Influence of immunoglobulin G-glycan and subclass variation on antibody effector functions
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IgG2
Chapter 6

Affinity of human IgG subclasses to mouse Fc gamma receptors

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Human IgG is the main antibody class used in antibody therapies because of its efficacy and longer half-life, which are completely or partly due to FcγR-mediated functions of the molecules. Preclinical testing in mouse models are frequently performed using human IgG, but no detailed information on binding of human IgG to mouse FcγRs is available. The orthologous mouse and human FcγRs share roughly 60-70% identity, suggesting some incompatibility. Here, we report binding affinities of all mouse and human IgG subclasses to mouse FcγR. Human IgGs bound to mouse FcγR with remarkably similar binding strengths as we know from binding to human orthologue receptors, with relative affinities IgG3>IgG1>IgG4>IgG2 and FcγRI>>FcγRIV>FcγRIII>FcγRIIb. This suggests human IgG subclasses to have similar relative FcγR-mediated biological activities in mice.
Introduction

Immunoglobulin G (IgG) is the predominant antibody class present in mouse and human serum. It is also the main class used in development of antibody therapies, especially those for cancer\(^1\). IgG is also used to treat immune deficiencies and autoimmune diseases in the form of intravenous immunoglobulin (IVIg), generated from a plasma pool of thousands of donors\(^2,3\). The working mechanisms, efficacy and safety of immunoglobulin therapies must be investigated in \textit{in vivo} model systems before they can be studied in human trials. Therefore, a good understanding of the different model systems is required.

Most studies use mouse models to validate and investigate the efficacy of monoclonal antibody therapies\(^4\). Many mouse disease models of different settings are available or can be developed\(^5,6\). Antibody therapies currently in development are usually based on humanized or human antibodies to reduce the possibility of immunogenicity, increase half-life and increase efficacy\(^1\). Cancer therapies often depend not only on the neutralizing capacity of the antibody’s antigen binding fragments (Fabs) to eliminate pathogen or malignant cells, but also rely on interaction of the constant domain (Fragment crystallizable, Fc) with components of the immune system. This involves binding to molecules including C1q\(^7\), intracellular Fc receptor TRIM21\(^8\), neonatal Fc receptor FcRn\(^9\) and the family of Fc gamma receptors (FcγRs). For antibody-mediated cancer-therapies, the FcγRs (found on myeloid cells, natural killer (NK) cells, and also B cells) are generally considered to mediate the main effector mechanisms, and lead to phagocytosis, antibody-dependent cell-mediated cytotoxicity (ADCC) and other effector functions\(^10\).

Human IgG can be divided in four subclasses, IgG1, IgG2, IgG3 and IgG4, and mouse in IgG1, IgG2a, IgG2b and IgG3, with IgG2c being the equivalent of IgG2a in some mouse strains such as C47Bl/6 mice\(^11-13\). All subclasses mediate effector functions slightly different due to variable specificity and affinity for the different IgG binding partners mentioned above\(^14-16\). Most of the currently approved therapeutic monoclonal antibodies are IgG1, but also IgG2 and IgG4 are used\(^17-20\). IgG2 and IgG4 are preferred when Fc-mediated effector functions are not needed or not preferred, as these subclasses bind with lower affinity to the FcγR and activate complement less actively\(^14,15\).

To evaluate results obtained by testing human antibodies in mice, it is important to consider the differences in Fc-mediated binding to effector molecules. The general properties of the mouse and human FcγR have recently been reviewed by Bruhns and Jönsson\(^21\). The human FcγR locus contains five activating FcγR (FcγRIa, FcγRIIa, FcγRIIc, FcγRIIIa and FcγRIIIb) associated with an immunoreceptor tyrosine-based activation motif (ITAM, except FcγRIIIb) in the intracellular domain (FcγRIIa and FcγRIIc) or the associated common FcR γ-chain (FcγRIa and FcγRIIIa)\(^14\). Human granulocytes express FcγRIIIb, which is a GPI-linked receptor without direct signaling motif, but may still participate in signaling through association with other molecules in lipid rafts\(^14,22\). Humans also express one inhibitory FcγR (FcγRIIb) within this locus, which expresses an immunoreceptor tyrosine-
based inhibitory motif (ITIM) in the intracellular domain.

