

## Review

## Targeting FcRn to Generate Antibody-Based Therapeutics

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The MHC class I-related receptor FcRn serves multiple roles ranging from the regulation of levels of IgG isotype antibodies and albumin throughout the body to the delivery of antigen into antigen loading compartments in specialized antigen-presenting cells. In parallel with studies directed towards understanding FcRn at the molecular and cellular levels, there has been an enormous expansion in the development of engineering strategies involving FcRn to modulate the dynamic behavior of antibodies, antigens, and albumin. In this review article, we focus on a discussion of FcRn-targeted approaches that have resulted in the production of novel antibody-based platforms with considerable potential for use in the clinic.

### The Motivation behind Modulating Antibody Dynamics in the Body

The efficacy of current antibody-based therapeutics largely depends on homeostatic regulation of antibody levels at the appropriate site in the body. For example, for maximum efficacy of a therapeutic antibody in the treatment of cancer, it is necessary for sufficient antibody levels to be maintained over extended time periods at the tumor site [1]. By contrast, for antibody-mediated autoimmune diseases such as myasthenia gravis or idiopathic thrombocytopenic purpura, the levels of pathogenic antibodies are too high and approaches to reduce antibody concentrations are desirable [2]. Similarly, during diagnostic or **theranostic imaging** (see [Glossary](#)) with (radio)labeled antibodies, the background signal can be problematic due to the prolonged persistence of the antibody in the circulation, leading to poor contrast and possibly nonspecific organ damage [3,4]. A fundamental question is therefore what are the molecular and cellular processes that control antibody levels, particularly those of the IgG class that are typically used as therapeutics? Furthermore, how can this knowledge be used to tune the level and distribution of therapeutic or endogenous antibodies?

The neonatal crystallizable fragment receptor FcRn is an **MHC** class I-related receptor that interacts with antibodies of the IgG class as a heterodimer of a heavy ( $\alpha$ ) chain and  $\beta$ 2-microglobulin and that binds to the constant or fragment crystallizable (Fc) region of IgG (Figure 1A). FcRn is a key player in regulating the dynamic behavior, including distribution, transport, and persistence, of IgG antibodies throughout the body [5–7]. The identification of these activities extends the role of this Fc receptor well beyond its original identification as the transporter of IgG from mother to young (hence the name ‘n’, for neonatal), prompting extensive analyses of the molecular and cellular properties of FcRn. In parallel, the relevance of FcRn to antibody-based therapeutics has motivated the development of technologies to modulate the dynamic properties of these biologics and, in some cases, their cognate antigens *in vivo*. Given the enormous expansion of the use of antibodies in therapy over the past decade, this review article provides a brief overview of FcRn biology followed by a discussion of engineering approaches to generate improved, new generation therapeutics. Because of

### Highlights

Improved knowledge of FcRn at both molecular and cellular levels can be used towards better designing of novel classes of therapeutic antibodies.

Engineering antibodies to modulate their interaction with FcRn has resulted in several strategies to alter their dynamic behavior.

Half-life extended therapeutic antibodies with substantial increases in persistence in the body have been developed and are currently in clinical trials.

Antibodies that bind to their target antigen in a pH-dependent way (‘acid-switched’ antibodies) have been shown to be superior agents for the clearance of inflammatory cytokines, regulators of cholesterol levels, etc., from the body.

Engineered inhibitors that block the binding of antibodies to FcRn could provide a new generation of therapeutics to reduce the levels of pathogenic antibodies.

