Abstract

The binding sites on human IgG1 for human Fcγ receptor (FcγR) I, FcγRIIa, FcγRIIb, FcγRIIIa and neonatal FcR have been mapped. A common set of IgG1 residues is involved in binding to all FcγRs, while FcγRII and FcγRIII utilize distinct sites outside this common set. In addition to residues which abrogated binding to the FcγR, several positions were found which improved binding only to specific FcγRs or simultaneously improved binding to one type of FcγR and reduced binding to another type. Selected IgG1 variants with improved binding to FcγRIIIa were then tested in an in vitro antibody-dependent cellular cytotoxicity (ADCC) assay and showed an enhancement in ADCC when either peripheral blood mononuclear cells or natural killer cells were used.

Monoclonal antibodies (mAbs) elicit four main effector functions: antibody-dependent cellular cytotoxicity (ADCC), phagocytosis, complement-dependent cytotoxicity and half-life/clearance rate. ADCC and phagocytosis are mediated through interaction of cell-bound mAbs with Fcγ receptors (FcγRs), complement-dependent cytotoxicity by interaction of cell-bound mAbs with the complement system, and half-life by binding of free mAbs to the neonatal Fc receptor (FcRn). In addition to ADCC and phagocytosis, FcγRs play a critical role in linking other mAb-mediated immune responses with cellular effector functions, including release of inflammatory mediators, endocytosis of immune complexes and regulation of immune system cell activation [1–3]. For mAbs that recruit the immune system to kill a target cell, engineering the IgG Fc portion to improve effector function (through improved binding to FcγR, FcRn and/or complement) could be a valuable enhancement to antibody therapeutics.

Key words: antibody, antibody-dependent cellular cytotoxicity (ADCC), Fc receptor, neonatal Fc receptor (FcRn).

Abbreviations used: ADCC, antibody-dependent cellular cytotoxicity; FcγR, Fcγ receptor; FcRn, neonatal Fc receptor; HER2, human epidermal growth factor receptor-2; mAb, monoclonal antibody; NK, natural killer.

In some cases a full-length mAb is required for long half-life, but other effector functions may be unnecessary or even deleterious. Immune system recruitment can be ablated by using human IgG2 or IgG4 subclasses, altering IgG residues in the hinge or other regions of the Fc [4–9], or using antibody F(ab) or F(ab')2 fragments (though these may have rapid clearance rates). Absence of the carbohydrate attached to Asn-297 (Eu numbering scheme [10]) of the Fc also reduces binding of IgG to FcγRs and complement [8,11–15].

FcγRs are expressed on immune system effector cells [e.g. natural killer (NK) cells, neutrophils, monocytes/macrophages, B-cells] and comprise three classes: FcγRI (CD64), FcγRII (CD32) and FcγRIII (CD16). In humans, the latter two classes are further divided into FcγRIIa and FcγRIIb, FcγRIIIa and FcγRIIIb. The receptors differ in their affinity for IgG, e.g. FcγRII exhibits a high affinity for monomeric IgG1 whereas FcγRII and FcγRIII exhibit a relatively weak affinity for monomeric IgG1 and hence can only interact effectively with multimeric immune complexes. The complexity of the human FcR system is amplified by the presence of polymorphic forms of the receptors. FcγRIIa has two forms, Arg-131 and His-131, that differ in binding of IgG2 [16]. FcγRIIIa has polymorphisms at positions 48 (Leu/His/Arg) and 158 (Phe/Val), though only the latter has been reported to effect a binding and biological difference [17–19]. FcγRIIIb polymorphic forms NA1 and NA2 differ in four amino acids [20]. Possible clinical significance involving polymorphic forms for all three receptors has been reported [21–24].

The binding sites on human IgG1 for human FcγRI, FcγRIIa, FcγRIIb, FcγRIIIa and FcRn have been mapped [8]. All solvent-exposed residues in human IgG1 Fc were individually altered to alanine and each variant tested for binding to the different receptors. One group of IgG1 residues reduced binding to all FcγRs when altered to alanine: Pro-238, Asp-265, Asp-270, Asn-297 (loss of Fc carbohydrate) and Pro-329. All are in the IgG CH2 domain and clustered near the hinge joining CH1 and CH2; these residues, in conjunction with the hinge region (residues 216–238), previously shown to affect FcγR binding [4–6],
comprise the set of IgG1 residues utilized in binding all FcγRs.

While FcγRI utilizes only the common set of IgG1 residues for binding, FcγRII and FcγRIII interact with distinct residues in addition to the common set. Alteration of some residues reduced binding only to FcγRII (e.g. Arg-292) or FcγRIII (e.g. Glu-293). Unexpectedly, a number of variants showed improved binding to FcγRII or FcγRIII but did not affect binding to the other receptor (e.g. Ser-267Ala improved binding to FcγRII but binding to FcγRIII was unaffected; Figure 1). Other variants exhibited improved binding to FcγRII or FcγRIII with reduction in binding to the other receptor (e.g. Ser-298Ala improved binding to FcγRIII and reduced binding to FcγRII; Figure 1). IgG1 variants in which two or more residues were simultaneously altered to alanine exhibited additivity [8]. For FcγRIIIa, the best-binding IgG1 variants combined alanine substitutions at Ser-298, Glu-333 and Lys-334; Ser-298Ala/Glu-333Ala, Ser-298Ala/Lys-334Ala and Ser-298Ala/Glu-333Ala/Lys-334Ala all bound greater than 1.5-fold more strongly than native IgG1 in an ELISA-format assay. These variants exhibited a more pronounced improvement in binding to the FcγRIIIa(Val-158) polymorph compared with the FcγRIIIa(Val-158) polymorph. Though 1.5-fold might seem small, these variants also showed enhanced in vitro ADCC using purified human peripheral blood mononuclear cells or NK cells as effector cells. As with the ELISA-format binding assay, the increase in ADCC was more pronounced for effector cells from FcγRIIIa(Phe-158/Val-158) donors than those from FcγRIIIa(Val-158/Val-158) donors [8] (Figure 2).

