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Mini-Review

Theme: Investigation of the role of FcRn on the absorption, distribution, and elimination of IgG and Albumin
Guest Editor: Joseph Balthasar

Inhibitors of the FcRn:IgG Protein–Protein Interaction

Susan C. Low[1] and Adam R. Mezo[1,2]

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Abstract. The neonatal Fc receptor, FcRn, is responsible for controlling the half-life of IgG antibodies. As a result, inhibitors of FcRn have been investigated as a possible way to modulate IgG half-lives. Such inhibitors could have possible applications in reducing autoantibody levels in autoimmune disease states. To date, monoclonal antibodies, engineered Fc domains, and short peptides have been reported to inhibit FcRn function and modulate IgG half-lives *in vivo*.

KEY WORDS: engineered Fc; FcRn; IgG; inhibitors; monoclonal antibody; peptide.

INTRODUCTION

The existence of a receptor that is responsible for the long half-life of IgG was first proposed in the 1960s by Brambell (1). It was suggested that this receptor was saturable based on studies demonstrating increased metabolism of IgG with hyperimmunization (2). Decades later, this hypothesis was validated with the cloning of the neonatal Fc receptor, FcRn (3). FcRn is a 52-kDa membrane-bound heterodimeric glycoprotein comprising a heavy chain and a light chain (β2-microglobulin, β2m) (4). IgGs are taken up into cells most likely by fluid-phase pinocytosis since the steady-state location of FcRn is endosomal. FcRn binds Fc in the acidic environment of the endosome. IgG that binds FcRn is then recycled either apically or basolaterally to the plasma membrane, where upon exposure to a neutral pH it is released. In contrast, IgGs that do not bind to FcRn enter the lysosomal pathway and are degraded. As FcRn biology is not the focus of this review, the reader is directed to more detailed reviews for further FcRn background (4,5).

There are several lines of evidence suggesting that FcRn has a role in the regulation of IgG catabolism. For example, IgG has an abnormally short half-life in FcRn- (6) and β2m-deficient (7–9) mice compared to normal mice, and mutant murine IgG-Fc fragments have impaired binding to FcRn and abnormally short half-lives (10,11).

The fact that FcRn plays a critical role in the long half-life of IgG has prompted the proposal that inhibitors of FcRn may be useful in the treatment of autoimmune disease by competing with autoantibodies for FcRn binding, resulting in lysosomal degradation of the pathogenic antibodies (12,13). In support of this theory, it has been shown that FcRn-deficient mice are protected against arthritis (14). In addition, FcRn deficiency has been shown to protect against autoimmune blistering diseases (15,16). High doses of intravenous immunoglobulin (IVIg), which binds FcRn through its Fc domain, has also been shown to accelerate the catabolism of model antibodies (17) and ameliorate symptoms of idiopathic thromobocytopenia purpura (18), arthritis (14), and pemphigus (15) in rodent models. These data suggest that FcRn blockade may have effects on these and other IgG-mediated autoimmune diseases by saturating and inhibiting FcRn.

Each of these examples suggests that specific high-affinity FcRn inhibitors may have utility in the treatment of antibody-mediated autoimmune conditions. Several approaches to generating inhibitors of the IgG:FcRn interaction have been reported, and these will be the focus of this mini-review.

ANTI-FcRn MONOCLONAL ANTIBODIES

Over the course of studying FcRn biology, the monoclonal antibody 1G3 was generated by the Bjorkman group against the heavy chain of rat FcRn (19). 1G3 has an affinity for rat FcRn of 1.9 nM at pH 6 and 5.8 nM at pH 7.4. This is in comparison to the natural ligand IgG which does not bind to FcRn at pH 7.4. Liu *et. al.* exploited this property of the antibody and demonstrated that following two consecutive doses of 1G3 (30 mg/kg) 24 h apart, endogenous IgG levels in rats were reduced by 40%, and the effects of 1G3 appeared to last for 3 days (20). Interestingly, the pharmacokinetics of 1G3 were very short: after a 10-mg/kg i.p. injection, 1G3 had a similar Cmax to that of a mouse IgG control antibody (~50 μg/mL), but at 24 h, the serum concentration of 1G3 was less than 0.01 μg/mL. In contrast, the mouse IgG control antibody had a half-life of approximately 104 h. This shortened 1G3 antibody half-life may be the result of 1G3 binding tightly to FcRn at both pH 6 and 7.4, thus unable to recycle via FcRn (20).

