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# $Fc\gamma RIIIa-158V/F$ Polymorphism Influences the Binding of IgG by Natural Killer Cell $Fc\gamma RIIIa$ , Independently of the $Fc\gamma RIIIa-48L/R/H$ Phenotype

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We analyzed a genetic polymorphism of Fc $\gamma$  receptor Illa (CD16) that is present on position 158 (Phe or Val) in the membrane-proximal, IgG-binding domain. With a polymerase chain reaction-based allele-specific restriction analysis assay we genotyped 87 donors and found gene frequencies of 0.57 and 0.43 for *Fc* $\gamma$ *RIIIA-158F* and – *158V*, respectively. A clear linkage was observed between the *Fc* $\gamma$ *RIIIA-158F* and – *48L* genotypes on the one hand and the *Fc* $\gamma$ *RIIIA-158V* and – *48H* or –*48R* genotypes on the other hand ( $\chi^2$  test; *P* < .001). To determine the functional consequences of this Fc $\gamma$ RIIIa-158V/F polymorphism, we performed IgG binding experiments with natural killer (NK) cells from genotyped donors. All donors were also typed for the recently described triallelic Fc $\gamma$ RIIIa-48L/R/H polymorphism. NK cells were treated with lactic acid to remove cell-associated IgG. Fc $\gamma$ RIIIa<sup>NK</sup>.

**S** EVERAL IgG Fc receptor (Fc $\gamma$ R) polymorphisms that influence the binding of IgG have been described.<sup>1</sup> On neutrophils, the Fc $\gamma$ RIIIb-NA1 and -NA2 isoforms, which differ by four amino acids in the membrane-distal Ig-like loop of the receptor, interact differently with IgG-opsonized particles,<sup>2-4</sup> and influence the interaction of the receptor with Fc $\gamma$ RIIa.<sup>5</sup> On Fc $\gamma$ RIIa, the extensively investigated high responder/low responder polymorphism on amino-acid position 131 in the membrane-proximal, IgG-binding domain is critical for the interaction with human IgG2.<sup>6</sup> Moreover, the Fc $\gamma$ RIIa-131R/H polymorphism was found to be associated with several diseases, such as bacterial infections in children, heparin-induced thrombocytopenia, juvenile periodontitis, and systemic lupus erythematosus.<sup>7-12</sup>

Previously, Ravetch and Perussia described a polymorphism in the membrane-proximal domain of  $Fc\gamma RIIIa$ .<sup>13</sup> A nucleotide substitution at position 559 of  $Fc\gamma RIIIa$  predicts either a valine or a phenylalanine at amino-acid position 158 of  $Fc\gamma RIIIa$ . Because the IgG binding site is most probably located in this part of  $Fc\gamma RIIIa$ ,<sup>14,15</sup> we determined the gene frequency and functional consequences of this polymorphism in the context of the recently described  $Fc\gamma RIIIa$ -48L/R/H polymorphism. As expected, a clear although incomplete linkage was observed between the two polymorphisms. Moreover, we found that the previously described differences in binding between the  $Fc\gamma RIIIa$ -48 isoforms of IgG and of some of the CD16 monoclonal antibodies (MoAbs) are attributable to the  $Fc\gamma RIIIa$ -158 phenotype.

## MATERIALS AND METHODS

*MoAbs.* Anti-pan  $Fc\gamma RIII$  (CD16) MoAbs used were CLBFcRgran1 (mIgG2a) and MEM154 (mIgG1). B73.1 (mIgG1) reacts with NA1- $Fc\gamma RIIIb$  and with  $Fc\gamma RIIIa$ ,<sup>16</sup> and was kindly provided by Dr B. Perussia (Thomas Jefferson University, Philadelphia, PA). MEM154 was obtained through the 5th Leukocyte Typing Workshop. Phycoerythrin (PE)-labeled Leu19 (CD56; mIgG1) was purchased from Becton Dickinson (San Jose, CA). Fluorescein isothiocyanate (FITC)-conjugated goat–antimouse Ig and irrelevant control MoAbs of the IgG1 and IgG2a subclasses were from the Central Laboratory of the Netherlands Red Cross Blood Transfusion Service (CLB), Amsterdam, The Netherlands.

