A Novel Polymorphism of FcγRIIIa (CD16) Alters Receptor Function and Predisposes to Autoimmune Disease

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Abstract

A novel polymorphism in the extracellular domain 2 (EC2) of FcγRIIIA affects ligand binding by natural killer (NK) cells and monocytes from genotyped homozygous normal donors independently of receptor expression. The nonconservative T to G substitution at nucleotide 559 predicts a change of phenylalanine (F) to valine (V) at amino acid position 176. Compared with F/F homozygotes, FcγRIIIa expressed on NK cells and monocytes in V/V homozygotes bound more IgG1 and IgG3 despite identical levels of receptor expression. In response to a standard aggregated human IgG stimulus, FcγRIIIa engagement on NK cells from V/V (high-binding) homozygotes led to a larger rise in [Ca2+]i, a greater level of NK cell activation, and a more rapid induction of activation-induced cell death (apoptosis). Investigation of an independently phenotyped normal cohort revealed that all donors with a low binding phenotype are F/F homozygotes, while all phenotypic high binding donors have a V allele. Initial analysis of 200 patients with SLE indicates a strong association of the low binding phenotype with disease, especially in patients with nephritis who have an underrepresentation of the homozygous high binding phenotype. Thus, the FcγRIIIa polymorphism at residue 176 appears to impact directly on human biology, an effect which may extend beyond autoimmune disease characterized by immune complexes to host defense mechanisms. (J. Clin. Invest. 1997. 100:1059–1070.) Key words: receptors, Fc•polymorphism, genetics • macrophages • killer cells, natural • lupus erythematosus, systemic

Introduction

Genetic polymorphisms of human FcγRIIa and FcγRIIib have been characterized (1–13) and associated with certain disease risks (14–20). The two allelic forms of FcγRIIa differ by two nucleotides (nt), one in the first extracellular Ig-like domain (EC1) predicting a glutamine (Q) to tryptophan (W) at residue position 27 and one in the second extracellular Ig-like domain (EC2) predicting an arginine (R) to histidine (H) at residue position 131. The change at position 131 markedly alters the ability of the receptor to bind human IgG2 (10, 12), and this polymorphism has been associated with certain bacterial infections (14, 15, 19) and with SLE (16–18). The two allelic forms of neutrophil-specific FcγRIIib differ by five nucleotides which results in four amino acid differences in EC1 (21). Although binding of IgG does not seem to be affected (9), these two allelic forms do have different levels of quantitative function (9, 10), and the more active NA1 allele has been associated with severe renal disease in certain systemic vasculitides (20).

Several recent observations suggest that FcγRIIIa, which is expressed on natural killer (NK) cells, mononuclear phagocytes, and renal mesangial cells (22), might also be polymorphic in both its structure and quantitative expression. Vance and Guyre originally described a functional polymorphism in FcγRIIIa on NK cells among normal donors (23). Based on some differences both in IgG binding and in anti-CD16 reactivity, they suggested that variations in receptor expression might explain their observations. More recently, de Haas and colleagues have described a triallelic sequence polymorphism at nt 230 in FcγRIIIa (24). This single nucleotide substitution in the third exon encoding EC1 predicts an amino acid change from leucine (L) to arginine (R) or from leucine (L) to histidine (H) and reportedly influences the binding of human IgG and several anti-CD16 mAbs (24, 25). Such structural variants of FcγRIIIa, recognized by altered patterns of anti-CD16 mAb binding, may be related to a clinical phenotype of repeated infections (26).

