Evaluation of Human FcγRIIA (CD32) and FcγRIIIB (CD16) Polymorphisms in Caucasians and African-Americans Using Salivary DNA

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Two classes of low-affinity receptors for the Fc region of immunoglobulin G (IgG) (FcγR) are constitutively expressed on resting human neutrophils. These receptors, termed FcγRIIA (CD32) and FcγRIIIB (CD16), display biallelic polymorphisms which have functional consequences with respect to binding and/or ingestion of targets opsonized by human IgG subclass antibodies. The H131-R131 polymorphism of CD32 influences binding of human IgG2 and, to a lesser extent, human IgG3 to neutrophils. The neutrophil antigen (NA1-NA2) polymorphism of CD16 influences the efficiency of phagocytosis of bacteria opsonized by human IgG1 and IgG3. These polymorphisms may influence host susceptibility to certain infectious and/or autoimmune diseases, prompting interest in the development of facile methods for determination of CD32 and CD16 genotype in various clinical settings. We previously reported that genomic DNA from saliva is a suitable alternative to DNA from blood in PCR-based analyses of CD32 and CD16 polymorphisms. In the present study, we utilized for the first time this salivary DNA-based methodology to define CD32 and CD16 genotypes in 271 Caucasian and 118 African-American subjects and to investigate possible linkage disequilibrium between certain CD32 and CD16 genotypes in these two ethnic groups. H131 and R131 gene frequencies were 0.45 and 0.55, respectively, among Caucasians and 0.59 among African-Americans. NA1 and NA2 gene frequencies were 0.38 and 0.62 among Caucasians and 0.39 and 0.61 among African-Americans. Since FcγRIIA and FcγRIIIB synergize in triggering neutrophils, we also assessed the frequency of different CD32 and CD16 genotype combinations in these two groups. In both groups, the R/R131-NA2/NA2 genotype combination was more common than the H/H131-NA1/NA1 combination (threelfold for Caucasians versus sevenfold for African-Americans). Whether individuals with the combined R/R131-NA2/NA2 genotype are at greater risk for development of infectious and/or autoimmune diseases requires further investigation, which can be conveniently performed using DNA from saliva rather than blood.

Membrane receptors for the Fc region of immunoglobulin G (IgG) (FcγR) provide an important link between the humoral and cellular elements of the immune system. Three main classes of leukocyte FcγR are currently recognized, including FcγRI (CD64), FcγRII (CD32), and FcγRIII (CD16). FcγRI is a high-affinity receptor capable of binding human IgG1, IgG3, and IgG4 in monomeric form. FcγRII and FcγRIII, on the other hand, are low-affinity receptors which bind IgG1 and IgG3 in complexed or aggregated form. Among FcγR, only the FcγRII class is capable of binding human IgG2 efficiently (41).

Each class of FcγR is encoded by multiple genes, all of which are located on the long arm of chromosome 1 (26). In addition, alternative RNA splicing results in the generation of multiple transcripts, including soluble and membrane-bound receptor forms. Circulating neutrophils, a key element of host defense against acute bacterial infection, constitutively express FcγRIIa, a 40-kDa integral membrane glycoprotein, as well as FcγRIIIb, a 50- to 80-kDa phosphatidylinositol-linked glycoprotein, the latter of which is numerically predominant on these cells (9, 17). Both of these receptors display genetically defined structural polymorphisms which affect phagocytosis of IgG-opsonized targets.

A biallelic polymorphism in the A gene encoding FcγRII results in the generation of two distinct allotypes whose structures differ at amino acid residues 27 and 131. Only the amino acid substitution at position 131 significantly affects the ligand binding affinity and specificity of FcγRIIa. The allotype containing histidine at position 131 (H131) binds human IgG2 efficiently, whereas the allotype containing arginine (R131) at this same position does not (3, 24, 32, 36, 45). FcγRIIa-H131 also binds human IgG3 more efficiently than does FcγRIIa-R131 (5, 24).