Similarly, mice have three activating receptors containing an ITAM motif (FcγRIa, FcγRIII and FcγRIV) and also one inhibitory FcγR containing an ITIM motif (FcγRIIb)\textsuperscript{12}. These receptors are orthologues to the human FcγRI, FcγRIIa, FcγRIIIa and FcγRIIb, respectively\textsuperscript{12}. They differ slightly from the human system in expression level and function, although their relative affinities for IgG is generally similar within the two species\textsuperscript{12,21}. This has been well documented for both human\textsuperscript{14}, and mouse IgG subclasses for their corresponding FcγR within species\textsuperscript{12}.

To date, the affinity of human antibodies to mouse FcγR has not been thoroughly investigated, hampering interpretation of findings using human antibodies in mice. We know that human IgG (hereafter referred to as hlgG) subclasses activate FcγR-bearing mouse cells for phagocytosis and ADCC\textsuperscript{23,24}. Furthermore, it has been shown that hlgG subclasses compete for binding to mouse FcγR, with hlgG3 generally showing more competitive binding than hlgG1, followed by hlgG2 and then hlgG4\textsuperscript{23}. \textit{In vitro} and \textit{in vivo} functional assays have revealed, however, that hlgG1 effector functions dominate over hlgG3, and hlgG4 shows surprisingly good effector functions, perhaps explained by the higher FcγRI affinity as, for example, IFNγ-activated macrophages bear this receptor\textsuperscript{23,24}. Attempts have been made to circumvent this gap in knowledge of their exact affinities by using humanized mouse models lacking endogenous FcγR but expressing human FcγR\textsuperscript{25}, but these also have limitations because the FcγR-expression levels and expression patterns do not completely represent the human situation, still warranting the use of wild-type mice. For those cases, detailed information on the affinity of the hlgG subclasses for mouse FcγR is crucial to fully understand and rationalize the use of hlgG in mouse models. We therefore assessed the binding affinity of hlgG subclasses for mouse FcγR and compared that with endogenous affinity in a surface plasmon resonance (SPR) system.

**Material and methods**

To generate anti-human-rhesus D (anti-D) human IgG subclasses, the sequence of anti-human Rhesus D heavy and light chain variable domains of clone 19A10\textsuperscript{42} were codon optimized and synthesized from Geneart (Life Technologies). IgG constant domains with flanking 3’ NheI and 5’ EcoRI restriction sites were designed, codon optimized and ordered at Mr Gene and cloned as described previously\textsuperscript{43}. The variable region of the heavy chain (anti-D VH) was subcloned into pEE6.4 (Lonza) expression vector containing either human IgG1, 2, 3 or 4 constant domains. The variable region of the light chain (anti-D VL) was subcloned into pEE14.4 (Lonza) expression vector containing a kappa light chain. Heavy and light chain vectors to express anti \textit{Streptococcus pneumoniae} serotype 6A/B IgG1, IgG2 and IgG3 named GDob1 (classed switched variants derived from clone Dob1\textsuperscript{44}) were described by Saeland et al\textsuperscript{41}. Heavy and light chain vectors to express anti-BetV1 IgG4 directed against major birch allergen from birch pollen (Betula verrucosa) were previously described\textsuperscript{45}.

To generate anti-Kell mouse IgG subclasses, we used PUMA1 variable domain specific for Kell
antigen. The specifics of PUMA1 isolation sequencing and manipulation are reported elsewhere. Briefly, wild-type mice were transfused with KEL1 RBCs to induce immunity and a monoclonal anti-KEL1 antibody was isolated by standard myeloma fusion (PUMA1 antibody). VH and VL regions of PUMA1 were isolated by 5' RACE, codon optimized and synthesized from Genearth (Life Technologies). The variable region of the heavy chain (VH) was subcloned into pFuse expression vectors for mouse IgG1, IgG2a, IgG2b and IgG3 (Invivogen, pfuse-mg1fc1, pfuse-mg2afc1, pfuse-mg2bfc1, pfuse-mg3fc1); the variable region of the kappa light chain (VL) was subcloned into pFuse expression vector for mouse kappa light chain (Invivogen, pfuse2-mclk).

