IgG2m4, an engineered antibody isotype with reduced Fc function

Zhiqiang An,1 Gail Forrest,2 Renee Moore,3 Michael Cukan,1 Peter Haytko,1 Lingyi Huang,1 Salvatore Vitelli,1 Jing Zhang Zhao,1 Ping Lu,1 Jin Hua,1 Christopher R. Gibson,4 Barrett R. Harvey,1 Donna Montgomery,1 Dennis Zaller,1 Fubao Wang1,* and William Strohl1

Departments of 1Biologics Research; 2Drug Metabolism and Pharmacokinetics; West Point, PA USA; 3Department of Immunology; Rahway, NJ USA; 4GlycoFi, Merck & Co., Inc.; Lebanon, NH USA

Key words: IgG2m4, IgG, isotype, benign blocker, Fcγ receptors, Clq

Abbreviations: ADCC, antibody-dependent cell-mediated cytotoxicity; CDC, complement-dependent cytotoxicity; IgG, immunoglobulin G; Fc, fragment crystallizable; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; HPLC-SEC, high performance liquid chromatography-size exclusion chromatography

The Fc region of an antibody mediates effector functions such as antibody-dependent cell-mediated cytotoxicity (ADCC) and complement-dependent cytotoxicity (CDC), and plays a key role in the in vivo half-life of an antibody. In designing antibody therapeutics, it is sometimes desirable that the antibody has altered Fc-mediated properties. In the case of a “benign blocker” antibody, it is often desirable to diminish or abolish the ADCC and CDC functions while retaining its PK profile. Here, we report a novel engineered IgG isotype, IgG2m4, with reduced Fc functionality. IgG2m4 is based on the IgG2 isotype with four key amino acid residue changes derived from IgG4 (H268Q, V309L, A330S and P331S). An IgG2m4 antibody has an overall reduction in complement and Fcγ receptor binding in in vitro binding analyses while maintaining the normal in vivo serum half-life in rhesus.

Introduction

Therapeutic proteins, including monoclonal antibodies (mAbs), are playing an increasingly important role in combating critical medical conditions. Currently, there are more than 20 therapeutic proteins approved for clinical use, and the trend predicts that the growth of mAb therapeutics will continue.1 Among the therapeutic proteins that are marketed or in late stage clinical development, an overwhelming majority contains antibody Fc domains in one of two formats: full-size mAbs or Fc fusion proteins. The Fc region is an integral part of an antibody or Fc-fusion molecule, and can play a role in mediating effector functions such as antibody-dependent cell-mediated cytotoxicity (ADCC), complement dependent cytotoxicity (CDC), opsonization and transcytosis.2 This is accomplished by the Fc domain binding to either a cell surface receptor (e.g., Fcγ receptor) or Clq of the complement complex. In addition, FcRn binding mediated by the Fc domain plays an important role in the antibody’s half-life.3-5 For therapeutic mAbs that are marketed or in development, IgG is the overwhelming choice as the backbone of the drug molecule. Four subclasses of naturally occurring IgG (IgG1, IgG2, IgG3 and IgG4 in humans) elicit different in vivo biological responses due to their sequence and structural differences. The differential biological activities mediated by the various IgG subclasses are a result of their differences in binding to the Fcγ receptors6-9 and complement component Clq.10 Interactions between Fc motifs or residues and cellular components have been extensively studied using both human and mouse IgGs. The key amino acid residues in the Fc region that interact with Fcγ receptors (CD64 for FcγRI, CD32 FcγRII and CD16 FcγRIII), complement Clq and neonatal FcRn receptors have been determined. Earlier work using a domain swapping approach has defined regions that are required for Fcγ receptor (~230–238 amino acids11 according to the EU numbering system) and complement binding (292–340 amino acids12). Further studies have identified individual amino acid residues that interact with Fcγ receptors and complement Clq protein. For example, residues H268,13,14 A330 and P331 were shown to be critical for Fcγ receptors and Clq bindings.15-20

IgG1, IgG2 and IgG4 isotypes are the choices for therapeutic antibodies currently in clinical use.21-23 Key features of the three natural isotypes and a desired isotype with altered Fc function are summarized in Table 1. Though the different isotypes share some common characteristics such as long serum half life, significant differences exist among them. For example, IgG1 and IgG2, but not IgG4, can activate complement. IgG1 mediates ADCC effectively, while IgG2 and IgG4 have little, if any, activity. While these differences can help select a suitable isotype for a particular therapeutic treatment, sometimes the natural isotypes have undesirable properties. For example, it is known that IgG4 can form half antibodies, and potential side effects could occur.

