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Different Adaptations of IgG Effector Function in Human and Nonhuman Primates and Implications for Therapeutic Antibody Treatment

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Safety of human therapeutic Abs is generally assessed in nonhuman primates. Whereas IgG1 shows identical FcγR interaction and effector function profile in both species, fundamental differences in the IgG2 and IgG4 Ab subclasses were found between the two species. Granulocytes, the main effector cells against IgG2- and IgG4-opsonized bacteria and parasites, do not express FcγRIIb, but show higher levels of FcγRIIa in cynomolgus monkey. In humans, IgG2 and IgG4 adapted a silent Fc region with weak binding to FcγR and effector functions, whereas, in contrast, cynomolgus monkey IgG2 and IgG4 display strong effector function as well as differences in IgG4 Fab arm exchange. To balance this shift toward activation, the cynomolgus inhibitory FcγRIIb shows strongly increased affinity for IgG2. In view of these findings, in vitro and in vivo results for human IgG2 and IgG4 obtained in the cynomolgus monkey have to be cautiously interpreted, whereas effector function-related effects of human IgG1 Abs are expected to be predictable for humans. The Journal of Immunology, 2012, 188: 000–000.

The complex interplay of Fc receptors and Abs has been an area of scientific interest for the last decades. More than 30 years ago, we and others could show that different IgG Fc receptors (FcγRs) exist that are highly selective in terms of subclass specificity and affinity (1, 2). The high complexity of this system is increased by the existence of activating and inhibitory FcγRs (3) and the fact that the different effector cell populations are decorated with different types and different densities of Fc receptors (4). Interaction of monomeric Abs or Ab immune complexes with Fc receptors triggers Ab-dependent cellular cytotoxicity (ADCC) and Ab-dependent cellular phagocytosis, whereas interaction with complement results in complement-dependent cytotoxicity (CDC) (5). From a signaling standpoint, activation of the cellular effector functions is mediated by ITAM-containing FcγRs (6) and effector functions, whereas, in contrast, cynomolgus monkey IgG2 and IgG4 display strong effector function as well as differences in IgG4 Fab arm exchange. To balance this shift toward activation, the cynomolgus inhibitory FcγRIIb shows strongly increased affinity for IgG2. In view of these findings, in vitro and in vivo results for human IgG2 and IgG4 obtained in the cynomolgus monkey have to be cautiously interpreted, whereas effector function-related effects of human IgG1 Abs are expected to be predictable for humans. The Journal of Immunology, 2012, 188: 000–000.

The online version of this article contains supplemental material.

Abbreviations used in this article: ADCC, Ab-dependent cellular cytotoxicity; CDC, complement-dependent cytotoxicity; CRP, C-reactive protein; GSH, reduced glutathione; LC-MS, liquid chromatography-mass spectrometry; NHP, nonhuman primate.
and toward IgG4, which (together with IgE) is generated in antiparasite immunity and responses to allergens. In contrast, IgG1 and IgG3 subclasses, which are mainly involved in other types of the humoral immune response, show a very similar pattern of FcγR binding and effector function in the two species. To our knowledge, this study describes for the first time the complete set of FcγRI/II/III and FcγRs in comparison with the human system, and will therefore be very helpful in interpreting the data generated with therapeutic Abs in NHP and their translation to humans.