All IgGs were produced by transient transfection of HEK-freestyle cells (Thermo Fisher Scientific), as previously described by Vink et al. and Dekkers et al. After 5 days, IgG-containing cell supernatant from these cells was harvested by spinning twice at maximum speed (>4000 g) and subsequent filtration with 0.45 nm puradisc syringe filter (Whatmann, GE Healthcare, 10462100).

IgG was isolated from cell supernatant with affinity chromatography columns HiTrap Protein A HP (GE Healthcare, 29-0485-76) for human IgG1, IgG2, and IgG4 or HiTrap Protein G HP (GE Healthcare, 29-0485-81) for human IgG3 and mouse IgG1, IgG2a, IgG2b and IgG3 on ÄKTA prime (GE Healthcare) according to standard procedures. Purified antibody fractions were concentrated to concentration >1 mg/mL using Protein Concentrators, 9K MWCO (Pierce, Thermo Fisher Scientific, 89884A) and subsequently dialysed against phosphate-buffered saline (PBS) overnight using Slide-A-Lyzer™ Dialysis Cassettes, 10K MWCO (Thermo Fisher Scientific, 66384). Antibody concentration was determined using Nanodrop 2000c UV/VIS spectrophotometer (Thermo Fisher Scientific).

Surface plasmon resonance (SPR) measurements were carried out on a IBIS MX96 (IBIS technologies) as described. Biotinylated mouse FcγRI (50086-M27H-B-50), FcγRIIB (50030-M27H-B-50) and FcγRIIV (50036-M27H-B-50) were purchased from SinoBiologicals; biotinylated mouse FcγRIII was not available, therefore His-tag conjugated FcγRIII (SinoBiologicals, 50326-M08H-50) was used. All FcγR were spotted using a Continuous Flow Microspotter (Wasatch Microfluidics) onto a single SensEye G-streptavidin sensor (Senss, 1-08-04-008) allowing for binding affinity measurements of each antibody to all FcγR simultaneously on the IBIS MX96 (IBIS technologies) as described. The biotinylated FcγR were spotted in duplicate and in three-fold dilutions, ranging from 30 nM to 1 nM for FcγRI, FcγRIIB and FcγRIIV in PBS 0.075% Tween-80 (VWR, M126-100 mL), pH 7.4. For the His-tagged FcγRIII, biotinylated anti-His IgG1 (GenScript, A00613) was spotted in duplicate and 3-fold dilution onto the sensor and 100 nM his-FcγRIII (equally diluted in PBS 0.075% Tween-80, pH 7.4) was loaded onto the sensor before each antibody injection.

The IgGs were then injected over the IBIS at dilution series in PBS supplemented with 0.075% Tween-80 ranging from 3.9 nM to 1140 nM for all mouse IgG subclasses, ranging from 1.5 nM to 3000 nM for human IgG1 and IgG3, ranging from 15.6 nM to 2000 nM for human IgG2, or ranging from 23.4 nM to 3000 nM for human IgG4. Regeneration after each sample was carried out with acid buffer (10 mM Gly-HCl, pH 2.2). The system was referenced for slight changes in refractive index due to environmental factors by buffer composition or temperature referencing the signals on spots to signals outside spots. Calculation of the dissociation constant ($K_D$) was performed by fitting a 1:1

6
Chapter 6. Human subclasses mouse FcγR

Fig. 1

a

FcγR conc.: 30 nM 10 nM 3 nM 1 nM

Time (s)

IgG Concentration (M)

b

FcγR conc.: 30 nM 10 nM 3 nM 1 nM

Time (s)

IgG Concentration (M)

c

FcγR

Time (s)

IgG Concentration (M)