*Correspondence to: Fubao Wang; Email: fubao_wang@merck.com
Submitted: 07/09/09; Accepted: 09/26/09
Previously published online: www.landesbioscience.com/journals/mabs/article/10185

©2009 Landes Bioscience. Do not distribute.
were introduced at positions that have been shown to play an important role in Fcγ receptor and complement C1q binding. The amino acid residues adjacent to the mutated positions are homologous to the IgG4 natural sequences. As a result, any stretch of sequences with eight amino acids containing each changed amino acid in IgG2m4 can be found in either IgG2 or IgG4. Two sequence stretches (300FRV VSVLTVL309 and 330SSIEKTISKT339) in IgG2m4 are absent in IgG2 and IgG4. An in silico study did not suggest that they are novel human T-cell epitopes (data not shown), but this remains to be verified in future T cell activation assays or in vivo studies.

IgG2m4 expression. To test the expressability of IgG2m4 antibodies in mammalian cells, a number of constructs with different variable regions were expressed in human embryonic kidney (HEK) 293 cells transiently or in Chinese Hamster Ovary (CHO) stably. The corresponding IgG1 constructs were expressed in parallel for comparisons. The IgG2m4 and IgG1 antibodies were purified using identical purification procedures and subjected to a wide range of tests for their expression level, yield, purity and biophysical properties. Typical and identical patterns were observed for an IgG2m4 and IgG1 pair under reducing and non-reducing SDS-PAGE conditions (Fig. 2). HPLC-SEC analysis confirmed that the antibodies were present in solution with greater than 98% monomer and no observed aggregates or clippings were associated with the amino acid changes described here (data not shown). The glycosylation pattern of IgG2m4 and IgG1 antibodies expressed in stable CHO cell lines were also analyzed. The following glycan forms were found in the IgG2m4 antibody: G0F (51.7%), G1F (34.8%), G2F (7.2%), and other minor glycan forms. Similar glycan patterns were observed for the IgG1 glycoforms (data not shown).

FcγR binding. A CHO expressed IgG2m4 antibody was compared to its IgG1 counterpart for the ability to bind to the three types of Fcγ receptors in an ELISA binding assay (Fig. 3). The IgG1 antibody binds strongly to all three Fcγ receptors as expected: FcγRI (Fig. 3A), FcγRIIa and b/c (Fig. 3B and C), and

---

Table 1. Features of naturally occurring IgG isotypes and desired isotype as a benign blocker

| Property                              | IgG1 | IgG2 | IgG4 | Desired |
|---------------------------------------|------|------|------|---------|
| Normal serum T½ (days)                | ~23  | ~23  | ~23  | ~23     |
| Normal serum conc. (mg/mL)            | 9    | 3    | 0.5  | n/a     |
| Activate classical complement         | ++   | +    | -    | -       |
| ADCC                                  | +++  | +/-  | +/-  | -       |
| Opsonization                          | +++  | +/-  | +    | -       |
| Cross placenta                        | ++   | +    | +    | -       |
| Diffusion-extravascular               | +++  | +++  | +++  | +++     |
| Structural stability                  | +++  | +++  | +/-  | +++     |
| Bind FcγRI (CD64; high affinity 1 x 10⁻⁹ M) | +++  | +    | +    | -       |
| Bind FcγRIIa.b (CD16; low affinity 5 x 10⁻⁹ M) | ++   | +/-  | +/-  | -       |
| Bind FcγRIIIa (CD16; low affinity 2 x 10⁻⁹ M) | ++   | +/-  | +    | -       |
both FcγRIIIa V158 and F158 alleles (Fig. 3D and E) in a concentration dependent manner and reached plateau at high antibody concentrations. In contrast, the engineered IgG2m4 did not bind to FcγRI (Fig. 3A) or either the FcγRIIIa V158 or F158 alleles (Fig. 3D and E). A slight signal increase was observed for IgG2m4 binding to FcγRIIIa-F158 allele at high antibody concentrations (Fig. 3E), but this is likely most non-specific assay background because the same increase was observed in the blank control (Fig. 3E). IgG2m4 binding affinity to FcγRIIa is weaker than that of IgG1, but it binds to FcγRIIa (Fig. 3B) in a concentration dependent manner and reached plateau at 1 μg/ml (Fig. 3B). In contrast, IgG2m4 showed minimal binding to FcγRIIb/c (Fig. 3C). Both blank control and IgG2m4 curves rose slightly as antibody concentration increased and the IgG2m4 line was slightly above the blank control line. It is unclear if the marginal increase represents true binding activity or background noise. In summary, the results indicate that IgG2m4 does not bind to FcγRI and FcγRIII, binds to FcγRIIa with an affinity weaker than IgG1 and demonstrates little or no binding to FcγRIIb/c.

