FcγRIIA Alleles Are Heritable Risk Factors for Lupus Nephritis in African Americans

Jane E. Salmon,* Sean Millard,* Leah A. Schachter,* Frank C. Arnett,‡ Ellen M. Ginzler,§ Mark F. Gourley,‖ Rosalind Ramsey-Goldman,¶ Margaret G.E. Peterson,* and Robert P. Kimberly*

**Department of Medicine, The Hospital for Special Surgery and The New York Hospital, Graduate Program in Immunology, The Cornell University Medical College, New York 10021; ‡Department of Medicine, University of Texas Health Science Center, Houston, Texas 77225; §Department of Medicine, State University of New York Health Science Center, Brooklyn, New York 11203; ‖National Institutes of Health, National Institute of Arthritis, Musculoskeletal and Skin Diseases, Bethesda, Maryland 20892; and ¶Department of Medicine, Northwestern University School of Medicine, Chicago, Illinois 60611*

Abstract

Allelic variants of FcγR confer distinct phagocytic capacities providing a mechanism for heritable susceptibility to immune complex disease. Human FcγRIIa has two codominantly expressed alleles, R131 and H131, which differ substantially in their ability to ligate human IgG2. The FcγRIIa-H131 is the only human FcγR which recognizes IgG2 efficiently and optimal IgG2 handling occurs only in the homozygous state. Therefore, since immune complex clearance is essential in SLE, we hypothesized that FcγRIIA genes are important disease susceptibility factors for SLE, particularly lupus nephritis. In a two-stage cross-sectional study, we compared the distribution of FcγRIIA alleles in African Americans with SLE to that in African American non-SLE controls. A pilot study of 43 SLE patients and 39 controls demonstrated a skewed distribution of FcγRIIA alleles, with only 9% of SLE patients homozygous for FcγRIIa-H131 compared with 36% of controls (odds ratio, 0.18; 95% CI, 0.05–0.69, $P = 0.009$). This was confirmed with a multicenter study of 214 SLE patients and 100 non-SLE controls. The altered distribution of FcγRIIA alleles was most striking in lupus nephritis. Trend analysis of the genotype distribution showed a highly significant decrease in FcγRIIA-H131 as the likelihood for lupus nephritis increased ($P = 0.0004$) consistent with a protective effect of the FcγRIIA-H131 gene. The skewing in the distribution of FcγRIIA alleles identifies this gene as a risk factor with pathophysiologic importance for the SLE diathesis in African Americans. (*J. Clin. Invest.* 1996. 97:1348–1354.) Key words: Fcγ receptors • immunoglobulin • nephritis • phagocytes • systemic lupus erythematosus

Introduction

Systemic lupus erythematosus (SLE), the prototype human immune complex disease, is characterized by tissue deposition of circulating antigen-antibody complexes leading to release of inflammatory mediators, influx of inflammatory cells, and clinically apparent disease, most prominently glomerulonephritis (1). The efficiency of the mononuclear phagocyte system clearance of circulating immune complexes depends upon the function of receptors which recognize the Fc portion of Ig (FcγR)[1] and receptors for complement. Through in vivo and in vitro studies with SLE patients, it is clear that inherited and acquired components of Fcγ receptor-dependent dysfunction may contribute to disease susceptibility and mechanisms of tissue injury (2–7).

Human receptors for IgG are diverse in structure and function. There are three families of FcγR (FcγRI, FcγRII, and FcγRIII), each containing multiple distinct genes and alternative splicing variants (reviewed in references 8–11). Additionally, there are allelic variants of FcγR which confer distinct functional capacities to phagocytes (12, 13). For example, FcγRIIa has two codominantly expressed alleles, R131 and H131 (previously known as HR and LR), which differ at amino acid position 131 and differ substantially in their ability to ligate human IgG2 and IgG3 (13–18). In fact, FcγRIIa-H131 is the only human FcγR which recognizes IgG2 efficiently. Even in the context of a polyclonal IgG opsonin, FcγRIIA alleles affect the ability of phagocytes to bind and internalize IgG-opsonized particles indicating that immune complexes containing IgG2 in combination with other IgG subclasses may be handled differentially according to host FcγRIIA genotype (13). Evidence that allelic variants of FcγR confer distinct functional capacities to phagocytes provides a mechanism for heritable differences of FcγR.

