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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 FcγRII 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 (FcγR) 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 interaction with IgG immune complexes (e.g., opsonized bacteria or parasites). These activities are counterregulated by the ITIM-containing FcγRIIb (CD32b), which plays a critical role in modulating IgG-mediated effector responses, such as in humoral immunity (6, 7). The relative densities of inhibitory (i.e., FcγRIIb) versus activating FcγRs, and in particular their corresponding selectivity and affinity toward different IgG isotypes, determine whether an Ab will generate an activating proinflammatory or an inhibitory anti-inflammatory response. For a given IgG isotype, Ravetch and Nimmerjahn (8) have found that the ratio of affinities of the activating ITAM-bearing FcγRs to the inhibitory FcγRIIb is a key determining factor. In the case of therapeutic Abs, IgG/FcγR interactions contribute significantly to both therapeutic effect and toxicity of these drugs (5). Due to their proximal evolutionary relationship to humans, the most appropriate preclinical model to analyze the therapeutic effect and potential toxicity of human Abs are nonhuman primates (NHP), in particular the cynomolgus monkey (9). However, recent clinical trial results have questioned whether NHP are an appropriate model. A striking example is the outcome of the TGN1412 trial in 2006, in which an anti-CD28 Ab (TGN1412) did not show severe adverse side effects in NHP, but generated a dramatic cytokine storm in all six treated human subjects (10). Possible contributions to this differential response in NHP and humans in this trial include differences in the signaling domain of CD28, the loss of Siglec-5 expression during human evolution (11, 12), or a different expression pattern of CD28 on a specific T cell subset (13). However, because triggering of a cytokine storm by TGN1412 is dependent on FcγR interaction (14), it has also been speculated that the human IgG4 Fc portion of the TGN1412 Ab might bind less efficiently to monkey than to human FcγRs (15).

In light of the growing use of IgG mAbs as biotherapeutic compounds, we further investigated the interaction of human and cynomolgus IgG subclasses and FcγRs to assess the appropriateness of cynomolgus monkeys in evaluating Fc-dependent efficacy and toxicity profiles of therapeutic IgGs. Our study revealed that the effector function of humans and NHP is an adaptable system, which is shaped by species-specific constraints. We have found clear differences in FcγR expression on granulocytes, allotypic variation of IgG4 Fab arm exchange, and enhanced activity of IgG2 and IgG4 subclasses in cynomolgus monkeys compared with the human system, which may have been evolved by different needs in phagocytosis and neutralization of bacteria and parasites. Interestingly, this overall enhanced activation function in cynomolgus monkeys is counterbalanced by a stronger inhibitory capacity of cynomolgus FcγRIIb toward IgG2, an isotype with preference for repetitive carbohydrate Ags on bacteria (16, 17),
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/IIA interactions and functional consequences in the cynomolgus monkey 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 FcγRI and Abs
Extracellular domains of human and cynomolgus FcγRI were cloned into expression vectors harboring a 4-aa purification tag (4APP; Novartis) and an Avi biotinylation tag (GLNDIFEAQKIEWHE; Avidity). The following receptor sequences were synthesized by Geneart: human FcγRIIA (CD16a) (Uniprot: P09637, 17-199), human FcγRIIB (UniProt: P08637, 15-199), human FcγRIIA (CD32a) (UniProt: N067674, 34-217), human FcγRIIA 131R (UniProt: N067674, 34-217, aa 131 = R), human FcγRIIA (UniProt: AAL29096, 1-210), human FcγRIIB (CD32b) (UniProt: P31994, 42-223), and cynomolgus FcγRIIB (UniProt: AAL29097, 1-226). Receptors were site directed biotinylated with BirA (Avidity). Extracellular domain of human FcγRI (CD64) was produced from R&D Systems (Minneapolis, MN). Cynomolgus κ L chain (GenBank: JN984930) and γ H chain C regions (IgG1, GenBank: JN984927; IgG2, GenBank: JN984928; IgG4, GenBank: JN984929) were cloned from cynomolgus blood mRNA (primer κ L chain, 5′-ATCAACAGGCTGTGGCTGAGACA-3′, 5′-TTTACCGGAGACACGGAGTAG-3′). Genomic DNA from the blood of 10 cynomolgus monkeys was sequenced to identify the γ H chain C regions (primers: 5′-GCCGGGACTCTGTGGAGAG-3′, 5′-CCTGGGAAGTATGTACACGG-3′). Functional assays
ADCC activity was assessed by the depletion of B cells. PBMCs were purified by Ficol gradient, 100% Ficol was used for the human blood separation, and 90% Ficol plus 10% PBS for the cynomolgus blood separation. Human or cynomolgus PBMC were incubated for 1 h with the anti-FcγRIIb Abs, stained for B cells with anti-CD21 (B-ly4, BD Biosciences), and analyzed by flow cytometry. Killing was calculated as percentage of B cells without Ab to percentage of B cells with Ab.

