Allelic Polymorphisms of Human Fcγ Receptor IIA and Fcγ Receptor IIIB

Independent Mechanisms for Differences in Human Phagocyte Function

Jane E. Salmon, Jeffrey C. Edberg, Nina L. Brogle, and Robert P. Kimberly

Department of Medicine, The Hospital for Special Surgery and The New York Hospital,
The Cornell University Medical College, New York 10021

Abstract

Two different allelic polymorphisms among the isoforms of human Fcγ receptors have been defined: the low-responder (LR)–high-responder (HR) polymorphism of huFcγRIIA expressed on both PMN and monocytes and the NA1–NA2 polymorphism of the neutrophil FcγRIII (huFcγRIIB). To address the issues of whether the LR–HR polymorphism has a significant impact on FcγR-mediated functions in human blood cells and whether any differences in LR–HR might be related to higher FcγR-mediated phagocytosis in NA1 donors, we examined FcγR-specific binding and internalization by donors homozygous for the two huFcγRIIA alleles. PMN from LR homozygotes showed consistently higher levels of internalization of erythrocytes opsonized with pooled human IgG (E-hlgG). The absence of an LR–HR phagocytic difference by erythrocytes opsonized with either anti-FcγRIIA MAb IV.3 or rabbit IgG, as opposed to E-hlgG, suggested that the Fc piece of the opsonin might be important for this LR–HR difference. Accordingly, we studied HR and LR homozygotes with human IgG subclass-specific probes. Both PMN (independent of huFcγRIIB phenotype) and monocytes from LR donors bound and internalized erythrocytes coated with human IgG2 (E-hlgG2) efficiently, whereas phagocytes from HR donors did so poorly. E-hlgG2 internalization was completely abrogated by blockade of the ligand binding site of huFcγRIIA with IV.3 Fab, indicating that huFcγRIIA is essential for the handling of hlgG2 and that the mechanism of the LR–HR phagocytic difference is at the level of ligand binding to huFcγRIIA. In contrast, the difference in internalization of E-hlgG between NA1 and NA2 homozygous donors was independent of the huFcγRIIA phenotype and did not manifest differences in ligand binding. Thus, the two known allelic polymorphisms of human FcγR have distinct and independent mechanisms for altering receptor function, which may influence host defense and immune complex handling. (J. Clin. Invest. 1992. 89:1274–1281.) Key words: IgG subclasses • phagocytosis • monocyte • neutrophil

Introduction

Two different allelic polymorphisms among the isoforms of human Fcγ receptors have been defined. The NA1–NA2 polymorphism of the neutrophil FcγRIII (huFcγRIIB) reflects several differences in amino acid sequence and two potential glycosylation sites (1–5). The low-responder (LR)–high-responder (HR) polymorphism of huFcγRIIA reflects two amino acid substitutions at positions 27 and 131, with an arginine residue at amino acid position 131 being critical for efficient binding of mlgG1 (6–9). For each of these allelic systems, there has been substantial interest in identifying their functional consequences and the molecular mechanisms underlying any differences. We have shown that individuals homozygous for the NA1 and NA2 alleles of huFcγRIIB have distinct phagocytic capacities (10).

The recent observation by Warmerdam et al. (8) that position 131 of FcγRIIA expressed in transfected fibroblasts influences the capacity for binding human IgG2 (hlgG2) suggests that this polymorphism may also be important in human systems. Using a large donor population with defined huFcγRIIA alleles, we examined the effect of this polymorphism on quantitative phagocytosis by human phagocytes. Our results revealed that homozygous LR donors have a higher capacity to internalize erythrocytes (E) opsonized with human IgG (E-hlgG) than homozygous HR donors. Further analysis of these homozygous donors indicated that, when studied with human IgG (hlgG) subclass-specific probes, PMN and monocytes from both LR and HR subjects are able to bind and internalize E-IgG1 comparably. With E-IgG2, both cell types from homozygous LR subjects bind and internalize efficiently whereas cells from HR donors do so poorly. Thus, with intact human phagocytes, the LR–HR polymorphism influences both ligand binding to huFcγRIIA and subsequent FcγR-mediated phagocytosis.