Materials and Methods

Recombinant FcγRs and Abs

Extracellular domains of human and cynomolgus FcγR were cloned into expression vectors harboring a 4-aa purification tag (4APP; Novartis) and an Avi biotinylation tag (GLNDIFEAQKIEWHE; Avidity), expressed in E. coli and purified with the natural leader and the CD33 leader showed an Avi biotinylation tag (GLNDIFEAQKIEWHE; Avidity), expressed in 293 cells, and purified by protein G and size exclusion chromatography. The human receptors were expressed with a CD33 signal leader (P20138, 1-16) instead of the natural leader sequence to enhance protein expression. Receptor proteins expressed with the natural leader and the CD33 leader showed identical amino acid sequences as analyzed by mass spectrometry. The following receptor sequences were synthesized by Geneart: human FcγRIIA (GenBank: JN984926), 158F (Uniprot: P08637, 17-199), cynomolgus FcγRIIA; human FcγRIIA (CD32a) 131H (Uniprot: NP_067674, 34, 217), human FcγRIIA 131R (Uniprot: NP_067674, 34, 217, aa 131 = R), cynomolgus FcγRIIA (Uniprot: P31994, 42-223), and cynomolgus FcγRIIB (Uniprot: AAL92096, 1-226). Receptors were site directed biotinylated with BirA (AviTag). Extracellular domain of human FcγRI (CD64) protein was purchased from R&D Systems (Minneapolis, MN). Cynomolgus κ L chain (GenBank: JN984930) and γ H chain C regions (IgG1, GenBank: JN984927; IgG2, GenBank: JN984926; IgG4, GenBank: JN984929) were cloned from cynomolgus blood mRNA (primer: 5′-GCCACCGCCATGGTTCGCC-3′, 5′-AATACAGCTCTCCCTGTTGCA-3′, γ H chain, 5′-GCCCTC- CACCCAGGGCGCCCATCG-3′, 5′-TTTACCCGGAGACACGGAGAG-3′). Genomic DNA from the blood of 10 cynomolgus animals was sequenced for the CDR3 (CD64) region, and 90% Ficoll plus 10% PBS for the human blood separation, and 90% Ficoll plus 10% PBS for the cynomolgus blood separation.

Highly purified monomeric Abs with VH and VL derived from the X. laevis light chain and H chain C regions (IgG1, GenBank: JN984927; IgG2, GenBank: JN984928) were cloned from cynomolgus blood. Different FcγRs were expressed with the natural leader and the CD33 leader showed identical amino acid sequences as analyzed by mass spectrometry. The following receptor sequences were synthesized by Geneart: human FcγRIIA (GenBank: JN984926), 158F (Uniprot: P08637, 17-199), cynomolgus FcγRIIA (CD32a) 131H (Uniprot: NP_067674, 34-217), human FcγRIIIA (CD64) protein was purchased from R&D Systems (Minneapolis, MN). Cynomolgus κ L chain (GenBank: JN984930) and γ H chain C regions (IgG1, GenBank: JN984927; IgG2, GenBank: JN984926; IgG4, GenBank: JN984929) were cloned from cynomolgus blood mRNA (primer: 5′-GCCACCGCCATGGTTCGCC-3′, 5′-AATACAGCTCTCCCTGTTGCA-3′, γ H chain, 5′-GCCCTC- CACCCAGGGCGCCCATCG-3′, 5′-TTTACCCGGAGACACGGAGAG-3′). Genomic DNA from the blood of 10 cynomolgus animals was sequenced for the CDR3 (CD64) region, and 90% Ficoll plus 10% PBS for the human blood separation, and 90% Ficoll plus 10% PBS for the cynomolgus blood separation.