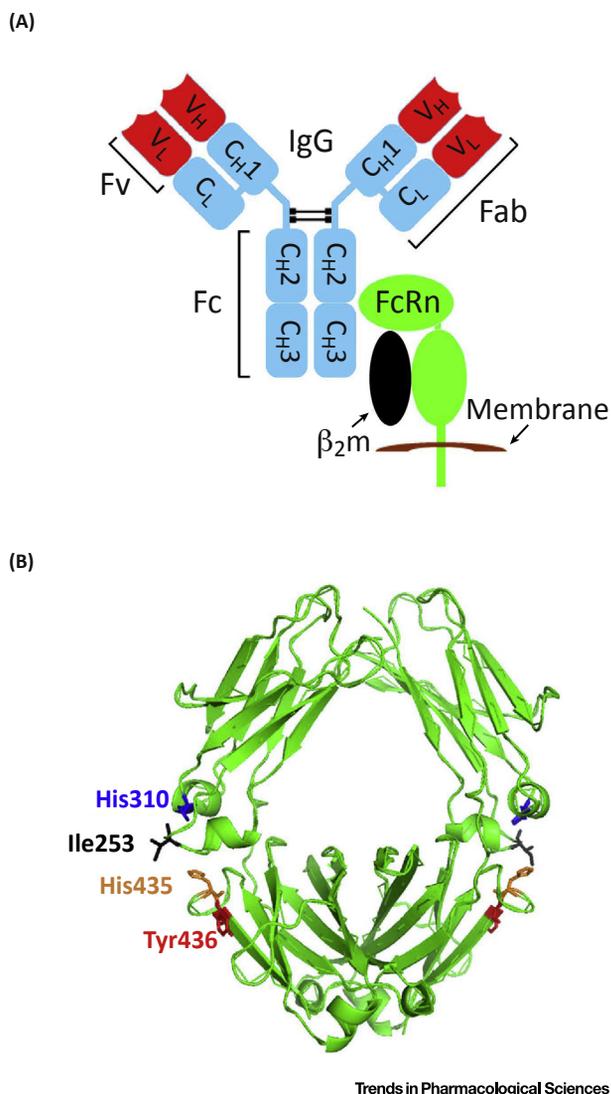
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**Figure 1. The Molecular Nature of the IgG–FcRn Interaction.** (A) Schematic representation of the IgG–FcRn interaction. The constant regions and variable domains (Fv) of the antibody IgG are colored blue and red, respectively. FcRn is a heterodimer of an  $\alpha$  chain (green) and  $\beta$ 2-microglobulin ( $\beta$ <sub>2</sub>m, black) and is shown in its transmembrane form. Fc, the constant or fragment crystallizable (Fc) region. (B) The location of the residues (Ile253, His310, His435, and Tyr436) at the CH2–CH3 domain interface of the Fc region of human IgG1. These residues play a central role in binding to FcRn. The figure was drawn using the X-ray crystallographic structure of human IgG1-derived Fc fragment [100] and Pymol (The PyMOL Molecular Graphics System, Version 2.0 Schrödinger, LLC).

space limitations, we do not discuss the role of FcRn in regulating albumin levels and the related implications for therapy (see [Box 1](#) for a brief overview of this topic).

### The Molecular Details of FcRn–IgG Interactions

IgG residues that are critical for the binding of mouse or human IgG1 to FcRn include Ile253, His310, His435, His436 (mouse) or Tyr436 (human) that are located on the exposed loops at the CH2–CH3 domain interface of IgG1 [5,8–10] (Figure 1B). In general, these amino acids are well conserved across IgG isotypes and species, although amino acids such as residues 435

#### Box 1. Role of FcRn in Regulating Albumin Levels and Related Implications for Therapy

Beyond the IgG-related functions of FcRn, the identification of this receptor as a transporter of albumin has resulted in the engineering of albumin–FcRn interactions, or albumin binding proteins, to generate therapeutics [6,96,97], in addition to the recent use of FcRn inhibitors to accelerate the clearance of toxic drug–albumin complexes [98]. Through the ability of FcRn to bind and recycle albumin away from lysosomal degradation, this receptor can also regulate nutrient supply to cells by controlling the intracellular supply of albumin-derived amino acids [99].

### Glossary

**Allotype:** a polymorphic variant of an antibody gene. Antibody alleles frequently vary between individuals within a species, giving rise to allotypic variation.

**Cross-presentation:** the loading of peptides derived from exogenous proteins onto MHC class I molecules following the internalization of the protein into the antigen presenting cell.

**Fluid phase pinocytosis:** the internalization of molecules into cells via the pinching off of vesicles that contain extracellular fluid. Internalization is not mediated by binding of the molecules to receptors but instead is dependent on the extracellular concentration of the molecule of interest.

**Intravenous immunoglobulin (IVIg):** a preparation of IgG prepared from plasma pooled from multiple human donors. This preparation is used to treat autoimmune and immunodeficiency diseases in the clinic.

**Isoelectric point (pI):** the pH at which a protein (or other molecule) has no net positive or negative charge.

**MHC:** a group of proteins (class I or class II) that is recognized by immune cells called T cells and are involved in inducing an immune response to destroy pathogens, or in the case of autoimmunity, to cause tissue damage. MHC proteins can vary from one person to the next; consequently, they are major players in transplant rejection if the donor and recipient are not matched. A minority of MHC class I-related molecules, such as FcRn, do not play a role in activating T cells, but instead serve other functions.

**Off-rate:** the rate at which two proteins or other molecules dissociate from each other.

**On-rate:** the rate with which two proteins or other molecules associate with each other. The ratio of the off-rate to on-rate yields the dissociation constant of the interaction.