**C1q binding.** C1q is the first complement component that binds to the Fc domain to initiate the complement cascade. Lack of C1q binding results in the absence of CDC. As shown in Figure 4, IgG2m4 was subject to C1q binding analysis in an ELISA similar to the FcγR assay described earlier. IgG1 can bind to C1q in a concentration dependent manner whereas IgG2m4 does not possess any detectable C1q binding activity within the concentration range analyzed here (up to 10 μg/ml).
is of great interest for a therapeutic antibody that does not require Fc mediated effector functions, or where Fc mediated functions would be deleterious. The IgG2m4 isotype does not bind to FcγRI and III receptors and C1q, it still binds to FcγRIIa, and it has little or no binding to FcγRIIb/c. An IgG2m4 antibody was compared with IgG1 in a variety of assays since this is the preferred isotype when Fc mediated effector functions are desired. Not only does the IgG2m4 antibody show normal half-life in the rhesus PK, it also expresses well in transiently transfected HEK293 cells or in CHO stable cell lines. For all the antibodies and Fc fusion proteins tested in our laboratories to date, IgG2m4 based molecules exhibited good production yields and structural stability (see results section, IgG2m4 expression). No difference has been observed for antigen binding when antibodies with identical variable regions are tested in IgG1 and IgG2m4 backbones (data not shown).

It has been reported that IgG2 binds to FcγRIIa with high affinity. Here, we observed significant IgG2m4 binding to FcγRIIa, but with little or no FcγRIIb/c binding. Previous work suggested different functions for residue H268. In the work reported by Medgyesi et al. H268 was shown to be important in FcγRIIb binding while in another study by Brekke et al. the residue did not seem to play a significant role. These experiments were carried out in different contexts, and the results should be interpreted with caution. It should be noted that FcγRIIa with ITAM motif and FcγRIIb with ITIM motif play different roles, but they share a high degree of sequence homology in their extracellular domains, and it is a challenge to further differentiate their functions by perturbing the homologous sequences.

Progress has been made in engineering the Fc domains to further differentiate the binding to FcγRIIa vs. FcγRIIb. It is known that IgG2 binds to the H131 allele of FcγRIIa with higher affinity than the R131 allele, which may explain the significant binding we detected because FcγRIIa H131 allele was used in the experiment shown in Figure 3B. The results reported here suggest that further work is needed to understand the differential binding profiles among different types of Fcγ receptors. Manipulation of additional residue(s) in IgG2m4 is warranted in order to abolish the FcγRIIa binding completely. It is interesting to point out that the introduction of L234F/L235E/P331S triple mutation into the Fc of human IgG1 resulted in an almost complete loss of binding to CD32A (FcγRIIa), consistent with earlier work that showed that L234/L235 play an important role in IgG1 binding to FcγRIIa. It is interesting to see if the triple mutation (L234F/L235E/P331S) can bind to FcγRIIb/c.

The marketed IgG2 and IgG4 hybrid (IgG2/4, eculizumab, which closely resembles the IgG2m4 described in this work, has been successfully developed. The two constructs are similar in design, but each possesses distinctive amino acid residues in the CH domains. They were both designed to reduce or eliminate FcγR and

Figure 2. IgG2m4 expression. Both IgG1 and IgG2m4 antibodies with identical variable regions were expressed in stable CHO cells. An aliquot of each antibody was loaded onto SDS-PAGE, and run under reduced and non-reduced conditions. Lane A1, IgG1 antibody (non-reduced conditions); Lane B1, IgG2m4 antibody, (non-reduced conditions); Lane A2, IgG1 antibody (reduced conditions); Lane B2, IgG2m4 antibody (reduced conditions); Lane M, protein molecular weight markers in kilodaltons.