Rmax

KD (M)
Langmuir binding model to the RU \(_{360}\) values at each antibody concentration. This furthermore resulted in a \(R_{\text{max}}\) value reflecting the functional amount of receptor molecules on the sensor (see Fig. 1a). For consistent reporting, these fits were carried out at each receptor density, and final reported \(K_D\)s were calculated by interpolating to \(R_{\text{max}}=500\) (see Fig. 1c). In the case of FcyRIII, his-FcyRIII association and dissociation curves on anti-His were subtracted before calculation of IgG-binding affinity using SPRINT 1.9.4.4 software (IBIS technologies). In case of very low affinity (hIgG2 and hIgG4 binding to FcyRIIb and FcyRIII, and IgG4 binding to FcyRIV), fits were performed by fixing \(R_{\text{max}}\) values obtained using IgG1/3 with the same receptor densities, as the theoretical maximal number of available IgG-binding places on a sensor surface is independent of affinity and therefore subclass. For binding of human IgG clone 19A10 (anti-RhD) to FcyRIII, fits based on \(R_{\text{max}}\) obtained for IgG1 binding to FcyRIII were consistently lower than \(R_{\text{max}}\) obtained for IgG3-binding to the same receptor. The values for this receptor in Fig. 3 therefore represent the average and SEM of both calculations. Analysis and calculation of all binding data was carried out with Scrubber software version 2 (Biologic Software).

All statistical analyses were performed using Graphpad Prism software. Error bars represent one standard deviation and significance was determined by a p-value <0.05.

# Results

Binding affinity of mouse and human IgG for the four mouse FcyR (FcyRI, FcyRIIb, FcyRIII and FcyRIV) was measured using SPR on the IBIS MX96. The IBIS MX96 is a multiplex biosensor with the ability to measure up to 96 different ligand - analyte interactions simultaneously. All the FcyR, and controls, were spotted at various concentrations using a continuous flow microspotter onto a single sensor array. During measurement in the IBIS MX96, the analytes (IgG) were injected over the sensor in increasing concentration. RU values at 360 s were plotted vs concentration of antibody, and data were fitted to a 1:1 Langmuir binding model, in agreement with previous literature, although the binding characteristics are intrinsically complex\(^{26–28}\). This is consistent with many other studies of IgG-FcyR binding; for some of those interactions, association/dissociation kinetics fall outside of the range that is accurately quantifiable with SPR. The complexity of the IgG-FcyR-interactions, with IgG having two mutually exclusive binding sites for FcyRs and the possible involvement of glycans on both FcyR and IgG, introduces additional heterogeneity. To minimize other sources of heterogeneity we used C-terminally biotin tagged FcyRI, FcyRIIb and FcyRIV and anti-HIS was coupled to a streptavidin-coated sensor at four different concentrations. The anti-HIS was used to capture c-terminally HIS tagged FcyRIII onto the chip. All subclasses were V-region matched for either mouse or human IgG subclasses, with anti-Kell or anti-D...
specificity, respectively. Each subclass was injected in several concentrations (shown for human IgG1 in Fig. 1), and association and dissociation was monitored (Fig. 1a). These data were used to generate affinity plots calculating affinity ($K_D$) for each receptor concentration (Fig. 1b). This method was chosen because the association-dissociation profiles did not fit simple 1:1 kinetic binding models, but more heterogeneous binding models. This result is in accordance with findings from other groups\textsuperscript{26-29}, but hampered accurate estimation of association and dissociation constants. The obtained dissociation constants ($K_D$) were interpolated to calculate the affinity at the receptor concentration giving $R_{max}$=500 as previously described\textsuperscript{30}, allowing fair affinity comparison at one bioactive concentration between all the receptors (Fig. 1c). As previously observed\textsuperscript{30}, the apparent $K_D$ was independent of the receptor density on the sensor for lower affinity receptors, but did vary slightly for the high affinity FcγRI, underlining the importance of reporting the $K_D$ at a fixed receptor density.

The corresponding data sets were also generated for the other human and mouse IgG subclasses (supplementary Fig. 1). The calculated dissociation constants in $K_D$ (M) for the mouse IgG subclasses at $R_{max}$=500 are displayed in Fig. 2. We detected no binding of mIgG1 to FcγRI and FcγRIV, no binding of mIgG2b to FcγRIII and no binding of mIgG3 to any of the mouse FcγR. mIgG1 bound equally well to both FcγRIIb and FcγRIII. mIgG2a bound all receptors with FcγRI > FcγRIV > FcγRIII > FcγRIIb. Similarly, mIgG2b bound all receptors except mouse FcγRI with similar preference.