**Phage display library:** the generation of bacteriophage particles, using molecular biology methods, that bear a ‘library’ (i.e., large number) of different recombinant proteins such as antibody variable domains or Fv/Fab

and 436 show higher variability than Ile253 and His310 [11]. The His residues of IgG interact with acidic residues of FcRn [9,12]. The interaction of the protonated imidazole side chain of His ( $pK_a \sim 6$ ) with these acidic residues at pH  $\sim 6.0$  confers the characteristic pH-dependent binding (relatively high affinity at acidic pH, with very weak to negligible binding at pH 7.3–7.4) that is observed for the majority of IgGs [13,14]. Consistent with this, isotypes such as mouse IgG2b and a human IgG3 **allotype** that contain Tyr and Arg, respectively, at position 435 do not go through protonation/deprotonation cycles between pH 6.0 and pH 7.4 and have significantly higher binding affinity for FcRn at near-neutral pH compared with other isotypes or IgG3 allotypes containing His at position 435 of IgG [13–15]. This gain of binding at pH  $\sim 7.3$ –7.4 has marked functional consequences that are discussed in detail below.

A further confounding factor concerning FcRn–IgG interactions relates to the cross-species differences for FcRn–IgG interactions, interactions for which the molecular basis has been defined [16,17]. These observations have led to the conclusion that mouse models have limitations for the preclinical pharmacokinetic analyses of therapeutic antibodies, particularly if they have been engineered to increase their affinity for FcRn [18]. This issue has motivated the development of mice that transgenically express human FcRn instead of mouse FcRn [19,20]. The use of such mice provides valuable insight into the pharmacokinetic behavior of engineered antibodies that can be translated to non-human primates and humans.

### The Subcellular Trafficking Behavior of FcRn and IgG

Understanding the subcellular trafficking behavior of FcRn is important for understanding the mechanisms that maintain IgG homeostasis, in addition to providing insight into the design of engineered antibodies with altered dynamic properties in the human body. This has led to multiple studies directed towards elucidating the cell biology of FcRn [21–29]. FcRn is ubiquitously expressed in both parenchymal (endothelial, epithelial) and hematopoietic cells [30–32], and the majority of cellular trafficking studies of FcRn and its IgG ligand have been carried out in endothelial or epithelial cells [21–29]. Live cell imaging of endothelial cells following transfection with FcRn tagged with GFP (to track FcRn in live cells) demonstrates that FcRn–IgG complexes are internalized primarily by **fluid phase pinocytosis** (due to the very low affinity of FcRn for most IgG subclasses at extracellular, near-neutral pH), sorted in early (sorting) endosomes away from lysosomal degradation and recycled back to the cell surface for exocytic release [24–26] (Figure 2). The acidic pH in early endosomes (pH  $\sim 6.0$ ) is permissive for FcRn–IgG interactions, whereas the extracellular, near-neutral pH (pH  $\sim 7.4$ ) for most cell types enables efficient dissociation of IgG from FcRn during fusion of exocytic compartments with the plasma membrane. The primary pathway for internalization of IgG into cells involves fluid phase pinocytosis, although receptor (FcRn)-mediated uptake becomes dominant for IgG molecules that naturally bind, or are engineered to bind, to FcRn at near-neutral pH [33,34]. In contrast to IgGs that interact with FcRn, those that are either mutated to ablate binding, or naturally do not bind due to cross-species differences (e.g., mouse IgG1 to human FcRn [16]), fail to be sorted intracellularly and enter lysosomes [25]. These endosomal sorting processes provide a framework for the cellular mechanisms through which FcRn regulates the levels of IgGs in the body. For example, when the intracellular levels of IgG increase, all FcRn molecules are in the bound state and the excess free antibodies are degraded in lysosomes. Furthermore, IgG recycling assays using transiently transfected, FcRn–GFP-expressing endothelial cells can serve as a predictor of *in vivo* dynamics [18] and have been followed by the development of a stably transfected endothelial cell system to obtain correlates with behavior in mice transgenically expressing human FcRn [35].

FcRn can be distinguished from many other receptors through its ability to undergo both recycling and **transcytosis** [21]. The complex intracellular processes involved in FcRn

fragments. The phage bearing antibody fragments or domains that bind to target antigen can be selected, with the selected phage harbouring the genes encoding the displayed fragment or domain.

**Plasmapheresis:** treatment of the blood of a patient to remove plasma components such as autoreactive (pathogenic) antibodies. This procedure involves the separation of plasma from blood cells, followed by the replacement of the plasma with saline, albumin, and/or specially prepared plasma.

**Rab GTPase:** a class of G coupled proteins that are involved in the regulation of intracellular trafficking pathways, such as the movement of vesicles along microtubules or in membrane fusion. Typically, different cellular compartments have specific Rab GTPases associated with them, allowing Rab GTPases to be used as markers of early endosomes, late endosomes, etc.