In vivo pharmacological kinetics. The in vivo pharmacokinetics (PK) of a human antibody with an IgG2m4 isotype was evaluated in rhesus monkey following a single intravenous 10 mg/kg dose. The serum concentration-time profiles of IgG2m4 conformed to the two-compartmental PK behavior that is frequently observed for mAbs. The individual and mean PK parameters for the IgG2m4 are summarized in Table 2, and the individual serum concentration-time model fits are shown in Figure 5. Overall, the IgG2m4 antibody had low clearance, relative to hepatic blood-flow, and a volume of distribution that approximates total serum volume. The terminal half-life of the IgG2m4 was relatively consistent between the two monkeys and averaged 8.8 days. Additional paired IgG1 and IgG2m4 antibodies with identical variable regions showed comparable PK in rhesus (data not shown), which collectively indicates that the IgG2m4 retains the normal serum half life.

Discussion

The work reported here describes an IgG isotype that was engineered to have reduced C1q and FcγR binding. Such an isotype is of great interest for a therapeutic antibody that does not require Fc mediated effector functions, or where Fc mediated functions would be deleterious. The IgG2m4 isotype does not bind to FcγRI and III receptors and C1q, it still binds to FcγRIIa, and it has little or no binding to FcγRIIb/c. An IgG2m4 antibody was compared with IgG1 in a variety of assays since this is the preferred isotype when Fc mediated effector functions are desired. Not only does the IgG2m4 antibody show normal half-life in the rhesus PK, it also expresses well in transiently transfected HEK293 cells or in CHO stable cell lines. For all the antibodies and Fc fusion proteins tested in our laboratories to date, IgG2m4 based molecules exhibited good production yields and structural stability (see results section, IgG2m4 expression). No difference has been observed for antigen binding when antibodies with identical variable regions are tested in IgG1 and IgG2m4 backbones (data not shown).

It has been reported that IgG2 binds to FcγRIIa with high affinity. Here, we observed significant IgG2m4 binding to FcγRIIa, but with little or no FcγRIIb/c binding. Previous work suggested different functions for residue H268. In the work reported by Medgyesi et al. H268 was shown to be important in FcγRIIb binding while in another study by Brekke et al. the residue did not seem to play a significant role. These experiments were carried out in different contexts, and the results should be interpreted with caution. It should be noted that FcγRIIa with ITAM motif and FcγRIIb with ITIM motif play different roles, but they share a high degree of sequence homology in their extracellular domains, and it is a challenge to further differentiate their functions by perturbing the homologous sequences.

Progress has been made in engineering the Fc domains to further differentiate the binding to FcγRIIa vs. FcγRIIb. It is known that IgG2 binds to the H131 allele of FcγRIIa with higher affinity than the R131 allele, which may explain the significant binding we detected because FcγRIIa H131 allele was used in the experiment shown in Figure 3B. The results reported here suggest that further work is needed to understand the differential binding profiles among different types of Fcγ receptors. Manipulation of additional residue(s) in IgG2m4 is warranted in order to abolish the FcγRIIa binding completely. It is interesting to point out that the introduction of L234F/L235E/P331S triple mutation into the Fc of human IgG1 resulted in an almost complete loss of binding to CD32A (FcγRIIa), consistent with earlier work that showed that L234/L235 play an important role in IgG1 binding to FcγRIIa. It is interesting to see if the triple mutation (L234F/L235E/P331S) can bind to FcγRIIb/c.

The marketed IgG2 and IgG4 hybrid (IgG2/4, eculizumab, which closely resembles the IgG2m4 described in this work, has been successfully developed. The two constructs are similar in design, but each possesses distinctive amino acid residues in the CH domains. They were both designed to reduce or eliminate FcγR and
complement binding, but maintain normal FcRn binding. By doing so, reduced effector functions such as ADCC and CDC and normal serum half-life are expected. The CH2 and CH3 domains come from IgG4 in the IgG2/4 design whereas the CH2 and CH3 domains come from IgG2 in the IgG2m4 design. Several issues are associated with the IgG4 isotype in therapeutic antibody development. It is known that IgG4 forms half antibody, which could have serious implications when used in antibody therapeutics. Other IgG4 related issues include reduced avidity effect due to its functional monovalency, shorter half-life when compared to that of IgG1 and IgG2, and possible IgG4 connection with Wegener granulomatosis. It is not clear how much IgG4 function still remains in IgG2/4 due to its intact IgG4 CH2 and CH3.