The FcγRIIIA sequence polymorphism on NK cells which reportedly influences ligand binding (24) raised the possibility that this sequence polymorphism might explain previously described differences in NK FcγRIIIa and NK cell function (23, 26). To test this hypothesis, we identified several of the normal donors studied by Vance and Guyre (23) and characterized the nucleotide sequence of their FcγRIIIA. Contrary to our expectation, these donors were monomorphic at nt 230 and nt 248 (amino acid positions 66 and 72). However, they were polymorphic at nt 559, a site noted by Ravetch and Perussia as...
potentially polymorphic (21). This nonconservative T to G substitution predicts a change of phenylalanine (F) into valine (V) at position 176 in the membrane-proximal EC2. Since several studies suggest that the second Ig-like domain strongly influences ligand binding (27–32), we pursued further characterization of this 176F/V polymorphism by identifying normal donors homozygous at position 176 (and homozygous at positions 66 and 72). Compared with F/F homozygotes, FcγRIIIa expressed in V/V homozygotes bound more IgG1 and IgG3 despite identical levels of receptor expression. These observations indicate that the sequence polymorphism at nt position 559 alters the apparent affinity of FcγRIIIa on both NK cells and monocytes for IgG. This difference affects the ability of the receptor to initiate a range of cell programs in response to and monocytes for IgG. This difference affects the ability of

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A. FcγRIIIA Genomic Primers

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B. FcγRIIIA cDNA Primers

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FcyRIIIA gene. The reverse primer (5′-TGT AAA ACG ACG GCC AGT ATG GAC TTC TAG CTG CAC-3′) corresponds to nt 348 to 331 in exon 3. The PCR product was purified and sequenced as described above.

Flow cytometric assay for human IgG binding. Human IgG binding assays were performed using anticoagulated, washed whole blood. Mouse mAbs were used either for direct immunofluorescence (Leu11a-FITC, 3G8-FITC) or for indirect immunofluorescence (3G8, Gran1, AGT ATG GAC TTC TAG CTG CAC-3′) and CD56-PE at 5°C, PE, and FITC.

Fluorescence using TC, PE, and FITC. right angle light scattering in combination with three-color immunofluorescence of individual cell populations was based on forward and

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Iden...nnt to the tissue culture plated (precoating for 2 h at 37°C) before the addition of cells. Since induction of CD25 expression was observed within 1 h of stimulation as previously reported (40, 41), we examined incubation periods ranging from 1 to 48 h. CD25 expression was determined by flow cytometry using anti-CD25-FITC (Caltag Laboratories).

Figure 1. Schematic representation of the FcγRIIIA genomic structure and cDNA structure showing the relative location of primers used for PCR-based sequencing (sequencing sets 1–4) and allele-specific PCR (PCR set 1). For sequencing analysis, an M13-based dye-primer sequencing strategy was used. FcγRIIAs encoding cDNA was prepared from purified MNC. Relative positions of nt 230 and 559 are shown. S, Signal sequence (encoded in two exons); TM/CY, transmembrane/cytoplasmic domains; 3′-UT, 3′ untranslated sequence.
The viability of purified and 24-h IL-2–primed (100 U/ml) NK cells after FcγRIIIa-mediated stimulation was determined by quantitation of propidium iodide (PI) uptake in the FACScan® and by direct visual assessment of trypan blue exclusion. Significant changes in cell viability could be detected within 1 h of stimulation with mAb or aggregated IgG (42–44). To determine if FcγRIIIa-mediated stimulation was inducing cell death via apoptosis, in selected experiments cells were fixed, permeabilized, and analyzed for quantitative DNA content. Subdiploid uptake of PI reflects cell death via apoptosis (45).

Additionally, we examined stimulated cells for apoptotic morphology (chromatin condensation and nuclear fragmentation) (43) after fixation (2% paraformaldehyde plus 0.5% glutaraldehyde in 0.05 M cacodylate buffer, pH 7.2, followed by post-fixation with 1% osmic acid in cacodylate buffer, pH 7.2). Cells were suspended in 1.5% agar, embedded in Spurr’s resin, and the thin sections were viewed in a Philips CM-12 electron microscope.

**Statistical analysis.** Differences in ligand binding and mAb binding were analyzed by Student’s *t* test. The χ² test was used to analyze

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**Figure 2.** Sequence analysis of a portion of FcγRIIIa cDNA from three normal donors. FcγRIIIa encoding cDNA was prepared from purified MNC and an M13-based dye-primer sequencing strategy was used (see Methods). Donors homozygous for nt 559-T (A), homozygous for nt 559-G (C), and a donor heterozygous for nt 559-T/G (B) are shown. In each tracing, nt 531 (*) is shown to indicate the presence of cDNA encoding the FcγRIIIA gene (C at nt 531) and not the FcγRIIIB gene (which is T at nt 531).
the distribution of FcγRIIIA genotypes (corresponding to 176-V/V, 176-F/F, and 176F/V) in SLE and non-SLE controls. The null hypothesis was rejected at the 95% confidence level ($P < 0.05$).