**Theranostic imaging:** the use of imaging to identify patients that are likely to respond to treatment due to the presence of the molecular target of a specific therapeutic agent.

**Transcytosis:** the transport of proteins or other macromolecules across cellular barriers, such as polarized epithelial or endothelial cells. For some receptors such as FcRn, this process can occur in both directions across the cells and is therefore bidirectional.

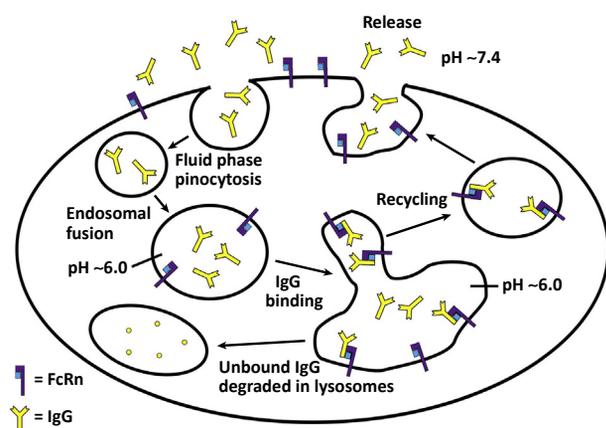


Figure 2. Model for the Activity of FcRn as an IgG Homeostat. IgG enters cells by fluid phase pinocytosis in small tubulovesicular transport carriers that fuse with larger, FcRn-positive early (sorting), acidic endosomes in which binding to FcRn can occur. Bound IgG molecules are recycled and released by exocytosis involving fusion of recycling compartments with the plasma membrane. By contrast, IgG that does not bind to FcRn in sorting endosomes enters lysosomes and is degraded.

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trafficking, along with their molecular coordinators such as the **Rab GTPases** [36,37], have been investigated in both endothelial and polarized epithelial cells [28,38]. Live cell imaging of endothelial cells has been used to track highly motile FcRn+ tubulovesicular transport carriers (TCs) and reveals a dynamic network of TCs that can be identified by the presence of different complements of Rab GTPases [23]. These TCs mediate the transport of vesicles between different subcellular compartments or the plasma membrane [23], thereby playing a central role in determining the intracellular fate of internalized IgG. Analyses in endothelial cells have revealed that the Rab GTPase Rab11A is associated with recycling compartments that lead to exocytosis of FcRn-IgG complexes, indicating that Rab 11A plays a central role in IgG salvage and recycling [38]. Interestingly, although Rab11A is involved in exocytosis in polarized epithelial cells, it is not required for transcytosis [28]. Instead, Rab25 and the microtubular motor protein myosin Vb regulate the bidirectional transcytosis of FcRn [28]. Recent studies have resulted in the identification of multiple effectors that differentiate the transcytotic and recycling pathways in epithelial cells [39,40]. Combined with electron microscopy studies in rat intestinal epithelial cells, the subcellular trafficking analyses demonstrate that early (sorting) and recycling endosomes are structurally diverse, forming a network that is connected via small, highly motile TCs [41,42].

### The Subcellular Trafficking of Multimeric IgG-Antigen Complexes

In contrast to monomeric IgG molecules that, with or without bound antigen, are typically recycled by FcRn, multimeric immune complexes (ICs) comprising antigen-antibody complexes have very short *in vivo* persistence due to internalization and trafficking into lysosomes [43]. This internalization is typically mediated by other Fc receptors, Fc $\gamma$ Rs, on Fc $\gamma$ R-expressing cells that, in contrast to FcRn, can bind to ICs at near-neutral pH [44]. The delivery of ICs to lysosomes has several important consequences for host defence. First, this pathway leads to the destruction of antibody-opsonized pathogens. Second, this may prevent the transcytosis of pathogens bound to antibody across epithelial barriers, although the delivery of 'small' ICs across the intestine can stimulate protective immunity [45]. Third, for hematopoietic cells, internalization of multimeric ICs by Fc $\gamma$ Rs in antigen-presenting cells is followed by 'handover' to FcRn, which is not typically involved in uptake due to the near-neutral extracellular pH, in early endosomes leading to antigen delivery into both the **cross-presentation** (involving MHC class I) and MHC class II presentation pathways to stimulate T cell-mediated immunity [46,47]. Consistent with a role for FcRn in antigen presentation, overexpression of this receptor

in transgenic mice increases T cell responses [48]. The contribution of FcRn to antigen presentation pathways is not discussed further here, but for a comprehensive review of this topic, see [49].