Isolation of cells. Fresh, EDTA-anticoagulated blood from

158F bound significantly less IgG1, IgG3, and IgG4 than did FcγRIIIa<sup>NK</sup>-158V, irrespective of the FcγRIIIa-48 phenotype. Moreover, freshly isolated NK cells from FcγRIIIa-158VV individuals carried significantly more cytophilic IgG than did NK cells from FcγRIIIa-158FF individuals. In addition, CD16 monoclonal antibody (MoAb) MEM154 bound more strongly to FcγRIIIa-158V, compared with -158F, again independently of the FcγRIIIa-48 phenotype. The binding of MoAb B73.1 was not influenced by the FcγRIIIa-158V/F polymorphism, but proved to depend solely on the amino acid present at position 48 of FcγRIIIa. In conclusion, the previously reported differences in IgG binding among the three FcγRIIIa-48L/R/H isoforms are a consequence of the linked, biallelic FcγRIIIa-158V/F polymorphism at amino-acid position 158. (© 1997 by The American Society of Hematology.

healthy volunteers was diluted with two volumes of phosphate-buffered saline (PBS) and was centrifuged over a Ficoll gradient (Pharmacia Fine Chemicals AB, Uppsala, Sweden) with a specific gravity of 1.076 g/mL. Peripheral blood mononuclear cells (PBMC) were obtained from the interphase and were washed twice with PBS, containing 0.2% (wt/vol) bovine serum albumin (BSA).

*Flow cytometry.* PBMC were incubated with CD16 MoAbs for 25 minutes at room temperature. The cells were washed with PBS/BSA, and were incubated with FITC-labeled goat–antimouse-Ig for 25 minutes at room temperature. Free  $F(ab')_2$  regions of the conjugate were blocked with a mixture of irrelevant mIgG1 and mIgG2a. Thereafter, the cells were incubated with PE-labeled CD56. Only CD56<sup>+</sup> lymphocytes were analyzed in a FACScan flowcytometer (Becton Dickinson).

 $Fc\gamma RIIIA$ -48L/R/H genotyping. Genotyping for the Fc $\gamma$ RIIIA-48L/R/H polymorphism was performed as previously described,<sup>17</sup> with a polymerase chain reaction (PCR)-based allele-specific restriction analysis assay. Briefly, a 91-bp  $Fc\gamma RIIIA$ -specific fragment containing the polymorphic site was amplified from genomic DNA and digested with *Mnl* I. Digested fragments were electrophoresed in 10% acryl amide gels, stained with ethidium bromide, and visualized with UV light. Homozygous  $Fc\gamma RIIIA$ -48LL individuals showed 40-bp, 34-bp, and 17-bp bands, whereas PCR fragments of individuals carrying no or only one  $Fc\gamma RIIIA$ -48L allele showed a 51-bp band. The genotype of these latter individuals was determined by direct sequencing of the amplified fragments, using one of the PCR primers, end-labeled with <sup>32</sup>P (Amersham International, Buckinghamshire, UK), with the Life Technologies cycle sequencing kit, follow-

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From the Central Laboratory of the Netherlands Red Cross Blood Transfusion Service and Laboratory for Experimental and Clinical Immunology, University of Amsterdam, Amsterdam; and Academic Medical Centre, Amsterdam, The Netherlands.

ing the instructions of the manufacturer (Life Technologies, Gaithersburg, MD).