A second polymorphism, involving FcγRIIIB, is responsible for the biallelic neutrophil-specific antigen (NA1 and NA2) system (15). The NA1 and NA2 allotypes of FcγRIIIB differ by five nucleotides and four amino acids, with NA2 containing two additional N-linked glycosylation sites. These differences have been shown to influence the capacity of FcγRIIIB to interact with human IgG. Hence, neutrophils from individuals who are homozygous for the NA1 allele display greater phagocytosis of IgG-opsonized targets than do neutrophils from NA2-homozygous donors (30, 32). Both IgG1 and IgG3 antibodies appear to react more readily with the NA1 allotype than with the NA2 allotype (5).

Recent evidence suggests that certain FcγRIIA and/or FcγRIIIB allotypes may contribute to increased susceptibility to certain infectious or autoimmune diseases (4, 11, 34, 35). This has spawned interest in the development of rapid methods for determining FcγRIIA and FcγRIIIB genotypes in various clinical settings. The majority of techniques reported to
date have employed genomic DNA from peripheral blood in the performance of such analyses. We recently reported that DNA isolated from saliva is a satisfactory alternative to DNA from blood in PCR-based analyses of FcγRIIA and FcγRIIB genotype (43). To date, however, neither we nor other groups have employed salivary DNA to define CD32 and/or CD16 genotype in various ethnic groups. Hence, the focus of the present study was to employ salivary DNA to determine the distribution of FcγRIIA and FcγRIIB genotypes in a large population of Caucasian and African-American subjects. Moreover, inasmuch as FcγRIIA and FcγRIIB can function synergistically in triggering neutrophil responses (10, 31, 33, 40, 44), we considered the possibility that certain genotype combinations may be less favorable than others in supporting IgG-mediated neutrophil responses. Accordingly, we also compared the frequencies of different FcγRIIA and FcγRIIB genotype combinations in our Caucasian and African-American populations.

### MATERIALS AND METHODS

#### Subjects.

Whole-saliva samples were obtained from 118 unrelated African-American (43 male, 75 female) and 271 Caucasian (132 male, 139 female) adult subjects using a collection method described previously (43). All participants were randomly recruited, nonweighted volunteers, selected without regard to oral or general health status. Seventy-eight of the African-American samples were generously provided by Jonathan Korostoff, University of Pennsylvania, again without regard to health status. Informed written consent was obtained from each donor prior to sample acquisition. Caucasian and African-American subjects participating in this study confirmed (by self-reporting) that all four of their grandparents were of Caucasian and African-American descent, respectively. The saliva specimens were either stored at 4°C and DNA extracted within 2 h of isolation or stored at 70°C until processed.

#### Isolation of DNA from saliva.

DNA was isolated from saliva using a commercial DNA purification kit (QIAamp blood kit; Qiagen, Inc., Chatsworth, Calif.) and a commercial DNA purification kit (QIAamp blood kit; Qiagen, Inc., Chatsworth, Calif.) is a suitable alternative to DNA isolated from blood as a template for PCR-based analysis of FcγRIIA and FcγRIIB genotype, as described in detail elsewhere (43). We previously demonstrated that DNA isolated from whole blood DNA. Moreover, genotype results were concordant with those obtained using DNA polymerase. The NA1-specific amplification protocol, which amplifies a 142-bp product, included 1 cycle of 95°C for 5 min; followed by 30 cycles of 95°C for 30 s, 57°C for 30 s, and 72°C for 45 s; and then 72°C for 7 min to facilitate primer extension. In the NA2-specific PCR, which generates a 169-bp product, included 1 cycle of 95°C for 5 min; followed by 30 cycles of 95°C for 30 s, then 60°C for 30 s, and 72°C for 45 s; and then 72°C for 7 min to facilitate primer extension. The products of the two allele-specific PCR assays were resolved in a 2.5% agarose gel, stained with ethidium bromide, and visualized under UV illumination.

#### Statistical analyses.

The distributions of FcγRIIA (H131/H131, H131/R131, R131/R131) and FcγRIIB (NA1/NA1, NA1/NA2, NA2/NA2) genotypes among Caucasian and African-American subjects were compared using the chi-square test (contingency table analysis). Gene frequencies were compared to the Hardy-Weinberg equilibrium according to the method described by Smith.