FcγRIIa is an important receptor mediating phagocytic function on monocytes, macrophages, and neutrophils (19, 20). As the only human FcγR with the potential to efficiently bind human IgG2, an IgG subclass which does not activate complement-dependent mechanisms effectively, FcγRIIA-H131 is essential for handling IgG2 immune complexes. Because autoantibodies of IgG2 subclass may play an important role in the pathogenesis of proliferative glomerulonephritis in SLE (21, 22), we explored the possibility that FcγRIIA alleles may be

Address correspondence to Jane E. Salmon, The Hospital for Special Surgery, 535 East 70th Street, New York, NY 10021; Phone: 212-606-1422; FAX: 212-717-1192.

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1. *Abbreviations used in this paper:* CLR, collagen-like region; E-hIgG2, erythrocyte coated with human IgG2; FcγR, receptors for Fc portion of IgG in human cells; FcγRIIa, a 40-kD FcγR expressed on human mononuclear phagocytes and neutrophils; FcγRIIA, the gene encoding for FcγRIIa; PE, phycoerythrin; PMN, neutrophils.

inherited susceptibility factors in SLE and particularly in lupus nephritis.

For this study, African Americans were selected because of the increased incidence and severity of SLE in this population (23). To test the hypothesis that FcγRIIA alleles might influence risk for SLE or organ involvement, particularly nephritis, we compared distribution of FcγRIIA alleles in African Americans with SLE to that of non-SLE African American control subjects.

Methods

Study subjects. Patients meeting the revised American College of Rheumatology criteria for SLE (24) were recruited along with non-SLE control subjects. All subjects were African American. In the pilot stage of the study, 43 patients and 39 controls were recruited from the University of Texas Health Science Center, Houston, TX. In the second stage, subjects from four other medical centers in different regions of the United States were studied (Hospital for Special Surgery-Cornell University Medical Center, New York, NY; SUNY-Health Science Center, Brooklyn, NY; Northwestern University School of Medicine, Chicago, IL; National Institutes of Health (NIH), Bethesda, MD). The number of non-SLE controls from each center was related proportionally to that center's share of the SLE sample. In the two New York centers, only individuals with four black grandparents were recruited. In the other centers, patients and controls who self-declared themselves as African American were recruited. SLE patients were classified as having nephritis if they fulfilled American College of Rheumatology criteria for renal involvement (persistent proteinuria $>$ 500 mg/24 h [or $>$ 3+] or cellular casts) (24). The study was approved by the Institutional Review Board of each participating center.

FcγRIIA phenotyping and genotyping. Phenotyping of donors for the R131 and H131 alleles of FcγRIIa was performed by quantitative flow cytometry of peripheral blood neutrophils and monocytes using mAbs 41.H16 (recognizing FcγRIIa-R131) and IV.3 (recognizing both FcγRIIa alleles) as previously described (13, 25). For genotyping, DNA was isolated from peripheral blood (Puregene kit; Gentra Systems, Minneapolis, MN). A 1-kb portion of the FcγRIIA gene containing exon 4, parts of exon 5, and the intervening intron, was amplified by PCR in a gene-specific fashion using primers described by Osborne et al. (26). Exon 4 encodes the second extracellular domain of FcγRIIA which contains the functionally important polymorphism resulting from a single base substitution (A or G) at nucleotide 494. This encodes amino acid 131 in second extracellular domain which determines affinity for specific IgG subclasses. Histidine is in the H131 allele variant (494A) which is characterized by a high affinity for human IgG2 and a low affinity for murine IgG1, whereas arginine is in the R131 allele variant (494G) which has the opposite binding properties (13–17). The PCR products were denatured and applied to a Hybond-N in duplicate using a Bio-Dot apparatus (Bio-Rad Laboratories, Hercules, CA). The blots were probed with allele-specific oligonucleotides 3′-end labeled with digoxigenin, immunodetected with an alkaline phosphatase–conjugated antidigoxigenin antibody and visualized with a colormetric substrate system (Boehringer-Mannheim Biochemicals, Indianapolis, IN) as described by Osborne et al. (26). In 22 of 22 subjects tested, phenotypes determined by quantitative flow cytometry were confirmed to be concordant with genotypes.

