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Role of neutrophil FcγRIIa (CD32) and FcγRIIIb (CD16) polymorphic forms in phagocytosis of human IgG1- and IgG3-opsonized bacteria and erythrocytes

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SUMMARY

The four subclasses of IgG have different biological activities associated with their Fc regions. Fcγ receptors on leucocytes (FcγR) mediate binding and phagocytosis of opsonized particles. Two structurally and functionally distinct allelic polymorphisms of the FcγR have been defined: the H/R131 forms of FcγRIIa (CD32), and the neutrophil antigen 1 (NA1)/NA2 forms of FcγRIIIb (CD16). In this study the activities of allotypes of CD16 are analysed with antibacterial IgG subclass antibodies and with IgG1 and IgG3 anti-Rhesus D, and the activities of CD32 with IgG1 and IgG3 anti-Rhesus D. With respect to the allotypes of CD16, polymorphonuclear leucocytes (PMN) homozygous for FcγRIIIb-NA2 exhibited a lower (21–25%) IgG1-mediated phagocytosis of *Staphylococcus aureus* strain Wood (STAW), *Haemophilus influenzae* type b (Hib), and *Neisseria meningitidis* group B (NMen) than IIIb-NA1 PMN. The difference was apparent only when the micro-organisms were opsonized in the absence of complement, and was furthermore enhanced (34–52%) upon blockade of FcγRIIa. In addition, monoclonal IgG3 anti-D-mediated rosette formation and phagocytosis was consistently found to be lower (16%) with FcγRIIIb-NA2 than with IIIb-NA1 PMN. For the allotypes of CD32 we now show that IgG3 anti-D sensitized erythrocytes formed more (50%) rosettes and were phagocytosed at a higher rate with PMN carrying FcγRIIa-H131 than with PMN carrying IIa-R131. Heterozygous FcγRIIa-H/R131 PMN exhibited intermediate phagocytic activity in this respect. This study illustrates a critical role of FcγR allotypes in functional interactions with biologically relevant IgG subclass antibodies.

INTRODUCTION

IgG subclass antibodies have different properties with respect to binding to IgG Fc receptors (FcγR) on leucocytes.[1] For a better understanding of the relation between IgG subclass deficiencies and increased susceptibility to bacterial infections, we have studied this effector function of (antibacterial) IgG subclass antibodies in more detail.

Fcγ receptors, expressed on a broad range of blood cells, play an important role in the binding and phagocytosis of IgG-opsonized particles.[2,3] The most abundantly present phagocyte, the polymorphonuclear leucocyte (PMN), expresses constitutively two of the three FcγR classes: FcγRIIa (CD32) and FcγRIIIb (CD16). FcγRI (CD64), expressed on activated PMN, binds monomeric IgG with high affinity. FcγRIIa and IIIb are low-affinity receptors, interacting with complexed or aggregated IgG.

Both FcγRIIa and IIIb exhibit a genetically determined polymorphism. FcγRIIa may occur as FcγRIIa-H131 or FcγRIIa-R131 allotype, previously called Low-Responder and High-Responder allotypes, respectively, on the basis of their interaction with murine IgG1.[2–6] The substitution of just one amino acid, His or Arg at position 131, was found to be critical for IgG binding. FcγRIIIb occurs as neutrophil antigen 1 (NA1) or 2 (NA2) allotypes, which differ in number of glycosylation sites at amino acid positions 65 and 82.[2,3,7]

Human IgG1 and IgG3 subclasses interact with all FcγR classes.[1–3] FcγRIIa has been shown to be the sole FcγR class capable of binding human IgG2, IIa-H131 having a much

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Abbreviations: AGS, agammaglobulinaemic serum; EA-IgG, erythrocyte coated with IgG; Hib, *Haemophilus influenzae* type b; NA, neutrophil antigen; NMen, *Neisseria meningitidis*; PMN, polymorphonuclear leucocytes; STAW, *Staphylococcus aureus*, strain Wood 46.

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higher affinity for IgG2 than the IIa-R131 allotype.[2–5,8,9] With respect to the FcγRIIIb polymorphism, Salmon *et al.* showed a functional difference in the activity of PMN expressing the IIIb-NA1, or IIIb-NA2 allotypic forms.[8,10] Evidence is emerging for an *in vivo* role of the FcγRIIa polymorphism.[3,11,12] We found that individuals with a deficiency of a component of the terminal complement pathway (C6 or C8) in combination with both the FcγRIIa-R/R131 and the FcγRIIIb-NA2/NA2 allotype had experienced more meningococcal infections than C6- or C8-deficient family members with other combinations of these FcγR allotypes.[11] This indicates that both the FcγRIIa and IIIb polymorphisms may influence host susceptibility.

