

Soluble Fc γ RIIIa^{M ϕ} Levels in Plasma Correlate with Carotid Maximum Intima-Media Thickness (IMT) in Subjects Undergoing an Annual Medical Checkup

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Macrophages play a major role in the development of vascular lesions in atherogenesis. The cells express Fc γ RIIIa (CD16) identical to that in NK cells, but with a cell type-specific glycosylation, and these soluble forms (sFc γ RIIIa) are present in plasma. We measured sFc γ RIIIa^{M ϕ} derived from macrophages in plasma from subjects undergoing an annual medical checkup. The levels of sFc γ RIIIa^{M ϕ} increased with age, and correlated positively with body mass index, blood pressure, LDL cholesterol to HDL cholesterol ratio, triglycerides, hemoglobin A1c, and creatinine, but negatively with HDL-cholesterol levels. The sFc γ RIIIa^{M ϕ} levels were related to the number of risk factors for atherosclerosis: such as aging, current smoking, diabetes, hypertension, hyper-LDL-cholesterolemia, hypo-HDL-cholesterolemia, and family history of atherosclerotic diseases. In addition, the sFc γ RIIIa^{M ϕ} levels were correlated with carotid maximum intima-media thickness (IMT). These findings indicate the macrophages are activated during the incipient stage of atherosclerosis, and suggest sFc γ RIIIa^{M ϕ} may be used as a predictive marker for atherosclerosis.

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INTRODUCTION

Fc γ receptor type III (Fc γ RIII: CD16), one of the low-affinity receptors for the Fc region of IgG, exists in two alternative forms. Fc γ RIIIa is an integral membrane protein expressed on natural killer cells (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). Fc γ RIIIb is a glycosylphosphatidylinositol (GPI)-linked protein expressed exclusively on neutrophils and it can be induced on eosinophils (3). Both Fc γ RIIIa and Fc γ RIIIb are released from the cell surface. Fc γ RIIIa is released by the action of a metalloprotease upon *in vitro* activation of NK cells and macrophages (4,5). Fc γ RIIIb is released upon activation and during

neutrophil apoptosis by proteolytic activity (6–8).

Atherosclerosis pathogenesis is characterized by increased adhesion of monocytes to the injured endothelium, followed by their extravasation into the vessel wall (9). Within the wall, monocytes differentiate into macrophages and then turn into lipid-laden foam cells, which lead to the development of macroscopic fatty streaks (10). Monocytes also are transformed into activated macrophages which secrete cytokines and modify lipoproteins at least in part by oxidation (10). The expression of Fc γ RIIIa in macrophages is indicative of a functional subset of cells able to participate in humoral and cellular immune responses. Fc γ RIIIa is expressed only in a

minor subset of peripheral blood monocytes (11) and has been shown to be present *in vivo* in human atherosclerotic plaques (12). Binding of LDL-immune complexes to Fc receptors on monocyte/macrophages activates responses that promote atherosclerotic processes (13). In addition, several members of the matrix metalloproteinase (MMP) family are produced by macrophages in human atherosclerotic plaques (14).

Recently, we have succeeded in raising a new anti-Fc γ RIII monoclonal antibody (mAb), MKGR14, which specifically recognizes Fc γ RIIIa^{M ϕ} (15). Using this antibody, we measured sFc γ RIIIa^{M ϕ} in plasma and found that the level of sFc γ RIIIa^{M ϕ} were increased significantly in patients with coronary artery diseases (CAD), but not in patients with vasospastic angina (VSA) or intact coronary artery, compared with age-matched healthy donors (16). These findings indicate that the macrophages are activated during the process of atherosclerosis. The next question is whether the macrophages are activated during the

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incipient stage of atherosclerosis, and whether sFcyRIIIa^{Mφ} may serve as a marker for atherosclerosis. In the present study, to determine the activity of macrophages in incipient atherosclerotic status, we measured sFcyRIIIa^{Mφ} in plasma from subjects whose atherosclerosis is not yet serious with an annual medical checkup.