Assay of FcγRIIa-mediated phagocytic function. Fresh anticoagulated human peripheral blood was separated by centrifugation through a discontinuous two step Ficoll-Hypaque gradient (12). Neutrophils (PMN) were isolated from the lower interface and washed with HBSS. Erythrocyte target particles were coupled to human IgG2 myeloma protein by a biotin-avidin technique (E-hIgG2) and used as probes of FcγRIIa-specific internalization (27). Density of opsonization of E-hIgG2 was determined by flow cytometry as previously described (13, 27). Cells were stained with phycoerythrin (PE)-conjugated rabbit anti–human IgG $F(ab')_2$ (Tago Immunochemicals, Burlingame, CA) and cell-associated immunofluorescence was quantitated.

Quantitation of phagocytosis by PMN was performed as previously described (13). PMN (100 μl at 5×10^6-ml) were combined with E-hIgG2 (125 μl at 1×10^9 E/ml), centrifuged at 44 *g* for 3 min, and then incubated at 37°C for 15 min to allow for maximum internalization. After hypotonic lysis of noninternalized E, phagocytosis was quantitated by light microscopy. At least 400 cells per slide were counted in duplicate without knowledge of the donor FcγRIIA genotype. The data are expressed as phagocytic index (number of ingested erythrocytes per 100 phagocytes). In the FcγRIIa blocking experiments, the PMN were preincubated with IV.3 Fab for 15 min and the mAb was present throughout the assay of phagocytosis.

Quantitation of FcγRIIa expression. Mononuclear cells were isolated from fresh anticoagulated blood by centrifugation through a discontinuous Ficoll-Hypaque gradient (5). For each experiment, monocytes from SLE patients and at least one non-SLE control were simultaneously stained and analyzed. Fresh leukocytes were incubated with saturating amounts of both anti-CD14 mAb MO2 (murine IgM) directly conjugated to FITC (Coulter Clone, Miami, FL) and anti-FcγRII (CD32) mAb IV.3 (murine IgG2b), followed by incubation with PE-conjugated goat anti–mouse IgG $F(ab')_2$ (Tago Immunochemicals). Parallel samples were stained with directly conjugated isotype controls for each mAb (murine IgM-FITC [Sigma Chemical Co., St. Louis, MO] and murine IgG1-PE [Becton Dickinson & Co., Mountainview, CA], respectively). Cell-associated immunofluorescence was quantitated on a Cytofluorograf IIS with a 2151 computer as previously described (5). For each experiment, the instrument was calibrated with quantitative FITC and PE microbead standards (Flow Cytometry Standards Corp., Research Triangle Park, NC) to allow for assessment of both absolute and relative levels of immunofluorescence. Blood monocytes were identified by characteristic forward and right angle scatter and by MO2-FITC immunofluorescence. The intensity of IV.3-PE fluorescence of the MO2-positive population was recorded and converted to mean channel linear fluorescent units.

Statistical analysis. For assessment of the relative phagocytic capacity of individuals with each of the three different FcγRIIA genotypes, all experiments were performed in a matched-triplet design. Accordingly, in each experiment neutrophils from donors of all three genotypes were studied simultaneously. Phagocytic capacities of the different FcγRIIA genotypes were compared using ANOVA with contrasts between adjacent groups (R131/R131 vs R131/H131 and R131/H131 vs H131/H131). A probability of 0.05 was used to reject the null hypothesis that there was no difference among the genotypes.

The distribution of FcγRIIA genotypes (R131/R131, R131/H131, and H131/H131) in SLE and non-SLE controls was compared by the chi-square test (3×2 contingency table). A probability of 0.05 (2-tailed) was used to reject the null hypothesis that there is no difference in the distribution of genotypes between the groups. To compare the frequency of FcγRIIA-H131 homozygosity (the hypothesized protective genotype) in the two groups, the chi-square test was also used (2×2 contingency table: H131/H131 vs R131/R131 and R131/H131). The odds ratios and 95% confidence intervals were calculated to provide an estimate of the risk of homozygosity for FcγRIIA-H131 in the SLE patients compared with non-SLE controls. Similar analyses were used to compare lupus nephritis patients with the non-SLE controls. A contingency table test for trend (Jonckeere-Terpstra Test) was used to evaluate the association of FcγRIIA genotype distribution with disease manifestations (lupus nephritis vs non-nephritis vs non-SLE) (28). No correction has been made for multiple comparisons except where results are noted for contrasts.