Although in adults IgG2 is often the predominant antibody isotype in the response against polysaccharide antigens, young children exhibit a slow maturation of this isotype.[13] In addition, antibody responses against some (viral) protein antigens may be restricted to IgG1 and IgG3 subclasses.[14,15] Therefore, we felt it important to examine the interaction of FcγRIIa and FcγRIIIb with polyclonal human IgG1 antibacterial antibodies, and with human IgG3 in phagocytosis experiments, taking into account the functional polymorphisms of these receptors. In addition, we addressed the activity of PMN heterozygous for the FcγRIIa allotypes, as this may be relevant for interpretation of clinical models. To study FcγR-mediated binding and phagocytosis, we used human polyclonal IgG1 and IgG2 antibodies to opsonize whole bacteria:[9] *Staphylococcus aureus* strain Wood (STAW), *Haemophilus influenzae* type b (Hib) and *Neisseria meningitidis* group B (NMen). In addition, red blood cells (RBC) sensitized with human monoclonal IgG1 and IgG3 anti-Rhesus D were used as indicator cells. Our studies demonstrate that the differences in activity of the allotypic forms for IgG subclass proteins extend to polyclonal antibacterial antibodies and to human IgG1 and IgG3 anti-Rhesus D antibodies.

MATERIALS AND METHODS

Monoclonal antibodies (mAb) and opsonins

The following murine mAb were used: anti-FcγRI (CD64) mAb 197,[16] anti-FcγRII (CD32) mAb IV.3[17] (Medarex, Annandale, NJ), mAb 41H16[18] (generously provided by Dr B. M. Longenecker, Edmonton, Canada), and highly purified $F(ab')_2$ fragments of mAb AT10[19] (donated by Dr M. Glennie, Tenovus Research Laboratory, Southampton, UK); anti-FcγRIII (CD16) mAb CLB/FcRGran1, CLB/FcRGran11 (from the Central Laboratory of the Netherlands Red Cross Blood Transfusion Service (CLB), Amsterdam, the Netherlands), GRM1[20] (provided by Dr M. Garrido, Granada, Spain) and 3G8 (Medarex).[21] We used mAb 3D9 (provided by Dr M. Krych, St Louis, MO) and B2.12 (CLB), against CR1 and CR3, respectively.[9] Irrelevant isotype-matched mAb (mouse IgG1, IgG2a and IgG2b) were from Southern Biotechnology (Birmingham, AL), and anti-IgG subclass mAb (MH161-1-ME2, MH162-1-ME2, MH163-1-ME3 and MH164-4-ME3) were from the CLB.

In inhibition studies, PMN were preincubated for 30 min at 4° with blocking anti-FcγR and/or anti-CR mAb: 10 μg/ml 197; 5 μg/ml IV.3; 0·5 μg/ml AT10; 10 μg/ml CLB/FcRGran1; 12 μg/ml 3G8; 10 μg/ml 3D9; 10 μg/ml B2.12; or 10 μg/ml isotype-matched irrelevant mAb.[9]

For sensitization of RBC (see below), we used human monoclonal IgG1 anti-Rhesus D (2B6) and IgG3 anti-Rhesus D (1A3-3) antibodies (kindly provided by Dr B. Kumpel, Bristol, UK).[22,23] Bacteria were opsonized with polyclonal IgG1 and IgG2 preparations obtained from the serum of healthy persons.[9,24] The IgG subclass preparations contained less than 5% of contaminating subclasses.[25] Specific antibody activities to whole STAW and Hib were determined by enzyme-linked immunosorbent assay (ELISA).[25] Anti-NMen antibodies were analysed with the same ELISA,[25] using freshly cultured *N. meningitidis* serogroup B (strain H4464). These bacteria were harvested in the late log phase, washed and coated onto microtitre plates (10^7/ml). IgG subclass binding was detected with subclass-specific horseradish peroxidase-conjugated mAb. As a source of complement we used agammaglobulinaemic serum (AGS) with almost undetectable (less than 1% of pooled normal serum) antibacterial IgG and a normal haemolytic complement activity (as determined by CH-50 and AP-50 titres).