[1] Syntonix Pharmaceuticals, Inc., A subsidiary of Biogen Idec, 9 Fourth Ave, Waltham, MA 02451, USA.
[2] To whom correspondence should be addressed. (e-mail: amezo@syntnx.com)

Myasthenia gravis (MG) is an autoimmune disease that is predominantly mediated by autoantibodies. The disease symptoms include muscle weakness and fatigability which are due to antibodies generated against the acetylcholine receptor (AChR) and other neuromuscular antigens. Depending on disease severity, MG patients can be categorized into two groups: patients who have developed myasthenic crisis and patients who have generalized MG but are not in crisis (21). A rat model of passive experimental autoimmune myasthenia gravis (EAMG) in which the disease is induced by administering the anti-acetylcholine receptor antibody, mAb35, resembles the disease characteristics of MG crisis, in that it is severe and has a fast onset. The disease symptoms that occur in the passive EAMG model include a decrease in body weight and a loss of grip strength due to muscle weakness. When 1G3 was administered 24 or 2 h before mAb35 injection, a dose of 30 mg/kg almost completely prevented the symptoms of EAMG in this rat model. Importantly, there was a dose-dependent decrease in serum mAb35 levels at 48 h after 1G3 treatment, indicating that the mechanism of 1G3 action was due to enhanced clearance of mAb35 by FcRn blockade. To investigate the effects of FcRn blockade on chronic MG, rats were immunized with AChR in Freund's Complete Adjuvant (11). At the onset of disease symptoms (approximately 21 days after administration of the AChR), 1G3 was administered and resulted in significantly suppressed disease symptoms.

The Bjorkman group also developed a monoclonal antibody, 4C9, directed against the light chain of FcRn, β2m. This antibody was found to block the binding of IgG to FcRn *in vitro* (19). Getman and Balthasar (22) treated rats with 4C9, at doses of 3 to 60 mg/kg, and found that 4C9 induced a transient and dose-dependent increase in the elimination of an exogenously administered anti-methotrexate IgG (AMI). In particular, the AMI clearance rate was increased from 0.99 mL h^{-1} kg^{-1} (control) to 1.97 mL h^{-1} kg^{-1} in rats dosed with 60 mg/kg 4C9, and the effects of 4C9 appeared to last for approximately 2 days. One caveat with 4C9 is that the effect of targeting β2m, which is also present in other major histocompatibility complex class I proteins, renders 4C9 less selective than inhibitors that target the heavy chain of FcRn. Nevertheless, these experiments demonstrate that inhibitors targeting the light chain of FcRn can impact the pharmacokinetics of IgG antibodies.

MUTANTS OF THE Fc REGION OF IgG1 ANTIBODIES

IgG has the longest half-life in circulation of all immunoglobulin classes, ranging from 7 to 21 days in healthy humans (23). The Fc region of IgG has been implicated as the domain responsible for the long half-life of IgG through binding to FcRn (5).

Petkova *et al.* (24) engineered Fc mutants of the humanized monoclonal antibody Herceptin (Hu4D5-IgG1) which is directed against human epidermal growth factor receptor 2. The two mutants, N434A (NA) and T307A/E380A/N434A (TENAAA) had 3.4-fold and 11.8-fold improved binding to human FcRn, respectively, using enzyme-linked immunosorbent assay-based assays (25) and 1.6-fold and 3.3-fold improved binding to human FcRn, respectively, in cell-based assays (16) compared to the wild-type antibody. The improved binding of the mutant Hu4D5-IgG antibodies to human FcRn resulted in a 2.2-2.5-fold increased half-life in mice and correlated with the ability to increase the catabolism of human IgG in human FcRn mice. Two 2 mg doses of NA or TENAAA 24 h apart decreased the half-life of human IgG to 3.37±0.81 days and 3.26±0.19 days, respectively, compared to the wild-type Hu4D5-IgG (5.24±0.64 days), and the effects of these inhibitors appeared to last for several days.

Plasma or purified antibodies from patients with rheumatoid arthritis have been found to cause inflammatory joint lesions in mice that are deficient in the Fc inhibitory receptor, FcγRIIB (26). When human FcRn transgenic mice that have been crossed with FcγRIIB deficient mice were treated with plasma from a rheumatoid arthritis patient followed by treatment with 2.5 mg injections of TENAAA, there was reduced ankle swelling in comparison to mice treated with the wild-type Hu4D5-IgG control (16). These data suggest that the high-affinity IgG-Fc mutants NA and TENAAA can block FcRn from binding autoimmune antibodies responsible for diseases such as rheutmatoid arthritis. As a result, these autoantibodies are cleared more rapidly, and symptoms of the disease are ameliorated in this mouse model.