Results

The effect of R131/H131 heterozygosity in FcγRIIa function. The R131-H131 polymorphism is codominantly expressed with

the total number of FcγRIIa receptors on the surface of phagocytes similar among individuals (12, 13, 25). Using homozygotes, we and others have shown that the FcγRIIA-H131 is the only FcγR which recognizes human IgG2, while in R131 homozygotes there is minimal binding of IgG2 model immune complexes (13). To establish the functional phenotype of the heterozygous state in which half of the receptors on the phagocyte reflect the FcγRIIA-R131 gene product and half the FcγRIIA-H131 gene product, we took two approaches. First, we developed a model to study the internalization of human IgG2 by PMN with half the number of surface FcγRIIa-H131 molecules. Neutrophils from homozygous FcγRIIA-H131 donors were treated with mAb against the FcγRIIa ligand binding site (IV.3 Fab fragments) at a concentration sufficient to block 50% of the FcγRIIa (0.1 μg/ml) and 100% of FcγRIIa (1 μg/ml), as determined by flow cytometry. There was a significant difference in internalization of erythrocytes coupled to IgG2 for the three conditions (ANOVA F = 45.8, DF = 2,10, $P < 0.0001$; Contrasts for IV.3 Fab treatments: 1 μg/ml vs 0.1 μg/ml $P < 0.05$; 0.1 μg/ml vs 0 μg/ml $P < 0.05$). Internalization of erythrocytes coupled to human IgG2 was inhibited by 58±19% in the cells with half the number of available FcγRIIa and inhibited by 94±9% with FcγRIIa completely blocked suggesting that R131/H131 heterozygotes would have an intermediate capacity for handling IgG2 as compared to the homozygous states.

As a second approach, we directly measured phagocytosis of E-hIgG2 by PMN from normal donors homozygous or heterozygous for R131 and H131. In a series of experiments performed in matched-triplet design, donors of each of the three genotypes were studied simultaneously. The results, shown in Fig. 1, demonstrate that heterozygotes have a distinct phenotype. They confirm that heterozygotes have an intermediate capacity to recognize IgG2 as predicted from the FcγRIIa blocking experiments. These data demonstrate that the FcγRIIA-R131 gene product is associated with deficient IgG2 handling in both homozygotes and heterozygotes, whereas the FcγRIIA-H131 gene product provides optimal IgG2 handling only in the homozygous state. Therefore, all non-FcγRIIA-H131 homozygotes have potentially decreased efficiency of immune complex clearance and thus increased risk for immune complex deposition.

Pilot study of FcγRIIA allele distribution. In the pilot study to test our hypothesis, we compared the distribution of FcγRIIA alleles between 43 African American SLE patients and 39 African American non-SLE controls from Houston, TX. FcγRIIA genotypes of patients and non-SLE controls were determined by PCR amplification of genomic DNA and allele specific probes. There was a significant skewing in the distribution of the three genotypes between the two groups (3×2 contingency table, $\chi^2 = 8.45$, $P = 0.015$) (Table I). In the SLE patients there was a relative overrepresentation of the FcγRIIA-R131 allele and corresponding reduction in the FcγRIIA-H131 allele (Table I). Homozygosity for the FcγRIIA-H131 allele was found in only 9% of the 43 SLE patients, compared to 36% in the 49 non-SLE control subjects (odds ratio: 0.18; 95 percent confidence interval, 0.05 to 0.69, $\chi^2 = 6.96$, $P = 0.009$) suggesting a protective effect of the FcγRIIA-H131 gene with a decreased risk for SLE.