PMN

PMN were isolated from blood obtained from healthy volunteers selected on the basis of their FcγRIIa and FcγRIIIb allotypes (see next section). PMN were separated from platelets and mononuclear cells by density gradient centrifugation over 1·076 g/ml Percoll (Pharmacia, Uppsala, Sweden) as described.[26] Erythrocytes were removed by isotonic lysis at 0°.

FcγRIIa and FcγRIIIb polymorphisms

FcγR polymorphisms were determined with indirect immunofluorescence on monocytes and PMN.[5] Fluorescence was quantified with a flow cytometer (FACScan, Becton Dickinson, San Jose, CA). For FcγRIIa allotyping we used mAb IV.3 (binds both IIa-R131 and IIa-H131) and mAb 41H16 (reactive with IIa-R131 only).[17,18,27] The FcγRIIa H/R131 allotypes were further confirmed by T-cell proliferation assays using murine IgG1 and human IgG2 anti-CD3 mAb,[5] or by allele-specific oligonucleotide (ASO) hybridization using IIa-H131 and IIa-R131-specific oligonucleotides.[28] For FcγRIIIb allotyping we used specific mAb CLB/FcRGran11 (selective for IIIb-NA1) and GRM1 (selective for IIIb-NA2).[10,20] FcγRIIIb NA1-NA2 allotypes were confirmed serologically with a radioimmunoassay.[20,29]

Phagocytosis assay

The phagocytic capacity of PMN was analysed by a flow cytometric phagocytosis assay as previously described.[9] Briefly, bacteria were labelled with fluorescein isothiocyanate (FITC; Sigma), washed and opsonized with either purified IgG1 or IgG2 preparations in the presence or absence of AGS.[9] IgG1 or IgG2 antibodies were used in concentrations that led to 0·1 μg/ml, 0·2 μg/ml and 0·1 μg/ml IgG bound to STAW, Hib and NMen, respectively. Complement (AGS) was added in a final concentration of 0·5% (v/v), 2% (v/v) and 2% (v/v) for STAW, Hib and NMen, respectively. PMN and opsonized bacteria were added together and incubated in a shaking water bath at 37°. At selected times, samples were taken and added to ice-cold buffer to stop phagocytosis. Fluorescence of adherent or ingested FITC-labelled bacteria was quantified by flow cytometry. Trypan blue [0·064% (w/v), 5 min, 4°; Flow

Laboratories, Irvine, UK] was used to quench fluorescence from adherent FITC-labelled bacteria.[30]

Phagocytosis results were calculated from the area under the curve (time and PMN fluorescence) and expressed as a percentage of that obtained with control heat-inactivated normal human serum plus complement (AGS).[9]

EA-rosette formation and EA-phagocytosis

Rosette formation between IgG-sensitized RBC (EA-IgG) and PMN was performed essentially as described.[31,32] We used -D-RBC, which have a high expression of the Rhesus D polypeptide on the RBC (≈150 000 D sites/cell).[31] Briefly, neat (7 μg/ml), and serial dilutions of IgG1 and IgG3 anti-D antibodies were used to opsonize -D- RBC.[22,23] Antibodies and RBC (5×10^8/ml) were incubated for 2 hr at 37° with regular shaking. Sensitization levels obtained with 4·5 μg/ml IgG anti-D were approximately 100 000 molecules IgG/RBC.[31] The cells were washed twice and pellets were resuspended at 2×10^8 cells/ml. EA-IgG were added to PMN (1×10^6/ml) in a ratio of ≈50 to 1. Suspensions were mixed, centrifuged for 4 min at 33 *g* and incubated for 1 hr at 37°. PMN were scored microscopically for rosette formation (rosette: defined as three or more EA bound per PMN) and after isotonic lysis for phagocytic activity (defined as one or more EA per PMN). For some experiments RBC were labelled with FITC (0·1 μg/ml 30 min at 37°) before incubation with the anti-D antibodies,[32] to allow flow cytometric analysis.

Statistical analysis

Data were statistically analysed using the Mann–Whitney *U* test (MWU), or the Wilcoxon Matched Pair Signed Rank test (WSR); $P < 0·05$ was considered significant.