### RESULTS

We previously demonstrated that DNA isolated from whole blood DNA. Moreover, genotype results were concordant with phenotype results obtained by flow cytometric analysis. The NA1-specific PCR assays included 54 ng (sample or genotype controls) of DNA, 200 μM dNTPs, 1.75 mM MgCl2, 0.5 μM sense and antisense primer, and 0.49 U of Expand enzyme in a volume of 18 μl. The NA1-specific PCR protocol, which generates a 169-bp product, included 1 cycle of 95°C for 5 min; followed by 30 cycles of 95°C for 30 s, then 60°C for 30 s, and 72°C for 45 s; and then 72°C for 7 min to facilitate primer extension. The products of the two allele-specific PCR assays were resolved in a 2.5% agarose gel, stained with ethidium bromide, and visualized under UV illumination.

#### Distribution of CD32 and CD16 genotypes in Caucasians and African-Americans.

The distributions of the H131 and

### Table 1. Distribution of FcγRIIA (CD32) and FcγRIIB (CD16) genotypes among Caucasian and African-American subjects

| Subject group | CD32a | CD16b |
|---------------|-------|-------|
|               | H/H131 | H/R131 | R/R131 | NA1/NA1 | NA1/NA2 | NA2/NA2 |
| African-Americans (n = 118) | 22 | 43 | 22 | 48 | 48 |
| % T* | 18.6 | 44.9 | 18.6 | 40.7 | 40.7 |
| Caucasians (n = 271) | 64 | 115 | 92 | 38 | 129 | 104 |
| % T | 23.6 | 42.4 | 33.9 | 14.0 | 47.6 | 38.4 |

* T, percentage of total subjects of indicated ethnicity.

b Distribution of CD32 genotypes did not differ significantly between Caucasian and African-American subjects (χ2 = 1.183, P = 0.255).

c Distribution of CD16 genotypes did not differ significantly between Caucasian and African-American subjects (χ2 = 2.166, P = 0.347).

### References

(43) and (44) We considered the possibility that certain genotype combinations may be less favorable than others in supporting IgG-mediated neutrophil responses. Accordingly, we also compared the frequencies of different FcγRIIA and FcγRIIB genotype combinations in our Caucasian and African-American populations.
R131 alleles of CD32 and the NA1 and NA2 alleles of CD16 among Caucasians and African-Americans are shown in Table 1. Frequencies of the H/H131, H/R131, and R/R131 genotypes among 118 African-Americans were 18.6, 44.9, and 36.4%, respectively. A similar distribution was observed among 271 Caucasian subjects. Despite a slight increase in the frequency of the H/H131 genotype in Caucasians compared with African-Americans (23.6 versus 18.6%, respectively), differences in the distribution of genotypes between the two ethnic groups were not significant (as determined by a chi-square test employing a 2 × 2 contingency table). In each ethnic group, the R/R131 genotype was more common than the H/H131 genotype.

We also examined the distribution of CD16 genotypes in these two ethnic groups. Once again, no significant differences were observed (as determined by chi-square test) between Caucasians and African-Americans. In both groups, the NA2/NA2 genotype was 2-fold more common than the NA1/NA1 genotype (40.7 versus 18.6%, respectively, for African-Americans, and 38.4 versus 14.0% for Caucasians).

Several methods have been used to determine the distribution of CD32 and/or CD16 genotypes in various ethnic groups, including Caucasians and African-Americans. These methods included DNA sequence analysis, single-stranded conformational polymorphism, PCR-based analysis using allele-specific oligonucleotide probes, and PCR-based analysis using allele-specific primers (2, 23, 28, 34), in each instance employing genomic DNA obtained from peripheral blood. We compared CD32 and CD16 genotype results, obtained using salivary DNA, with those reported previously using peripheral blood DNA. As indicated in Table 2, reported frequencies of the H131 and R131 alleles of CD32 among Caucasian subjects exhibited only minor variation (Table 3) among three studies, one of which (6) included a population of German descent. Similar NA1 and NA2 gene frequencies (0.365 and 0.635, respectively) were reported in a Danish population (39). Somewhat greater, albeit modest, variation was observed in the NA1 and NA2 gene frequencies reported herein and those previously reported by Hessner and coworkers with respect to subjects of African-American descent (13). For both the Caucasians (P = 0.8516), and African-Americans (P = 0.1337), the Hardy-Weinberg equilibrium was met.