FcyRIIIA-158V/F genotyping. Genotyping of the FcyRIIIA-158V/F polymorphism was performed by means of a (nested) PCRbased allele-specific restriction analysis assay. Two FcyRIIIA genespecific primers (sense A013: 5'-ATA TTT ACA GAA TGG CAC AGG-3'; antisense A012: 5'-GAC TTG GTA CCC AGG TTG AA-3'; italic characters denote mismatches that were introduced to increase specificity) were used to amplify a 1.2-kb fragment containing the polymorphic site. This PCR assay was performed with 5 ng of genomic DNA, 150 ng of each primer, 200 µmol/L of each dNTP, and 2 U of Taq DNA polymerase (Promega, Madison, WI), diluted in a buffer recommended by the manufacturer in a total volume of 50  $\mu$ L in a Perkin Elmer Cetus cycler (Norwalk, CT). The first PCR cycle consisted of 10 minutes denaturation at 95°C, 1½ minute primer annealing at 56°C, and 1<sup>1</sup>/<sub>2</sub> minute extension at 72°C. This was followed by 35 cycles in which the denaturing time was decreased to 1 minute. The last cycle was followed by 8 minutes at 72°C to complete extension. The sense primer in the second PCR contained a mismatch that created an NlaIII restriction site only in FcyRIIIA-158V-encoding DNA (A014: 5'-atc aga ttc gAT CCT ACT TCT GCA GGG GGC AT-3'; uppercase characters denote annealing nucleotides, lowercase characters denote nonannealing nucleotides), the antisense primer was chosen just 5' of the fourth intron (A016: 5'-acg tgc tga gCT TGA GTG ATG GTG ATG TTC AC-3'). This "nested" PCR was performed with 1  $\mu$ L of the amplified fragment, 150 ng of each primer, 200 µmol/L of each dNTP, and 2 U of Taq DNA polymerase, diluted in the recommended buffer. The first cycle consisted of 5 minutes' denaturing at 95°C, 1 minute primer annealing at 64°C, and 1 minute extension at 72°C. This was followed by 35 cycles in which the denaturing time was 1 minute. The last cycle was followed by  $9\frac{1}{2}$  minutes at 72°C to complete extension. The 94-bp fragment was digested with NlaIII, and digested fragments were electrophoresed in 10% polyacrylamide gels, stained with ethidium bromide, and visualized with UV light. Cycle sequencing of first-round fragments to check for specificity was performed with <sup>32</sup>P end-labeled primer A016 with the Life Technologies cycle sequencing kit. Genomic DNA of individuals whose FcyRIIIa encoding cDNA sequence was known were used to optimize the PCR assav.

IgG-binding experiments. Quantification of IgG binding to natural killer (NK) cells of genotyped donors was performed as previously described.<sup>17</sup> In short, mononuclear cells were pretreated with 0.1% (wt/vol) lactic acid (pH 3.9) to remove NK-cell–associated IgG. This treatment did not alter Fc $\gamma$ RIIIa expression as measured with CD16 MoAb CLBFcRgran1 (not shown). The cells were incubated with saturating amounts of human IgG subclasses purified from sera of patients suffering from multiple myeloma. The antibodies were at least partially aggregated, because binding to PMN was observed with the IgG1 and IgG3 preparations (not shown). After washing, bound IgG was detected by FITC-labeled F(ab')<sub>2</sub> fragments of goat–antihuman-IgG (Kallestad, South Austin, TX), and NK cells were identified with PE-labeled CD56. Only CD56<sup>+</sup> lymphocytes were analyzed in a FACScan flowcytometer.

*Statistical analysis.* The data were analyzed with the Student's *t*-test and the  $\chi^2$ -test. Data with different standard deviations (SDs) were compared with the Welch's approximate *t*-test. *P* values below .05 were considered significant.

#### RESULTS

 $Fc\gamma RIIIA$ -158V/F genotyping. An  $Fc\gamma RIIIA$ -derived fragment containing the polymorphic site was amplified from genomic DNA with two  $Fc\gamma RIIIA$  gene-specific primers. Subsequently, a nested PCR was performed in which an

NlaIII restriction site was created only in the  $Fc\gamma RIIIA-158V$ allele. Figure 1 shows digestion of the 94-bp PCR fragment with NlaIII. In lanes 1 to 3, PCR fragments from homozygous  $Fc\gamma RIIIA-158FF$  donors are not digested by NlaIII. In lanes 4 to 6, three bands of 94 bp, 61 bp, and 33 bp are visible, indicating the  $Fc\gamma RIIIA-158VF$  heterozygosity of these individuals. Although the fragments in lanes 7 to 9 were from homozygous  $Fc\gamma RIIIA-158VV$  donors, a 94-bp band of low intensity remained, which was not removed by longer digestion, higher enzyme concentrations, or use of isoschizomer Nsp I. The 94-bp band in these donors was not a result of amplification of  $Fc\gamma RIIIB$  fragments due to unspecificity of the first-round PCR, because the  $Fc\gamma RIIIB$ gene carries a G at nucleotide position 559, which results in digestion by NlaIII. Direct sequencing of several first-round PCR products confirmed the  $Fc\gamma RIIIA$  gene specificity of the assay and the  $Fc\gamma RIIIA-158$  genotype of the donors. Genotyping of 87 healthy white individuals yielded gene frequencies of 0.57 and 0.43 for  $Fc\gamma RIIIA-158F$  and -158V, respectively.