RESULTS

Phagocytic activity of PMN expressing FcγRIIIb-NA1 and IIIb-NA2 allotypes

Homozygous FcγRIIIb-NA1 and IIIb-NA2 subjects were compared for phagocytosis of STAW, Hib and NMen opsonized with IgG1 or IgG2 (Fig. 1). In these experiments, the subjects were matched for FcγRIIa H/R131 allotypes. Opsonization of bacteria was performed either in the absence or presence of an exogenous complement source (AGS). Bacteria opsonized with antibacterial IgG2 antibodies, served as control, and showed PMN from NA1 and NA2 subjects to be equally active (Fig. 1).[9] For all three types of bacteria, we observed a significant difference between NA1 and NA2 PMN in phagocytosis of IgG1-opsonized bacteria (Fig. 1). A similar relative difference ((NA1 − NA2)/NA1) was observed between NA1 and NA2 PMN when trypan blue was used to quench FITC from adherent bacteria: IgG1 in the absence of complement gave a relative difference between NA1 and NA2 of 35%, 29% and 29% for STAW, Hib and NMen, respectively. To exclude any contribution by FcγRIIa, we added anti-FcγRII blocking mAb IV.3 or F(ab')$_2$ fragments of AT10, in amounts that almost completely blocked phagocytosis of IgG2-opsonized bacteria in parallel experiments (not shown).[9] Figure 1 shows that after blocking of FcγRIIa the differences in activity by NA1 and NA2 PMN for phagocytosis of IgG1-opsonized bacteria were increased. Opsonization with

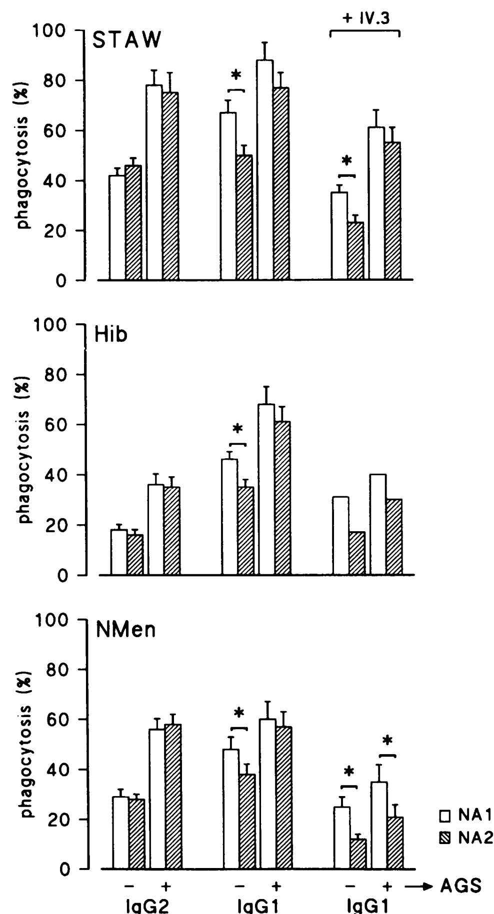

Figure 1. Higher phagocytosis (adhesion and ingestion) activity of PMN expressing the NA1 (open bars) compared to the NA2 (hatched bars) allotypic marker of FcγRIIIb. Subjects were matched for their FcγRIIa H/R131 allotype, and PMN from homozygous FcγRIIIb-NA1/NA1 and IIIb-NA2/NA2 were compared. PMN (1×10^6/ml) were incubated with opsonized STAW, Hib or NMen. Opsonizations were performed in the presence or absence of a complement source (AGS). Values represent means ± SD of at least four different donors. Each donor was tested twice. FcγRIIa blocking (mAb IV.3) of phagocytosis of Hib was done with only two donors (means are shown). Data are those of non-quenched samples, and are expressed as percentage of that with normal human serum plus complement. Asterisks mark significant difference (MWU, $P < 0·05$) between the two PMN populations.

IgG1 antibodies in the presence of complement, did not result in significantly different phagocytosis activity between NA1 and NA2 PMN (Fig. 1). However, after blocking of FcγRIIa phagocytic activity with IgG1-opsonized NMen in the presence of complement was significantly higher in NA1 compared to NA2 PMN. Moreover, a trend towards a difference between NA1 and NA2 was evident with STAW and Hib.