This difference in FcγR-mediated phagocytosis, coupled with recent data localizing the genes for both huFcγRII and huFcγRIII to human chromosome 1 (11–13), raised the possibility that an association between the NA alleles of huFcγRIIB and the LR–HR alleles of huFcγRIIA might explain the difference in FcγR-mediated phagocytosis between NA1 homozygous and NA2 homozygous donors. Thus, we also examined

1. Abbreviations used in this paper: AI, adherence index; ANOVA, analysis of variance; E, erythrocytes; EA, IgG-sensitized bovine erythrocytes; Eα, biotinylated erythrocytes; EαG, streptavidin-coated Eα; E-hlgG, E coated with pooled hlgG; E-hlgG1, E coated with human IgG1; E-hlgG2, E coated with human IgG2; E-hlgG3, E coated with human IgG3; E-hlgG4, E coated with human IgG4; E-IV.3, E coated with IV.3 Fab(ab')2; hlgG, human IgG containing mixed subclasses; HR, high-responder allele of huFcγRIIA; huFcγR, receptors for Fc portion of IgG in human cells; huFcγRIIB, 40-kD receptor on human neutrophils and monocytes for Fc portion of IgG; huFcγRIIB, 50–78-kD receptor on human neutrophils for Fc portion of IgG; LR, low-responder allele of huFcγRIIA; MCF, mean channel log fluorescence units; mlgG1, murine IgG1 myeloma protein; PI, phycoerythrin; PI, phagocytic index.
the differences in phagocytosis for NA1 and NA2 homozygotes matched for expression of the LR–HR polymorphism. Phagocytosis of E coated with hlgG was higher for NA1 donors independent of the LR–HR phenotype. Furthermore, there were no differences in E-hlgG binding by cells expressing either NA1 or NA2 alleles. The mechanism of the phagocytic difference, therefore, appears to be distal to ligand binding. These data demonstrate that the two recognized allelic polymorphisms of human Fcγ receptors have independent mechanisms for mediating functionally apparent differences in FcγR-mediated phagocytosis. These differences may have important implications for human disease.

Methods

Subjects. Peripheral blood was collected from 32 disease-free volunteers who ranged in age from 20 to 56 yr (34±8 yr [mean±SD]). Protocols for these studies were approved by the Institutional Committee on Human Rights in Research.

Determination of NA antigens by leukoagglutination. Typing for neutrophil-specific antigens, NA1 and NA2, was performed by leukoagglutination as described by Lalezari (2, 14), with a panel of anti-NA1 and anti–NA2 allosera kindly provided by Dr. David Stonecek of the American Red Cross Neutrophil Serology Reference Laboratory, St. Paul, MN. The assignment of NA type was confirmed by immunoprecipitation and flow cytometry with MAbs CLB-FcR gran 1, CLB-gran 11, and GRM1 (15, 16).

Determination of LR–HR alleles by flow cytometry. Phenotyping of donors for the LR–HR alleles of huFcγRIIA was performed by quantitative flow cytometry using MAbs 41.H16 and IV.3 as described by Gosselin et al. (17). Phenotypic assignment was corroborated by anti-CD3 mitogenesis assays as described below.

Reagents. HBSS, RPMI 1640, and FCS were from Gibco Laboratories, Grand Island, NY. FCS was heat inactivated at 56°C for 60 min before use. FITC-conjugated rabbit anti-human IgG F(ab')2, FITC-conjugated goat anti-rabbit IgG F(ab')2, and phycoerythrin (PE)-conjugated goat anti–mouse IgG F(ab')2, were purchased from Tago, Inc., Burlingame, CA. All conjugated reagents were absorbed against human mononuclear cells before use. Sulfoconcanamidobiotin (Sulfo-NHS-biotin), sulfoconcanamidyl-6-(biotinamido) hexaoxide (NHS-LC-biotin), and streptavidin were obtained from Pierce Chemical Co., Rockford, IL. FMLP (Sigma Chemical Co., St. Louis, MO) was dissolved in American Chemical Society grade DMSO for a stock concentration of 10−3 M and stored at −20°C.

MAb 3G8 (mlgG1) recognizing huFcγRIII (CD16) was kindly provided by Dr. J. Unkeless, Mt. Sinai Medical Center, New York (18). The MAb IV.3 (mlgG2b) and IV.3 Fab fragments recognizing huFcγRII (CD32) and 3G8 Fab(ab')2 were purchased from Medarex, Inc., (West Lebanon, NH) (19). Silver stain analysis of the IV.3 Fab fragment preparation and the 3G8 Fab(ab')2 preparation indicated that there was no intact IgG. The MAb 41H.16 (mlgG2a) recognizing huFcγRIII was generously provided by Dr. Thomas Zipf, University of Texas Cancer Center, Houston, TX (20). Leu4 and OKT3 (IgG1 and IgG2a anti-CD3, respectively) were provided by Dr. Robert Evans, Memorial Sloan-Kettering Cancer Center, New York. Isotype controls (mlgG1 [MOPC21] and mlgG2a [UPC10]) were purchased from Sigma Chemical Co.