Vaccaro *et al.* (27) developed an antibody that enhances IgG degradation which has been named an "Abdeg." This Abdeg (MST-HN) is a mutant of IgG1 in which the amino acids Met252, Ser254, Thr256, His433, and Asn434 were mutated to Tyr252, Thr254, Glu256, Lys433, and Phe434, respectively. MST-HN has an increased affinity for human FcRn binding at pH 6.0 (K_{D1}=15.5 nM for MST-HN compared to 370 nM for wild-type human IgG1) and retains significant binding activity at pH 7.2 unlike wild-type IgG1 which does not bind to FcRn at neutral pH. In mice treated with 200 or 500 μg of MST-HN, the clearance of ^{125}I-IgG1 was increased by 80% to 88% after 120 h and, interestingly, significantly lowered endogenous IgG concentrations. In addition, the effects of MST-HN appeared to last for approximately 2 days. The same group also studied a mutant of IgG1 (named "HN"), where only amino acids His433 and Asn434 of IgG1 were mutated to Lys433 and Phe 434, respectively (28). HN possessed weaker binding affinity to both mouse and human FcRn than MST-HN but was still effective as an Abdeg in mice. In mice treated with 500 μg of HN, serum levels of a tracer ^{125}I-IgG1 were reduced by 59% after 120 h.

ANTI-FcRn PEPTIDES

Recently, a family of peptides was identified capable of binding to human FcRn and inhibiting the binding of hIgG (29). These peptides were discovered by peptide phage display and contain no homology to the Fc domain of IgG. The consensus motif consisted of GHFGGXY, where X is preferably a hydrophobic amino acid and the motif is enclosed by a disulfide loop. One member of the peptide family was chemically optimized (30) into a 3.1-kDa peptide homodimer named SYN1436. SYN1436 binds to human FcRn with subnanomolar affinity in surface plasmon resonance affinity binding experiments. This peptide binds to human FcRn and not to rodent FcRn; therefore, initial *in vivo* activity experiments were performed in transgenic mice

where the mouse FcRn and β2m genes have been replaced with their human homologs (TG32B mice). SYN1436 was found to accelerate the catabolism of exogenously administered human IgG in doses as low as 1 mg kg^{-1} day^{-1}. Lastly, treatment of cynomolgus monkeys with repeated doses of 5 mg/kg SYN1436 three times per week was found to reduce endogenous IgG levels by approximately 80%, providing the first evidence that FcRn inhibitors can affect IgG levels in nonhuman primates. In addition, the peptide effects appeared to last for several days in monkey groups that were dosed with a frequency of once per week.

CONCLUSION

There has been an increasing interest over the last several years in generating inhibitors of FcRn in order to better understand the biology and therapeutic potential of inhibiting FcRn function *in vivo*. To date, inhibitors have included monoclonal antibodies, mutant Fc (IgG) proteins, and peptides. Data are now accumulating that each of these types of molecule can bind effectively to FcRn *in vitro*, and some are capable of alleviating symptoms of autoimmune disease in rodent models and/or increase the catabolism of IgG. Peptide inhibitors of FcRn have also been shown to reduce endogenous IgG levels in cynomolgus monkeys. Collectively, the *in vitro* and *in vivo* FcRn inhibitor data in rodents and nonhuman primates indicates an intriguing and novel potential for future treatments of autoimmune diseases.