Table 1 displays the correlation between the Fc $\gamma$ RIIIa-48 and the Fc $\gamma$ RIIIa-158 phenotypes. A clear linkage was observed between the *Fc\gammaRIIIA-158V/F* and the *Fc\gammaRIIIA-48L/R/H* polymorphisms ( $\chi^2$ -test, P < .001). All *Fc\gammaRIIIA-158FF* individuals were homozygous *Fc\gammaRIIIA-48LL*, whereas all donors with heterozygous *Fc\gammaRIIIA-48LR* or -48LH genotypes carried at least one *Fc\gammaRIII-158V* allele.

Binding of CD16 MoAbs to NK cells of genotyped donors. To determine the influence of the Fc $\gamma$ RIIIa-158V/F and -48L/R/H polymorphisms on the binding of a panel of CD16 MoAbs, we investigated binding patterns by NK cells of genotyped donors (Table 2). The binding of MoAb CLBFcRgran1 was not significantly different among NK cells from all donors. In contrast, Fc $\gamma$ RIIIa-158VF-positive NK cells from donors homozygous positive for Fc $\gamma$ RIIIa-48L bound more MEM154 than did NK cells of Fc $\gamma$ RIIIa-158FF-positive donors (Welch's approximate *t*-test, *P* =.01). In turn, Fc $\gamma$ RIIIa-158VF NK cells from donors who had only one *Fc\gammaRIIIa-48L* allele bound less MEM154 than did Fc $\gamma$ RIIIa-158VV NK cells (*P* = .04).

Comparison of NK cells of  $Fc\gamma RIIIa-158VF$  phenotyped donors who were either homozygous  $Fc\gamma RIIIa-48LL$ , or heterozygous -48LR or -48LH showed different binding patterns with MoAb B73.1. NK cells positive for the  $Fc\gamma RIIIa-48R$  or -48H isoforms showed reduced binding of B73.1 compared with  $Fc\gamma RIII-48LL$  homozygous NK cells (P = .05). Binding of MoAb MEM154 was equal among these donors. Three  $Fc\gamma RIIIa-158VV$  individuals who were either homozygous  $Fc\gamma RIIIA-48RR$  or -48HH were tested. Binding of MEM154 to cells of these donors was equal compared to the binding to NK cells from  $Fc\gamma RIIIa-158VV$ individuals with a heterozygous  $Fc\gamma RIIIa-48$  phenotype, whereas the binding of B73.1 was diminished (Table 2).

Binding of IgG subclasses by  $Fc\gamma RIIIa^{NK}$ -158 isoforms in the context of  $Fc\gamma RIIIa^{NK}$ -48 phenotype. To determine the functional consequences of the  $Fc\gamma RIIIa$ -158V/F polymorphism, we compared the IgG binding capacity of NK cells from genotyped donors. We studied the differences in IgG binding between individuals who had different

Fig 1. Nlalll restriction analysis of the 94-bp FcyRIIIA-specific fragment, containing the polymorphic nucleotide 559. A 1.2-kb FcyRIIIA-specific fragment was amplified from genomic DNA, followed by a nested PCR. The sense primer of this nested PCR contained a mismatch that introduced a NIallI restriction site only in the FcvRIIIA-158V (559G) allele. Homozygous FcyRIIIA-158FF fragments were not digested (lanes 1 through 3). Three bands (94 bp, 61 bp, and 33 bp) were visible in heterozygous individuals, whereas homozygous Fc<sub>2</sub>/RIIIA-158VV fragments were maximally digested (lanes 7 through 9). A 92-bp fragment of low intensity remained in homozygous 559G fragments (lanes 7 through 9).



