγRIII	–0.189	—	0.447**	—

Significant correlations, * p < 0.05, ** p < 0.01. RF: rheumatoid factor; WBC: white blood cell count; Hb: hemoglobin.

RA. However, in some RA patients only sFcγRIIIa^{NK} but not sFcγRIIIa$^{M\phi}$ was detected. In addition, as shown in lines R56 and R61, the ratio of sFcγRIIIa^{NK} to sFcγRIIIa$^{M\phi}$ varied in the same patient at different periods. These results also indicated that CD16 GRM1 captured sFcγRIIIa but not NA1-sFcγRIIIb in our immuno-PCR assay.

DISCUSSION

FcγRIII (CD16) exists in 2 alternative forms. NK cells and macrophages express FcγRIIIa[1] and neutrophils express FcγRIIIb[4]. Both FcγRIII are released from the cell surface by proteolytic cleavage[4,6,8-12], and these soluble forms are present in plasma[3,10,20]. We measured sFcγRIIIa in plasma of NA(1+,2–) phenotype donors with CD16 GRM1, which recognizes not only NA2-FcγRIIIb but also FcγRIIIa[7]. In RA patients, the concentrations of sFcγRIIIa, as well as the total sFcγRIII level, were significantly higher than in healthy controls (Figure 1). Both sFcγRIII levels were increased in parallel with the concentrations of IgG, IgA, and IgM, and with ESR or Lansbury Index (Table 2, Figure 2).

It was found that the level of total sFcγRIII in plasma correlates with the production of neutrophils in the bone

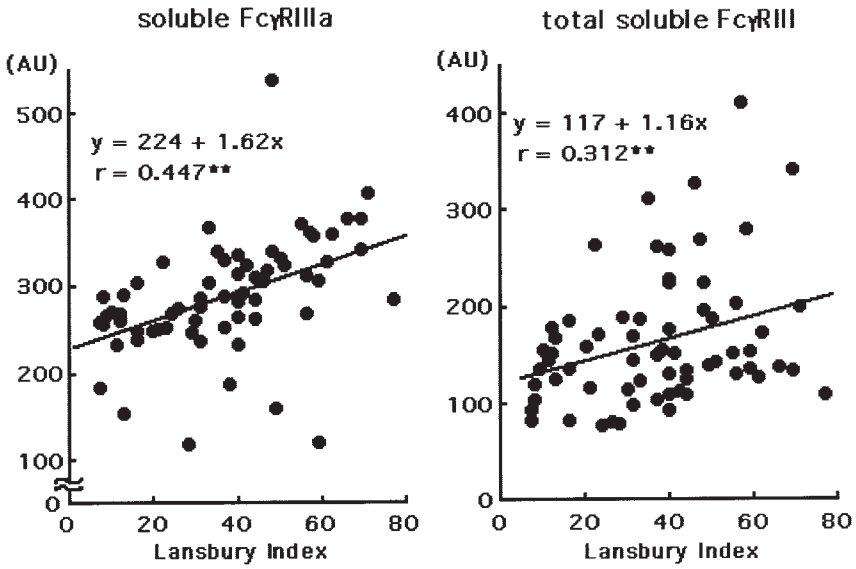

Figure 2. Correlation between sFcγRIII levels and Lansbury index in patients with RA. The sFcγRIIIa (left) or total sFcγRIII (right) concentrations were measured by immuno-PCR or ELISA, respectively, and are presented as the percentage of sFcγRIII compared to the amount of sFcγRIII in pooled plasma. Significant correlations, **$p < 0.01$.

marrow and not with the number of circulating neutrophils[11]. In our study, total sFcγRIII levels correlated with the number of circulating neutrophils in healthy controls, but not in patients with RA (Table 2). These conflicting findings may be due to the variation in total sFcγRIII levels associated with the NA phenotype[20]. The homogeneous population of NA(1+,2–) donors selected for this study had a lower range of sFcγRIII concentrations. The sFcγRIIIa levels in plasma also correlated with the number of circulating NK cells in healthy controls, but not in patients with RA, which supports observations that the sFcγRIIIa is mainly derived from NK cells[3].

FcγRIIIa is mainly expressed on NK cells and macrophages, but also on a subset of T lymphocytes[1]. Release of FcγRIIIa from activated T lymphocytes has not yet been reported, but may well be possible. Presumably, sFcγRIIIa derived from actived T lymphocytes has a similar glycosylation pattern as FcγRIIIa^{NK}. CD16 GRM1 precipitated one protein from culture supernatant of LGL, which resemble activated T lymphocytes (Figure 3).

Studies have shown that plasma sFcγRIII is mainly derived from neutrophils and to a lesser extent from NK cells[3,10]. In our study, sFcγRIII concentrations are given as the percentage of sFcγRIII compared with the amount of sFcγRIII in a standard plasma pool. It is interesting to note the difference between sFcγRIIIa and sFcγRIIIb levels. We calculated the relative levels of FcγRIIIa and FcγRIIIb from the recovery of sFcγRIIIa. sFcγRIIIa was present in amounts about 50 times lower than sFcγRIIIb in pooled plasma from healthy NA(1+,2–) phenotyped donors.

Large amounts of various MMP were detected in joints of patients with RA, and these MMP might play an important role in the pathological processes of RA associated joint destruction. Among all MMP, MMP-3 (stromelysin-1) has been considered to be the critical enzyme for cartilage matrix breakdown in RA, since it can act directly on native type II collagen, a principal collagen type in joint cartilage[26]. Although at levels less than those from patients with RA, high levels of MMP-3 are found in synovial fluid from patients with OA[27]. In contrast, MMP-3 serum levels are significantly increased in patients with active inflammatory rheumatic diseases (i.e., RA), but are normal in noninflammatory rheumatic diseases (OA)[28]. Similarly, both sFcγRIIIa and total sFcγRIII levels were significantly higher in patients with RA but normal in patients with OA.