Interaction of PMN with IgG1 and IgG3 anti-D-sensitized RBC

To evaluate further phagocytosis by PMN with different

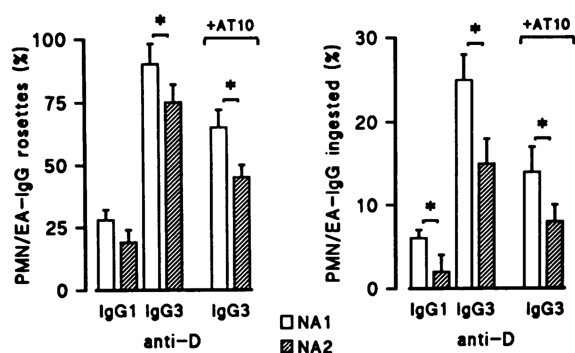

Figure 2. Effect of FcγRIIIb allotypes on rosette formation (left panel) and phagocytosis (right panel) of EA-IgG anti-D. Subjects were matched for their FcγRIIa H/R131 allotype, and PMN from homozygous FcγRIIIb-NA1/NA1 (open bars) and IIIb-NA2/NA2 (hatched bars) donors were compared. -D- RBC were sensitized with human IgG1 and IgG3 mAb anti-D (4·5 μg/ml) and incubated with PMN. Blocking of FcγRIIa was performed by addition of mAb AT10 [F(ab')$_2$ fragments]. Results are expressed as percentage (mean ± SD) PMN with ≥3 EA/PMN, or after isotonic lysis, with ≥1 ingested EA/PMN. Values were obtained from four different donors. Each donor was tested twice. Unsensitized RBC did not form rosettes. Asterisks mark significant difference (MWU, $P < 0·05$) between the two PMN populations.

FcγRIIIb NA1 and NA2 allotypes, we used -D- RBC as indicator cells. These indicator cells are suitable for the analysis of interactions with low-affinity FcγR because of their high expression levels of Rhesus D.[31] At all sensitization levels, EA rosette formation with EA-IgG1 was low (15–35%). In contrast, at EA-IgG3 sensitization levels between 1 and 7 μg/ml, between 65 and 100% of rosettes were observed with PMN from different donors. Approximately 20% of PMN with EA-IgG rosettes ingested EA-IgG3. The ability of FcγRIIIb-NA1 PMN to form rosettes with EA-IgG3 was significantly higher than that of IIIb-NA2 PMN, the relative difference (NA1 − NA2)/NA1 being 16% (Fig. 2). This difference increased to 30% when FcγRII-blocking mAb (AT10) were added (Fig. 2). The relative difference in internalization of EA-IgG3 by IIIb-NA1 and IIIb-NA2 PMN was 40%. The number of ingested EA-IgG1 was also higher with FcγRIIIb-NA1 compared to IIIb-NA2 PMN (6% and 2%, respectively). FITC-labelled RBC sensitized with anti-D IgG3 were used to analyse the blocking capacity of mAb against FcγR and CR (Table 1). The percentage PMN with bound EA-IgG3 was inhibited by mAb against FcγRIII from 60 to 22%, while anti-FcγRII mAb showed much less inhibition. Anti-CR3 mAb (B2.12) also blocked binding of EA-IgG3, in contrast to anti-CR1 mAb (3D9). Upon addition of a combination of anti-FcγRII and III mAb, the percentage PMN with EA-IgG3 rosettes decreased to 15%. Inhibition was nearly complete when both FcγRII and III, and CR3 were blocked. No inhibition was observed with anti-FcγRI mAb 197 in the experiments (data not shown). After quenching of FITC fluorescence from adherent EA-IgG essentially the same results were found (not shown).

Table 1. Effect of blocking mAb against FcγRIIa, FγRIIIb, CR1 and CR3 on phagocytosis of FITC-labelled EA-IgG3 to PMN*

mAb	Specificity	Positive PMN (%) †
Irrelevant mAb (10 μg/ml)‡	–	60 ± 7
IV.3 (5 μg/ml)	FcγRII	34 ± 4
AT10 [0·5 μg/ml, F(ab')$_2$]	FcγRII	37 ± 4
CLB/FcRGran1 (10 μg/ml)	FcγRIII	22 ± 4
3G8 (12 μg/ml)	FcγRIII	21 ± 2
AT10 + 3G8	FcγRII, III	15 ± 2
3D9 (10 μg/ml)	CR1	62 ± 5
B2.12 (10 μg/ml)	CR3	32 ± 3
AT10 + 3G8 + 3D9 + B2.12	FcγRII, III, CR1, CR3	3 ± 3

*PMN were from an FcγRIIa-H/H131/FcγRIIIb-NA1/NA1 donor, providing the combination of FcγR for the most effective binding. PMN were blocked with mAb for 30 min at 4° before incubation with EA-IgG3.