Multicenter study of FcγRIIA allele distribution in SLE. To confirm the association of FcγRIIA-R131 with SLE and the potentially protective effects of FcγRIIA-H131 homozygosity, we performed a larger cross-sectional study with subjects from four centers. For this second stage, 214 African

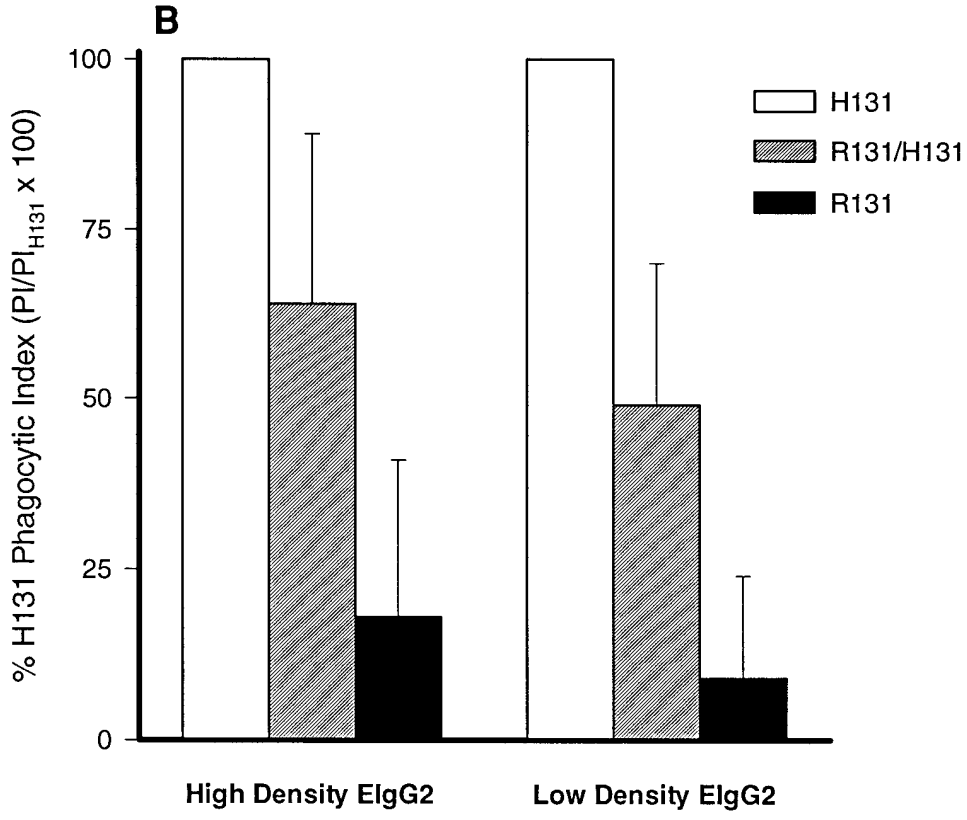

Figure 1. The effect of R131/H131 heterozygosity in FcγRIIa-mediated handling of human IgG2. PMN from normal donors homozygous for the FcγRIIA-H131 or FcγRIIA-R131 alleles or heterozygous were studied simultaneously for internalization of E-hIgG2 (opsonized at two densities). Phagocytic index (number of ingested erythrocytes/100 phagocytes) was quantitated by light microscopy. Data are presented as percent H131/H131 phagocytic index (PI/$PI_{H131/H131} \times 100$). The results reflect mean±SD for six experiments. In each of six experiments, internalization of both E-IgG2 probes was greatest for the H131/H131 subjects, followed by heterozygotes, and minimal for R131/R131 (ANOVA F = 26, DF = 5,25, $P < 0.0001$; contrasts at high density E-hIgG2: H131/H131 vs R131/H131 $P = 0.02$; R131/H131 vs R131/R131 $P = 0.005$; contrasts at low density E-hIgG2: H131/H131 vs R131/H131 $P = 0.002$; R131/H131 vs R131/R131 $P = 0.009$).