The calculated dissociation constants for human IgG to the mouse FcγRs at $R_{max}$=500 are displayed in Fig. 3 and summarized in Table 1. To estimate/calculate the affinity of weak binding interactions (mainly for hlgG2 and hlgG4) more precisely, curve fitting was performed using values of $R_{max}$ based on fits for the other subclasses of the respective receptors. Increasing the concentration of these IgG to achieve more accurate results was impractical. We used an average of at least three replicate experiments, and this yielded consistent results, with all IgG binding to FcγRI and FcγRIV for example showing similar $R_{max}$. One notable exception was FcγRIII, where fits based on $R_{max}$ for hlgG1 were consistently lower than based on $R_{max}$ for hlgG3. Therefore, the $K_D$ values in Fig. 3 for hlgG1, hlgG2 and hlgG4 represent the average and SEM of both calculations (see Materials & Methods section for details). hlgG1 and hlgG3 bound all receptors with affinities that were

<table>
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<th></th>
<th>mFcγRI</th>
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<th>mFcγRIII</th>
<th>mFcγRIV</th>
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<td>11 (2)</td>
<td>21 (12)</td>
<td>26 (6)</td>
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</table>

Table 1. Affinity in $K_D$ (μM) of human IgG subclasses for mouse FcγRs
S.E.M. in brackets behind $K_D$, -/- no binding detected.
remarkably similar to those of mouse IgG2a to all the receptors. hIgG4 also bound all mouse receptors, but with significantly lower affinity. Binding of hIgG2 was only observed for FcγRIIb and FcγRIII, with a similar reactivity pattern as mouse IgG1, albeit with a ~5-60x lower affinity. These affinity ranges were confirmed using another set of human IgG subclasses (supplementary Fig. 3). Furthermore, using the same setup in IBIS MX96, the human IgG subclasses bind the human FcγR with affinities comparable to those reported in literature (supplementary Fig. 4).

**Discussion**

Here, we report for the first time the binding affinities of hIgG of all subclasses to all mouse FcγRs and compare this to normal binding of endogenous mouse IgG. We found that human IgG subclasses bind both with similar relative affinity to the mouse FcγR as to orthologue receptors in humans. In addition, we found that human IgG binds mouse FcγR with strikingly similar affinities as mouse IgG, which was highly analogous to that seen for human IgG binding to human FcγR, with only a marginal decrease in affinity. This suggests that preclinical testing of human IgG1 in mouse models may mimic FcγR-mediated effector functions surprisingly well.

We also report the affinities of IgG to Fc-receptors, using a more sophisticated method than previously attempted, by performing a more fair comparison between FcγR using affinity ranking by calculating the $K_D$ at a fixed maximal response in the biosensor. This was possible because we simultaneously measured IgG binding to all receptors, the latter being fixed to a biosensor-array through a site-specific c-terminal tag at different concentrations. Injecting the ligand at different concentrations allowed us to calculate...
affinity for all at ligand densities of the receptors. The final affinity constant was then calculated for an interpolated theoretical $R_{\text{max}}$, identical for all the receptors, resulting in a fair comparison between the receptors. This is important, especially for the higher affinity receptors FcγRI and FcγRIV, because the calculated affinities depend on the exact ligand density, which is partly due to interfering effects, including rebinding effects30.

The affinities we obtained for mouse IgG for mouse FcγR and human IgG for human FcγR are in agreement with those previously reported 12,14,16,21,31–34. We have compared the mouse data obtained in this research with that found in literature in a summarizing table (Supplementary table 1). Some minor differences were noted, with our data falling well within the range of the affinities found by other groups. The minor discrepancies noted could be due to in the different methods used, as the previous efforts relied on IgG being randomly coupled to the biosensor measuring binding of soluble FcγR, whilst in our study IgG was titrated and flowed over spotted FcγRs coupled in homogenous orientation through a site-specific c-terminal tag.