†Percentage (mean ± SD, $n = 4$) of total PMN with FITC-fluorescence was quantified flow cytometrically. Unsensitized FITC-labelled EA gave less than 5% positive PMN.

‡Mouse IgG1, IgG2a and IgG2b were used as irrelevant isotype-matched mAb.

Interaction between PMN of different FcγRIIa allotypes and IgG3 anti-D-sensitized RBC

We next studied EA-rosette formation of EA-IgG1 and EA-IgG3 with PMN expressing different FcγRIIa H/R131 allotypes (Fig. 3). Rosette formation occurred more efficiently between EA-IgG3 and PMN from FcγRIIa-H/H131 than with PMN from IIa-R/R131 donors (WSR, $P < 0·05$). Rosette formation with EA-IgG1 proved to be not significantly different between different FcγRIIa allotypes. Heterozygous FcγRIIa-H/R131 showed an intermediate level of rosette formation with EA-IgG3 (Fig. 3) (WSR, $P < 0·05$). The percentage of total PMN with ingested EA-IgG3 was 24 ± 4%, 12 ± 2% and 6 ± 0% ($n = 8$) (WSR, $P < 0·05$) for

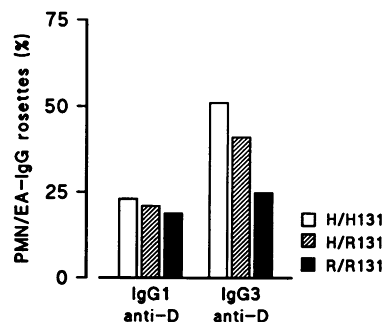

Figure 3. Rosette formation between PMN from donors with FcγRIIa-H/H131, IIa-H/R131 and IIa-R/R131 allotypes and EA-IgG1 and EA-IgG3. All three PMN donors were FcγRIIIb-NA1/NA2. -D- RBC were sensitized with human IgG1 and IgG3 mAb anti-D (4·5 μg/ml) and incubated with PMN. Eight experiments with different FcγRIIa H/R131 allotypes were performed using PMN from five different donors (see also Results section). The data are from a representative experiment, performed in duplicate: mean percentage PMN with ≥3 EA/PMN is shown.

FcγRIIa-H/H131, IIa-R/H131 and IIa-R/R131 subjects, respectively. No difference in phagocytosis, nor EA-IgG3 rosette formation was observed between FcγRIIa-H131 and IIa-R131 PMN in the presence of blocking anti-FcγRII mAb. The difference between FcγRIIa-H131 and IIa-R131 PMN was not enhanced in the presence of anti-FcγRIII mAb. Flow cytometric results paralleled those of rosette formation and phagocytosis (not shown).

DISCUSSION

Both the H/R131 FcγRIIa and the NA1/NA2 FcγRIIIb allotypes differ in their functional capacity. We have shown here that these functional differences extend to biologically relevant IgG1 and IgG3 antibodies. Previously, others have analysed (dimers of) myeloma IgG subclass preparations, myeloma IgG-sensitized RBC or transfected cell lines.[8,10,32,33]