**Results**

**FcγRIIIA sequence polymorphisms.** The recent observation of an FcγRIIIA sequence polymorphism on NK cells which influences ligand binding (24) raised the possibility that this sequence polymorphism might explain previously described differences in NK FcγRIIIa and NK cell function (23). To test this hypothesis we identified several of these normal donors and characterized the nucleotide sequence of their FcγRIIIA. The cell type specific expression of FcγRIIIA and FcγRIIIB in NK cells/mononuclear phagocytes and in neutrophils, respectively, provides a strategy for selective sequencing of cDNAs derived from these two highly homologous genes. Furthermore, within the coding region of FcγRIII, there are 10 nucleotide differences between FcγRIIIA and FcγRIIIB that can be used to confirm the presence of only FcγRIIIA or FcγRIIIB sequence. Using this approach, two normal donors, one with the low binding FcγRIIIA phenotype and one with a high binding FcγRIIIA phenotype (23), were both shown to be T/T/230 homozygotes. Interestingly, however, while the low FcγRIIIA phenotype showed no differences from the conventional sequence, the donor characterized phenotypically as high binding showed amino acid residue 176F in the membrane proximal EC2 of FcγRIIIA might affect ligand binding and receptor function.

Sequence analysis of the entire coding region for MNC FcγRIIIA cDNA from a total of 30 normal donors revealed variation in nt position 559 (T or G). In this group, cDNAs from three individuals contained only G559 while six donors contained only T559. The remaining 21 donors were found to contain both T559 and G559 (Fig. 2). All 30 normal donors were homozygous T at nt 230 and homozygous C at nt 248 (24, 25). There were no other sequence differences throughout the whole FcγRIIIA gene except that two donors were heterozygous at position 249 for a conservative G559 to A249 substitution, a silent variation at the third position of the codon for serine. These data demonstrate that the sequence variation in the FcγRIIIA gene at nt 559 (amino acid 176) is not a rare mutation, but rather a common polymorphism.

**Characterization of CD16 epitopes.** To determine if the 176F to V change affects the binding of anti-CD16 mAb which might explain previously reported variations in anti-CD16 mAb reactivity (28, 30, 31, 46, 47), the reactivity of FcγRIIIA on peripheral blood NK cells was characterized using a panel of anti-CD16 mAb. Donors homozygous for 176F or 176V and homozygous for 66L and 72S were examined by flow cytometry. Using the well characterized anti-CD16 mAb CLB-Gran1, identical CD16 fluorescence intensities were observed on CD56 positive NK cells from donors of both genotypes (Fig. 3 A and Table I). Similar results were evident with six additional anti-CD16 mAbs (Table I) including mAb B73.1 which is affected by the polymorphism at nt 230 (24, 25). mAbs 1D3 and MEM154 showed differential binding to NK cells from donors homozygous for F compared with V. In both instances, these mAb bound well to 176F/V donors but only poorly to 176F/F donors (Fig. 3 C and D, and Table I). mAb 3G8 showed subtle differences which did not reach statistical significance with our sample size (Fig. 3 B). These data indicate that although donors homozygous for either the F or the V alleles express the...
same level of CD16 protein on the surface of NK cells, the reactivities for some anti-CD16 mAbs differ, suggesting that these allelic proteins have different three-dimensional structural characteristics.

We then examined the mAb epitopes expressed on CD16 positive circulating monocytes. This population, typically a small percentage of circulating monocytes (35), was identified by multicolor fluorescence. Because FcγRIIIa expression by monocytes is variable among donors, we sought donors homozygous for 176F or 176V (and homozygous for both 66L and 72S) expressing comparable levels of mAb CLB-Gran1 reactivity on their peripheral blood monocytes. In paired experiments, B73.1 showed identical reactivity while both 1D3 and MEM154 showed less reactivity with the 176 F/F donor (results not shown).