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measure their binding against the different human and cynomolgus FcγR by surface plasmon resonance. In contrast to human IgG2 and IgG4 isotypes, which show weak interaction with the human and cynomolgus activating receptors FcγRI and FcγRIII, cynomolgus IgG2 and IgG4 showed substantial binding to corresponding FcγR of both species (Fig. 2). Human and cynomolgus FcγRI do not bind to human IgG2, but show nanomolar affinity to cynomolgus IgG2 (Fig. 2A, 2B, Supplemental Figs. 2, 3, Table I). The binding of FcγRI to cynomolgus IgG4 has a 10× higher affinity due to a slower off-rate in comparison with human IgG4 (Fig. 2B). Human and cynomolgus FcγRIII bind cynomolgus IgG2 and IgG4 with a 5–10× higher affinity than human IgG2 and IgG4 (Fig. 2D–G). Overall, human and cynomolgus FcγRI and FcγRIII show a qualitatively similar binding profile toward the different human and cynomolgus IgG isotypes (IgG1–IgG4). In humans, FcγRIII has an allotypic variation and FcγRIII-158V has a higher affinity than FcγRIII-158F (23). Interestingly, cynomolgus FcγRIII contains the more valine-like amino acid isoleucine at position 158 and accordingly shows a high-affinity phenotype (Table I). Distinct amino acid substitutions in the cynomolgus IgG sequences at the FcγR binding region of the activating human FcγRIIb (Fig. 3). This difference in binding is directly correlated with a different amino acid pattern in the Fc binding region of cynomolgus FcγRIIb (Fig. 3C). There is a polymorphism in the Fc binding region of the activating human FcγRIIa, which has a higher affinity to IgG2 if the amino acid at position 131 is a histidine. Accordingly, human FcγRIIa-131H and cynomolgus FcγRIIa (131H) show a higher affinity to IgG2 than human FcγRIIa-131R (Fig. 2C). In contrast to human FcγRIIb, in which the amino acid at position 131 is an arginine leading to low-affinity binding of IgG2, cynomolgus FcγRIIb has a high-affinity conferring histidine at this position (Fig. 3C). Accordingly, cynomolgus FcγRIIb shows a 20-fold higher affinity to human IgG2 and a 10-fold higher affinity to cynomolgus IgG2 compared with human FcγRIIb (Fig. 2C).

IgG4 Fab arm exchange

Another important feature that distinguishes cynomolgus IgG4 from human IgG4 is its inability to perform Fab arm exchange. It is now well established that human IgG4 can efficiently perform

**FIGURE 1.** FcγRII expression on human and cynomolgus granulocytes (A) and monocytes (B) distinguished by forward and sideward scatter intensity. Human donors were simultaneously analyzed for the different FcγRII allotypes (FcγRIIa 131H, HR, RR). Cynomolgus granulocytes expressed significantly higher levels of FcγRII in comparison with all human individuals and to each subgroup of the FcγRIIa allotypes (**p < 0.0001**). No difference was found in FcγRII expression between human and cynomolgus monocytes.

**FIGURE 2.** Binding of human and cynomolgus IgG isotypes to FcγR. (A–G) Binding of the different isotypes to (A) human FcγRI, equilibrium-binding constants (Kₐ); (B) human FcγRI, dissociation rates (kₐ); (C) FcγRIIa, Kₐ, and FcγRIIb, Kₐ; (D–G) binding levels at equilibrium: (D, E) human FcγRIIa; (F, G) cynomolgus FcγRIIa. Human IgG1 (black circle), human IgG2 (green circle), human IgG3 (blue circle), human IgG4 (red circle), cyno IgG1 (black triangle), cyno IgG2 (green triangle), cyno IgG3 (blue triangle), cyno IgG4 (red triangle), 3 = 2 for all experiments; (A–C) mean values of two independent experiments; (D–G) values in duplicates; one representative experiment of two is shown.
Table 1. Overview of binding affinities to FcγR and ADCC potency of the different anti-CD20–based human and cynomolgus IgG isotype Abs