MATERIALS AND METHODS

Subjects

Three hundred and fourteen subjects (35 and 40 to 62 years) received a routine medical checkup for adult diseases. All subjects were staff members at our hospital, received a medical checkup at 35 years of age and one each year after they reached 40 years of age. Each subject gave written informed consent. Ten subjects with hepatic diseases were excluded from this study because sFcyRIIIa may be catabolized in the liver (17). Furthermore, two subjects with renal diseases also were excluded because sFcyRIIIa are excreted from the kidney (6). In support of this, we detected sFcyRIIIa^{Mφ}, as well as total sFcyRIIIa, in urine at about 10% to 20% concentration of plasma levels in healthy donors (unpublished data). Twelve of 302 subjects were excluded for inflammatory disease (CRP ≥ 40 mg/L or leukocyte count ≥ 110 × 10⁹/L), because inflammation activates macrophages as well as neutrophils. Clinical characteristics and laboratory findings in the remaining 290 subjects are shown in Tables 1 and 2. The subjects were divided into four groups (0, I, II, III) which had no, one, two, or three and over risk factors: such as aging, current smoking, diabetes, hypertension, hyper-LDL-cholesterolemia, hypo-HDL-cholesterolemia, and family history of atherosclerotic diseases. Age was set at more than 45 years old for males and for post-menopausal females. Smokers were defined as those currently smoking any tobacco. Diabetes mellitus was defined as those subjects who were under an active treatment with insulin or oral hypoglycemic agents, or who had a hemoglobin A1c of ≥ 6.5%. The subjects

Table 1. Clinical characteristics of subjects with an annual medical checkup.

	subject category group ^a			
	0	I	II	III ^b
<i>n</i> (M/F)	10/60	30/64	42/30	35/19
Age (years) ^{c,d}	43.9 ± 4.4	47.5 ± 6.6**	51.4 ± 5.6***#	54.1 ± 5.2***#§§
Body mass index (kg/(m) ²)	21.6 ± 2.5	22.1 ± 2.6	22.7 ± 2.6*	23.6 ± 2.7***#
Systolic blood pressure (mmHg)	115 ± 11	116 ± 12	126 ± 16***#	145 ± 21***#§§
Diastolic blood pressure (mmHg)	70 ± 9	73 ± 10	79 ± 11***#	89 ± 16***#§§
Hypertension, <i>n</i> (%)	—	5 (5)	19 (26)	32 (59)
Diabetes, <i>n</i> (%)	—	—	—	10 (19)
Hyper-LDL-cholesterolemia, <i>n</i> (%)	—	3 (3)	10 (14)	12 (22)
Hypo-HDL-cholesterolemia, <i>n</i> (%)	—	2 (2)	3 (4)	10 (19)
Smoking, <i>n</i> (%)	—	17 (18)	20 (28)	23 (43)
Familial medical record, <i>n</i> (%)	—	26 (28)	29 (40)	31 (57)

^aThe subjects were divided into four groups (0, I, II, III) which had no, one, two, or three and over risk factors such as aging, current smoking, diabetes, hypertension, hyper-LDL-cholesterolemia, hypo-HDL-cholesterolemia, and family history of atherosclerotic diseases.

^bThe subjects with diabetes were classified into category group III, even if they did not have any other risk factor.

^cData is presented as the mean ± SD or *n* (%), as indicated.

^dSignificant differences compared with group 0 (*: *P* < 0.05, **: *P* < 0.01), compared with group I (‡: *P* < 0.05, #‡: *P* < 0.01), compared with group II (§§: *P* < 0.01).

Table 2. Laboratory findings in subjects with an annual medical checkup.

	subject category group ^a			
	0	I	II	III ^b
Total cholesterol (mmol/L) ^{c,d}	5.24 ± 0.69	5.26 ± 0.69	5.44 ± 0.83	5.60 ± 0.92***#
LDL cholesterol (mmol/L)	2.96 ± 0.56	3.01 ± 0.71	3.14 ± 0.80	3.40 ± 0.83***#§§
HDL cholesterol (mmol/L)	1.82 ± 0.37	1.74 ± 0.38	1.62 ± 0.46**	1.41 ± 0.39***#§§§
LDL/HDL ratio ^e	1.72 ± 0.56	1.85 ± 0.66	2.09 ± 0.77***#	2.59 ± 0.94***#§§§
Triglycerides (mmol/L)	1.02 ± 0.69	1.17 ± 0.65	1.58 ± 1.28***#	1.84 ± 1.14***#
Glucose (mmol/L)	5.31 ± 0.49	5.23 ± 0.46	5.53 ± 1.08	6.29 ± 3.21***#§§§
Hemoglobin A1c (%)	4.66 ± 0.26	4.74 ± 0.32	4.85 ± 0.36*	5.30 ± 1.02***#§§§
Leukocytes (10 ⁹ /L)	57.2 ± 12.9	57.0 ± 13.1	58.3 ± 12.8	60.7 ± 14.8
hs-CRP ^f (mg/L)	0.52 ± 0.50	0.56 ± 0.57	0.72 ± 0.68*	0.76 ± 0.63*
< 0.2 mg/L, <i>n</i> (%)	14 (20)	21 (22)	5 (7)	5 (9)
Creatinine (μmol/L)	52 ± 8	55 ± 13*	60 ± 12***#	62 ± 12***#
ALT (IU/L) ^g	17.1 ± 7.2	22.2 ± 10.8**	25.0 ± 11.1**	25.1 ± 11.2**
AST (IU/L) ^h	20.4 ± 4.2	23.0 ± 5.5**	25.0 ± 6.4***#	24.1 ± 7.0**

^aThe subjects were divided into four groups (0, I, II, III) which had no, one, two, or three and over risk factors such as aging, current smoking, diabetes, hypertension, hyper-LDL-cholesterolemia, hypo-HDL-cholesterolemia, and family history of atherosclerotic diseases.