Purified hlgG1 and hlgG2 myeloma proteins were obtained from Dr. Elliot Osserman of Columbia University, New York. hlgG3 and hlgG4 myeloma proteins were obtained from The Binding Site, Inc. (San Diego, CA). A second set of preparations of hlgG1, hlgG2, hlgG3, and hlgG4 were obtained from Calbiochem-Behring Corp., San Diego, CA. Normal pooled hlgG was obtained from Sigma Chemical Co.

Preparation of cells. Fresh anticoagulated human peripheral blood was separated by centrifugation through a discontinuous two-step Ficoll-Hyapque gradient (10). PMNs were isolated from the lower inter-face and washed with HBSS. Contaminating E were lysed with hypotonic saline (0.02% NaCl) for 20 s followed by 0.16% NaCl and a final wash with HBSS. Mononuclear cells were isolated from the upper Ficoll-Hyapque interface and washed with HBSS. The percent monocytes within the mononuclear cell layer was determined by peroxidase staining. After final washes, all cells were resuspended to 5 × 10^6 PMNs or monocytes/ml.

Preparation of E. Antibody-coated erythrocytes (EA) were prepared by incubating bovine E with rabbit IgG anti–bovine E antibody (Cappel Laboratories, Cochranville, PA) for 1 h at 37°C. The cells were washed and resuspended at 10^6 cells/ml in RPMI and 20% FCS (21). The standard amount of antibody used was a fourfold dilution of the minimum agglutinating titer and resulted in maximum phagocytosis of EA by monocytes.

E were coupled to pooled or subclass-specific hlgG by a biotin–avidin technique (22). To prepare biotinylated E (Eba), 0.5 ml of E (1 × 10^6 cells/ml) in 0.1 M carbonate buffer (pH 8.6) was incubated with sulfon-NHS-biotin (500 μg/ml) for 20 min at 4°C with mixing, followed by three washes with buffer containing divalent cations as follows (mM): (2.5 veronal buffer, pH 7.4; 146 NaCl, 0.05% gelatin, 0.15 CaCl2, and 0.05 MgCl2). Eba at 1 × 10^6/ml were incubated with an equal volume of streptavidin (250 μg/ml) for 30 min at 4°C with mixing. The streptavidin-coated Eba (Ea) were then washed three times with buffer and resuspended to 1 × 10^6 E/ml for immediate use. Pooled hlgG or purified human myeloma proteins of specific IgG subclasses were biotinylated with NHS-LC-biotin (0.01 mg biotin/mg protein) for 60 min at room temperature with occasional mixing. To bind the biotinylated hlgG to the Eba, Ea (12.5 μl at 1 × 10^6/ml) were combined with varying amounts of biotinylated protein (200 μg/ml to 1 mg/ml) for 30 min at 4°C with mixing. After three washes, the hlgG–coated Ea (EhlgG) were then resuspended in 125 μl (1 × 10^6 E/ml) and used immediately. For certain experiments, the Ea were coupled to biotinylated IV.3 Fab. The results of experiments using E-IV.3 prepared by the biotin–avidin sandwich technique were comparable to those using E-IV.3 Fab heteroantibodies (23–25).

Assay of phagocytosis. Quantitation of phagocytosis by PMNs and monocytes was performed as previously described (10, 21, 26). Briefly, cells were resuspended in RPMI with 20% heat-inactivated IgG-free FCS (Gibco Laboratories), at 5 × 10^6 cells/ml. In certain experiments, the PMNs were preincubated with either IV.3 Fab or 3G8 Fab(ab')2 (30 μg/ml) for 5 min; the MAbs were present throughout the assay of phagocytosis. For experiments with FMLP-treated PMN (10−7 M), FMLP was added simultaneously with the phagocytic particle.

To assess internalization of E target particles, cells (100 μl) were combined with EA (250 μl E IV.3 (250 μl), or E-hlgG (125 μl). The leukocyte–erythrocyte mixtures were 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/slide were counted in duplicate without knowledge of the donor huFcγRIII or huFcγRIIIb allotype. The data are expressed as phagocytic index (PI, number of ingested E/100 phagocytes). For assays with monocytes, PI was corrected for the percent peroxidase positivity of mononuclear cells.

Assay of adherence. To quantitate adherence, PMN and E target particles were prepared and combined as described in the assay of phagocytosis above. After centrifugation at 44 g for 3 min, the PMN–E mixtures were maintained at room temperature for 10 min and then gently resuspended. Adherence of E to PMN was quantitated by light microscopy. Data are expressed as adherence index (AI, number of adherent or internalized erythrocytes/100 PMN). Under these conditions, <1% of E had internalized E.