<table>
<thead>
<tr>
<th></th>
<th>Human IgG1</th>
<th>Human IgG2</th>
<th>Human IgG3</th>
<th>Human IgG4</th>
<th>Cyno IgG1</th>
<th>Cyno IgG2</th>
<th>Cyno IgG3</th>
<th>Cyno IgG4</th>
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<tr>
<td><strong>Cellular binding</strong> (n = 2)</td>
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<tr>
<td>Cyto FcyRI EC50 (nM)</td>
<td>0.20 ± 0.09</td>
<td>&gt;30⁷</td>
<td>0.13 ± 0.05</td>
<td>0.29 ± 0.04</td>
<td>0.14 ± 0.03</td>
<td>0.40 ± 0.11</td>
<td>0.31 ± 0.07</td>
<td>0.30 ± 0.18</td>
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<td>Biaxore binding (n = 2)</td>
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<tr>
<td>Human FcyRI Kd (nM)</td>
<td>0.17 ± 0.01</td>
<td>No binding</td>
<td>0.13 ± 0.03</td>
<td>1.24 ± 0.37</td>
<td>0.17 ± 0.02</td>
<td>0.48 ± 0.12</td>
<td>0.23 ± 0.04</td>
<td>0.35 ± 0.1</td>
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<td>Kd (nM)</td>
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<tr>
<td>Human FcyRIIIA 158V</td>
<td>0.37 ± 0.03</td>
<td>17 ± 0.2⁷</td>
<td>0.42 ± 0.02</td>
<td>13.3 ± 0.4</td>
<td>0.46 ± 0.0</td>
<td>3.32 ± 0.15</td>
<td>0.62 ± 0.03</td>
<td>2.87 ± 0.06</td>
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<tr>
<td>Human FcyRIIIA 158F</td>
<td>1.71 ± 0.1</td>
<td>49 ± 6⁷</td>
<td>1.47 ± 0.06</td>
<td>42 ± 4⁴</td>
<td>1.12 ± 0.07</td>
<td>5.98 ± 0.04</td>
<td>1.79 ± 0.02</td>
<td>7.7 ± 0.56⁷</td>
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<tr>
<td>Cyto FcyRIII</td>
<td>0.36 ± 0.01</td>
<td>11.4 ± 0.8⁴</td>
<td>0.4 ± 0.04</td>
<td>11.2 ± 0.4⁴</td>
<td>0.48 ± 0.01</td>
<td>2.38 ± 0.07</td>
<td>0.62 ± 0.02</td>
<td>2.84 ± 0.02</td>
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<tr>
<td>Human FcyRIIIA 131H</td>
<td>0.56 ± 0</td>
<td>0.37 ± 0.06</td>
<td>1.69 ± 0.02</td>
<td>4.01 ± 0.29</td>
<td>0.28 ± 0.03</td>
<td>1.13 ± 0.31</td>
<td>1.03 ± 0.1</td>
<td>2 ± 0.13</td>
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<td>Human FcyRIIIA 131R</td>
<td>1.12 ± 0.11</td>
<td>21.8 ± 0.21</td>
<td>2.11 ± 0.16</td>
<td>3.23 ± 0.1</td>
<td>0.43 ± 0.02</td>
<td>6.2 ± 0.2⁴</td>
<td>0.45 ± 0.04</td>
<td>1.08 ± 0.06</td>
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<tr>
<td>Human FcyRIIB</td>
<td>4.88 ± 0.02</td>
<td>10.89 ± 0.1²</td>
<td>6.5 ± 0.2⁴</td>
<td>3.75 ± 0.14</td>
<td>2.08 ± 0.05</td>
<td>19.7 ± 0.8⁶</td>
<td>3.71 ± 0.09</td>
<td>4.42 ± 0.3</td>
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<tr>
<td>Cyto FcyRIIB</td>
<td>1.84 ± 0.05</td>
<td>0.47 ± 0</td>
<td>4.21 ± 0.13</td>
<td>4.79 ± 0.1</td>
<td>1.17 ± 0.05</td>
<td>1.92 ± 0.02</td>
<td>3.59 ± 0.05</td>
<td>4.94 ± 0.34</td>
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<td>ADCC EC50 (μg) (n = 4)</td>
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<tr>
<td>Human PBMC</td>
<td>0.04 ± 0.04</td>
<td>11 ± 9⁶</td>
<td>0.09 ± 0.10</td>
<td>3.6 ± 4.9</td>
<td>0.02 ± 0.01</td>
<td>0.16 ± 0.05</td>
<td>0.03 ± 0.01</td>
<td>0.23 ± 0.27</td>
</tr>
<tr>
<td>Cyto PMBC</td>
<td>0.03 ± 0.02</td>
<td>13 ± 10⁶</td>
<td>0.09 ± 0.04</td>
<td>0.94 ± 0.60</td>
<td>0.05 ± 0.03</td>
<td>0.40 ± 0.20</td>
<td>0.07 ± 0.04</td>
<td>0.16 ± 0.01</td>
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⁷Value is extrapolated and is outside the range of the used concentrations.