^bThe subjects with diabetes were classified into category group III, even if they did not have any other risk factor.

^cData is presented as the mean ± SD or *n* (%), as indicated.

^dSignificant differences compared with Group 0 (*: *P* < 0.05, **: *P* < 0.01), compared with group I (‡: *P* < 0.05, #‡: *P* < 0.01), compared with group II (§: *P* < 0.05, §§: *P* < 0.01).

^eLDL/HDL ratio indicates LDL cholesterol to HDL cholesterol ratio.

^fhs-CRP, high-sensitivity C-reactive protein.

^gALT, alanine aminotransferase.

^hAST, aspartate aminotransferase.

with diabetes were classified into category group III, even if they did not have any other risk factor. Arterial hypertension was defined in the presence of active treatment with antihypertensive agents or otherwise as a systolic blood pressure of ≥ 140 mmHg and/or diastolic blood pressure of ≥ 90 mmHg on at least two separate occasions. Hyper-LDL-cholesterolemia was defined as a documented LDL-cholesterol value ≥ 4.14 mmol/L (≥ 160 mg/dl). Hypo-HDL-cholesterolemia was defined as a documented HDL-cholesterol value < 1.03 mmol/L (< 40 mg/dl). Familial medical record of myocardial infarction, angina, or cerebral infarction was ranked. The trial was approved by the ethical committee at Kansai Medical University, Osaka, Japan.

Carotid ultrasonography was performed using an ultrasound imaging system (SONOS 5500; Philips, The Netherlands). The scanning and reading were performed by two well-trained physicians (K Amano and M Fukui). The physicians adopted the same ultrasonography protocol with the same system in the same lab. The imaging protocol involved obtaining a single longitudinal lateral view of the distal 10 mm of the right and left common carotid arteries. Maximum intima-media thickness (IMT) was defined as the single thickest wall among near and far wall on both sides of the common carotid arteries, carotid sinus, and internal carotid artery. "Thickening" of the wall was defined as a maximum IMT ≥ 1.1 mm in our hospital, according to the literature (18–20). Prior to the survey, the physicians discussed in detail, and verified, all the procedures to be used.

ELISA for Total sFcγRIII and sFcγRIIIa^{MΦ}

The total sFcγRIII concentrations were measured by enzyme-linked immunosorbent assay (ELISA) as described before (21). Briefly, an ELISA plate (Nunc Immunoplate Maxisorp, Roskilde, Denmark) was coated with an anti-FcγRIII mAb, CLBFCrgranI, which recognizes all types of FcγRIII and was used as a

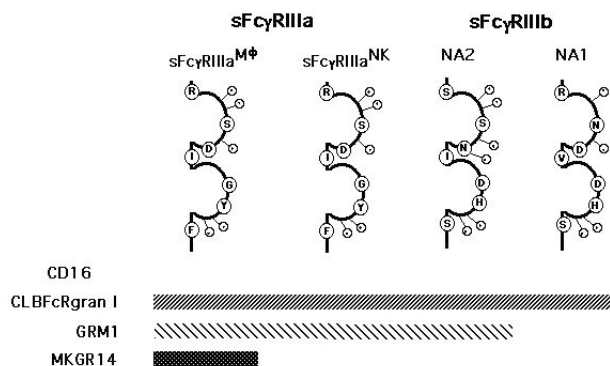


Figure 1. Schematic representation of sFcγRIII and binding specificity of anti-FcγRIII mAb. ⊙— represents potential N-linked glycosylation site.

capture antibody (Figure 1). After unbound sites had been blocked with 2% milk in phosphate-buffered saline (PBS), diluted ethylene diamine tetraacetic acid (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 0.02% (v/v) Tween-20, the plates were

incubated with a biotin-labeled rabbit anti-FcγRIII antibody, which recognizes all types of FcγRIII and was used as detection antibody. After incubating with horseradish-peroxidase-labeled streptavidin, the amount of sFcγRIII was detected with tetramethylbenzidine and H₂O₂.

The sFcγRIIIa^{MΦ} concentrations were measured with a sensitive chemiluminescent

Table 3. Simple correlation between sFcγRIII levels and laboratory findings in subjects with annual medical checkup.