Immunofluorescent flow cytometry. Fresh leukocytes (5 × 10^5 in PBS with 0.1% FCS) were incubated with saturating amounts of specific MAb or isotype controls for 30 min at 4°C. After two washes with cold PBS containing 1% FCS, cells were incubated with saturating concentrations of phycoerythrin (PE)-conjugated goat anti–mouse IgG F(ab')2, for 30 min at 4°C followed by washing twice with cold PBS/1%
FCS. E-hlG were stained with FITC-conjugated rabbit anti-human IgG F(ab')2, E coated with IV.3 Fab were stained with PE-conjugated goat anti-mouse IgG F(ab')2, and E opsonized with rabbit anti-ox E antibodies were stained with FITC-conjugated goat anti-rabbit IgG F(ab')2, followed by washes with cold PBS/1% FCS.

After staining, cell-associated immunofluorescence was quantitated on a Cytofluorograf II (Becton Dickinson Immunocytochemistry Systems, Mountain View, CA) with a 2151 computer as previously described (10, 15, 16). For each experiment, the instrument was calibrated with FITC-conjugated calf thymus nuclei (Fluorotrol-GF, Becton, Dickinson & Co., Mountain View, CA) and quantitative PE microbead standards (Flow Cytometry Standards Corp., Research Triangle Park, NC) to allow for assessment of both absolute and relative levels of immunofluorescence.

Anti-CD3 proliferation assay. Peripheral blood mononuclear cells, separated by Ficoll-Hypaque as described above, were resuspended at 1 × 10⁶ cells/ml in RPMI 1640 with 10% FCS (supplemented with glucose, penicillin, and streptomycin) and aliquoted into 96-well plates at 1 × 10⁶ cells/well. OKT3 (IgG2α anti-CD3, 5 μg/ml final concentration), Leu4 (IgG1 anti-CD3, 5 μg/ml final concentration), nonspecific control isotype antibodies, or media alone were added to triplicate wells and the cells were incubated for 4 d at 37°C. For the final 8 hr, [³H]thymidine (Amersham Corp., Arlington Heights, IL) 2 μCi/well was added, after which the cells were harvested, washed, and [³H]-thymidine incorporation measured in a beta counter (27-29).

Data analysis. For assessment of phagocytic capacity, all experiments were performed in a matched-pairs experimental design. Accordingly, each subject, homozygous for a given FcγR isoform (huFcyRIIA or huFcyRIIIB), was studied in comparison to a second subject, homozygous for the other allele of the same isoform and matched for the same phenotype of the other FcγR (e.g., HR=NA1 vs. LR=NA1).

Data are displayed as mean±SEM. PI for specific probes were compared using a paired t test (two-tailed). Differences in phagocytic capacity between the groups for E opsonized over a range of densities were compared by repeated measures analysis of variance (ANOVA). A probability of 0.05 was used to reject the null hypothesis that there is no difference between the groups.

Results

Characterization of huFcyRIIA and huFcyRIIIB alleles. The allotypic characteristics of our normal population for huFcyRIIIB were determined by serological typing and quantitative flow cytometry of PMN using NA1- and NA2-specific M Abs (10, 15, 16). The LR-HR alleles of huFcyRIIIB in our population were identified by quantitative flow cytometry with MAb 41H.16, which recognizes the LR allele of huFcyRIIIB, and MAb IV.3, which recognizes both HR and LR alleles (17). Using the ratio of fluorescence intensity of 41H.16/IV.3 in both monocytes and PMN, we assigned the homozgyous HR phenotype to donors having a ratio of 0.88-1.1 (n = 8), the heterozygous HR/LR phenotype to donors having a ratio of 0.42-0.59 (n = 11), and the homozygous LR phenotype to donors having a ratio of < 0.13 (n = 13). The phenotypic assignment of homozygous individuals was corroborated by proliferation assays with anti-CD3 M Abs of both mIgG1 and mIgG2a isotypes. In all cases, the results of the mitogenesis assays were in agreement with the flow cytometry assignment for huFcyRIIIB.

Quantitative expression of huFcyRII as determined by flow cytometry using MAb IV.3 showed small differences between the phenotypes (PMN: HR 129±2, HR/LR 128±3, LR 121±2 mean channel log fluorescence units [MCF]; monocytes: HR 130±4, HR/LR 122±3, LR 118±4 MCF; for PMN, HR vs. LR P < 0.02, Mann-Whitney U test; all other comparisons, P > 0.1). The expression of huFcyRIII as determined by flow cytometry using MAb 3G8, which recognizes the NA1 and NA2 alleles, was the same for all phenotypes (NA1 154±4; NA1/NA2 150±2; NA2 152±6; P > 0.1 for all comparisons).