 $Fc\gamma RIIIA-158V/F$  genotypes, but were identical regarding the  $Fc\gamma RIIIA-48L/R/H$  genotype. Preincubation of PBMC with F(ab') fragments of CD16 MoAb CLBFcRgran1 reduced the binding of IgG to less than 10% of control values, indicating that the interaction was FcyRIIIa-mediated (data not shown). As shown in Table 3, lactic acid-treated homozygous  $Fc\gamma RIIIa$ -48LL NK cells from individuals heterozygous at position 158 of  $Fc\gamma RIIIa$  bound more IgG3 than did NK cells from Fc $\gamma$ RIIIa-158FF phenotyped donors (P = .02). Although a trend was observed, the difference in binding of IgG1 was not statistically significant. In one single experiment, NK cells from the one available donor who was homozygous  $Fc\gamma RIIIA-48LL$  as well as -158VV bound more IgG3 than did  $Fc\gamma RIIIA$ -8LL-matched homozygous  $Fc\gamma RIIIA-158FF$  or heterozygous donors, without clear differences in binding of IgG1 (data not shown). In individuals who were heterozygous for the FcyRIIIa-48L/R/H polymorphism, FcyRIIIa-158VV-positive NK cells bound more IgG1 compared with FcyRIIIa-158VF-positive NK cells (Table 3; Welch's approximate *t*-test, P = .03). For these donors, the differences in binding of IgG3 were not statistically significant, although a trend was observed. When we determined the levels of NK-cell–associated IgG of freshly isolated NK cells, we observed that  $Fc\gamma RIIIa-158FF$ -positive cells carried less IgG than did  $Fc\gamma RIII-158VV$ -positive cells. Comparing NK cells from donors of the same  $Fc\gamma RIIIa-48L/R/H$  phenotype, we observed a trend toward higher levels of cytophilic IgG bound by  $Fc\gamma RIIIa-158VF$  and -158VV-positive cells, as compared with  $Fc\gamma RIIIa-158FF$ -positive cells (Table 3). The levels of NK-cell–associated IgG of  $Fc\gamma RIIIa-48LL$  and -158VF-positive NK cells did not significantly differ from that of  $Fc\gamma RIIIa-48LR$  or -48LH NK cells of the same -158VF phenotype.

To exclude any effect of the triallelic  $Fc\gamma RIIIa-48L/R/H$ polymorphism on IgG binding, we compared  $Fc\gamma RIIIa-48LL$ positive NK cells with  $Fc\gamma RIIIa-48LR$ -or -48LH-positive NK cells from donors who were all heterozygous  $Fc\gamma RIIIa-$ 158VF. As shown in Table 3, no statistically significant differences were observed in either the amount of NK-cell– associated IgG or in the binding of IgG subclasses to lactic acid–treated NK cells. Therefore, we depicted the results of IgG-binding experiments, comparing  $Fc\gamma RIIIa-158FF$  NK-

FcγRIIIa-158			FcγRIIIa-48L/R/H Phenotype							
Genotype	Phenotype	Total	48LL	48LR	48LH	48RR	48HH	48RH		
559T/T	158FF	28	28	*	_	_	_	_		
559G/T	158VF	44	34	4	6	_	_	_		
559G/G	158VV	15	4	6	4	1	—†	_		

Table 1. Fc $\gamma$ RIIIA-158V/F Genotypes in White Individuals (n = 87), Typed for Fc $\gamma$ RIIIA-48L/R/H

Gene frequencies are 0.57 and 0.43 for Fc $\gamma$ RIIIa-158F and Fc $\gamma$ RIIIa-158V, respectively. A clear linkage was observed between the Fc $\gamma$ RIIIA-158F and the Fc $\gamma$ RIIIA-48L alleles, as well as between the Fc $\gamma$ RIIIa-158V and either the Fc $\gamma$ RIIIa-48R or -48H alleles ( $\chi^2$  test; P < .001 in both cases).

\* A dash (—) denotes that in this study none of the tested donors had this genotype.

t In a previous study we described one healthy individual and two unrelated patients suffering from recurrent bacterial and viral infections who were homozygous FcγRIIIa-48H/H (De Vries, unpublished data, 1996 and ref 18). These three donors were found to be homozygous FcγRIIIa-158VV.

Genotype			MoA	b	
FcγRIIIA-48	FcγRIIIA-158	Control mlgG2a	CLBFcRgran1	MEM154	B73.1
48LL	158FF	12 ± 6	1,539 ± 257	42 ± 20	303 ± 176
48LL	158VF	8 ± 4	1,713 ± 719	460 ± 218*	248 ± 105
48LR or 48LH	158VF	6 ± 2	1,443 ± 504	323 ± 182	121 ± 51†
48LR or 48LH	158VV	10 ± 5	1,102 ± 298	718 ± 341‡	151 ± 117
48HH§	158VV	7	1,694	892	55
48RR <sup>  </sup>	158VV	15	1,443	642	103

Table 2. CD16 MoAb Binding (mean fluorescence intensities ± SD) of NK Cells From Individuals Genotyped for FcyRIIIA Polymorphisms

At least three donors of each genotype were tested. Data were analyzed with the Student's *t*-test the and Welch's approximate *t*-test. *P* values below .05 were considered significant.