### FcRn as a Regulator of the *In Vivo* Half-Lives of IgG

The identification of FcRn as a global regulator of IgG levels more than two decades ago has led to studies directed towards engineering antibodies for longer *in vivo* persistence [5,6]. This was initially achieved by mutagenesis of IgG residues in the vicinity of the FcRn interaction site to generate a library of engineered Fc fragments derived from mouse IgG1. These mutated genes were used to produce a **phage display library** from which Fc fragments with mutations that conferred higher affinity for FcRn at pH 6.0 (early endosomal pH) were selected [50]. Subsequently, similar approaches, or molecular modelling based on structural data, have been used to generate human IgG (IgG1 or IgG4) variants with increased affinity for human FcRn, resulting in two- to fivefold increases in half-life in monkeys and humans [51–58]. For example, the analysis of antibodies specific for staphylococcal  $\alpha$ -toxin with the 'YTE' half-life extending mutations [Met252 to Tyr, Ser254 to Thr, Thr256 to Glu that are all in proximity to the key residues of IgG that interact with FcRn (Figure 1B) [59]] during Phase II clinical trials demonstrated half-lives ranging from 80 to 112 days, compared with  $\sim$ 20 days for wild-type (WT) human IgG1 [52]. Importantly, half-life extended antibodies have been shown to have superior efficacy as therapeutics against tumor xenografts in mice and in the prophylaxis of simian-HIV infection in non-human primates [57,60].

A challenge associated with the engineering of antibodies for increased affinity for FcRn at acidic pH (pH  $\sim$ 6.0) is to retain sufficiently low affinity for FcRn at near-neutral pH (pH 7.4), to enable efficient exocytic release following recycling or transcytosis. The possibility of a negative effect of increased binding of IgG to FcRn at near-neutral pH (pH 7.4) on the *in vivo* IgG persistence was first introduced in 1997 [50]. As the affinity of the FcRn–IgG interaction at pH 6.0 is enhanced, the binding at pH 7.4 tends to increase in parallel in most cases [56,61]. Furthermore, although increased binding of an engineered antibody at near-neutral pH can be offset by higher affinity at endosomal pH (pH  $\sim$ 6.0) to result in slower IgG clearance, analyses of mutated respiratory syncytial virus-specific antibodies with a range of binding properties have revealed a requirement for the dissociation constant of IgG–FcRn interaction to be greater than  $\sim$ 400 nM at pH 7.4 for half-life extension to be achieved [61]. By contrast, pharmacokinetic and modelling analyses of a panel of vascular endothelial growth factor-specific antibodies demonstrated that as the dissociation constant of the IgG–FcRn interaction at pH 7.4 decreases below  $\sim$ 10<sup>4</sup> nM, further improvements in persistence are not observed [62]. This apparent discrepancy between the two studies is due to the consideration of different affinity thresholds, namely, the threshold for a reduction in persistence below that of the WT parent versus the threshold where the affinity starts to counteract the improved interaction at acidic pH (pH  $\sim$ 6.0), while still maintaining a longer half-life than the WT. Nevertheless, in both cases, at the cellular level the increased binding at near-neutral pH (pH  $\sim$ 7.4) results in decreased exocytic release of IgG at the cell surface.

Although the nature of binding of the Fc region of the antibody with FcRn is a major determinant of its pharmacokinetic behavior, the antigen binding fragment (Fab) arms (Figure 1B) can also modulate IgG–FcRn interactions [63–66]. This effect can be attributed to electrostatic interactions of regions of high density of positively charged residues in the complementarity determining regions (CDRs) located in the variable fragment (Fv) of the antibody [65,66]. Such interactions can slow dissociation of the antibody from FcRn at near-neutral pH, which can be quantitated using pH gradient FcRn affinity chromatography [64]. Clearly, this secondary,

Fv-mediated interaction can also be modified by the binding of the antigen to the antibody. This regulation of IgG–FcRn interactions via antigen binding represents an additional pathway to alter antibody pharmacokinetics. Furthermore, other factors such as the **isoelectric point (pI)** of the antibody that can also be related to the presence of charged residues in the CDRs can affect internalization into cells [65–69], thereby contributing to the regulation of the dynamic behavior of antibodies.