**TABLE 2. Distribution of FcγRIIA genotypes in Caucasians and African-Americans**

| Ethnic group       | No. of subjects with FcγRIIA genotype (%) | Gene frequency |
|--------------------|------------------------------------------|----------------|
|                    | H/H131 | H/R131 | R/R131 | H131 | R131 |
| Caucasians         |        |        |        |      |      |
| Present study (n = 271) | 64 (23.6) | 115 (42.4) | 92 (33.9) | 0.45 | 0.55 |
| Osborne et al. (23) (n = 35) | 8 (23.0) | 19 (54.0) | 8 (23.0) | 0.50 | 0.50 |
| Reilly et al. (28) (n = 47) | 14 (30.0) | 24 (51.0) | 9 (19.0) | 0.55 | 0.45 |
| Botto et al. (2) (n = 259) | 57 (22.0) | 120 (46.3) | 82 (31.7) | 0.45 | 0.55 |
| African-Americans  |        |        |        |      |      |
| Present study (n = 118) | 22 (18.6) | 53 (44.9) | 43 (36.4) | 0.41 | 0.59 |
| Salmon et al. (34) (n = 100) | 27 (27.0) | 50 (50.0) | 23 (23.0) | 0.52 | 0.48 |
| Reilly et al. (28) (n = 50) | 7 (14.0) | 30 (60.0) | 13 (26.0) | 0.44 | 0.56 |

**TABLE 3. Distribution of FcγRIIB genotypes in Caucasians and African-Americans**

| Ethnic group       | No. of subjects with FcγRIIB genotype (%) | Gene frequency |
|--------------------|------------------------------------------|----------------|
|                    | NA1/NA1 | NA1/NA2 | NA2/NA2 | NA1 | NA2 |
| Caucasians         |        |        |        |      |      |
| Present study (n = 271) | 38 (14.0) | 129 (47.6) | 104 (38.4) | 0.38 | 0.62 |
| Hessner et al. (13) (n = 90) | 10 (11.0) | 46 (51.0) | 34 (38.0) | 0.37 | 0.63 |
| Bux et al. (6) (n = 160) | 19 (11.9) | 73 (45.6) | 68 (42.5) | 0.35 | 0.65 |
| African-Americans  |        |        |        |      |      |
| Present study (n = 118) | 22 (18.6) | 48 (40.7) | 48 (40.7) | 0.39 | 0.61 |
| Hessner et al. (13) (n = 99) | 16 (16.0) | 30 (30.0) | 53 (54.0) | 0.31 | 0.69 |

Distribution of combined CD32-CD16 genotypes in Caucasians and African-Americans. Both CD32 and CD16 play a role in phagocytosis of IgG-opsonized targets by human neutrophils and may act synergistically in promoting neutrophil function. Neutrophils obtained from individuals who are homozygous for the H131 allele of CD32 manifest greater phagocytic activity toward IgG2- and IgG3-opsonized targets than do neutrophils from individuals who are homozygous for the R131 allele. Similarly, neutrophils from donors who are homozygous with respect to the NA1 allele of CD16 display greater phagocytosis of IgG1- and IgG3-opsonized targets than do neutrophils from NA2 homozygous donors. It might be anticipated, therefore, that certain combinations of CD32 and CD16 genotypes may be more favorable than others in supporting phagocytosis of IgG-coated targets. This prompted us to examine the