\* Statistically significant difference from mean fluorescence intensity of NK cells from Fc $\gamma$ RIIIa-158FF individuals of the same *Fc\gammaRIIIA-48* genotype.

† Significantly different from Fc $\gamma$ RIIIa-48LL NK cells of the same *Fc\gammaRIIIA-158* genotype.

 $\ddagger$  Significantly different from Fc $\gamma$ RIIIa-158VF NK cells of the same *Fc\gammaRIIIA-48* genotype.

§ Two individuals were tested; one healthy control and one patient suffering from recurrent viral infections.

<sup>||</sup> One healthy individual was tested.

cells with Fc $\gamma$ RIIIa-158VV NK cells, disregarding the Fc $\gamma$ RIIIa-48 phenotype (Fig 2). Significant differences in levels of IgG1 (P = .007), IgG3 (.005), IgG4 (.02), and cytophilic IgG (.004) were observed.

#### DISCUSSION

Recently we identified a triallelic polymorphism in the membrane-distal Ig-like domain of  $Fc\gamma RIIIa$ , in which a leucine, an arginine, or a histidine can be present at amino acid position 48.17 In the present report, we functionally characterized a previously described genetic polymorphism of  $Fc\gamma RIIIA$ , encoding either a phenylalanine (F) or a valine (V) at amino acid position 158 in the membrane-proximal Ig-like loop of  $Fc\gamma RIIIa$ .<sup>13</sup> We determined that the gene frequencies for FcyRIIIA-158F and FcyRIIIA-158V were 0.57 and 0.43, respectively. The  $Fc\gamma RIIIA-158F$  genotype was shown to be clearly linked to the  $Fc\gamma RIIIA-48L$  genotype, whereas the  $Fc\gamma RIIIA-158V$  was found to be linked to  $Fc\gamma RIIIA-48R$  and -48H. A linkage between allotypes of the  $Fc\gamma RIIIA-158V/F$  and the  $Fc\gamma RIIIA-48L/R/H$  polymorphisms was expected because the two polymorphisms are located within the same gene.

In our previous work, we attributed differences in binding of several CD16 MoAbs to the amino acid present on position 48 of  $Fc\gamma RIIIa$ . We now show that the binding of MEM154 depends on the presence of a valine at amino acid position 158 of  $Fc\gamma RIIIa$  and is not influenced by the amino acid polymorphism at position 48. These findings are in conformity with data from Tamm and Schmidt,<sup>19</sup> who found that the epitope recognized by MEM154 is located in the membrane-proximal domain. We did not observe any effect of the  $Fc\gamma RIIIa$ -158V/F polymorphism on B73.1 binding but confirmed our initial observations that only the amino acid at position 48 influenced the binding of MoAb B73.1.<sup>17,18</sup>

The membrane-proximal domain, carrying the  $Fc\gamma RIIIa$ -158V/F polymorphism, is generally accepted to contain the IgG-binding site in  $Fc\gamma Rs$ , and experiments with mutants of  $Fc\gamma RIIIb$  showed that the membrane-proximal loop is essential for IgG binding in Fc<sub>γ</sub>RIIIb.<sup>14,15</sup> To study the functional consequences of the Fc $\gamma$ RIIIa-158V/F polymorphism in the context of the FcyRIIIa-48L/R/H polymorphism, we performed IgG binding experiments with NK cells from genotyped donors. NK cells from homozygous FcyRIIIa-158VV-positive individuals bound more IgG1 and IgG3 than did Fc $\gamma$ RIIIa-158FF-positive NK cells, irrespective of the amino acid present on position 48 of the receptor. NK cells of  $Fc\gamma RIIIa-158VF$  heterozygous donors showed intermediate levels of IgG binding, indicating a gene-dosis effect. These data indicate that the donor-dependent differences in IgG binding of FcyRIIIa isoforms are attributable to the

Table 3. Binding of Human IgG Subclasses of NK Cells From Individuals Genotyped for  $Fc\gamma RIIIA$  Polymorphisms (MFI  $\pm$  SD)

Genoty	уре	Subclass					
FcγRIIIA-48	FcγRIIIA-158	lgG1	lgG2	lgG3	lgG4	Cytophilic IgG	
48LL	158FF	913 ± 317	58 ± 33	390 ± 131	106 ± 104	337 ± 159	
48LL	158VF	1,257 ± 608	$124\pm126$	912 ± 286*	413 ± 178	$695~\pm~405$	
48LR or 48LH	158VF	1,243 ± 194	155 ± 133	$746 \pm 230$	$548 \pm 202$	1,139 ± 542	
48LR or 48LH	158VV	1,814 $\pm$ 507†	$173\pm98$	1,053 $\pm$ 487	$487\pm286$	$\textbf{1,788} \pm \textbf{796}$	

At least three donors of each genotype were tested. Data were analyzed with the Student's *t*-test and the Welch's approximate *t*-test. *P* values below .05 were considered significant.