Phagocytosis by PMN from LR homozygotes is higher than HR homozygotes. Given the difference in ligand binding by FcγRIIA alleles transfected into fibroblasts or COS cells (7-9), we considered the possibility that the FcγR-mediated phagocytic capacity of blood cells expressing these alleles might differ. Accordingly, we examined quantitative phagocytosis by PMN from normal donors who are homozygous for the LR or HR alleles. To control for differences due to huFcyRIIIB, each pair of homozygous donors was matched for NA alleles. Although not different when probed with anti-FcγRII Fab-coated E (E-IV.3, a ligand-independent huFcyRII-specific probe), LR homozygotes showed significantly higher phagocytosis than HR homozygotes when probed with E-hlG (PI [E-hlG internalized/100 PMN]: LR vs. HR, 50±7 vs. 27±10, P < 0.003; Fig. 1). Over a broad range of opsonization densities, LR subjects had a greater level of phagocytosis of E-hlG (P < 0.02, repeated measures ANOVA). In contrast, phagocytosis EA and E-IV.3 prepared at a broad range of opsonization densities was indistinguishable between the two groups (P > 0.1, repeated measures ANOVA). The absence of the difference in phagocytosis between LR and HR donors with EA and E-IV.3 suggested that the nature of the ligand might be important in defining the LR-HR functional difference.

Higher phagocytosis by FcγRII A LR homozygotes: relationship to hlG subclasses. Since the alleles of huFcyRIIA differentially bind murine IgG1 (9, 27-32) and hlG2 in a transfected fibroblast system (8), we examined the capacity of the LR-HR alleles expressed on blood cells to recognize and bind subclasses of hlG. In each of seven pairs of homozygous LR and HR subjects matched for huFcyRIIIB alleles, there was a dramatic difference between the HR and LR individuals in

![Figure 1. Phagocytosis of E-hlG by PMNs from LR homozygotes is higher than that by HR homozygotes. PMNs from normal donors, homozygous for the LR or HR alleles and matched for identical NA alleles of huFcyRIIIB, were studied simultaneously for internalization of E-IV.3 and E-hlG in a matched-pairs design. PI was quantitated by light microscopy. In each of six pairs, internalization of E-hlG was greater for the homozygous LR subjects (P < 0.003, paired t test). In contrast, phagocytosis of E-IV.3 was indistinguishable between the two groups (P = NS). (c) LR; (a) HR.](image)
phagocytosis of E-hlgG2 (PI [E internalized/100 PMN]: LR vs. HR, 47±7 vs. 3±1, P < 0.0004), whereas internalization of E-hlgG1, E-hlgG3, and E-hlgG4 was similar for both donor types (Fig. 2 A). The lack of internalization of E-hlgG2 reflected an inability of the E-hlgG2 to bind to PMNs from HR subjects (AI for E with intermediate density of opsonization: 0–3 E attached/100 PMN; Fig. 2 B). Identical results were obtained with a second and different myeloma preparation of each hlG subclass. A series of blocking experiments confirmed that huFcγRIIA was responsible for the phagocytosis of E-hlgG2. Pretreatment of PMN with MAb IV.3 Fab (30 μg/ml) to block huFcγRII completely abrogated internalization of E-hlgG2 (Fig. 3). Indeed, pretreatment with 3G8 F(ab')2 to block huFcγRIIIB did not block, but rather enhanced, phagocytosis of E-hlgG2 consistent with our previous findings that cross-linking huFcγRIIIB primes phagocytosis mediated by huFcγRIIIB in PMN (PI: control vs. 3G8 F(ab')2, 47±16 E internalized/100 PMN vs. 72±16, P < 0.05) (25).

The capacity of the LR allele of huFcγRIIA to bind mlgG1 and form subclass-specific rosettes or induce T cell proliferation (32, 33) is dependent in part on the concentration of MAb presented to receptor-bearing cells. A similar relationship is apparent for the capacity of the HR allele of huFcγRIIA on PMN to bind hlG2. As shown in Figure 4 A, higher densities of hlG2 on E resulted in a higher PI in both LR and HR donors. At all levels of hlG2, however, a marked difference between the LR and HR groups was evident (P < 0.001, repeated measures ANOVA). In contrast, the phagocytosis of E-hlgG1 showed no difference between the two groups at any level of E surface hlG1 (Fig. 4 B). Similarly, there was no difference in internalization of E-hlgG4 opsonized over a range of densities between both groups. For example, the PI for E-hlgG4 with MCF 170–179 was 29±10 E internalized/100 PMN for LR donors and 27±6 for HR donors.