Table I. Pilot Study: Distribution of FcγRIIA Alleles in African American SLE Patients and Non-SLE Controls

	SLE patients	Non-SLE controls
	n = 43	*n = 39*
Genotype*		
No. of subjects (% of group)		
R131/R131	16 (37)	10 (26)
R131/H131	23 (53)	15 (38)
H131/H131	4 (9)	14 (36)
Allelic frequency (%)‡		
R131	64	45
H131	36	55

*Odds ratio for the risk of SLE in FcγRIIA-H131/H131 homozygotes compared with H131/R131 and R131/R131: 0.18 (95% C.I. 0.05-0.69); $\chi^2 = 6.96$, $P < 0.009$; ‡$\chi^2 = 5.27$, $P < 0.022$.

American SLE patients were recruited from Hospital for Special Surgery-Cornell University Medical College, New York; SUNY-Health Science Center, Brooklyn, NY; Northwestern University School of Medicine, Chicago, IL; National Institutes of Health, Bethesda, MD (60, 88, 40, and 26 patients, respectively). Each center provided non-SLE African American control subjects in proportion to that center's share of SLE patients, with a total of 100 non-SLE controls recruited from these centers. The distribution of FcγRIIA alleles in the non-SLE control populations from the centers which recruited only individuals with four black grandparents (Hospital for Special Surgery, New York and SUNY-Health Science Center, Brooklyn, NY) was similar to that of the centers which recruited individuals who were self-declared African Americans.

In this multicenter study, the skewing in the distribution of FcγRIIA genotypes in SLE patients compared to the non-SLE controls was confirmed (3 × 2 contingency table, $\chi^2 = 7.77$, $P = 0.021$) (Table II). Similar to the pilot study, in the SLE patients there was an increased frequency of the FcγRIIA-R131 allele and corresponding underrepresentation in FcγRIIA-H131 ($\chi^2 = 7.55$, $P = 0.006$, Table II). Of the 214 SLE patients, 37% were homozygous for FcγRIIA-R131 compared with 23% of 100 non-SLE controls. In contrast, the number of FcγRIIA-H131 homozygotes was again reduced suggesting a protective effect of this genotype. Thus, this second series of patients confirmed the hypothesis tested in the initial pilot sample.

Because a diminution in the number of FcγRIIa-H131 receptors results in a clearly detectable decrease in FcγRIIa-specific function (Fig. 1), it was necessary to exclude the possibility of alterations in receptor expression in SLE patients. The relative expression of FcγRIIa on peripheral blood monocytes, determined by two-color flow cytometry, was similar for SLE patients ($n = 25$) and non-SLE controls ($n = 14$; 132±27 mean linear channel fluorescence vs 152±19, P = NS, respectively). Therefore, we could compare the distribution of alleles in the two groups without the confounding variable of an acquired expression polymorphism.

Multicenter study of FcγRIIA allele distribution in lupus nephritis. Patients with lupus nephritis have the most profound defect in immune complex clearance. This defect is not a fixed property, but rather a dynamic deficit which correlates with disease activity (3–5). Disease-induced dysfunction superimposed upon inherited polymorphisms of FcγRIIA with decreased functional capacity, such as FcγRIIA-R131 homozygosity, may provide the milieu for the development of immune complex glomerular deposition and lupus nephritis. In contrast, FcγRIIA-H131 homozygosity may be protective in this situation.

To determine whether FcγRIIA alleles are risk factors for SLE in general or relate specifically to the development of nephritis, we separated the 214 patients into two groups: those with ACR criteria for nephritis ($n = 103$) and those without ($n = 111$). The percent of patients with nephritis from New York, Chicago, and NIH was 44, 38, and 92%, respectively. Interestingly, among the NIH patients ($n = 26$) there were no FcγRIIA-H131 homozygotes. Evaluation of the entire multicenter group demonstrated a strong association between FcγRIIA genotypes and renal disease in SLE. The skewing in the distribution of three FcγRIIA genotypes was most profound in the nephritis group compared to non-SLE controls (3 × 2 contingency table, $\chi^2 = 11.83$, $P = 0.003$) (Table III). This subset of patients had the greatest increase in the frequency of the FcγRIIA-R131 allele and greatest reduction in

Table II. Multicenter Study: Distribution of FcγRIIA Alleles in African American SLE Patients and Non-SLE Controls

	SLE patients	Non-SLE controls
	n = 214	*n = 100*
Genotype*		
No. of subjects (% of group)		
R131/R131	80 (37)	23 (23)
R131/H131	97 (45)	50 (50)
H131/H131	37 (17)	27 (27)
Allelic frequency (%)‡		
R131	60	48
H131	40	52

*Odds ratio for the risk of SLE in FcγRIIA-H131/H131 homozygotes compared with H131/R131 and R131/R131: 0.57 (95% C.I. 0.31-1.03); $\chi^2 = 3.38$, $P < 0.066$; ‡$\chi^2 = 7.55$, $P < 0.006$.