	Correlation coefficient	
	sFcγRIIIa ^{MΦ}	total sFcγRIII
Age	0.137** ^a	0.082
Body mass index	0.181** ^a	0.063
Systolic blood pressure	0.270**	-0.017
Diastolic blood pressure	0.249**	-0.035
Total cholesterol	0.003	0.002
LDL cholesterol	0.048	0.083
HDL cholesterol	-0.216**	-0.028
LDL/HDL ratio ^b	0.200**	0.046
Triglycerides	0.120*	-0.130*
Glucose	0.024	-0.015
Hemoglobin A1c	0.237**	-0.027
Leukocytes	0.042	0.116*
hs-CRP ^c	0.081	0.062
Creatinine	0.191**	-0.038
ALT	0.110	-0.087
AST	0.067	-0.055
Total sFcγRIII	-0.048	—

^aSignificant correlations (*: *P* < 0.05, **: *P* < 0.01).

^bLDL/HDL ratio indicates LDL cholesterol to HDL cholesterol ratio.

^chs-CRP, high-sensitivity C-reactive protein. Correlation between sFcγRIII levels and hs-CRP were calculated in 245 subjects, and the other 45 subjects showed less than 0.2 mg/L, limit of measure of hs-CRP.

ELISA with an anti-Fc γ RIIIa^{M ϕ} mAb MKGR14, which specifically recognizes Fc γ RIIIa^{M ϕ} (15). MKGR14 bound to cultured monocytes, a subpopulation of monocytes, and peritoneal monocytes/macrophages, but not to NA1NA2-neutrophils or NK cells. Immunoblotting assay showed that MKGR14 precipitated Fc γ RIIIa^{M ϕ} from the lysate of cultured monocytes, but did not precipitate Fc γ RIIIb or Fc γ RIIIa^{NK} from the lysate of NA1NA2 neutrophils or large granular lymphocytes, respectively. In addition, MKGR14 precipitated only 50–60 kDa protein from the lysate of biotin-labeled cultured monocytes. All these results show that MKGR14 specifically recognizes Fc γ RIIIa^{M ϕ} .

A white ELISA plate (LumiNunc Plates, Roskilde, Denmark) was coated with MKGR14, which was used as capture antibody (see Figure 1). After unbound sites had been blocked with 0.2% highly purified casein in PBS, diluted EDTA plasma in HPE buffer was incubated in the wells overnight at 4° C. After washing, the plates were incubated with a biotin-labeled anti-Fc γ RIII mAb GRM1, which recognizes NA2-Fc γ RIIIb and Fc γ RIIIa and was used as a detection antibody (see Figure 1). After incubating with alkaline phosphatase-labeled streptavidin, the amount of sFc γ RIII was detected with CDP-Star Substrate and Sapphire-II (Applied Biosystems, Bedford, MA, USA) in a MicroLumat Plus microplate luminometer (Berthold Technologies, Bad Wildbad, Germany).

A calibration curve was constructed with pooled plasma from healthy donors and the concentration of sFc γ RIII in this pool was set at 100 arbitrary units (AU), as described before (15,16,21). So the sFc γ RIIIa^{M ϕ} or total sFc γ RIII concentrations are presented as the percentage of sFc γ RIII compared with the amount of sFc γ RIII in the pooled plasma.

Statistical Analysis

Differences in sFc γ RIII levels or laboratory data among the groups were tested by analysis of variance (ANOVA) with Fisher's protected least resistance significant

difference (PLSD) post hoc test. Correlations of sFc γ RIII levels with laboratory data were tested by Fisher's z-transform test and multiple regression of carotid maximum IMT was tested by ANOVA.

RESULTS

Using Fc γ RIIIa^{M ϕ} -specific anti-Fc γ RIII mAb (15), we measured sFc γ RIIIa^{M ϕ} derived from macrophages in plasma. The level of sFc γ RIIIa^{M ϕ} increased with age, and correlated positively with body mass index, blood pressure, LDL cholesterol to HDL cholesterol ratio, triglycerides, hemoglobin A1c, and creatinine-but negatively with HDL-cholesterol levels (Table 3). In contrast, the total sFc γ RIII levels correlated positively with leukocyte counts but negatively with triglycerides. No correlation between the levels of two sFc γ RIIIs was observed in subjects with an annual medical checkup.