Interestingly, internalization of E-hlgG and E-hlgG1 by HR PMN was identical over most of the range of opsonization, whereas internalization of E-hlgG by LR PMN was higher than that of E-hlgG1 with similar densities of opsonization. This difference is presumably due to recognition of hlG2 on the E-hlgG by the LR allele, and this observation suggests that functional differences between the huFcγRIIA alleles may be apparent even with polyclonal antibody responses.

Detection of the differential capacity of huFcγRIIA alleles to bind hlG2 is not restricted to PMNs. Human monocytes, which express both FcγRIIA and FcγRI, show efficient internalization of E-hlgG2 when derived from LR subjects but little internalization when derived from HR subjects (PI [E internalized/100 monocytes]: LR vs. HR, 145±14 vs. 21±3, P < 0.0005). In contrast, phagocytosis of both E-hlgG and E-hlgG1 was comparable for both groups (PI [E internalized/100 monocytes]: LR vs. HR, 152±10 vs. 146±25 and 149±13 vs. 153±37, respectively, P > 0.1). Blockade of huFcγRII with IV.3 Fab blocked internalization of E-hlgG2 by monocytes in LR subjects (PI [E internalized/100 monocytes]: 169±43 vs. 10±6, P < 0.03), similar to the findings in PMN (Fig. 3).

Figure 2. PMNs from LR homozygotes internalize E-hlgG2. PMNs from pairs of homozygous HR and LR donors with identical NA alleles were studied simultaneously with all probes. (A) For the assay of phagocytosis, PMNs were incubated with E-hlgG1, E-hlgG2, E-hlgG3, or E-hlgG4 opsonized with similar intermediate densities of IgG (MCF 150–160). In each of seven pairs, there was minimal internalization of E-hlgG2 by HR donors (LR vs. HR, P < 0.0004). No significant difference in phagocytosis of the other probes was noted. (B) For the

Figure 3. Blockade of huFcγRIIA with IV.3 Fab completely inhibits internalization of E-hlgG2 in both LR and HR PMNs. PMNs from HR and LR donors were pretreated with IV.3 Fab (30 μg/ml) or control medium for 10 min and then combined with E-hlgG2 prepared at lower (intermediate density; MCF 150–160) or higher (MCF 170–180) density opsonization. In each experiment both an HR and an LR donor were studied simultaneously (n = 3–4 pairs). Pretreatment of PMN with IV.3 Fab blocked phagocytosis of both E-hlgG2 probes in LR donors (control vs. IV.3 Fab, P < 0.03). In HR donors, IV.3 Fab blocked phagocytosis of the high density E-hlgG2 (control vs. IV.3 Fab, P < 0.006). (c) LR; (a) HR.
Figure 4. The lack of internalization of E-hlgG2 by PMNs from homozygous HR subjects is relative, not absolute. PMNs from HR and LR were combined with E opsonized with varying amounts of hlgG2 (A) or hlgG1 (B), as determined by flow cytometry (n = 3–5 pairs/level). Phagocytosis of all probes was directly related to degree of opsonization. (A) Internalization of E-hlgG2 was higher for LR donors at all levels of opsonization (P < 0.001, repeated measures ANOVA), whereas that for E-hlgG1 remained similar for both groups. (c) LR; (●) HR.

Figure 5. The difference in E-hlgG2 internalization between LR and HR donors persists in primed PMNs. PMNs from HR and LR donors were treated with FMLP (10^{-7} M), 3G8(Fab')2 (30 μg/ml), or control medium before quantitation of phagocytosis of E-hlgG2 prepared at lower (intermediate density; MCF 150–160) and higher (MCF 170–180) density opsonization. Phagocytosis of the (A) lower density E-hlgG2 (A) and the (B) higher density E-hlgG2 was greater for LR donors (n = 3–9). Low density E-hlgG2, LR vs. HR: control P < 0.01; FMLP P < 0.03, 3G8(Fab')2, P < 0.01; High density E-hlgG2, LR vs. HR: control P < 0.001, FMLP P < 0.03, 3G8(Fab')2 P < 0.03. (c) LR; (●) HR.