Characterization of ligand binding. A single amino acid change at residue 131 in the membrane proximal domain of FcγRIIIa (CD32) results in a nearly 10-fold alteration in quantitative ligand binding of human IgG2 (10–12). To determine if the 176F/V polymorphism in the homologous extracellular domain of FcγRIIIa altered ligand binding, we examined the binding of pooled human IgG and of human IgG myeloma proteins to peripheral blood leukocytes from our homozygous donors. FcγRIIIa has a higher affinity for IgG than FcγRIIa and FcγRIIib. This higher affinity (reported to be in the range of 1–7 × 10^7 M^-1) is less than the affinity of IgG binding to FcγRIa, but is sufficient to allow binding of monomer IgG at physiological concentrations. Binding of pooled human IgG to NK cells was observed in all donors, but the level of binding was substantially different between our homozygous donor groups. Individuals homozygous for both 176V and 66L bound significantly more IgG1 and IgG3 than did donors homozygous for both 176F and 66L (Fig. 4). The difference in binding of IgG1 and IgG3 was observed at both concentrations of IgG (15 and 30 μg/ml) used in these studies. Binding of the myeloma proteins to CD56 positive NK cells was completely blocked by the anti-CD16 mAb CLB-Gran1 (results not shown). A difference in binding of IgG4 (30 μg/ml) was also observed; there was detectable but low binding to donors homozygous for 176V but not 176F. mAb CLB-Gran1 was used to confirm identical levels of CD16 protein on the NK cell surface (Fig. 3). No binding of IgG2 (30 and 15 μg/ml) or IgG4 (15 μg/ml) to NK cells from either donor type was observed.

In a number of experiments, we were also able to observe IgG myeloma protein binding to FcγRIIIa on the small subset of human monocytes expressing CD16. Using preincubation with anti-CD64 mAb 197 to block the high affinity FcγRIa, binding of human IgG to FcγRIIIa could be quantitated. Complete blockade of FcγRIa was confirmed by showing that the binding of IgG2a (30 μg/ml) was reduced to background autofluorescence levels in the presence of mAb 197 (Fig. 5 C). In paired experiments with donor monocytes matched for CLB-Gran1 reactivity and with FcγRIa blocked by mAb 197, 176V homozygous donors bound more IgG1 than did donors homozygous for 176F (Fig. 5, A and B). These data document that the nt 559 polymorphism of FcγRIIIA which changes a single amino acid in EC2 results in a change in apparent affinity for ligand binding independent of the cell type in which it is expressed.

Functional implications of the 176F/V polymorphism. To determine if the difference in quantitative binding of ligand to the 176F and 176V alleles results in differences in receptor
characteristic light scatter properties, by reactivity with anti-CD14 shown (Fig. 5). Complete blockade of ligand binding (mIgG2a) is shown (40, 41). Stimulation with mAb 3G8 and F(ab')2 GAM induced rapid upregulation of expression of CD25 (IL-2R) on the surface of purified NK cells. Using purified NK cells from homozygous donors, engagement and cross-linking of CD16 with IgG aggregates also resulted in rapid upregulation of CD25 expression. However, donors homozygous for 176V showed significantly higher levels of CD25 expression relative to donors homozygous for 176F (Fig. 7).

FcγRIIIa on NK cells is important in regulating NK cell survival through receptor-mediated activation-induced cell death (42–44). Both anti-CD16 mAb plus GAM cross-linker and IgG aggregates decreased NK cell survival quite rapidly. However, while comparable levels of cell survival were apparent after anti-CD16 mAb stimulation, there was a marked difference between 176F/F and 176V/V donors in the degree of NK cell death after stimulation with IgG aggregates (Fig. 8). Nuclear fragmentation and chromatin condensation, characteristic of apoptosis and assessed by transmission electron microscopy, was observed in NK cells stimulated via FcγRIIIa (with cross-linked mAb or aggregated IgG) (results not shown). In addition, quantitative PI staining of fixed and permeabilized cells demonstrated a distinct population of apoptotic cells with subdiploid DNA content in aggregated IgG stimulated but not control cells (Fig. 9).