We did not observe binding of mIgG2b or mIgG3 to mFcγRI, although some groups have reported positive interactions21,34,35. One likely explanations are allelic variations of FcγRI within mouse, which increases binding of this receptor to these two subclasses34,35. For this study, we only had access to a single allelic variant of FcγRI. In addition, a recent report has demonstrated that mouse IgG subclasses are also subject to allotypic variations, which may also have affected these interactions36.
The relative affinities of human IgG for the mouse receptors have also been studied by Overdijk et al.\textsuperscript{23}, using competition assays. They observed a higher affinity of hlgG3 to the mouse FcγR that did not translate in higher efficacy in their cell-based and in vivo assays. We also found that hlgG3 generally bound slightly better to mouse FcγR than hlgG1, but these were small differences. Even though we find similar binding affinities and patterns of hlgG1 and hlgG3 to the set of mouse FcγR, several studies showed less efficacy of hlgG3 in mouse cell and in mouse tumor models\textsuperscript{23,24}. hlgG3 has, however, been found to be effective in mediating complement-dependent cytotoxicity, particularly against tumor targets with low epitope densities, which is probably due to the extraordinary long hinge of hlgG3 that may form a hexameric docking platform for C1q under those conditions\textsuperscript{37–39}. Similarly, hlgG3 has been found highly efficient in protecting against pneumococcal infections in vivo, also through complement\textsuperscript{40,41}.

hlgG2 has a comparable binding pattern for the mouse Fc receptors as mlgG1, but with an estimated 5- to 50-fold lower affinity than mlgG1. This is in agreement with the results found by Overdijk et al., who showed hlgG2 to compete moderately with mlgG1 binding for mFcyRIIb and mFcyRIV\textsuperscript{23}.

Interestingly, hlgG4 bound most mouse FcγR with relative low affinities, but with medium affinity to mFcyRI. Studies from Overdijk et al. and Steplewski et al. showed high hlgG4 efficacy in mouse xenograft models, which are explained by the higher FcyRI affinity and hlgG4 efficacy in ADCC assays where cells, such as IFNγ activated macrophages, bear this receptor\textsuperscript{23,24}.

For the low affinity interactions (with a \(K_D\) above ca. 1×10\textsuperscript{-6} M), absolute \(K_D\) values become less precise, as can be appreciated by comparing results for clones 19A10 (anti-D) and GDob1 (Fig. 3 and Supplementary Fig. 3), although the overall binding patterns were fairly consistent.

In this study, we have described binding of human IgG to all mouse FcγR in much more detail than previously reported. The relative binding patterns are strikingly similar as we know from the orthologue interaction with the human receptors, with the general relative affinities IgG3>IgG1>IgG4>IgG2. The mouse FcγR also displayed similar preference for human IgG as they do for mouse IgG, with FcγRI>>FcγRIV>FcγRIIb>FcγRIII (hlgG1 and hlgG3 mainly), which is close to what we know for the human orthologue receptors, hFcyRIIa>>FcyRIIa>FcyRIIb (where mFcyRIV is orthologue to hFcyRIIIa and mFcyRIII is orthologue to hFcyRIIa\textsuperscript{12}).

This high conservation of both interspecies affinity and reactivity pattern suggests that using human IgG in mouse models to test FcγR-effector mechanism is feasible, and the use of humanized models of Tg/KO mice, which do not yet completely reflect human expression patterns and levels\textsuperscript{25}, may not be necessary.
Acknowledgements

We would like to thank Pleuni Heer-Ooijevaar for supplying the BetV1 constructs and Remco Visser for BetV1 and Gdob1 purification.

Disclosure of Potential Conflicts of Interest

The authors declare no potential conflict of interest.

Author contributions

G.D, A.E.H.B, H.L.H, T.S. and S.L.T performed experiments. G.V. and G.D. devised the study. All authors designed the experiments. G.D. and G.V. wrote the manuscript, which was co-edited by all authors.
References


Supplementary Figure 1. Sensorgram of mouse IgG subclasses binding to mouse FcγR
Each mouse IgG subclasses in concentration ranges from 3.9 nM to 1140 nM flown over different spotted mouse FcγR, only the sensorgrams for the highest ligand concentration of the different mouse FcγR are shown; FcγRI 30nM, mFcγRIIb 100nM, FcγRIII 100nM and mFcγRIV 30nM.
Supplementary Figure 2. Sensorgram of human IgG subclasses binding to mouse FcγR
Each human IgG subclasses in concentration ranges from 1.5 nM to 3000 nM for human IgG1 and IgG3, from 15.6 nM to 2000 nM for human IgG2, or from 23.4 nM to 3000 nM for human IgG4, flown over different spotted mouse FcγR, only the sensorgrams for the highest ligand concentration of the different mouse FcγR are shown; FcγRI 30nM, mFcγRIIb 100nM, FcγRIII 100nM and mFcγRIV 30nM.