As shown in Figure 2, the sFc γ RIIIa^{M ϕ} levels were related to a number of risk factors for atherosclerosis including aging, current smoking, diabetes, hypertension, hyper-LDL-cholesterolemia, hypo-HDL-cholesterolemia, and familial history of myocardial infarction, angina, or cerebral infarction. It was not clear which factor was most effective in increasing levels. Subjects were selected who had no risk factor for atherosclerosis except targeted risk factor or aging. Each control group was matched for age. As shown in Table 4, aging, smoking, diabetes, hypertension, or hyper-LDL-cholesterolemia itself affected on plasma sFc γ RIIIa^{M ϕ} levels, but family history of atherosclerotic diseases did not influence the plasma levels. In contrast, the total sFc γ RIII levels did not change with the number of risk factors. Effects of gender on sFc γ RIII levels also were analyzed and higher sFc γ RIIIa^{M ϕ} and lower total sFc γ RIII levels were shown in males than in females (Table 4).

It is known that IMT is an early marker for atherosclerosis (22), and maximum IMT generally was related to the number of risk factors for atherosclerosis. Fifty-four subjects (M/F = 22/32,

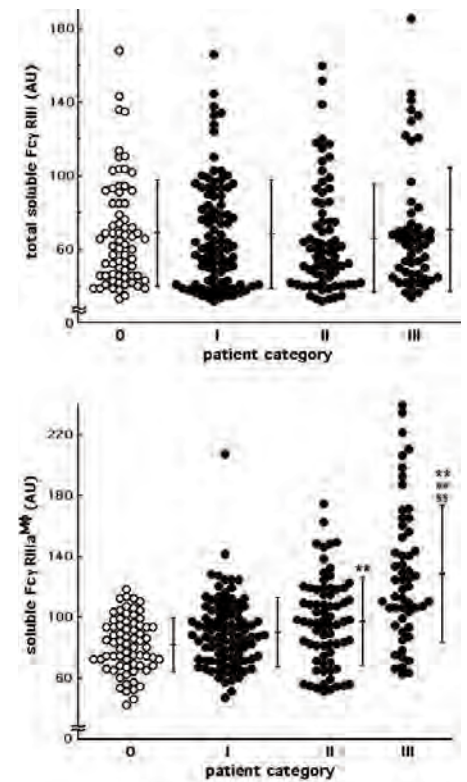


Figure 2. Concentration of sFc γ RIIIa^{M ϕ} (upper) and total sFc γ RIII (lower) in plasma from subjects with an annual medical checkup. The subjects were divided into four groups (0, I, II, III) which had no, one, two, or three and over risk factors including aging, current smoking, diabetes, hypertension, hyper-LDL-cholesterolemia, hypo-HDL-cholesterolemia, and family history of atherosclerotic diseases. Each horizontal bar with a vertical line is the mean \pm SD. Significant differences compared with group 0 (**: $P < 0.01$), compared with group I (#: $P < 0.01$), and compared with group II (§§: $P < 0.01$).

51.3 \pm 5.8 years) were recruited randomly and examined using an ultrasound wall tracking system for IMT measurements. As shown in Figure 3, the sFc γ RIIIa^{M ϕ} levels were well correlated with carotid maximum IMT ($n = 54$, $r = 0.702$, $P < 0.01$). The sFc γ RIIIa^{M ϕ} levels were compared with the other markers on contribution of maximum IMT by multiple regression method. As shown in Table 5, the sFc γ RIIIa^{M ϕ} levels were correlated more with maximum IMT than hs-CRP or the other markers.

Table 4. Effects of aging, smoking, diabetes, hypertension, lipidemia, or familial medical record on concentration of sFcγRIIIa^{MΦ} in plasma from subjects with an annual medical checkup.^a