Characterization of the 176F/V polymorphism in donors with disparate antibody-dependent cellular cytotoxicity (ADCC) activity. Because of the clearly defined differences in FcγRIIIa-induced function in our homozygotes, we considered the possibility that the differences in quantitative ADCC by NK cells of different donors, previously described by Vance (23), might reflect the 176F/V polymorphism. This possibility was reinforced by the original observation that the difference in ligand binding among these donors was much greater than the difference in mAb 3G8 binding. Accordingly, we made cDNA from MNC preparations from six previously characterized individuals. Four low binding FcγRIIIa homozygous for 176V/V donors were heterozygous and homozygous for 176F/V and 176F/F, respectively; Fig. 6). When aggregated human IgG was used as the stimulus, a rapid rise in [Ca²⁺]i was also observed in the homozygous donors, but the magnitude of the rise in NK cells from the 176V homozygote donor was more than threefold greater than the rise observed in NK cells from the 176F homozygote donor (56 and 189 nM in the 176F and 176V homozygous donors, respectively; Fig. 6).

To examine the impact of the 176F/V polymorphism on integrated cell functions, upregulation of surface CD25 (IL-2 receptor) on IL-2–treated NK cells after engagement of FcγRIIIa was assessed (40, 41). Stimulation with mAb 3G8 and F(ab')2 GAM induced rapid upregulation of expression of CD25 (IL-2R) on the surface of purified NK cells. Using purified NK cells from homozygous donors, engagement and cross-linking of CD16 with IgG aggregates also resulted in rapid upregulation of CD25 expression. However, donors homozygous for 176V showed significantly higher levels of CD25 expression relative to donors homozygous for 176F (Fig. 7).

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Association of the 176F allele with autoimmune disease. We have shown previously that the low binding allele of FcγRIIIa (131H) is associated with SLE and nephritis in African-American patients (16). To determine if skewing of the normal 176F/V allelic system might also be associated with SLE, we developed a genotyping assay based on allele-specific PCR.
The fidelity of this assay was established using a genomic template from our initial group of FcγRIIIa sequenced normal donors \( (n/H11005 = 30) \) and confirmed by the sequencing of selected SLE patients \( (n/H11005 = 38) \) and additional normal donors \( (n/H11005 = 11) \). Using this assay, we genotyped a population of 200 ethnically diverse patients with documented SLE and a cohort of 113 ethnically diverse normal individuals. There was a significant skewing in the distribution of the three genotypes \( (2^2 \text{ contingency table}, \chi^2 = 9.87, P < 0.01; \text{Table II}) \) and in the allelic frequency \( (2^2 \text{ contingency table}, \chi^2 = 6.13, P < 0.015; \text{Table II}) \) between the two groups. In the SLE patients, there was an increase in homozygosity for 176F; 44% of the 200 SLE patients but only 23% in the 112 non-SLE control subjects were 176F homozygous. In contrast, only 4% of the 79 SLE patients with nephritis were 176V/V homozygotes compared with 15% of the 121 nonrenal SLE patients (15% in normal controls). These results suggest that the presence of the FcγRIIIa 176F allele is a significant risk factor for development of SLE, especially with nephritis.

**Discussion**

The recent observation of an FcγRIIIa sequence polymorphism on NK cells which influences ligand binding \( (24) \) raised the possibility that this sequence polymorphism might explain previously described differences in NK FcγRIIIa and NK cell function \( (23) \). To test this hypothesis we identified several of these normal donors and characterized the nucleotide sequence of their FcγRIIIa. Contrary to our expectation, these donors were monomorphic at nt 230 and nt 248 but polymorphic at nt 559. This polymorphism predicts an F to V substitution in position 176 of EC2 of FcγRIIIa, the domain which is critical for ligand binding. Normal donors, homozygous for F and for V at position 176 and homozygous for L at position 66 and for S at position 72 in all cases, were characterized for ligand binding and for FcγRIIIa function. NK cells and monocytes from donors with 176V/V bound more IgG1 and IgG3 than the corresponding cells from 176F/F donors. FcγRIIIa-176V/V elicited a larger flux in \([\text{Ca}^{2+}]_i\) \( \), a greater degree of cell activation, and a more pronounced program of activation-induced cell death than FcγRIIIa-176F/F. FcγRIIIa-176F/F individuals were overrepresented in a population of 200 SLE patients while FcγRIIIa-176V/V individuals were underrepresented among patients with nephritis. These data, coupled with other observations \( (14–20) \), suggest an important role for FcγR polymorphisms in human disease.