Table III. Multicenter Study: Distribution of FcγRIIA Alleles in African American Lupus Nephritis Patients and Non-SLE Controls

	SLE patients	Non-SLE controls
	n = 103	*n = 100*
Genotype*		
No. of subjects (% of group)		
R131/R131	43 (42)	23 (23)
R131/H131	48 (47)	50 (50)
H131/H131	12 (12)	27 (27)
Allelic frequency (%)‡		
R131	65	48
H131	35	52

*Odds ratio for the risk of SLE in FcγRIIA-H131/H131 homozygotes compared with H131/R131 and R131/R131: 0.36 (95% C.I. 0.16-0.80); $\chi^2 = 6.75$, $P < 0.01$; ‡$\chi^2 = 11.33$, $P < 0.0008$.

FcγRIIA-H131 (χ^2 = 11.33, P = 0.0008; Table III). The homozygous FcγRIIA-R131 genotype was particularly enriched in the nephritis group (42%) while FcγRIIA-H131 homozygosity was uncommon (12%). Analysis of the trend of the distribution of the three FcγRIIA genotypes across the three groups of study subjects (non-SLE, non-nephritis SLE, and SLE nephritis) showed a highly significant decrease in FcγRIIA-H131 homozygosity as the prevalence of lupus nephritis increased (Jonckheere-Terpstra Test L JT(x) = −3.351, P = 0.0004). There was a symmetrical trend for increased FcγRIIA-R131 homozygosity with increased prevalence of renal involvement.

Because it appeared that FcγRIIA-H131 homozygosity was protective for renal disease among SLE patients, we measured FcγRIIa expression among the few FcγRIIA-H131 homozygotes with nephritis to exclude a decrease in monocyte receptor expression. We considered the possibility that some of these individuals were heterozygous for FcγRIIA-H131 and a null allele of FcγRIIA and thus, expressed half a complement of FcγRIIa-H131. Two color flow cytometric analysis demonstrated that FcγRIIa on monocytes from FcγRIIA-H131/H131 nephritis patients (n = 12) was similar to that for non-SLE controls (n = 14) (143±25 mean linear channel fluorescence vs 153±30, respectively) providing no evidence that a null allele was leading to deficient immune complex handling. Taken together, these data and the FcγRIIA distribution data support a model of relative, but not absolute, protection from lupus nephritis in patients homozygous for FcγRIIA-H131.

Discussion

African Americans and Afro-Caribbeans are recognized as a group with greater prevalence and more severe SLE than Caucasians (23, 29). Though the basis for these differences is likely to reflect both genetic and environmental factors, recognition of the relative importance of inherited factors among various ethnic groups may provide insights into the clinical differences described for ethnically defined populations and enhance our understanding of the mechanisms of disease in SLE. In this study, we found an association between FcγRIIA alleles and lupus nephritis in African Americans. There was an increased frequency of FcγRIIA-R131 gene in the SLE patients. The skewing in FcγRIIA gene distribution was more marked in the nephritis group as compared with non-SLE controls (Tables II and III). In contrast, FcγRIIA-H131, the only human FcγR with the ability to efficiently recognize human IgG2, was correspondingly decreased in frequency in lupus patients. The H131/H131 genotype was uncommon among nephritis patients, suggesting that FcγRIIA-H131 plays a protective role in the homozygous state. This idea is supported by our in vitro experiments showing that the FcγRIIA-R131 gene product is characterized by deficient IgG2 handling in both homozygotes and heterozygotes (Fig. 1). Thus, it appears that FcγRIIA-R131/R131 homozygotes and R131/H131 heterozygotes have the potential for less efficient immune complex clearance and are at a greater risk for immune complex deposition.