	<i>n</i>	age	sFcγRIIIa ^{MΦ}	total sFcγRIII	sFcγRIIIa ^{MΦ}	total sFcγRIII	TG ⁿ	sFcγRIIIa ^{MΦ}	total sFcγRIII
Control ^{b,c}	70	43.9 ± 4.4 ^d	79.8 ± 17.9	67.3 ± 28.9					
Aged	37	52.9 ± 5.2**	88.5 ± 17.7*	72.5 ± 29.9					
Male	25	46.9 ± 6.9	89.6 ± 16.6	58.5 ± 25.9					
Female	82	47.0 ± 6.2	80.7 ± 18.4*	72.3 ± 29.5*					
Control	98	47.5 ± 6.5	82.1 ± 18.7	69.3 ± 30.3					
Former	13	45.3 ± 5.8	78.8 ± 20.2	64.5 ± 17.9					
Smoking	33	45.3 ± 5.5	103.9 ± 29.3*** ^{†††}	57.8 ± 25.0					
Control	51	52.7 ± 4.5	5.13 ± 0.39	4.82 ± 0.28	82.1 ± 20.3	71.5 ± 33.3			
DM ^f	10	55.4 ± 4.2	8.95 ± 5.88**	7.33 ± 1.26**	133.6 ± 47.9**	68.8 ± 41.8			
Control	92	47.9 ± 5.6	111.5 ± 9.4	68.8 ± 7.4	79.5 ± 17.8	66.6 ± 27.7			
Border	11	50.7 ± 6.2	132.4 ± 3.2**	82.5 ± 6.9**	92.1 ± 22.0*	87.6 ± 39.7			
HT ⁱ	20	49.7 ± 6.1	139.0 ± 16.0**	88.9 ± 8.1** [†]	95.2 ± 26.1**	66.5 ± 39.9			
Control	88	47.6 ± 6.2	5.21 ± 0.63	2.98 ± 0.54	1.83 ± 0.32	1.68 ± 0.43	0.90 ± 0.34	80.3 ± 17.5	68.5 ± 28.8
Low HDL ^o	3	44.7 ± 3.1	4.47 ± 0.74*	2.75 ± 0.73	0.92 ± 0.08**	2.97 ± 0.67**	1.89 ± 0.56**	86.3 ± 18.0	52.0 ± 7.5
High LDL ^p	12	50.7 ± 6.7	6.71 ± 0.52** ^{§§}	4.44 ± 0.24** ^{§§}	1.66 ± 0.40 ^{§§}	2.81 ± 0.66**	1.35 ± 0.59** [§]	98.8 ± 23.8**	68.0 ± 19.9
High L/H ^q	6	47.8 ± 5.3	5.82 ± 0.44** ^{§§††}	3.73 ± 0.21** ^{§§††}	1.16 ± 0.06**	3.20 ± 0.08**	2.03 ± 0.82** ^{††}	83.0 ± 33.4	90.8 ± 42.9
High TG ^r	13	47.2 ± 7.3	5.51 ± 0.75 ^{§††}	3.01 ± 0.50 ^{††††}	1.45 ± 0.20** ^{§§††}	2.09 ± 0.38** ^{§§††††}	2.49 ± 0.50** ^{§†††}	81.0 ± 15.9 [†]	64.3 ± 25.3
Control	104	48.1 ± 5.8	81.0 ± 18.5	68.6 ± 29.7					
Familial medical record	48	49.6 ± 6.4	82.5 ± 24.0	68.1 ± 30.9					

^aSubjects for annual medical checkup have no risk factor for atherosclerosis except indicated risk factor or aging.

^bSignificant differences compared with controls or male (*: *P* < 0.05, **: *P* < 0.01), compared with former smokers (^{††}: *P* < 0.01) or compared with border hypertension ([†]: *P* < 0.05), or compared with low HDL ([§]: *P* < 0.05, ^{§§}: *P* < 0.01), or compared with high LDL ([†]: *P* < 0.05, ^{††}: *P* < 0.01), or compared with high L/H ([†]: *P* < 0.05, ^{††}: *P* < 0.01).

^cControl indicates age-matched healthy controls.

^dData is presented as the mean ± SD.

^eHbA1c, hemoglobin A1c.

^fDM, diabetes.

^gsBP, systolic blood pressure.

^hdBp; diastolic blood pressure.

ⁱHT, hypertension.

^jT-Chol, total cholesterol.

^kLDL-C, LDL cholesterol.

^lHDL-C, HDL cholesterol.

^mL/H, LDL cholesterol to HDL cholesterol ratio.

ⁿTG, triglycerides.

^olow HDL, hypo-HDL-cholesterolemia.

^phigh LDL, hyper-LDL-cholesterolemia.

^qhigh L/H, high LDL cholesterol to HDL cholesterol ratio.

^rhigh TG, hyper-triglyceridemia.

In Figure 3, the data of four subjects appear to be outliers driving the apparent high correlation in the linear regression analysis. Therefore, the analysis of this data was set when the

four subjects who appear to be outliers were excluded. The sFcγRIIIa^{MΦ} levels still were correlated with carotid maximum IMT (*n* = 50, *r* = 0.327, *P* = 0.02).