\* Statistically significant difference from IgG3 binding by NK cells from FcyRIIIa-158F/F individuals of the same FcyRIIIA-48 genotype.

† Significantly different from Fc $\gamma$ RIIIa-158V/F cells of the same *Fc\gammaRIIIA-48* genotype.



Fig 2.  $Fc\gamma RIIIa^{NK}$ -158V binds more IgG than does  $Fc\gamma RIIIa^{NK}$ -158F. IgG binding by NK cells from individuals either homozygous  $Fc\gamma RIIIA$ -158FF (**I**) or homozygous  $Fc\gamma RIIIA$ -158VV ( $\Box$ ) was compared, irrespective of the  $Fc\gamma RIIIa$ -48L/R/H genotype. At least three different donors of each genotype were tested. The level of cytophilic IgG and the binding of IgG1, IgG3, and IgG4 was significantly higher in NK cell from  $Fc\gamma RIIIA$ -158VV individuals (P < .05 in all cases).

FcγRIIIa-158 polymorphism and not to the FcγRIIIa-48L/ R/H polymorphism, as we previously suggested.<sup>17</sup> Due to the linkage between the two *FcγRIIIA* polymorphisms, the FcγRIIIa-48LL-positive donors described in our previous work were all homozygous FcγRIIIa-158FF and therefore their NK cells showed low IgG binding. In contrast, the tested FcγRIIIa-48LR or -48LH-positive donors all carried one or two FcγRIIIa-158V alleles and therefore bound high levels of IgG.

Vance et al<sup>20</sup> described a donor-dependent difference in binding of CD16 MoAb 3G8 and monomeric IgG to NK cells. Although we did not observe clear differences in 3G8 binding among our donors (not shown), our IgG binding experiments suggest that the polymorphism described by these investigators might be the result of different Fc $\gamma$ RIIIa-158V/F phenotypes.

Hulett and Hogarth<sup>21</sup> extrapolated Fc $\gamma$ RII data to Fc $\gamma$ RIII and suggested that the phenylalanine on position 158 of Fc $\gamma$ RIIIa might be partly responsible for the medium affinity of the receptor for IgG, thus attributing the low-affinity receptor of Fc $\gamma$ RIIIb to the valine at position 158. Our results are contradicting with this theory because Fc $\gamma$ RIIIa-158V bound more IgG than did Fc $\gamma$ RIIIa-158F. Recent studies suggest that association of Fc $\gamma$ RIIIa with the Fc $\epsilon$ RI- $\gamma$  chain might be responsible for the medium affinity, because association of Fc $\gamma$ RI with the  $\gamma$ -chain increases the affinity of the receptor for IgG, and results from experiments with Fc $\gamma$ RI-IIb mutants that could associate with the  $\gamma$ -chain pointed in the same direction.<sup>22</sup>

The clinical consequences of our findings remain to be established. A higher affinity for IgG could result in higher clearance of immune complexes in patients suffering from (auto)immune disease. On the other hand, a higher amount of cytophilic IgG might imply a decreased availability of Fc $\gamma$ RIIIa and, thus, obstruction of receptor-immune complex interaction. Recently we<sup>23</sup> and others<sup>18</sup> separately described children with a homozygous *Fc\gammaRIIIA-48HH* genotype suffering from recurrent viral infections. We genotyped the two

unrelated patients from the former study and found that they were both homozygous  $Fc\gamma RIIIA-158VV$ . Previous work has suggested that cytophilic IgG on NK cells has inhibitory effects on natural cytotoxicity.<sup>24,25</sup> It is tempting to speculate that in the described pediatric patients, NK cells are loaded with a high amount of  $Fc\gamma RIIIa-158V$ -bound IgG, thereby hampering a proper antiviral response.

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