Supplementary Figure 3. Binding of human IgG subclasses to mouse FcγR
The human IgG1, IgG2 and IgG3 anti-pneumococcal (Gdob1) and IgG4 anti birch antigen (BetV1) were assessed for their binding affinity in $K_D$ (M) to the mouse FcγRI, FcγRIIb, FcγRIII and FcγRIV by SPR. -/- denotes no binding detected, * denotes binding > 3 μM, represented by the horizontal dashed line which indicates the maximum concentration of IgG which is used. Data represents means and SEM of n=2.
Supplementary Figure 4. Affinity of human IgG subclasses to human FcγR

a, b) Affinity in $K_d$ (M) of human anti-RhD IgG subclasses to human FcγR family. -/- denotes no binding detected, * denotes binding > 2 μM, represented by the horizontal dashed line which indicates the maximum concentration of IgG which is used. Data is single representative of n=3.

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Supplementary table 1. Comparison of affinity in $K_D$ (μM) of mouse IgG subclasses for mouse FcγRs from this research versus literature.

Data obtained in this research in bold type, S.E.M. in brackets behind $K_D$; -- no binding detected.

<table>
<thead>
<tr>
<th></th>
<th>mFcγRI</th>
<th>mFcγRIIb</th>
<th>mFcγRIII</th>
<th>mFcγRIIV</th>
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<tr>
<td><strong>mIgG1</strong></td>
<td></td>
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</tr>
<tr>
<td>-/-</td>
<td>0.15 (0.05)</td>
<td>0.23 (0.03)</td>
<td>-/-</td>
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</tr>
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<td>-/- 1</td>
<td>0.30 1</td>
<td>3.2 1</td>
<td>-/- 1</td>
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<tr>
<td>-/- 2</td>
<td>0.83 2</td>
<td>4.8 2</td>
<td>-/- 2</td>
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<tr>
<td>-/- 3</td>
<td>0.17 3</td>
<td>0.32 3</td>
<td>-/- 3</td>
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</tr>
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<td></td>
<td>0.25 4</td>
<td>2.0 4</td>
<td></td>
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<tr>
<td><strong>mIgG2a</strong></td>
<td>0.012 (0.002)</td>
<td>0.69 (0.16)</td>
<td>0.34 (0.04)</td>
<td>0.060 (0.020)</td>
</tr>
<tr>
<td>0.006 4</td>
<td>2.4 1</td>
<td>1.5 1</td>
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<td>0.026 2</td>
<td>1.8 2</td>
<td>1.8 2</td>
<td>0.071 2</td>
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<tr>
<td>0.018 3</td>
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<td>0.14 3</td>
<td>0.010 3</td>
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<tr>
<td>0.033 5</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>0.022 6, b</td>
<td></td>
<td></td>
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<tr>
<td>0.0013 6, c</td>
<td></td>
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<tr>
<td><strong>mIgG2b</strong></td>
<td>-/-</td>
<td>0.83 (0.14)</td>
<td>0.45 (0.06)</td>
<td>0.12 (0.03)</td>
</tr>
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<td>0.45 1</td>
<td>1.6 1</td>
<td>0.059 1</td>
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<tr>
<td>-/- 2</td>
<td>0.91 2</td>
<td>1.6 2</td>
<td>0.063 2</td>
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<td>0.26 4</td>
<td>0.91 4</td>
<td>0.034 4</td>
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<td>10 5, a</td>
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<td>-/- 6, b</td>
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<td>0.021 6, c</td>
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<td>-/- 6, b</td>
<td>-/-</td>
<td>-/-</td>
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<tr>
<td>0.011 6, c</td>
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</tbody>
</table>

a, As provided in the review of Bruhns and Jönsson\(^5\), original source unknown.  
b, Affinity for Fc RI.1 (a) allele, as described by Gavin et al.\(^6\).  
c, Affinity for Fc RI.4 (d) allele, as described by Gavin et al.\(^6\).
Supplementary references