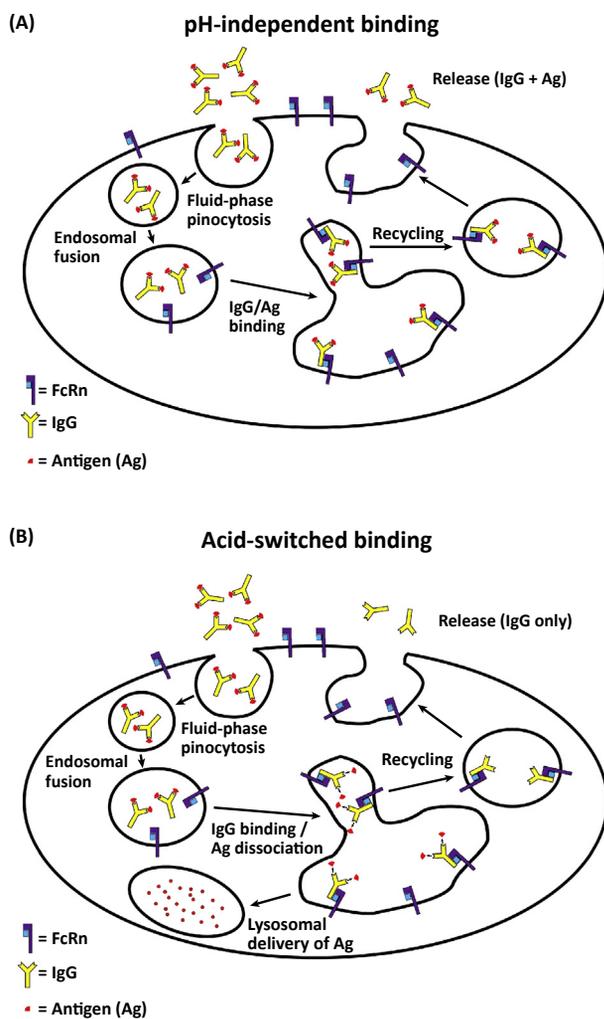
### Engineering Antibodies for Enhanced Antigen Clearance

Monoclonal antibodies are widely used to neutralize toxins or inflammatory molecules such as cytokines. However, these antibodies typically bind to one or two target molecules (due to the presence of two antigen binding sites per antibody molecule) and, unless the binding site on their target is repeated, delivery of these agents does not result in immune complex formation. Consequently, such antibodies can have the undesirable effect of acting as carriers for their targets, thereby prolonging rather than reducing the persistence of these unwanted molecules (Figure 3A) [70,71]. This effect can reduce the efficacy of antibodies that are delivered to neutralize such deleterious molecules. In addition, in some cases antigen binding to antibody can increase the clearance rate of the antibody, resulting in lower effective doses, through pathways that are not completely understood but that can be determined by the clearance behavior of the target antigen following entry of the antibody-antigen complex into cells [72,73]. To circumvent these problems, several groups have used antibody engineering approaches to generate novel antibody platforms that are discussed below.

#### Acid-Switched Antibodies

Acid-switched antibodies bind to their target with higher affinity at near-neutral pH than at acidic pH. These antibodies dissociate from their antigen in acidic endosomes following internalization into cells [72,74,75] (Figure 3B). The consequence of acid-switching is that while the antibody is recycled by FcRn, the antigen is delivered to lysosomes and degraded. Each antibody therefore avoids lysosomal degradation and can be loaded multiple times with an antigen, with the advantage that the iterative use of such antibodies confers a need for sub-stoichiometric doses relative to antigen load. This strategy has been used to generate acid-switched antibodies specific for targets such as the IL-6 receptor (IL-6R), IL-6, proprotein convertase subtilisin kexin type 9, complement component 5, C-X-C motif chemokine 10, and connective tissue growth factor [72–77]. Most acid-switched antibodies have been generated for antigens that exist in soluble form, with the exception of the IL-6R, which can be membrane bound or soluble [74].

The anticipated effects of acid-switching on endosomal dissociation have been corroborated by cell biological analyses using fluorescence microscopy [73,75]. These studies show the appearance of antigen in the endosomal lumen followed by delivery to lysosomes, whereas the antigen-specific antibody remains on the FcRn-positive membrane of the endosome and is recycled. Based on analyses of antibodies with a range of target binding properties, an upper estimate of 20–70 s for the half-life of the antibody-antigen complex at acidic pH has been estimated to enable endosomal dissociation [76,78]. Collectively, these studies have led to the conclusion that the dissociation rate ('**off-rate**') at acidic, endosomal pH is a dominant factor, rather than the relative ratios of dissociation constants at pH 6.0 to pH 7.4, in determining the efficiency of antigen clearance [78,79]. Nevertheless, for antibodies with a very fast **on-rate** for antigen binding at acidic pH, rebinding may mitigate the effects of rapid dissociation. Interestingly, the analysis of the effect of combining acid-switching with half-life extension through enhancement for FcRn binding has met with mixed results, showing benefit for target antigen clearance over acid-switching alone in some, but not other, studies [73,74,76,78]. Importantly, for antigens that can contribute to antibody clearance due to endo-lysosomal trafficking of