IgG2m4 was designed to resemble IgG2 and it is expected to behave mostly like IgG2. More studies are needed to compare the two constructs in multiple aspects, such as FcRn binding affinities under different pH conditions, inter- and intra-chain disulfate bond formation and stability and contribution of the CH2 and CH3 domains to their overall stability. Since the IgG2/4 format is used in eculizumab, information on its behavior in humans will help refine the overall effort in designing a complete “benign blocker.” The complete lack of detectable C1q binding activity positions IgG2m4 as an alternative to IgG4 when complement binding is not needed or allowed. Further confirmation is needed in in vivo models with IgG2m4 antibodies that can demonstrate lack of CDC.

In conclusion, the IgG2m4 isotype possesses unique features that facilitate its application as a platform Fc for both mAb and Fc protein fusion based therapeutics in which effector functionality is not desired. One would expect that an IgG2m4 based antibody with reduced Fc functions should show reduced effector functions in vivo.

**Figure 3.** Fcγ receptor binding. Purified IgG1 and IgG2m4 antibodies (Fig. 2), along with controls were serially diluted for an ELISA Fcγ receptor binding assay. Experimental details were described in the Materials and Methods section. (A) FcγRI; (B) FcγRIIa H131; (C) FcγRIIb/c; (D) FcγRIII V158 and (E) FcγRIII F158. Closed circle, IgG1; closed square, IgG2m4 and closed triangle, negative control.

**Materials and Methods**

**Gene synthesis and vector construction.** The antibody expression vectors consist of a pair of plasmids: one for the light chain and the other for the heavy chain. The overall organization of the two expression vectors is similar. Briefly, the heavy or light chain expression cassette comprises a human CMV (HCMV) promoter and leader sequence on the 5’ end of a cloning site, and the sequences for constant regions (kappa or lambda light chain and IgG2m4 for heavy chain, respectively) and bovine growth hormone (BGH) polyadenylation signal on the 3’ side.
of the other cloning site. The leader sequence at the amino termini of the proteins mediated the secretion of the expressed antibodies into the culture medium. DNA encoding the designed IgG2m4 heavy chain constant region, including the four amino acid changes, was chemically synthesized (DNA2.0, CA) and cloned into the antibody expression vector. The variable regions were in-frame cloned into the regions between the leader signal sequence and the corresponding constant region. The expression vectors carry oriP from the Epstein Barr virus (EBV) genome for prolonged expression in 293EBNA cells and the bacterial sequences for kanamycin selection marker and replication origin in E. coli. The open-reading frames (ORF) were verified by sequencing. Standard molecular cloning techniques were applied using reagents from commercial vendors (Roche Diagnostics, IN and Invitrogen, CA).

Antibody expression, purification and characterization. Antibody was expressed either transiently in HEK293 or stably in CHO cells. For transient transfection, a pair of plasmids carrying light and heavy chain ORFs was transfected using FuGene (Roche Diagnostics, IN) and Opti-MEM medium (Invitrogen, CA). Transfected cells were incubated at 37°C in a 5% CO₂ incubator. Medium was harvested between day 7–10 post transfection. Antibodies were purified using protein A/G affinity chromatography according to the manufacturer’s recommendation (Pierce, IL). The purified antibodies were analyzed for their concentration (absorption at OD 280 nm), purity and homogeneity (SDS-PAGE and HPLC-SEC), endotoxin level, glycan forms (mass spectrometry). The overall purity is greater than 95% with IgG monomer >98%. For glycosylation analysis, N-glycans were released by PNGase F digestion, and they were fluorescently labeled and separated by HPLC. The glycan identity and relative quantity was determined by MALDI-TOF and HPLC.