DISCUSSION

Macrophages play a major role in the development of vascular lesions in atherogenesis. Recently, we measured sFcγRIIIa^{MΦ} in plasma and found that the

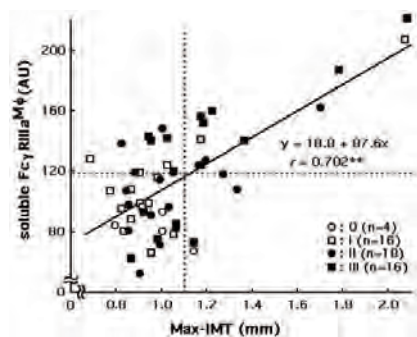


Figure 3. Correlation between sFcγRIIIa^{Mφ} levels and carotid maximum intima-media thickness (IMT) in subjects with an annual medical checkup. The subjects were divided into four groups (0, I, II, III) which had no, one, two, or three and over risk factors including aging, current smoking, diabetes, hypertension, hyper-LDL-cholesterolemia, hypo-HDL-cholesterolemia, and family history of atherosclerotic diseases. Dotted lines indicate reference value. Significant correlations (**: $P < 0.01$).

levels of sFcγRIIIa^{Mφ} were increased significantly and related to the number of significantly affected coronary arteries in patients with coronary artery diseases (CAD) (16). In addition, the levels of sFcγRIIIa^{Mφ} also increased with age in healthy donors ($n = 102$, 19–74 years, 41.8 ± 13.9 years). It is well known that atherosclerosis progresses gradually with age. To determine the activity of macrophages in incipient atherosclerotic status in the present study, we measured sFcγRIIIa^{Mφ} in plasma from subjects with an annual medical checkup. The levels of sFcγRIIIa^{Mφ} increased with age, and correlated positively with body mass index, blood pressure, LDL cholesterol to HDL cholesterol ratio, triglycerides, hemoglobin A1c, and creatinine, but negatively with HDL-cholesterol levels (Table 3). In addition, the levels of sFcγRIIIa^{Mφ} were related to the number of risk factors for atherosclerosis (Figure 2). sFcγRIIIa^{Mφ} is released by the action of a metalloprotease upon *in vitro* activation of macrophages (5). These findings indicate that the macrophage activation begins during the incipient stage of atherosclerotic process.

Table 5. Multiple regression of carotid maximum IMT with sFcγRIII^{Mφ} level and laboratory findings in subjects with annual health checkup.

	Standard regression coefficient	P
Age	0.145	0.1818
Body mass index	0.087	0.4382
Diastolic blood pressure	0.149	0.1827
HDL cholesterol	0.139	0.2375
Hemoglobin A1c	-0.092	0.4135
hs-CRP ^a	0.257	0.0198 ^b
sFcγRIII ^{Mφ}	0.716	< 0.0001

^ahs-CRP indicates high-sensitivity C-reactive protein.

^bSix subjects who showed less than the 0.2 mg/L limit of measure of hs-CRP, applied with 0.19 mg/L.

One of the characteristic features of atherosclerotic lesions is the infiltration of monocytes/macrophages and T lymphocytes into the subendothelial space (9), which shows immunologically competent cells playing a key role in chronic inflammation. It has been shown that the levels of inflammatory markers in serum, such as C-reactive protein, TNF α , and IL-6, are increased in patients with atherosclerosis (23). Because the FcγRIIIa is released from NK cells and macrophages, and FcγRIIIb is released from neutrophils on activation (4–8), the sFcγRIIIs in plasma also are a kind of marker for inflammation. In this study, the levels of sFcγRIIIa^{Mφ}, but not total sFcγRIII levels, increased with the number of risk factors for atherosclerosis (see Figure 2), and no correlation between the levels of two sFcγRIIIs (Table 3) was observed in subjects with an annual medical checkup.

Increased IMT is considered as an earlier morphological evidence of atherosclerosis before the formation of plaque and disturbance in blood flow (22). Fifty-four subjects were recruited randomly and examined with ultrasonography for IMT measurements. The sFcγRIIIa^{Mφ} levels were well correlated with carotid maximum IMT (Figure 3 and Table 5). Generally, maximum IMT was related to

the number of risk factors for atherosclerosis. In this study, a positive correlation also was shown between maximum IMT and the number of risk factors ($n = 54$, $r = 0.315$, $P = 0.02$). However, some subjects were excluded from this correlation, for example, one subject from Group I showed an extremely thick IMT, at 2.06 mm. In this case, the sFcγRIIIa^{Mφ} level also was high with a value of 205 AU.

In summary, the sFcγRIIIa^{Mφ} levels were related to the number of risk factors for atherosclerosis including aging, current smoking, diabetes, hypertension, hyper-LDL-cholesterolemia, and hypo-HDL-cholesterolemia. In addition, the sFcγRIIIa^{Mφ} levels were correlated with carotid maximum IMT. These findings may show that macrophages are activated during the incipient stage of atherosclerosis, and that sFcγRIIIa^{Mφ} may serve as a predictive marker for atherosclerosis.