Results
Different FcγRII expression on human and cynomolgus granulocytes
FcγRII fulfill diverse functions in the different immune cells from neutrophil Ab-mediated phagocytosis of bacteria to regulation of B cell activation. Human granulocytes express only FcγRIIb and FcγRIIA, and therefore become highly activated by IgG-opsonized bacteria or parasites. In contrast to humans, NHP do not express any form of FcγRII on granulocytes (21). Surprisingly, we found instead a 3-fold higher expression of FcγRII on cynomolgus granulocytes in comparison with humans (Fig. 1). This is a specific difference in the FcγR expression pattern of neutrophils as other populations such as monocytes (Fig. 1), B cells, or NK cells (data not shown) show no differences in the expression pattern and levels between human and cynomolgus monkey. As FcγRIII has a different binding profile in comparison with FcγRI, especially for the IgG2 subclass (22), we decided to perform a complementary analysis of the whole IgG/FcγR interaction set in humans and the cynomolgus monkey.

Cynomolgus IgG2 and IgG4 show increased binding to FcγRII and FcγRI
Highly purified monomeric Abs with VH and VL derived from the CD20-binding Ab rituximab (19) recombinantly expressed with the four human and cynomolgus IgG subclasses were used to

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measure their binding against the different human and cynomolgus FcγRs 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 FcγRII in 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 Fab binding region of cynomolgus IgG2 and IgG4 (Fig. 3). The “silent” effector function of human IgG2 and IgG4 can be explained by differences to IgG1 in residues of the hinge and Cγ2 domain that dramatically decrease their affinity for FcγRs and complement, therefore rendering them inactive for ADCC or CDC (26). This is not the case for cynomolgus IgG2 and IgG4, where these residues are identical to the full effector function bearing IgG1 and IgG3 isotypes (Fig. 3A). In summary, cynomolgus IgG2 and IgG4 show increased binding to human and cynomolgus FcγRIII and FcγRI in comparison with their human counterparts, whereas human and cynomolgus IgG1 and IgG3 show an almost identical binding to human and cynomolgus FcγRIII and FcγRI.

High-affinity binding of cynomolgus FcγRIIb to IgG2

Interestingly, all the activating ITAM-bearing FcγRs of human and cynomolgus origin show almost identical binding profiles. One striking difference is that the cynomolgus inhibitory FcγRIIb binds IgG2 with substantially higher affinity than human FcγRIIb (Fig. 2C). 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...
EFFECTOR FUNCTION OF NONHUMAN PRIMATES

Table I. Overview of binding affinities to FcγR and ADCC potency of the different anti-CD20–based human and cynomolgus IgG isotypes

<table>
<thead>
<tr>
<th>Cellular binding (n = 2)</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>
</tr>
</thead>
<tbody>
<tr>
<td>Cyto FcγRI 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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<tr>
<td>Biacore binding (n = 2)</td>
<td></td>
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<tr>
<td>Human FcγRI Kₐ (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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⁷Value is extrapolated and is outside the range of the used concentrations.

FIGURE 3. Molecular sequence of human and cynomolgus IgG and FcγR. (A) Amino acid alignment of selected regions of the hinge and C₃2 of the human and cynomolgus IgG subclasses. (B) Amino acid alignment of the upper C₃2 of human primate IgG2; the differences to human IgG1 are highlighted. (C) Amino acid alignment of the Fc binding site of human, human primate, and NHP FcγRIIb.