Fcγ receptor binding assay. Fcγ receptor binding assays were carried out as described with minor modifications. High binding plates (Corning Costar 96 well plate white flat bottom polystyrene high bind microplate, Cat#3922) were coated with 100 μl per well of Fcγ receptor solutions in PBS at the following concentrations: 1 μg/mL for FcγRI (R&D Systems, MN) and FcγRIIa (Pichia pastoris produced), 2 μg/mL for FcγRIIb/c (P. pastoris produced), 0.4 μg/mL for FcγRIIIa-V158, and 0.8 μg/mL for FcγRIIIa-F158 (both P. pastoris produced). Receptors were expressed using P. pastoris as described. For FcγRI, antibody was coated in assay diluent (1% BSA, PBS, 0.05% Tween-20) in monomeric form. For all other receptors, the antibody was coated after dimerization with alkaline phosphatase conjugated goat anti-human IgG F(ab')₂ (Jackson Immunoresearch, PA) for 1 hour at room temperature. FcγRI bound antibody was also detected using the same goat anti-human IgG F(ab')₂, and all plates were quantified by measuring excitation at 340 nm and emission at 465 nm after 18 hour incubation with SuperPhos (Virolabs, VA).

Figure 4. C1q binding. Purified IgG1 and IgG2m4 antibodies were serially diluted for an ELISA based C1q binding assay. Experimental details were described in the Materials and Methods section. Closed circle: IgG2m4 antibody, closed square: IgG1.

Table 2. Summary of rhesus monkey PK study

| Monkey | CL (mL/day/kg) | VDSS (mL/kg) | Alpha half-life (day) | Beta half-life (day) |
|--------|----------------|-------------|-----------------------|----------------------|
| 1      | 2.9            | 37.2        | 0.01                  | 9.0                  |
| 2      | 3.8            | 45.8        | 0.02                  | 8.5                  |
C1q binding assay. Serially diluted antibody was coated (100 µl per well in 50 mM Na₂HCO₃, pH 9.0) to clear high binding plates (Corning Costar 96 well EIA/RIA polystyrene high binding plates, Cat# 3590). Supernatants were aspirated, and the plate was then washed three times with 300 µl per well of 0.05% Tween-20 in PBS. 100 µl per well of 2 µg/ml Human C1q complement (US Biological, MA) in assay diluent (0.1% Bovine Gelatin, PBS, 0.05% Tween-20) was then added and incubated for 2 hours, followed by another wash. 100 µl of 1:2,000 HRP conjugated sheep polyclonal anti-human C1q antibody (AbDSerotec, NC) diluted in assay diluent was next added to each well and incubated for 1 hour. After a final wash, 100 µl per well of TMB (Virolabs, VA) was added to each well and incubated until color developed. Reaction was stopped by addition of 0.5 M H₂SO₄ and OD₄₅₀ nm was measured with a plate reader.

In vivo PK study. Two rhesus monkeys were administered IgG2m4 at 10 mg/kg in 20 mM L-Histidine, 150 mM NaCl, 0.01% PS-80, pH 7.4 by intravenous bolus injection. Serum samples were collected at various times up to 46 days post-dose for the purpose of pharmacokinetic analysis. Once collected, the serum samples were refrigerated at 4°C until analyzed by ELISA. 

Briefly, ELISA plates were coated with a solution of mAb that recognizes IgG2m4 and were stored at 4°C overnight. The plates were treated with blocking buffer (Tris buffered saline (50 mM Tris, pH 7.5 and 150 mM NaCl) supplemented with 1% bovine serum albumin (BSA), 0.01% sodium azide and 0.05% tween-20) to decrease non-specific binding, incubated for one hour at room temperature and washed with buffer (Tris buffered saline supplemented with 0.05% tween-20). Serum samples and IgG2m4 standards were added to the appropriate wells and the plates incubated for 2 hours at room temperature. The plates were washed to remove any unbound IgG2m4, treated with biotinylated mouse anti-human kappa antibody, and incubated for 1 hour at room temperature to allow the biotinylated antibody to bind to the anti-IgG2m4/IgG2m4 complex. The plates were washed again, treated with a streptavidin-Europium solution and incubated to allow the solution to react with the biotinylated antibody complex. The plates were then washed to remove any excess solution, incubated at room temperature for one hour following the addition of enhancement buffer purchased from vendor (Cat# 1244-105, Perkin-Elmer, MA) and read by time delayed fluorescence in an Envision plate reader. EC₅₀ values of the samples and standards were determined using the four parameter fit curve in Prism software. Antibody concentrations of the serum samples were calculated by multiplying the EC₅₀ of the standard curve by the dilution factor that gave the EC₅₀ of the serum sample. IgG2m4 serum concentration-time data were analyzed by non-linear regression using WinNonlin v5.0.1. (Mountain View, CA). Briefly, the data were used to fit a classical two-compartment open model with intravenous bolus administration. A weighting function of 1/Y was used for the model fits. Each monkey’s serum concentration-time data were analyzed individually and the resulting pharmacokinetic parameters summarized.