Studies comparing mice with and without specific FcγRs have shown that for some therapeutic mAbs the presence of FcγR is necessary for efficacy [25–28]. Though recruitment of the immune system to destroy the target cell could be the mechanism (via ADCC, phagocytosis or other pathways), it is also possible that in some cases the FcγR-bearing cells might function only as cross-linking agents. A full-length mAb can cross-link two target molecules as a consequence of its inherent bivalency, though additional cross-linking of cell-bound mAbs may enhance their function [29]. Rituximab, a chimaeric anti-CD20 mAb for non-Hodgkin’s lymphoma, requires cross-linking of CD20-bound mAb to effect efficient apoptosis of B-cells [30,31]. In vitro, cross-linking can be achieved using anti-human IgG-specific mAbs; in vivo, though, either FcγR-bearing cells or Clq must act as a cross-linking agent. When an IgG4

![Figure 1](image.png)

**Figure 1**

Binding of human IgG1 variants to human FcγRI, FcγRII and FcγRIII

Bar height represents binding of the IgG1 variant relative to the binding of native IgG1, where native IgG1 = 1.0. Error bars represent S.D. from the means of 4–16 assays [8]. FcγRI, dark grey; FcγRIIb, black; FcγRIIIa, light grey.
form of Rituximab was evaluated in monkeys it had no effect, whereas the IgG1 form was efficacious [32]. From this experiment alone one cannot discern whether FcγR and/or Clq act as the necessary cross-linking agent because IgG4 does not bind well to either. However, in a study comparing wild-type and FcγRI/FcγRIIa-IgG knock-out mice, Rituximab lost most of its efficacy in the knock-out mice, suggesting that FcγR-bearing cells are the major, if not only, cross-linking agent.

Regarding IgG1 variants with improved binding to FcγR, though these variants have not yet been tested in vivo, three important possibilities exist. First, if a therapeutic mAb utilizes FcγR-bearing cells as part of its mechanism of action (either as cross-linking agent or to elicit ADCC or phagocytosis), then mAbs with improved binding to FcγR (compared with native IgG) could exhibit increased efficacy. Second, IgG variants that have enhanced binding to activating FcγRs (FcγRI, FcγRIIa and/or FcγRIIa) and reduced binding to the inhibitory FcγRIIb might increase the efficacy of a therapeutic mAb even further, suggested by the improved efficacy of Herceptin in FcγRIIb-deficient mice [25]. Third, the human proteome contains a Val-158/Phe-158 polymorphism in FcγRIIa, and human IgG1 binds better to the FcγRIIa(Val-158) form than it does to the FcγRIIa(Phe-158) form [8,18,19]. Since about 90% of humans have at least one FcγRIIa(Val-158) allele (and ~45% are Phe-158/Phe-158 homozygotes) [21], use of a variant IgG that increased binding to both polymorphic forms, but even more so to the lower-affinity FcγRIIa(Phe-158) form, would mean enhanced efficacy for those that need it the most.

Another effector function of IgG involves its half-life (or, alternatively, clearance rate). Human IgG have a half-life of 7-16 days or longer [33]. An IgG receptor involved in maintaining IgG homeostasis in blood has been hypothesized [34], but only recently has a specific receptor been proposed to fulfil this role—the FcRn. In addition to regulating homeostasis, FcRn controls transcytosis across tissues [35]. This receptor is structurally related to MHC class I, comprising an α-chain that non-covalently associates with β2-microglobulin [36]. The IgG-FcRn interaction is pH-dependent, with IgG binding at pH 6 but not at pH 7.2 (the pH of blood), an observation integrated into a current model of how FcRn functions [35].

Many of the studies on FcRn have utilized the murine receptor. The epitope on murine IgG for murine FcRn has been mapped [35]. Notably, alteration of specific residues in murine IgG that improve binding to murine FcRn also effect an increased half-life in mice [37]. The epitope on human IgG for human FcRn has also been mapped and substituting specific IgG1 residues with alanine enhanced binding to FcRn [8]. The range of improvement in binding to human FcRn by alteration of individual IgG1 residues was 1.4- to 3.5-fold. Individual substitutions to alanine could be combined to further increase binding to FcRn up to 12-fold. Notably, these alterations improved binding only at pH 6.0, but not pH 7.2 [8], and therefore would not hinder dissociation of the IgG from FcRn. Hence, the half-life of therapeutic mAbs may be able to be extended by a varying degree dependent on which IgG1 amino acids are altered or in which combination. In some instances, it might be advantageous to either decrease or prolong the half-life, e.g. the latter
could conceivably reduce the dosage or frequency of administration without affecting the efficacy.

Finally, many of the IgG1 residues that are involved in binding to FcγRs and FcRn are different. As a consequence, it is possible to generate IgG1 variants with altered FcγR-binding characteristics (improved or debilitated) and altered half-life (prolonged or shortened). For example, if a therapeutic mAb required enhanced ADCC (or cross-linking ability) and a longer-than-normal half-life was needed, an Asp-265Ala/Asn-434Ala IgG1 variant might suffice. If a therapeutic mAb with ablated FcRn binding but longer-than-normal half-life was needed, an Asp-265Ala/Asn-434Ala IgG1 variant could be used.

References


Received 14 March 2002