The absence of the V allele in the phenotypic low binding donors and its presence in the high binding donors strongly suggests that the presence of the FcγRIIIa 176F allele is a significant risk factor for development of SLE, especially with nephritis.
suggest that this polymorphism explains the difference in NK FcγRIIIa originally described by Vance and colleagues (23). In that cohort, the tendency toward lower reactivity with mAb 3G8 among low binders is consistent with our data demonstrating the same subtle trend (Fig. 3 B and Table I). Most importantly, in the Vance study the ratio of IgG binding to mAb 3G8 reactivity clearly shows reduced ligand binding, even when mAb 3G8 is used to define receptor number. Less clear, however, is the relationship between the 176F/V polymorphism and the NK FcγRIIIa described in several patients with recurrent infections (26). Both of those patients showed markedly reduced reactivity with mAb B73.1. Based on our data that the B73.1 epitope is not influenced by position 176 and other data indicating that 66R/R donors have markedly reduced levels of B73.1 reactivity (24), we anticipate that these individuals with recurrent infections have some difference in NK cell FcγRIIIa other than variation at position 176. Indeed, the polymorphism at amino acid position 66 may contribute to this difference (26). Quantitatively, however, the approximate twofold increase in IgG1 binding reported for donors with 66R or 66H compared with 66L parallels the magnitude of the difference in IgG binding that we have seen with 176V compared with 176F. Although differences in ligand binding can influence susceptibility to infection (14, 15), this functional similarity between the polymorphisms at 66 and 176 and the prevalence of the 176F/V polymorphism makes this mechanism an unlikely basis for the rare patients described to date (26). However, the similarity in ligand binding raises the interesting question of whether the difference in ligand binding described for donors varying in position 66 might be explained by allelic association with position 176. To date, in more than 80 normal donors, we have identified only two individuals who are heterozygous T/G at nt 230 (66L/66R), one individual who is heterozygous T/A at nt 230 (66L/66H), and one individual who is homozygous at nt 230 for the uncommon A allele (66H). Therefore, we have been unable to test the hypothesis that 66R and 66H occur in association with 176V and that 176V may determine the ligand binding phenotype.

There are several interesting implications of the FcγRIIIa 176F/V polymorphism. Recent data have suggested that FcγR expressed on macrophages may play an important role in the regulation of serum IgG levels. Initial observations in the FcγRII knockout mouse demonstrated an impact on total IgG levels, but since the entire FcγRII gene with its various splice

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**Table II. Distribution of FcγRIIIa Alleles in SLE Patients and Non-SLE Controls**

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<td>n = 113</td>
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- **No. of subjects (% of group)**
  - 176F/F: 87 (44%) vs. 29 (26%)
  - 176F/V: 92 (46%) vs. 69 (61%)
  - 176V/V: 21 (10%) vs. 15 (13%)