Our results suggest that the biological differences in functional activity between the NA1 and the NA2 allotypic forms of FcγRIIIb also apply to polyclonal IgG1 antibacterial antibodies and to monoclonal IgG1 and IgG3 human anti-Rhesus D.[8,10] The phagocytosis of IgG1-opsonized bacteria, and anti-D IgG1- and anti-D IgG3-sensitized RBC, as well as the ability to form rosettes with anti-D IgG3-sensitized RBC was lower in NA2 homozygous subjects than in NA1 subjects. This difference was not a general property of PMN, and was not observed with IgG2-opsonized bacteria (Fig. 1). Blockade of FcγRIIa amplified differences in binding and phagocytosis between NA1 and NA2 subjects. Salmon *et al.* previously defined differences in phagocytic capacity of the different FcγRIIIb NA1/NA2 alleles, but found almost identical binding capacities for IgG oligomers.[10] Our experiments were not designed to differentiate between binding and ingestion of bacteria. However, after quenching the fluorescence of adherent bacteria we still observed more fluorescent bacteria in NA1 than in NA2 PMN, suggesting that indeed NA1 PMN are more active in internalization. Moreover, in the assay with EA-IgG, isotonic lysis of adherent EA-IgG3 or EA-IgG1 enhanced the relative differences in internalization activity between PMN from NA1 or NA2 donors, as compared to the binding (i.e. rosette formation) activity. In combination, these findings cannot exclude the possibility that differences in binding contribute to the overall difference between FcγRIIIb NA1/NA2 activity. It is still difficult to envisage how FcγRIIIb is involved in internalization differences, since FcγRIIIb on PMN is linked to the cell membrane via a glycosyl-phosphatidyl-inositol (GPI) anchor and does not possess transmembrane or cytosolic domains.[2] However, FcγRIIIb-induced intracellular responses have been demonstrated, indicating that (physical) interactions with other membrane proteins may take place.[2,34–37] Both FcγRIIa and FcγRIIIb are active in the phagocytosis of IgG-opsonized particles,[38] as was indicated by a reduced EA-IgG3 interaction with PMN in the presence of mAb against FcγRIIa or FcγRIIIb (Table 1). Anti-FcγRIII mAb influenced the binding of EA-IgG to a greater extent than did anti-FcγRII (Table 1), which is possibly due to the seven- to 10-fold higher expression of FcγRIIIb over FcγRIIa.[2] Interestingly, an anti-CR3 mAb (B2.12, IgM) also blocked the phagocytosis of EA-IgG3, suggesting an auxiliary role for CR3 in FcγR-mediated functions.[34,35] The rather large contribution of CR3 (Table 1) needs further study.

The clinical implications of the FcγRIIIb NA1/NA2 polymorphism are not yet clear. In C6 or C8-deficient individuals, the combined FcγRIIa-R/R131 and FcγRIIIb-NA2/NA2 phenotypes were found to be associated with susceptibility to Neisserial infections.[11] Although, a slightly lower (10–20%) phagocytic capacity of IgG1-opsonized bacteria was found with PMN from an FcγRIIIb^{neg} donor than with IIIb-NA2/NA2 PMN (data not shown), FcγRIIIb^{neg} donors do not appear to be prone to infections, provided no other components of the defence system are limiting.[39] In addition, it should be realized that the difference in functional capacity between PMN from NA1 or NA2 subjects was only apparent when purified IgG1 preparations (without complement) and homozygous FcγRIIIb-NA1 or IIIb-NA2 individuals were used, and not when bacteria were opsonized with native human serum (not shown). The NA1/NA2 difference in phagocytic capacity of bacteria opsonized by purified IgG1 in the presence of complement was significant with NMen when the FcγRII was blocked, but not with STAW or Hib (Fig. 1). This may point to differences in the contribution of the complement receptors to opsonization and phagocytosis of NMen, Hib and STAW.

With respect to the H-R131 allotype of FcγRIIa, we found that human IgG3 anti-D formed significantly more EA-rosettes with FcγRIIa-H131 compared to IIa-R131 PMN. This is in agreement with myeloma IgG3-dimer binding studies by Parren *et al.*[5]

The heterozygous FcγRIIa-H/R131 cell line K562 has been shown to have an intermediate activity in rosette formation with mouse IgG1 compared to that of transfected cell lines (3T6) homozygous for FcγRIIa H/R131 allotypes.[4] We confirmed this intermediate activity of heterozygous FcγRIIa-H/R131 with IgG3 anti-D sensitized RBC [and with IgG2-opsonized bacteria (R. G. M. Bredius, unpublished observations)].

Among other risk factor for infectious diseases, the FcγRIIa-R/R131 allotype may comprise an additional factor, particularly in the defence against encapsulated bacteria and against viruses, when these are IgG2[13] or IgG3[14,15] dependent, respectively. In addition, clearance of IgG2- and IgG3-containing immune complexes could be affected in FcγRIIa-R/R131 individuals. The latter was recently supported by the observation that an increased proportion of patients with systemic lupus erythematosus was FcγRIIa-H/R131 or IIa-R/R131 (i.e. responding to murine IgG1 anti-CD3 in a T-cell proliferation assay)[40] (and L. Duits, H. Bootsala, R. A. W. M. Derksen *et al.*, unpublished observations).

In conclusion, this study provides evidence for a functional role of FcγR polymorphisms in the binding and phagocytosis of opsonized particles: human IgG2 antibacterial antibodies and IgG3 anti-Rhesus D antibodies interact more readily with the FcγRIIa-H131 than IIa-R131 allotype, and IgG1 and IgG3 interact more readily with FcγRIIIb-NA1 than IIIb-NA2 allotype.

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