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REFERENCES

- Ravetch JV, Perussia BV. (1989) Alternative membrane forms of Fc gamma RIII (CD16) on human natural killer cells and neutrophils. Cell type-specific expression of two genes that differ in single nucleotide substitutions. *J. Exp. Med.* 170:481–97.
- de Haas M, Kleijer M, Minchinton RM, Roos D, von dem Borne AE. (1994) Soluble Fc gamma RIIIa is present in plasma and is derived from natural killer cells. *J. Immunol.* 152:900–7.
- Huizinga TW, Kleijer M, Tetteroo PA, Roos D, von dem Borne AE. (1990) Biallelic neutrophil NA-antigen system is associated with a polymorphism on the phospho-inositol-linked Fcγ receptor III (CD16). *Blood.* 75:213–7.
- Harrison D, Phillips JH, Lanier LL. (1991) Involvement of a metalloprotease in spontaneous and phorbol ester-induced release of natural killer cell-associated Fc gamma RIII (CD16-II). *J. Immunol.* 147:3459–65.

5. Levy PC *et al.* (1991) Characterization of human alveolar macrophage Fc gamma receptor III: A transmembrane glycoprotein that is shed under *in vitro* culture conditions. *Am. J. Respir. Cell Mol. Biol.* 5:307–14.
6. Huizinga TW *et al.* (1994) The plasma concentration of soluble Fc-gamma RIII is related to production of neutrophils. *Br. J. Haematol.* 87:459–63.
7. Homburg CHE *et al.* (1995) Human neutrophils lose their surface Fc gamma RIII and acquire Annexin V binding sites during apoptosis *in vitro*. *Blood.* 85:532–40.
8. Middelhoven PJ, van Buul JD, Hordijk PL, Roos D. (2001) Different proteolytic mechanisms involved in Fc gamma RIIIb shedding from human neutrophils. *Clin. Exp. Immunol.* 125:169–75.
9. Ross R. (1995) Cell biology of atherosclerosis. *Annu. Rev. Physiol.* 57:791–804.
10. van der Wal AC, Das PK, Tigges AJ, Becker AE. (1992) Macrophage differentiation in atherosclerosis. An *in situ* immunohistochemical analysis in humans. *Am. J. Pathol.* 141:161–8.
11. Rothe G *et al.* (1996) Peripheral blood mononuclear phagocyte subpopulations as cellular markers in hypercholesterolemia. *Arterioscler. Thromb. Vasc. Biol.* 16:1437–47.
12. Ratcliffe NR, Kennedy SM, Morganelli PM. (2001) Immunocytochemical detection of Fc gamma receptors in human atherosclerotic lesions. *Immunol. Letters.* 77:169–74.
13. Kiener PA *et al.* (1995) Immune complexes of LDL induce atherogenic responses in human monocytic cells. *Arterioscler. Thromb Vasc. Biol.* 15:990–9.
14. Dollery CM, McEwan JR, Henney AM. (1995) Matrix metalloproteinases and cardiovascular disease. *Circ. Res.* 77:863–8.
15. Masuda M *et al.* (2003) Measurement of soluble Fc gamma receptor type IIIa derived from macrophages in plasma: increase in patients with rheumatoid arthritis. *Clin. Exp. Immunol.* 132:477–84.
16. Masuda M *et al.* (2006) Increased soluble Fc gamma RIIIa (Mphi) in plasma from patients with coronary artery diseases. *Atherosclerosis.* 188: 377–83.
17. Koene HR, de Haas M, Kleijer M, Roos D, von dem Borne AE. (1996) NA-phenotype-dependent differences in neutrophil Fc gamma RIIIb expression cause differences in plasma levels of soluble Fc gamma RIII. *Br. J. Haematol.* 93:235–41.
18. Handa N *et al.* (1990) Ultrasonic evaluation of early carotid atherosclerosis. *Stroke.* 21:1567–72.
19. O'Leary DH *et al.* (1992) Distribution and correlates of sonographically detected carotid artery disease in the Cardiovascular Health Study. The CHS Collaborative Research Group. *Stroke.* 23: 1752–60.
20. Li R *et al.* (1994) B-mode-detected carotid artery plaque in a general population. Atherosclerosis Risk in Communities (ARIC) Study Investigators. *Stroke.* 25:2377–83.
21. Masuda M *et al.* (2003) Increase of soluble FcγRIIIa derived from natural killer cells and macrophages in plasma from patients with rheumatoid arthritis. *J. Rheumatol.* 30:1191–7.
22. Cheng KS, Mikhailidis DP, Hamilton G, Seifalian AM. (2002) A review of the carotid and femoral intima-media thickness as an indicator of the presence of peripheral vascular disease and cardiovascular risk factors. *Cardiovasc. Res.* 54: 528–38.
23. Plutzky J. (2001) Inflammatory pathways in atherosclerosis and acute coronary syndromes. *Am. J. Cardiol.* 88:10K–15K.