**Figure 3. Enhancement of Antigen Clearance by Acid-Switching.** (A) pH-independent binding. Antibodies that bind to antigen with similar affinity in the pH range 6.0–7.4 enter cells by fluid phase pinocytosis in small tubulovesicular transport carriers (TCs) that fuse with early (sorting) endosomes in which binding to FcRn can occur, followed by recycling of the IgG-antigen complex and exocytic release at the plasma membrane. This results in the buffering effect that prolongs the half-life of the targeted antigen. (B) Acid-switched binding. By contrast, pH-dependent or acid-switched antibodies have substantially higher affinity for antigen at near-neutral pH relative to acidic, endosomal pH. Following internalization into cells by fluid phase pinocytosis in small tubulovesicular TCs, the complexes enter acidic sorting endosomes in which antigen dissociates from antibody and enters the lysosomal pathway. The FcRn-bound antibody is recycled and exocytosed. This results in increased clearance of antigen combined with recycling of antibody for re-use.

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antibody-antigen-cognate receptor complexes, the interplay between antigen-receptor, antigen-antibody, and antibody-FcRn interactions is complex, possibly leading to different 'rules' for effective acid-switching in such systems.

#### Calcium-Switched Antibodies

An alternative approach to acid-switching has been described that exploits the substantially lower levels of calcium ( $\text{Ca}^{2+}$ ;  $\sim 2 \mu\text{M}$ ) in endosomes relative to the extracellular space ( $\sim 2 \text{mM}$ ) [80]. Specifically,  $\text{Ca}^{2+}$ -switched antibodies can be engineered that bind to their antigen with higher affinity at millimolar  $\text{Ca}^{2+}$  concentrations relative to micromolar concentrations. Significantly, the 1000-fold difference in  $\text{Ca}^{2+}$  concentration results in a larger 'dynamic range' for engineering endosomal dissociation relative to a pH change of  $\sim 1.5$  pH units (i.e.,  $\sim 30$ -fold difference in proton concentration).

### 'Sweeping' Antibodies

The knowledge that the cellular uptake of proteins by fluid phase processes is substantially less efficient than that of receptor-mediated internalization has prompted the generation of antibodies that are both acid-switched and engineered at the sites on IgG interacting with FcRn. These engineered antibodies have increased binding to FcRn at near-neutral pH (such mutations have been identified in previous studies using phage display or other approaches; see 'FcRn as a Regulator of the *In Vivo* Half-Lives of IgG') and are known as sweeping antibodies [76,79,81].

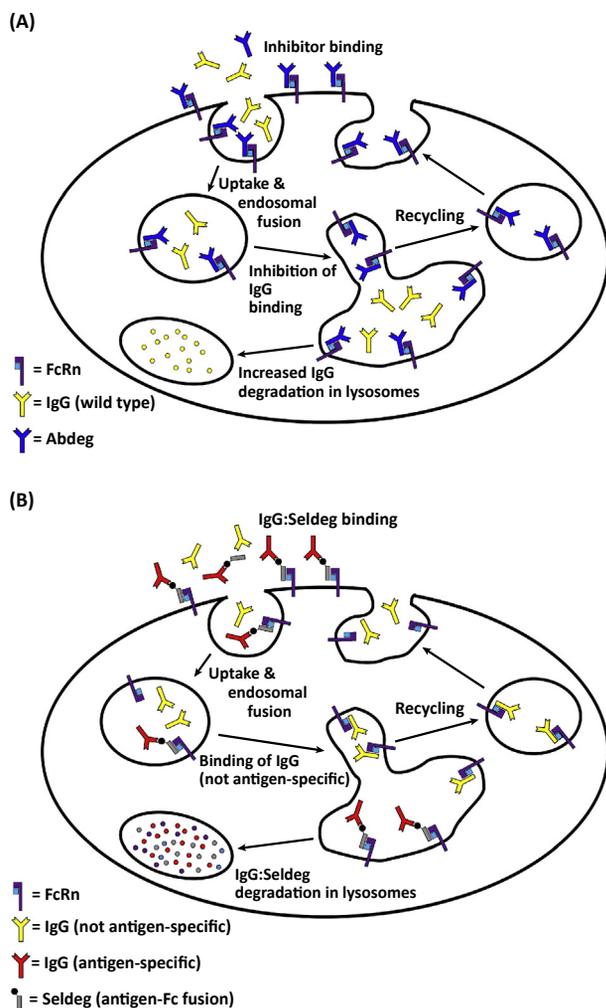
Live cell imaging shows that FcRn is exposed at the cell surface during exocytic release, followed by internalization to maintain low steady-state surface levels [24]. This behavior enables the rapid accumulation of sweeping antibodies within cells [33]. The increased internalization of these antibodies bound to cognate antigen into early endosomes results in more effective clearance of targeted antigen, due to increased endosomal delivery of antibody-antigen complexes [76,79,81]. However, as the binding affinity of an antibody for FcRn at near-neutral pH increases, its half-life decreases due to enhanced accumulation and retention within cells, culminating in lysosomal delivery [82,83]. Thus, there is a trade-off between increasing the delivery of an antibody-antigen complex and decreasing the *in vivo* persistence of the (therapeutic) antibody. How these two parameters interplay to achieve the required effect on target antigen will be dependent upon the dynamic behavior of the particular antigen and the longevity of the desired pharmacodynamic effect.