In contrast, combination of human and cynomolgus IgG4 Abs did not reveal an additional mass species, further providing evidence that cynomolgus IgG4 does not perform half-molecule exchange (Fig. 4C–F).

Cynomolgus IgG2 and IgG4 show enhanced effector function

To analyze whether the observed differences of human and cynomolgus IgG2 and IgG4 at the molecular and biochemical level are reflected in different function of these subclasses, we used human and cynomolgus PBMCs and serum to assess the ADCC and CDC potency of anti-CD20 Abs grafted on human and cynomolgus C region IgG subclasses. Cynomolgus B cells include two different populations characterized by different relative expression of CD20, CD40, and CD21 (i.e., CD20lowCD40highCD21+ and CD20highCD40lowCD21⁻) (29). Because the CD20lowCD40highCD21⁺ population in cynomolgus monkey shows similar properties as human B cells with respect to in vivo and in vitro sensitivity to rituximab treatment, we analyzed the in vitro depletion of CD40lowCD21⁺ B cells in both species to obtain comparable data. Human IgG2 and IgG4 are functionally silent not only toward human, but also toward cynomolgus B cells, because they show a similar low binding to human and cynomolgus FcyRIII (Fig. 2D, 2F), resulting in a corresponding similar low ADCC activity with human and cynomolgus NK cells (Fig. 5A, 5C). In contrast to human IgG2 and IgG4, cynomolgus IgG2 and IgG4 show potent ADCC with both human and cynomolgus PBMCs as well as potent CDC with human and cynomolgus serum (Fig. 5B, 5D, Supplemental Fig. 4). In contrast, IgG1 and IgG3, the subclasses with equivalent FcyRs binding, show a similar effector function profile on both human and cynomolgus PBMCs (Fig. 5).

In conclusion, the observed ADCC and CDC functional activities appear to directly correlate with critical amino acids and binding data differences of the human and cynomolgus IgG subclasses. In contrast to the human IgG2 and IgG4 subclasses, cynomolgus IgG2 and IgG4 are not silent, whereas IgG1 and IgG3 behave very similar in the two species.

Discussion

Despite the reported sequence homology of human and cynomolgus IgG subclasses and FcyRs (18), we could identify key differences between these two species on the level of molecular sequences, FcyR binding, receptor expression, distribution, and function. The identified differences induce changes, which can be explained by a single divergent evolutionary constraint, namely a different strategy to fight against bacteria and parasites. Cyno-
humans. The potential effector function of cynomolgus IgG4 is shown to be almost identical to that in both cynomolgus monkeys and human beings. The IgG1 and IgG3 subclasses are different in cynomolgus monkey, demonstrating potent FcR-mediated function of human effector function. In contrast to humans and human primates, this coevolution of activating-to-inhibitory ratio as proposed by Nimjerhahn and Ravetch (8) based on studies in the rodent system, as it seems to play a fundamental role in the evolution and function of human effector function.

Some of our findings are in contradiction with an earlier study by Jacobsen et al. (20) showing that cynomolgus IgG1 displayed the highest binding on cynomolgus monocytes compared with other human and cynomolgus IgG isotypes. In addition, cynomolgus IgG1 was far more potent in mediating ADCC in cynomolgus whole blood. The differences to the data presented in this report can be explained by the following improved technologies applied in this study. First, the size exclusion chromatography-

![Image](http://www.jimmunol.org/)