Our findings suggest a model in which the inability to clear immune complexes by way of FcγR increases the risk of SLE and particularly SLE nephritis. Given the known properties of FcγRIIa, these findings suggest that IgG2-containing immune complexes are important in lupus nephritis. IgG autoantibodies to the collagen-like region (CLR) of C1q are found in SLE patients (30–32), especially young patients with severe disease (33), and specifically correlate with the presence of proliferative glomerulonephritis (30). The titer of anti-C1q CLR has been related to the quantity of subendothelial glomerular deposition (34). The observation that human autoantibodies to C1q CLR bind to immune complexes containing human C1q in glomeruli of experimental animals is further evidence for their involvement in renal lesions (35). In two independent series of SLE patients with anti-C1q CLR antibodies, IgG2 was the predominant or exclusive IgG subclass (21, 36). From the perspective of the phagocyte in the context of immune complex disease, the efficiency of clearance of an immune complex with predominantly IgG2 depends upon FcγRIIA genotype. Human IgG2 is efficiently recognized only by FcγRIIA-H131 and does not activate complement well, precluding complement-dependent clearance mechanisms. The relative protective role of the homozygous FcγRIIA-H131 state in handling IgG2 immune complexes is readily apparent under these conditions. It is recognized, however, that the evidence for the role of IgG2 in the pathogenesis of lupus nephritis, while supported by these findings, remains speculative. In the absence of IgG2-containing immune complexes one would expect to find a minimal effect of FcγRIIA genes. This analysis implies that the role of specific genetic risk factors may vary with the qualitative nature of the immune response. Indeed, in the context of IgG1- and IgG3-containing immune complexes other mechanisms altering immune complex handling, such as complement deficiencies, complement receptor dysfunction, or other Fcγ receptor dysfunction, might be important in disease pathogenesis (37). However, in vitro studies have shown that even in the context of a polyclonal IgG opsonin, FcγRIIA alleles affect the ability of phagocytes to internalize IgG-opsonized particles indicating that the handling of immune complexes containing IgG2 in combination with other subclasses may vary according to host genotype (13). Of course, we cannot categorically exclude an alternative explanation for the association of FcγRIIA alleles with lupus nephritis, that this gene may be in linkage disequilibrium with other nearby candidate genes on chromosome 1, such as those for FcγRI, FcγRIII, or γ-chain.

Our initial study was restricted to a single ethnic group because the distribution of FcγRIIA alleles varies with ethnicity. The relative importance of disease-related polymorphic genes, such as MHC class II, C4A, IgG heavy chain variable region, may also vary among groups (26, 38–44). Among Hispanics in Venezuela, a study of 41 patients also suggested an increased frequency of a phenotype containing at least one FcγRIIA-R131 gene (45). Interestingly, although in a preliminary study of 94 Dutch Caucasian SLE patients Gmelig-Meyling et al. showed an association between FcγRIIA-R131 and nephritis (46), we have not replicated this finding in our series of 262 American Caucasian SLE patients and 103 non-SLE controls from the five centers contributing to the current work (R131/131, 22% of nephritis vs 24% of non-SLE; H131/H131, 20% of nephritis vs 24% of non-SLE; χ^2 = 0.1 $P > 0.05$). The basis for the contrasting results in these distinct Caucasian populations is unclear. Perhaps, there are differences in factors which influence production of IgG2 immune complexes or differences in the frequency of other polymorphic FcγR, complement receptors, or complement components which participate in immune complex handling. There may also be synergistic interactions between susceptibility factors such that deficiencies in one sys-

tem may amplify the importance of the other. Alternatively, a strong disease susceptibility factor may obscure the effects of a coexisting disease susceptibility gene, as has been described for C4A deletion (43). As with murine models which show polygenic inheritance with threshold effects, we recognize the importance of considering multiple genetic markers within each ethnic group (47–49). Even in the context of a polygenic disease, our studies demonstrate a critical role of FcγR in the pathogenesis of SLE and indicate that alleles with distinct function may be important heritable disease susceptibility factors. Definition of the roles of different FcγR and complement receptors raises the possibility of identifying risk profiles for severe disease which may help select patients requiring more aggressive therapies.

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