Acknowledgements

We thank colleagues at Merck Research Laboratories who supported the work. All authors are current or former Merck employees and own Merck stock and stock options.

References

1. Maggon K. Monoclonal antibody “Gold Rush”. Current Medicinal Chemistry 2007; 14:1978-87.
2. Goldsby RA, Kindt TJ, Osborne BA, Kuby J. Immunology 5th Edition 2003 W.H. Freeman and Company, New York.
3. Dall’Acqua Wf, Kiener PA, Wu H. Properties of human IgG1s engineered for enhanced binding to the neonatal Fc receptor (FcRn). J Biol Chem 2006; 281:23514-24.
4. Firan M, Bawdon R, Radu C, Ober RJ, Eaken D, Antohe F, et al. The MHC class I-related receptor, FcRn, plays an essential role in the maternofetal transfer of gamma-globulin in humans. Int Immunol 2001; 13:993-1002.
5. Roopenian DC, Akilesh S. FcRn: the neonatal Fc receptor comes of age. Nature Review Immunol 2007; 7:715-25.
6. Clark MR. IgG effector mechanisms. Chem Immunol 1997; 65:88-110.
7. Dzion M. Fc receptor biology. Annu Rev Immunol 1997; 15:203-34.
8. Ravetch JV, Kinet JP. Fc receptors. Annu Rev Immunol 1991; 9:457-92.
9. Nimmerjahn F, Ravetch JV. Fcgamma receptors as regulators of immune responses. Nat Rev Immunol 2008; 8:54-47.
10. Bindon CI, Hale G, Brüggemann M, Waldmann H. Human monoclonal IgG isotypes differ in complement activating function at the level of C4 as well as C1q. J Exp Med 1988; 168:127-42.
11. Chappel MS, Isenman DE, Everett M, Xu Y-Y, Dorrington KJ, Klein MH. Identification of the Fc receptor class I binding site in human IgG through the use of recombinant IgG1/IgG2 hybrid and point mutated antibodies. Proc Nad Acad Sci 1991; 88:9036-40.
12. Tao M-H, Canfield SM, Morrison SL. The differential ability of IgG4 to activate complement is determined by the COOH-terminal sequence of the CH2 domain. J Exp Med 1991; 173:1025-8.

13. Medgyesi D, Uray K, Sallai K, Hudecz F, Konecz G, Abramson J, et al. Functional mapping of the FcγRII binding site on human IgG1 by synthetic peptides. Eur J Immunol 2004; 34:1127-35.

14. Brekke OH, Michelsen TE, Aase A, Sandin RH, Sandlie I. Human IgG isotype-specific amino acid residues affecting complement-mediated cell lysis and phagocytosis. Eur J Immunol 1994; 24:2542-7.

15. Greenwood J, Clark M, Waldmann H. Structural motifs involved in human IgG antibody effector functions. Eur J Immunol 1993; 23:1098-104.

16. Armour KL, van de Winkel JG, Williamson LM, Clark MR. Differential binding to human FcγRIIa and FcγRIIb receptors by human IgG wildtype and mutant antibodies. Mol Immunol 2003; 40:585-93.

17. Thommesen JE, Michelsen TE, Loset GA, Sandlie I, Brekke OH. Lyssine 322 in the human IgG3 CH2 domain is crucial for antibody dependent complement activation. Mol Immunol 2000; 37:1099-1004.

18. Idusogie EE, Presta LG, Gazzano-Santoro H, Thompson K, Wong PY, Ultsch M, et al. Mapping of the C1q binding site on Rituxan, a chimeric antibody with a human IgG1 Fc. J Immunol 2000; 164:4178-84.

19. Canfield SM, Morrison SL. The binding affinity of human IgG for its high affinity Fc receptor is determined by multiple amino acids in the CH2 domain and is modulated by the hinge region. J Exp Med 1991; 173:1483-91.

20. Xu Y, Oomen R, Klein MH. Residue at position 331 in the IgG1 and IgG4 CH2 domains contributes to their differential ability to bind and activate complement. J Biol Chem 1996; 271:3469-74.