**FIGURE 4.** Fab arm exchange of TGN1412 with cynomolgus IgG4. (A) A total of 100 μg human rituximab-IgG4 and human TGN1412 (solid line), or 100 μg cynomolgus rituximab-IgG4 (dashed line) and human TGN1412, or 100 μg human rituximab-IgG4 alone (gray filled histogram) was injected into SCID mice (n = 2), and Fab arm exchange was analyzed 48 h later. (B) A total of 1 μg human rituximab IgG4 was incubated for 24 h at 37°C with different IgG4 variants of TGN1412 in the presence of GSH. The TGN1412 variants were constructed by exchanging the human IgG4 C region by rhesus IgG4, cynomolgus IgG4 (wild type), cynomolgus IgG4 S131C, cynomolgus IgG4 P228S, cynomolgus IgG4 with a human Cπ3 domain, and cynomolgus IgG4 with a rhesus Cπ3 domain. (C-F) LC-MS analysis of IgG4 Fab arm exchange. A total of 100 μg each Ab was incubated for 24 h at 37°C in 100 μl PBS and analyzed by LC-MS: (C) human rituximab-IgG4 + human TGN1412 without GSH; (D) human rituximab-IgG4 + human TGN1412 in the presence of GSH; (E) human rituximab-IgG4 + cynomolgus TGN1412 S131C without GSH; (F) human rituximab-IgG4 + cynomolgus TGN1412 S131C in the presence of GSH.

**FIGURE 5.** ADCC effector function of human and cynomolgus IgG isotypes. Lysis of B cells in the presence of human or cynomolgus monkey PBMC with different concentration of rituximab. (A) Human PBMC with human isotypes; (B) human PBMC with cynomolgus isotypes; (C) cynomolgus PBMC with human isotypes; (D) cynomolgus PBMC with cynomolgus isotypes. Human IgG1 (black circle), human IgG2 (green circle), human IgG3 (blue circle), human IgG4 (red circle), cyno IgG1 (black triangle), cyno IgG2 (green triangle), cyno IgG3 (blue triangle), cyno IgG4 (red triangle) (n = 4 for all experiments).

Of note, we found that the increase of activating function of cynomolgus IgG2 (i.e., higher binding to FcyRI and FcyRIII and higher effector function) is counterbalanced by a strongly increased affinity toward cognate antibodyFcγRIIb. Interestingly, in contrast to FcyRIIb in the NHP macaque family members cynomolgus and rhesus monkey, which contain a histidine at the position 131, all human primates analyzed (gorilla, orangutan, and chimpanzee) are characterized by an arginine at position 131. This change toward the low-affinity arginine variant of FcγRIIb is always accompanied by a corresponding low-affinity mutation in the Fc binding region of IgG2 similar to the mutation found in human IgG2 (Fig. 3B, 3C). We therefore hypothesize that the inhibitory receptor FcyRIIB and the silent isotypes coevolved and that macaque species such as the cynomolgus and rhesus monkey have developed a different strategy to balance IgG/FcγR interactions in contrast to humans and human primates. This coevolution of activating IgG isotypes and the inhibitory FcyR, FcyRIIB, supports the model of activating-to-inhibitory ratio as proposed by Nimjerhahn and Ravetch (8) based on studies in the rodent system, as it seems to play a fundamental role in the evolution and function of human effector function.

That recombinant Abs with rhesus IgG4 C regions display similar or even increased IgG4 Fab arm exchange than human IgG4. It has also been described that human IgG4 therapeutic Abs can perform Fab arm exchange with cynomolgus serum Abs, but the IgG4 molecular sequence or allotype was not analyzed in this study (35).
purified material used in our study guarantees a monomeric composition, whereas only protein A-purified material used by Jacobsen et al. (20) can result in a high percentage of aggregated, nonmonomeric Abs. For studying FcγR binding and effector function, the use of purely monomeric IgG is absolutely necessary, as FcγR shows 10- to 100-fold higher binding to aggregated IgG, and therefore, data generated with aggregated IgG would reflect the difference in the proportion of aggregated IgG rather than the mere difference in isotype selectivity. Furthermore, the affinity of IgG to the low-affinity FcγR (i.e., FcγRIIA, FcγRIIB, and FcγRIID) is micromolar, which obscures the detection of monomeric IgG in the FACS-based assay used by Jacobsen et al. (20) due to the fast off-rate displacing the IgG from the Fc receptor during the washing steps. In contrast, the surface plasmon resonance analysis of this interaction as used in our study is able to detect the low-affinity binding and is therefore the preferable method. In addition, the effector cells (i.e., monocytes) used in the Jacobson study (20) express a variety of different FcγR, and it is therefore not possible to clearly assign to which Fc receptors the different IgG subclasses bind. The functional activity of the different IgG isotypes was analyzed by Jacobsen et al. (20) in a whole blood B cell depletion assay using anti-CD20 Ab for depletion and CD19 as the marker for cytomolgus B cells. In contrast to humans, cytomolgus monkeys show two different populations of CD20-positive B cells. Only the CD40(high)CD21poscytomolgus monkey B cells are depleted by rituximab similar to human B cells and in a Fc-dependent manner (29, 36) (M. Warncke, unpublished observations), and therefore we used this population for our analysis. We think that the high quality of the material used in our study together with the precise methodology allowed us to identify differences between the human and cytomolgus IgG isotypes. The fact that the molecular sequence corresponds to the binding data and effector function further validates our approach.