**TABLE 3. Distribution of FcγRIIB genotypes in Caucasians and African-Americans**

| Ethnic group       | No. of subjects with FcγRIIB genotype (%) | Gene frequency |
|--------------------|------------------------------------------|----------------|
|                    | NA1/NA1 | NA1/NA2 | NA2/NA2 | NA1 | NA2 |
| Caucasians         |        |        |        |      |      |
| Present study (n = 271) | 38 (14.0) | 129 (47.6) | 104 (38.4) | 0.38 | 0.62 |
| Hessner et al. (13) (n = 90) | 10 (11.0) | 46 (51.0) | 34 (38.0) | 0.37 | 0.63 |
| Bux et al. (6) (n = 160) | 19 (11.9) | 73 (45.6) | 68 (42.5) | 0.35 | 0.65 |
| African-Americans  |        |        |        |      |      |
| Present study (n = 118) | 22 (18.6) | 48 (40.7) | 48 (40.7) | 0.39 | 0.61 |
| Hessner et al. (13) (n = 99) | 16 (16.0) | 30 (30.0) | 53 (54.0) | 0.31 | 0.69 |
distribution of CD32-CD16 genotype combinations in our Caucasian and African-American populations.

The nine possible combinations of CD32-CD16 genotypes were similarly distributed among Caucasians and African-Americans (Fig. 1). Consistent with the low frequency of the NA1 allele in both ethnic groups (Table 3), few NA1/NA1 homozygous individuals were represented, regardless of CD32 genotype. The majority of NA1 homozygotes identified in each group (68% of African-Americans and 50% of Caucasians) were heterozygous with respect to the H131 and R131 alleles of CD32. Among the least common genotypes found in either ethnic group was the combination of H/H131-NA1/NA1, which is considered the “most favorable” genotype on the basis of functional studies. In contrast, the “least favorable” combination of R/R131-NA2/NA2 was three- to sevenfold more common than the H/H131-NA1/NA1 combination in Caucasians and African-Americans, respectively. No statistically significant associations were found for either Caucasians or African-Americans.

**DISCUSSION**

Efficient phagocytosis and intracellular killing of bacteria typically require opsonization of the organism by specific IgG antibodies and complement, the former of which are recognized by FcγR expressed on the leukocyte membrane. Allelic polymorphisms of the two classes of low-affinity FcγR constitutively expressed on neutrophils have been shown to influence the ability of these receptors to bind human IgG subclass antibodies. Neutrophils from individuals who are homozygous for the H131 allele of FcγRIIA ingest and kill IgG2-opsonized bacteria more efficiently than do neutrophils from individuals homozygous for the R131 allele (3, 29, 32, 36, 46, 47). It has been suggested that the H131-R131 polymorphism may influence susceptibility to certain types of bacterial infection, particularly those in which IgG2 antibodies are thought to play an important protective role (42, 47). Indeed, FcγRIIA genotype has been reported to be associated with susceptibility to, and severity of, recurrent upper respiratory tract and meningococcal infections (4, 11, 25, 35). FcγRIIA genotype may also be a determinant of susceptibility to certain types of autoimmune disease, notably, systemic lupus erythematosus, at least in some ethnic groups (2, 21, 34, 38).

Functional differences have also been noted with respect to the NA1 and NA2 alleles of FcγRIIB, particularly as regards phagocytosis of targets opsonized by IgG1 and IgG3 subclass antibodies (5, 30, 32, 33). Neutrophils from NA1-homozygous individuals manifest greater phagocytic activity toward IgG-coated targets than do neutrophils from NA2 homozygotes, despite comparable ligand binding. Hence, the NA1-NA2 polymorphism appears to affect phagocytosis of IgG-coated targets via a ligand-independent mechanism (7). The extent to which the NA1-NA2 polymorphism of FcγRIIB influences susceptibility to infection is unclear. However, a recent report suggests that the NA2/NA2 genotype is associated with a higher rate of disease recurrence in patients with adult periodontitis (18). The NA2 allele also appears to be a risk factor for de-
development of serious gastrointestinal or genitourinary complications in patients with chronic granulomatous disease (12). Finally, in a recent study of patients with multiple sclerosis, Myhr and coworkers (19) observed that patients homozygous for the NA2 allele manifested a more benign course of disease than did patients who were heterozygous or homozygous for the NA2 allele.