21. An Z. Antibody therapeutics—a mini review. Trends in Bio/Pharmaceuticals Industry 2008; 4:24-9.

22. Peeters M, Balfour J, Arnold D. Review article: pan-Immunglobulin G (IgG) Fc receptor IIa (CD32): polymorphism and Fcγ2-mediated bacterial phagocytosis by neutrophils. Infect Immun 1995; 63:73-81.

23. Sivers EL, Appelbaum FR, Spielberger RT, Forman SJ, Flowers D, Smith FO, et al. Selective Ablation of Acute Myeloid Leukemia Using Antibody-Targeted Chemotherapy: A Phase I Study of an Anti-CD33 Calicheamicin Immunoconjugate. Blood 1999; 93:3678-84.

24. Adberse RG, Schuurman J. IgG4 breaking the rules. Immunology 2002; 105:9-10.

25. Lazar GA, Dang W, Karki S, Vafa O, Peng JS, Hyun L, et al. Engineered antibody Fc variants with enhanced effector function. Plos Natl Acad Sci 2006; 103:4005-10.

26. Mueller JP, Gianonni MA, Hartman SL, Elliott EA, Squinto SP, Mathis LA, et al. Humanized porcine VCAM-specific monoclonal antibodies with chimeric IgG2/G4 constant regions block human leukocyte binding to porcine endothelial cells. Mol Immunol 1997; 34:441-52.

27. Rother RP, Rollins SA, Mojicik CF, Brodsky RA, Bell L. Discovery and development of the complement inhibitor eculizumab for the treatment of paroxysmal nocturnal hemoglobinuria. Nature Biotechnol 2007; 25:1256-64.

28. Oganesyan V, Gao C, Shirinian L, Wu H, D’Alcaqua WF. Structural characterization of a human Fc fragment engineered for lack of effector functions. Acta Crystallogr D Biol Crystallogr 2008; 64:700-4.

29. Hougs L, Avejgaard A, Barington T. The first constant-region (CH1) exon of human IGHG2 is polymorphic and in strong linkage disequilibrium with the CH2 domain (CH1) exon polymorphism encoding the G2m(n+) allotype in Caucasians. Immunogenetics 2001; 52:242-8.

30. Kim TD, Cho S-E, Yang C-H, Kim J. Analysis of the C2 domain of human IgG subclasses in Wegener’s granulomatosis: a possible pathogenic role for the IgG4 subclass. Clin Exp Immunol 2000; 164:5313-92.

31. Salmon JE, Edberg JC, Brogle NL, Kimberly RP. Allelic variants of the human FcγRIIa gene and in strong linkage disequilibrium with the CH2 domain (CH1) exon of human IGHG2 is polymorphic and in strong linkage disequilibrium with the CH2 domain (CH1) exon polymorphism encoding the G2m(n+) allotype in Caucasians. Immunogenetics 2001; 52:242-8.

32. Sanders LA, Feldman RG, Voorhorst-Ogink MM, de Haan M, Rijkers GT, Caper PJ, et al. Human immuno-globulin G (IgG) Fc receptor IIa (CD32): polymorphism and Fcγ2-mediated bacterial phagocytosis by neutrophils. Infect Immun 1995; 63:73-81.

33. Armour KL, Clark MR, Hadley AG, Williamson LM. Recombinant IgG molecules lacking Fc receptor I binding and monocyte triggering activities. Eur J Immunol 1999; 29:2613-24.

34. Richards JO, Karki S, Lazar GA, Chen H, Dang W, Desjarlais JR. Optimization of antibody binding to FcgammaRIIa enhances macrophage phagocytosis of tumor cells. Mol Cancer Ther 2008; 7:2517-27.

35. Parren PW, Warmerdam PA, Boeije LC. On the interaction of IgG subclasses with the low affinity Fcgamma RI (CD32) on human monocytes, neutrophils, and platelets. Analysis of a functional polymorphism to human IgG2. J Clin Invest 1992; 90:1537-46.

36. Wines BD, Powell MS, Parren PW, Barnes N, Hogarth PM. The IgG Fc contains distinct Fc receptor (FcR) binding sites: the leukocyte receptors FcγRII and FcγRIIb bind to a region in the Fc distinct from that recognized by neonatal FcR and protein A. J Immunol 2000; 164:5313-8.