despite the observed differences of human and cynomolgus IgG1 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, 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...
further increased by mutations in the hinge and CH3 abrogating humans. The potential effector function of cynomolgus IgG4 is show an almost identical profile in both cynomolgus monkeys and binding and effector function, whereas IgG1 and IgG3 subclasses different in cynomolgus monkey, demonstrating potent Fc IgE) Abs (32, 33). Strikingly, only IgG2 and IgG4 properties are and allergens preferentially induce the generation of IgG4 (and specific IgG2 subclass Abs (16, 17), whereas helminth parasites bacteria are mainly opsonized by carbohydrate subclass specificity and Fc receptors (30, 31). Bacteria are also cross-reacted with carbohydrate-specific IgG2 subclass Abs (16, 17), whereas helminth parasites and allergens preferentially induce the generation of IgG4 (and IgE) Abs (32, 33). Strikingly, only IgG2 and IgG4 properties are different in cynomolgus monkey, demonstrating potent FcR binding and effector function, whereas IgG1 and IgG3 subclasses show an almost identical profile in both cynomolgus monkeys and humans. The potential effector function of cynomolgus IgG4 is further increased by mutations in the hinge and CH3 abrogating Fab arm exchange. Whether this is a general feature of all cynomolgus monkey subspecies and/or from different origins will require further investigation. This is in particular relevant, because, in the rhesus monkey, the critical amino acids involved in Fab arm exchange display allotypic variations (18, 34), and we could show

molgus granulocytes, consisting of neutrophils and eosinophils, do not express FcγRIIb, but instead display FcγRI at very high levels. Although FcγRIIb is a GPI-linked receptor, it still supports a variety of signaling events and is thought to enhance the overall avidity of IgG binding, thereby contributing to the IgG subclass specificity and FcγR-mediated function of human granulocytes (30, 31). Bacteria are mainly opsonized by carbohydrate-specific IgG2 subclass Abs (16, 17), whereas helminth parasites and allergens preferentially induce the generation of IgG4 (and IgE) Abs (32, 33). Strikingly, only IgG2 and IgG4 properties are different in cynomolgus monkey, demonstrating potent FcγR binding and effector function, whereas IgG1 and IgG3 subclasses show an almost identical profile in both cynomolgus monkeys and humans. The potential effector function of cynomolgus IgG4 is further increased by mutations in the hinge and CH3 abrogating Fab arm exchange. Whether this is a general feature of all cynomolgus monkey subspecies and/or from different origins will require further investigation. This is in particular relevant, because, in the rhesus monkey, the critical amino acids involved in Fab arm exchange display allotypic variations (18, 34), and we could show

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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).

Of note, we found that the increase of activating function of cynomolgus IgG2 (i.e., higher binding to FcγRI and FcγRIII and higher effector function) is counterbalanced by a strongly increased affinity toward cynomolgus FcγRIIb. Interestingly, in contrast to FcγRIIb 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 FcγRIIb 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 FcγR, FcγRIIb, 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.

Some of our findings are in contradiction with an earlier study from 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-

![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 human 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 Cγ3 domain, and cynomolgus IgG4 with a rhesus Cγ3 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).]
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 FcγRIII) 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 isoforms 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 cynomolgus B cells. In contrast to humans, cynomolgus monkeys show two different populations of CD20-positive B cells. Only the CD40highCD21+ cynomolgus 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 cynomolgus IgG isoforms. 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 FcγRIIA 131R than to human FcγRIIA 131H (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 cynomolgus 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 cynomolgus 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 cynomolgus FcγRIIB is different (Fig. 3), and therefore this mutant Fc variant (S267E and L328F) shows no enhancement in affinity to cynomolgus FcγRIIB (41).

In contrast to the differences identified in the silent IgG subclasses and FcγRIIB, the activating FcγR (FcγRI, FcγRIIA, FcγRIIIa) and the active IgG1 and IgG3 subclasses have comparable effector function in the human and the cynomolgus system: the binding characteristics of human and cynomolgus IgG1 and IgG3 toward human and cynomolgus FcγRs are comparable. IgG1 and IgG3 ADCC activity, which is mainly triggered in vitro by the ITAM-bearing receptor FcγRIIIa on NK cells (and to a lower extent FcγRIIA and FcγRI), is largely independent on FcγRIIB, is indistinguishable whether human or cynomolgus effector cell 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 cynomolgus 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 cynomolgus monkey as compared with humans, which qualifies the cynomolgus 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 FcγRII or FcγRIIB, which are increasingly being evaluated for therapeutic use, have to be evaluated very carefully, as the difference in FcγRII and FcγRII expression on human and cynomolgus granulocytes could influence their overall functional behavior (45). In particular, when evaluating Fc modifications altering the binding to FcγRII (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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