Evidence linking allelic polymorphisms of FcγRIIA and FcγRIIIB with increased susceptibility to infectious and/or autoimmune disease has stimulated interest in the development of methods for determining FcγR genotype in various patient populations. A number of methods for determining either FcγRIIA (16, 23, 28) or FcγRIIIB (6, 13) genotype have been reported, all of which employ DNA isolated from peripheral blood. We recently reported that DNA isolated from whole saliva can be utilized in place of DNA extracted from blood in PCR-based analyses of FcγRIIA and FcγRIIIB polymorphisms (43). In the present study, we employed salivary DNA to determine FcγRIIA and FcγRIIIB genotype in a population of Caucasian and African-American subjects. In both groups, gene frequencies for the H131 and R131 alleles of FcγRIIA, as well as the NA1 and NA2 alleles of FcγRIIIB, were similar to those reported previously (Tables 2 and 3). These results offer further support for the use of salivary DNA in the performance of such analyses.

Recent evidence suggests that FcγRIIA and FcγRIIIB may interact synergistically in triggering IgG-mediated neutrophil responses (40). Cross-linking of FcγRIIIB with F(ab')2 fragments of monoclonal antibody 3G8 (specific for FcγRIIib) enhances FcγRIIA-mediated phagocytosis (31). Moreover, simultaneously engaging both FcγRIIA and FcγRIIIB by means of receptor-specific monoclonal antibodies bound to erythrocytes produces a greater phagocytic response than is seen following ligation of either receptor alone, even when the total number of receptors ligated is equal (10). Conversely, blocking FcγRIIA through pretreatment with monoclonal antibodies results in depression of FcγRIIIB-mediated calcium fluxes, respiratory burst activity, and degranulation (1, 14, 20).

If FcγRIIA and FcγRIIIB cooperate in facilitating neutrophil responses, it might be anticipated that allelic polymorphisms of one or both of these two receptors might influence the outcome of such interactions. In this context, Salmon and coworkers demonstrated that cross-linking FcγRIIIB activates FcγRIIA for phagocytosis but that this effect is greater when employing neutrophils from donors homozygous for the NA1 allele than when using neutrophils from donors homozygous for the NA2 allele (33). Receptor cooperativity was observed even when employing erythrocytes opsonized with human IgG2, which does not bind to FcγRIIIB. These findings raise the possibility that certain combinations of FcγRIIA and FcγRIIIB alleles may be associated with greater or lesser susceptibility to infections, including those in which IgG2 plays a key protective role. Consistent with this hypothesis, it has been observed that the combination of homozygosity of the R131 allele of FcγRIIA and the NA2 allele of FcγRIIIB is associated with susceptibility to meningococcal infection in patients with terminal complement protein deficiency (11, 25).

The majority of studies performed to date have examined either FcγRIIA or FcγRIIIB genotype in various patient populations. Hence, there is little published information available regarding the frequency of various allelic combinations of FcγRIIA and FcγRIIIB in different ethnic groups. Functional studies characterizing the ability of different FcγR allotypes to bind human IgG subclasses would suggest that the most favorable genotype combination is FcγRIIA-H/H131–FcγRIIIB-N/A1/NA1, while the least favorable combination is FcγRIIA-R/R131–FcγRIIIB-NA2/NA2.

In the present study, we examined the distribution of FcγR allelic combinations in our Caucasian and African-American populations (Fig. 1). Given the predominance of the R131 allele of FcγRIIA and the NA2 allele of FcγRIIIB in both ethnic groups of subjects, a relatively small percentage of Caucasians and African-Americans, our finding that few individuals in either group exhibit the “preferred” genotype is not surprising. On the other hand, 12.5% of Caucasians and 17.8% of African-Americans genotyped exhibited the least favorable genotype combination. The reverse situation may apply among Japanese and Chinese subjects, among whom the H131 and NA1 alleles are predominant (18, 48). The significance of the interplay between specific alleles of FcγRIIA and FcγRIIIB in defining susceptibility to other infectious or autoimmune diseases has not been established and awaits further investigation.

The results of the present study indicate that such genotype analyses can be conveniently performed using DNA isolated from whole saliva, thus avoiding the need to collect peripheral venous blood.

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