The difference in E-hlgG2 internalization between LR and HR donors persists in primed PMNs. Simultaneous engagement of several receptors may be the most relevant physiological stimulus for phagocytes. For example, it is unlikely that microbes opsonized in vivo or endogenously formed immune complexes would be composed exclusively of hlgG2 and thus ligate only huFcγRII. The observation that treatment of PMNs with 3G8(Fab')2 to crosslink huFcγRIIIB augmented internalization of E-hlgG2 raised the possibility that primed or activated PMNs found in sites of infection or inflammation might show a difference between LR and HR donors. To examine this possibility, PMNs from LR and HR donors were pre-treated with 3G8(Fab')2, FMLP, or control medium. In spite of an increase in E-hlgG2 internalization, phagocytosis of E-hlgG2 by LR PMNs remained significantly higher than that by HR PMNs (Fig. 5). The increase in E-hlgG2 phagocytosis was associated with an increase in E-hlgG2 binding in both LR and HR donors, although this may not be the only mechanism for the increase in internalization (AI for LR [E-hlgG2 adherent/100 PMN]: control 134±34, FMLP 219±36, 3G8(Fab')2 150±42, n = 5–6, FMLP vs. control P < 0.02; AI for HR: control 8±3, FMLP 72±15, 3G8(Fab')2 40±10 n = 3–4, FMLP vs. control P < 0.04). Phagocytosis of E-hlgG2 by FMLP-stimulated PMNs from homozygous HR donors was also completely inhibited by blockade of huFcγRII with IV.3 Fab (PI [E internalized/100 PMN]: FMLP-treated PMNs = 37±9, FMLP-treated PMNs in the presence of IV.3 Fab = 5±2; n = 4; P < 0.05). Thus, in primed PMNs, hlgG2 binding and internalization is predominantly mediated by huFcγRII.

Higher phagocytic capacity of PMNs from NA1 subjects is not related to allelic polymorphisms of huFcγRII. Homozygous NA1 donors have higher phagocytosis of EA than homo-
zygous NA2 donors, which is FcγR specific rather than due to differences in generalized phagocytic capacity (10). Given the differences in FcγRIIA-mediated phagocytosis and the recent data localizing the genes for both huFcγRII and huFcγRIII to human chromosome 1 (11-13), we determined whether an association between the alleles of huFcγRIIA and the NA1-NA2 alleles of huFcγRIIIB might explain the higher phagocytic capacity of NA1 homozygotes. In each of six pairs of NA1 and NA2 homozygous individuals, each with identical huFcγRIIA alleles, internalization of EA and of E-hlgG was higher in the NA1 donor, independent of the huFcγRIIA phenotype of the donor pair (Fig. 6). The difference in phagocytic capacity was not related to alterations in binding of E-hlgG (AI for standard level of opsonization [E-hlgG adherent/100 PMN]; NA1 vs. NA2 = 265±69 vs. 280±66, n = 5). Simultaneous analysis of huFcγRII-specific phagocytosis in the NA1 and NA2 homozygous donors with E-IV.3 showed no significant differences (PI [E internalized/100 PMN]; NA1 vs. NA2 = 32±12 vs. 29±10, n = 5; Fig. 6). Thus, an huFcγRIIIB-specific mechanism, unrelated to the quantitative binding of ligand and unrelated to huFcγRIIA alleles, appears to be responsible for the difference in phagocytosis between NA1 and NA2 homozygous donors.

**Discussion**

Allelic polymorphisms of human FcγR have functionally distinct capacities that are important in mediating interactions between human phagocytes and IgG ligands. Using E-hlgG, we show consistently higher PMN phagocytosis by individuals homozygous for the LR allele of huFcγRIIA and by individuals homozygous for the NA1 allele of huFcγRIIIB. This difference is even more pronounced in LR homozygous subjects for hlgG2 subclass-specific phagocytosis, since, in contrast to HR, the LR allele of huFcγRIIA binds hlgG2 efficiently.