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REFERENCES

1. Brambell FW, Hemmings WA, Morris IG. A theoretical model of gamma-globulin catabolism. Nature. 1964;203:1352–4.
2. Fahey JL, Robinson AG. Factors controlling serum gamma-globulin concentration. J Exp Med. 1963;118:845–68.
3. Simister NE, Mostov KE. An Fc receptor structurally related to MHC class I antigens. Nature. 1989;337:184–7.
4. Roopenian DC, Akilesh S. FcRn: the neonatal Fc receptor comes of age. Nat Rev Immunol. 2007;7:715–25.
5. Ghetie V, Ward ES. Multiple roles for the major histocompatibility complex class I-related receptor FcRn. Annu Rev Immunol. 2000;18:739–66.
6. Roopenian DC, Christianson GJ, Sproule TJ, Brown AC, Akilesh S, Jung N, *et al.* The MHC class I-like receptor controls perinatal IgG transport, IgG homeostasis, and the fate of IgG-Fc-coupled drugs. J Immunol. 2003;170:3528–33.
7. Ghetie V, Hubbard JG, Kim JK, Tsen MF, Lee Y, Ward ES. Abnormally short serum half-lives of IgG in beta-2-microglobulin deficient mice. Eur J Immunol. 1996;26:690–6.
8. Israel EJ, Wilsker DF, Hayes KC, Schoenfeld D, Simister NE. Increaesd clearance of IgG in mice that lack beta 2-microglobulin: possible protective role of FcRn. Immunolgy. 1996;89:573–8.
9. Junghans RP, Anderson CL. The protection receptor for IgG catabolism is the beta2-microglobulin-containing neonatal intestinal transport receptor. Proc Natl Acad Sci U S A. 1996;93:5512–6.
10. Kim JK, Tsen MF, Ghetie V, Ward ES. Localization of the site of the murine IgG1 molecule that is involved in the binding to the murine intestinal Fc receptor. Eur J Immunol. 1994;24:2429–34.
11. Popov S, Hubbard JG, Kim J, Ober B, Ghetie V, Ward ES. The stiochiometry and affinity of the interaction of murine Fc fragments with the MHC class I-related receptor, FcRn. Mol Immunol. 1996;33:521–30.
12. Liu Z, Roopenian DC, Zhou X, Christianson GJ, Diaz LA, Sedmak DD, *et al.* Beta2-microglobulin-deficient mice are resistant to bullous pemphigoid. J Exp Med. 1997;186:777–83.
13. Yu Z, Lennon VA. Mechanism of intravenous immune globulin therapy in antibody-mediated autoimmune diseases. N Engl J Med. 1999;340:227–8.
14. Akilesh S, Petkova S, Sproule TJ, Shaffer DJ, Christianson GJ, Roopinean DC. The MHC class I-like Fc receptor promotes humorally mediated autoimmune diseases. J Clin Invest. 2004; 113:1328–33.
15. Li N, Zhao M, Hilario-Vargas J, Prisayanh P, Warren S, Diaz LA, *et al.* Complete FcRn-dependence for intravenous Ig therapy in autoimmune skin blistering diseases. J Clin Invest. 2005;115:3440–50.
16. Sesarman A, Sitaru AG, Olaru F, Zilliken D, Sitaru CJ. Neonatal Fc receptor deficiency protects from tissue injury in experimental epidermolysis bullosa acquisita. Mol Med. 2008;86:951–9.
17. Bleeker WK, Teeling JL, Hack CE. Accelerated autoantibody clearance by intravenous immunoglobulin therapy: studies in experimental models to determine the magnitude and time course of the effect. Blood. 2001;98:3136–42.
18. Hansen RJ, Balthasar JP. Effects of intravenous immunoglobulin on platelet count and antiplatelet antibody disposition in a rat model of immune thrombocytopenia. Blood. 2002;100:2087–93.
19. Raghavan M, Chen MY, Gastinel LN, Bjorkman PJ. Investigation of the interaction between the class I MHC-related Fc receptor and its immunoglobulin G ligand. Immunity. 1994;1:303–15.
20. Liu L, Garcia AM, Santoro H, Zhang Y, McDonnell K, Dumont J, *et al.* Amelioration of experimental autoimmune myasthenia gravis in rats by neonatal FcR blockade. J Immunol. 2007;178:5390–8.
21. Nicolle MW. Myathenia gravis. Neurologist. 2002;8:2–21.
22. Getman KE, Balthasar JP. Pharmacokinetic effects of 4C9, anti-FcRn antibody, in rats: implications for the use of FcRn inhibitors for the treatment of autoimmune and alloimmune conditions. J Pharm Sci. 2005;94:718–29.
23. Waldmann TA, Strober W. Metabolism of immunoglobulins. Prog Allergy. 1969;13:1–110.
24. Petkova SB, Akilesh S, Sproule TJ, Christianson GJ, Khabbaz HA, Brown AC, *et al.* Enhanced half-life of genetically engineered human IgG1 antibodies in a humanized FcRn mouse model: potential application in humorally mediated autoimmune disease. Int Immunol. 2006;18:1759–69.
25. Shields RL, Namenuk AK, Hong K, Meng YG, Rae J, Briggs J, *et al.* High resolution mapping of the binding site on human IgG1 for Fc gamma RI, Fc gamma RII, Fc gamma RIII, and FcRn and design of IgG1 variants with improved binding to the Fc gamma R. J Biol Chem. 2001;276:6591–604.
26. Petkova SB, Konstantinov KN, Sproule SJ, Lyons BL, Awwami MA, Roopenian DC. Human antibodies induce arthritis in mice deficient in the low affinity inhibitory IgG receptor Fc gamma RIIB. J Exp Med. 2006;203:275280.
27. Vaccaro C, Zhou J, Ober R, Ward ES. Engineering the Fc region of immunoglobulin G to modulate *in vivo* antibody levels. Nat Biotechnol. 2005;23:1283–8.
28. Vaccaro C, Bawdon R, Wanjie S, Ober RJ, Ward ES. Divergent activities of an engineered antibody in murine and human systems have implications for therapeutic antibodies. Proc Nat Acad Sci U S A. 2006;103:18709–14.
29. Mezo AR, McDonnell KA, Hehir CA, Low SC, Palombella VJ, Stattel JM, *et al.* Reduction of IgG in nonhuman primates by a peptide antagonist of the neonatal Fc receptor FcRn. Proc Nat Acad Sci U S A. 2008;105:2337–42.
30. Mezo AR, McDonnell KA, Castro A, Fraley C. Structure-activity relationships of a peptide inhibitor of the human FcRn: human IgG interaction. Bioorg Med Chem. 2008;16:6394–405.