Interestingly, position 131 of human FcγRIIA is also determining the high- and low-affinity binding of FcγRIIA to C-reactive protein (CRP). In contrast to IgG, CRP has a higher affinity to human FcγRIIA131R than to human FcγRIIA131H (37). As CRP is highly conserved in evolution and is mimicking important aspects of IgG, such as binding to FcγRs and complement (38), the discussed difference in NHP FcγRIIB may also affect the immune system beyond FcγR effector function.

Given the importance of FcγRIIB for the balance of the immune response, this remarkable shift in function of cytomolgus versus human FcγRIIB raises questions regarding the prediction of NHP effector function for human therapeutic Abs, as it has a direct influence on the toxicity assessment in cytomolgus or rhesus models of FcγRIIB-engaging agents (39, 40). For example, mutations in the Fc part of human IgG1 (S267E and L328F) led to >200-fold increased affinity to human FcγRIIB and FcγRIIA 131R, but not to FcγRIIA 131H. Thus, the key amino acid at position 131 is responsible for the increased affinity of this Fc variant (S267E and L328F) to FcγRIIB. However, this amino acid in cytomolgus FcγRIIB is different (Fig. 3), and therefore this mutant Fc variant (S267E and L328F) shows no enhancement in affinity to cytomolgus FcγRIIB (41).

In contrast to the differences identified in the silent IgG subclasses and FcγRIIB, the activating FcγR (FcγRI, FcγRIIA, FcγRIIB) and the active IgG1 and IgG3 subclasses have comparable effector function in the human and the cytomolgus system: the binding characteristics of human and cytomolgus IgG1 and IgG3 toward human and cytomolgus FcγRs are comparable. IgG1 and IgG3 ADCC activity, which is mainly triggered in vitro by the ITAM-bearing receptor FcγRIIA on NK cells (and to a lower extent FcγRIIA and FcγRI), and is largely independent on FcγRIIb, is indistinguishable whether human or cytomolgus effector cells are used. We therefore conclude that the observed species differences can be explained by key differences in the amino acid sequence composition at critical residues of the IgG2 and IgG4 Ab subclasses and the inhibitory receptor FcγRIIB in human and cytomolgus monkey. On the contrary, the activity profiles of the various activating FcγRs are by large and functionally conserved between the two species. Thus, human therapeutic Abs with a wild-type IgG1 C region are expected to have a very similar effector profile in the cytomolgus monkey as compared with humans, which qualifies the cytomolgus monkey as a relevant model for this isotype. However, modifications to the IgG1 wild type, resulting in decreased or enhanced binding (24, 42–44) to FcγRIII or FcγRIIB, which are increasingly being evaluated for therapeutic use, have to be evaluated very carefully, as the difference in FcγRIII and FcγRII expression on human and cytomolgus granulocytes could influence their overall functional behavior (45). In particular, when evaluating Fc modifications altering the binding to FcγRIIB (39, 40), equivalent FcγRIIB cross-reactivity for the selected animal model species is required to allow prediction to humans.

In summary, our findings shed new light on the evolution and plasticity of IgG effector function and allow a better extrapolation of human therapeutic Ab Fc-mediated functions from the NHP to humans.

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