- **Allelic frequency†**
  - 176F: 0.67 vs. 0.56
  - 176V: 0.33 vs. 0.44

A PCR-based genotyping assay (using genomic DNA) was developed using allele specific primers (see Results). 200 ethnically diverse patients with documented SLE (33) and 113 ethnically diverse normal volunteers were genotyped for FcγRIIIa alleles [nt 559G (176V) and/or nt 559T (176F)]. Allele and gene frequencies are shown. *SLE patients vs. normal controls; 3 × 2 contingency table, χ² = 9.87, P < 0.01; †SLE patients vs. normal controls; 2 × 2 contingency table, χ² = 6.13, P < 0.015.
isoforms was disrupted, the relative roles of FcyRIIb1 expressed on B cells as opposed to FcyRIIb2 expressed on macrophages could not be determined (50). Somewhat surprisingly, the naturally occurring disruption of the expression of the FcyRIIb2 isoform expressed on macrophages of NOD mice is strongly associated with upregulation of both IgG1 and IgG2b serum levels despite relatively normal expression of the B cell specific FcyRIIb1 (51). The possibility that macrophage FcR may be playing an important role in the regulation of IgG levels is further underscored by the observation in humans that different alleles of FcyRIIa are associated with different serum levels of IgG2 (14). This observation is particularly important because in humans, FcyRIIa is expressed on macrophages but not on B cells. Furthermore, this observation emphasizes that alleles with different capacities to bind human IgG2, not just presence or absence of receptor, are associated with different levels of IgG. Thus, it seems reasonable to extrapolate to the prediction that the FcyRIIa 176F/V alleles may influence the level of IgG1 and IgG3. How they influence specific responses to vaccination and net effective humoral immunity remains to be determined.

Of course the implications of the FcyRIIa 176F/V polymorphism extend beyond the regulation of serum IgG levels. Soluble FcyRIIa is clearly present in the circulation (52–54). In a number of systems, soluble receptor can influence the level of B cell activation presumably through binding surface immunoglobulin (55, 56). Since FcyRIIa binds ligand with higher affinity than FcyRIIb which is unable to bind ligand in monomeric form, FcyRIIa may play a particularly important role in mediating these effects. FcyRIIa may also play a critical role in the first-dose cytokine-release syndrome seen with some therapeutic monoclonal antibodies (57). Furthermore, our earlier studies in a primate model of immune complex handling demonstrated an essential role for FcyRIIa (58, 59), and more recent observations in mice with targeted disruption of murine FcyRII also support an important role in immune complex–mediated triggering of inflammatory reactions (60). Since each of these effects is dependent on binding of IgG, the potential for FcyRIIa 176F/V alleles to influence the biologic potential of both receptor and ligand is clearly evident.

To directly test this potential in human biology in vivo, we investigated the possibility that FcyRIIa 176F/V alleles might be abnormally represented in patients with SLE, a prototypic immune complex disease. A role for abnormal FcyR function in SLE has been described (61), and the skewing of FcyRIIa alleles in SLE has supported the hypothesis that the FcyRIIa allele with a low binding phenotype for human IgG2 would be overrepresented in SLE (16, 17). In most immune complexes, however, autoantibodies are not of the IgG2 isotype but rather the IgG1 and IgG3 isotypes. Thus, one might anticipate an overrepresentation of 176F and an underrepresentation of 176V in immune complex disease. In our study of 200 ethnically diverse SLE patients, this skewing was very apparent (Table II). Indeed, in patients with SLE and nephritis, the homozygous 176V was underrepresented by more than fourfold compared with those without nephritis. We recognize that these observations need confirmation in large independent populations, that further stratification by clinical phenotype and ethnicity may be insightful, and that studies of multiplex families will be informative. We also recognize that this association may result from linkage to a different gene at another locus. However, the biology of this polymorphism, its relevance to the pathophysiology of SLE, and the coincidence of FcyRIIa’s chromosomal location with a region of high interest in the microsatellite-based scanning of the genome in SLE patients (62, 63), all make FcyRIIa a likely gene for SLE disease risk.

Based on the biology of the FcyRIIa-176F/V polymor-
phism, one can imagine that it could influence many antibody-mediated responses involving IgG1 and IgG3. Since FcγRIIa is expressed on NK cells, mononuclear phagocytes, and renal mesangial cells, host defense against viral, bacterial, and other pathogens could be affected. Antibody-mediated immune surveillance could be altered as well as the interaction with immune complexes. Furthermore, the therapeutic response to intravenous gammaglobulin might vary in accordance with the FcγRIIa-176F/V polymorphism. Indeed, characterization of Fcγ receptor genotypes, in conjunction with other properties of the humoral immune response such as antibody subclass and complement status, may provide essential insights into vaccine effectiveness and disease risk.

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