Because the FcγRIIIa is released from NK cells and/or macrophages, and FcγRIIIb is released from neutrophils on activation[8-12], the sFcγRIII in plasma are a kind of marker for inflammation. It has been shown that the levels of inflammatory markers in serum, such as CRP, tumor necrosis factor-α (TNF-α), and interleukin 6 (IL-6), are increased in patients with atherosclerosis[29]. The sFcγRIIIa level and the total sFcγRIII level were also significantly increased in patients with coronary artery diseases, but not in patients with vasospastic angina or intact coronary artery, compared with age matched healthy donors (unpublished observation). In addition, the sFcγRIIIa level but not total sFcγRIII level was related to the number of significantly affected coronary arteries, and was correlated positively with an atherogenic index (low density lipoprotein to high

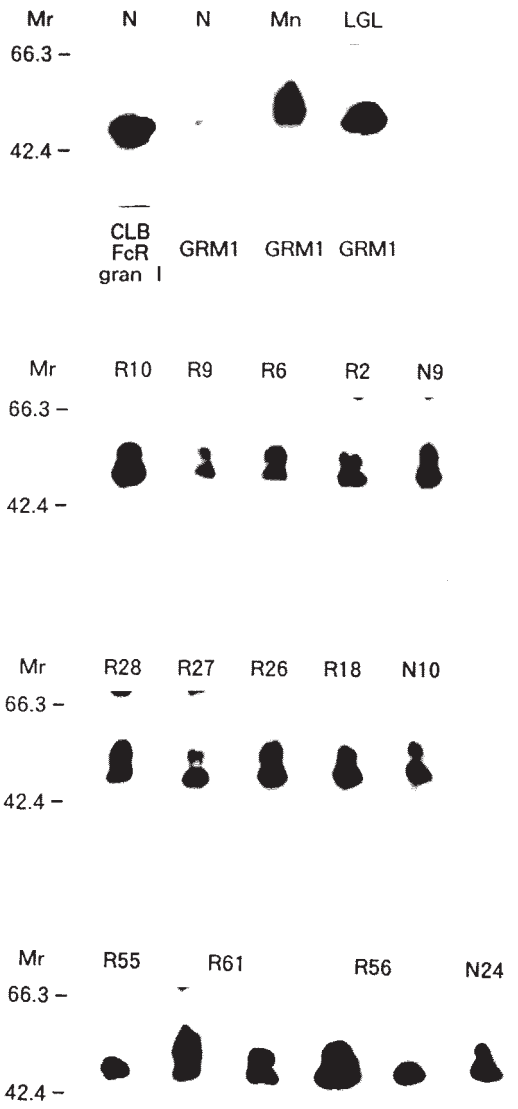

Figure 3. The origin of sFcγRIIIa in plasma. The immunoprecipitates with CD16 GRM1 prepared from plasma from RA patients (R) or healthy donors (N) were analyzed by SDS-PAGE under nonreducing conditions and transferred to nitrocellulose. The immunoprecipitates with CD16 CLBFcRgranI or GRM1 prepared from culture supernatant of NA1-neutrophils (6 hours: N), monocytes (4 days: Mn), or LGL (16 hours: LGL) were also used as controls. The FcγRIIIa and NA1-FcγRIIIb were detected by immunoblotting with CD16 CLB-LM6.30. Mr: relative molecular mass × 10^{-3}.

density lipoprotein (HDL) cholesterol ratio), but negatively with HDL cholesterol.

In patients with RA, sFcγRIIIa levels were correlated with inflammatory markers such as CRP and ESR (Table 2). However, these measures do not strictly convey the same information. CRP is produced by the liver in response to circulating IL-6, TNF-α, or IL-1[30], and is a marker of systemic inflammation. In contrast, sFcγRIIIa is produced from activated NK cells and/or macrophages, probably in the joint, in response to local MMP and also to local IL-6, TNF-α, and IL-1. In addition, NK cell and/or macrophage activation individually is different in each patient (Figure 3), and these differences may be caused by the conditions of each patient. An assay that discriminates sFcγRIIIa^{NK} from sFcγRIIIa$^{M\phi}$ may be superior to one that measures total sFcγRIIIa.

Several reports[14-16] have described reduced expression of FcγRIIIa on NK cells in RA. Macrophage FcγRIIIa is expressed at high levels only in the synovial intimal tissue and other tissues such as pericardium that are involved in RA[17]. To judge whether the increase of sFcγRIIIa in RA plasma indicated NK cell or macrophage activation, or both, we analyzed the precipitated sFcγRIIIa. In general, plasma sFcγRIIIa originates from both NK cells and macrophages (Figure 3). However, the ratio of sFcγRIIIa^{NK} to sFcγRIIIa$^{M\phi}$ varied between RA patients. This indicates that the increased sFcγRIIIa in these patients is caused by NK cell and/or macrophage activation that is individually different in each patient. In RA patients, sFcγRIIIa levels did not correlate with the number of circulating NK cells or lymphocytes (Table 2). It may be that the concentration correlates with the number of activated NK cells and/or macrophages. Indeed, sFcγRIIIa levels do correlate with the Lansbury index (Figure 2) and thus with disease severity.

We conclude sFcγRIIIa may serve as a marker for disease activity in RA.

ACKNOWLEDGMENT

We thank Dr. Dirk Roos for his valuable advice and discussions, Dr. Federico Garrido for his generous gifts of antibody, Marion Kleijer for her technical advice, and Yuichiro Imai, Takashi Yamaguchi and Jun Yamao for assistance with the genotyping assay.

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