The differences in functional capacity for the two alleles of huFcγRIIA appear to be mediated by a mechanism distinct from that involved in the differences between the two alleles of huFcγRIIIB. The biallelic HR–LR polymorphism of huFcγRIIA alters the affinity of the ligand binding site for some subclasses of IgG ligand. Originally distinguished by differences in binding of mlgG1 to human monocytes as assessed by T cell proliferation induced by anti-CD3 MAb (27-32), the functional HR–LR phenotype is determined by a single amino acid present at position 131, which plays a critical role in the binding capacity for mlgG1 (6, 7, 9). Warmerdam and colleagues (8) also showed that fibroblast transfectants expressing the LR allele (histidine residue at position 131) lack the capacity to bind mlgG1 but can bind hlgG2, whereas transfectants expressing the HR allele (arginine residue at position 131) bind mlgG1 but do not recognize hlgG2. Importantly, the functional impact of this difference is not restricted to transfected fibroblasts expressing a single receptor family but is evident in resting and activated human phagocytes. Our data indicate that for human PMN, which express both huFcγRII and huFcγRIIIB, submaximally opsonized E-hlgG are internalized more efficiently by LR donors. This difference reflects the ability of LR donors to bind hlgG2, which comprises ~20% of pooled hlgG (34, 35). E-hlgG2 are internalized efficiently by individuals homozygous for LR allele of huFcγRIIA but are internalized poorly by individuals homozygous for the HR allele (Fig. 2). Even in “primed” PMNs, which demonstrate enhanced binding and internalization of E-hlgG2, the difference between HR and LR donors persists (Fig. 5). This difference in E-hlgG2 phagocytosis is also evident in human monocytes that express both huFcγRII and huFcγRII. The inability of HR PMN to bind hlgG2 is relative rather than absolute, and at higher levels of opsonization there is a modest degree of internalization of E-hlgG2 (Fig. 4). A similar discriminative preference for the other human subclasses of IgG was not identifiable. Although absolute comparisons of efficiency of binding and internalization for each of the hlgG subclasses are not possible, in part because determinations of “comparable densities” of opsonization may be influenced by unique properties of individual myeloma proteins and by properties of the secondary antibody, our results suggest that in LR PMN the relative efficiency is hlgG1 = hlgG3 = hlgG2 > hlgG4 and, in HR PMN, hlgG1 = hlgG3 > hlgG4 > hlgG2.

The biallelic NA1-NA2 polymorphism of huFcγRIIIB, expressed only on human PMNs, is related to several amino acid differences, two of which alter potential N-linked glycosylation sites (1-5). Since huFcγRIIIB may function in part as a binding molecule to present ligand to huFcγRIIA (24), we considered the contribution of the LR–HR alleles of huFcγRIIA to the functional consequences of the huFcγRIIIB polymorphism. The current results demonstrate higher phagocytosis of E-hlgG by NA1 donors compared with NA2 donors, despite matching of donors for huFcγRIIA alleles (Fig. 6) and no difference in phagocytosis of E-IV.3 (an huFcγRII-specific probe) by NA1 and NA2 donors (Fig. 6). These data, along with our previous observation that blockade of huFcγRII with IV.3 Fab amplifies the difference in phagocytosis between NA1 and NA2 (10), support the conclusion that the difference in EA and E-hlgG phagocytosis between NA1 and NA2 homozygous donors is
independent of huFcγRIIA phenotype. The mechanism underlying the difference between NA1 and NA2 homozygotes remains undefined, although IgG-independent, allele-specific interactions with other surface membrane molecules provide provocative possibilities.

The implications of these two allotypic systems are intriguing. The NA1-NA2 functional polymorphism appears to be IgG-ligand independent. No differences in opsonized target binding have been demonstrated, and no subclass preference has been identified since, among HR homozygous donors, the inability to bind hIgG2 is equally apparent for both the NA1 homozygous and NA2 homozygous phenotypes. The difference in internalization is evident with concanavalin-treated E, which engage huFcγRIIB through nonclassical (IgG-ligand independent) carbohydrate-mediated interactions (10). The fact that the NA1-NA2 alleles are functionally distinct indicates, however, that huFcγRIIB serves as an integral participant in phagocytosis by PMNs. In contrast, the HR-LR functional polymorphism is directly ligand dependent, and the ability of LR PMNs and monocytes to bind hIgG2 efficiently suggests that a reexamination of the role of hIgG2 humoral response may be appropriate. For example, since the antibody response to bacterial polysaccharide antigens is predominantly hIgG2 isotype (36), the relationship between hIgG2 levels and susceptibility to infection with encapsulated bacterial pathogens (34, 35, 37) may be influenced by the HR-LR phenotype of the host. Thus, the increased risk of invasive Hemophilus influenzae infection in certain populations that have impaired antibody responses to H. influenzae and low hIgG2 levels (38, 39) may be greater for HR homozygotes than for LR homozygotes. Similarly, in cystic fibrosis, where bacterial colonization of the lungs by Pseudomonas aeruginosa is associated with high serum levels of hIgG2 anti-Ps. aeruginosa antibodies and immune complexes containing hIgG2 anti-Ps. aeruginosa antibodies, the potential for the hIgG2 antibodies to act as poor opsonins and inhibit clearance of the organisms (40-42) may vary in accord with the HR-LR phenotype of the host. Importantly, activation of PMNs does not abrogate this allelic difference in function. Indeed, one might speculate that the evolutionary pressure for the presence of the LR allele of huFcγRIIA (29) is related to more effective defense against microbial agents eliciting an hIgG2 humoral response.

The precedent for allelic differences in receptor structure having a significant impact on phagocyte function is of potential importance given the structural diversity of huFcγR. Definition of further such structure-function relationships among huFcγR may provide insights into both disease susceptibility and pathogenesis.

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