### Targeting FcRn to Modulate IgG Levels

The role of FcRn as a global regulator of IgG provides opportunities for the use of FcRn inhibitors to reduce the levels of antibodies that cause symptoms in diseases such as autoimmune disorders [33,84,85]. The FcRn inhibitors bind to FcRn with higher affinity than naturally occurring IgGs in the pH range 6.0–7.4 [33,84–89]. Consequently, they compete with endogenous antibodies for FcRn binding, driving such antibodies into degradative lysosomes [33]. Importantly, clinical studies have demonstrated that greater than 50% reductions in pathogenic antibody levels in several autoimmune diseases can result in therapeutic benefit [90,91], indicating that even partial reduction by FcRn inhibitors could be effective. A further application area is to use FcRn inhibitors to decrease background and improve contrast during diagnostic/theranostic imaging with (radio)labeled antibodies [89]. FcRn inhibition also offers an alternative to current strategies to reduce pathogenic antibody levels, such as **plasmapheresis, intravenous immunoglobulin (IVIG)** delivery, or B cell depletion, all of which can have undesirable side effects.

To date, FcRn inhibitors ranging from peptide/small protein to antibody-based blockers have been generated [33,84–89,92], and several of these are in various stages of clinical trials following preclinical analyses in animal models. One class of antibody-based inhibitors called Abdegs (for antibodies that enhance IgG degradation) (Figure 4A) involves the use of Fc engineering to generate competitive IgG molecules with substantially increased affinity for FcRn at both near-neutral and acidic pH [33]. Furthermore, several antibody-based FcRn inhibitors have been generated that induce similar effects on endogenous IgGs by binding to FcRn through their variable domains [86,87].

The competitive advantage of Abdegs and variable domain-based FcRn blockers over WT IgG occurs at several levels. First, they accumulate in FcRn-expressing cells via receptor-mediated processes rather than fluid phase pinocytosis [33]. Second, they have higher affinity for FcRn at acidic, endosomal pH. In this context, Abdegs also have higher affinity at



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**Figure 4. Antibody Engineering Strategies to Reduce Endogenous IgG Levels.** (A) In the presence of an inhibitor of FcRn such as an Abdeg (for antibodies that enhance IgG degradation) that binds to FcRn with increased affinity at both near neutral and acidic pH, the inhibitor is internalized by receptor (FcRn)-mediated processes and competes with endogenous IgGs for FcRn binding in acidic endosomes. Consequently, endogenous IgG molecules are driven into lysosomes and are degraded. The inhibitor shown binds through its Fc region to FcRn, whereas other classes of inhibitors can be peptide-based inhibitors or antibodies that bind through their variable domains [33,84–89,92]. (B) A specific class of engineered proteins comprising antibody Fc regions fused to an antigen (one antigen molecule per Fc fragment) can be used to deplete antigen-specific antibodies. This fusion protein, named a Seldeg (for selective degradation of antigen-specific antibodies), is engineered to bind to FcRn via its Fc region with increased affinity at both near neutral and acidic pH. Seldeg-antibody complexes are internalized into FcRn-expressing cells by receptor-mediated uptake and subsequently follow the constitutive degradation pathway of FcRn into lysosomes [83], leading to the breakdown of the targeted antibody. Because of their different mechanism of action, Seldegs can be used at lower doses than Abdegs and do not affect the levels of antibodies that are not specific for antigen.

endosomal, acidic pH than at extracellular, near-neutral pH for binding to FcRn, due to the use of the ‘natural’ interaction sites on FcRn and IgG that confer pH dependence (see ‘The Molecular Details of FcRn–IgG Interactions’). By contrast, antibodies that bind through their variable domains typically involve distinct sets of interacting residues and do not have this property [93]. These variable domain-binding antibodies interact with FcRn with affinities in the nanomolar range across pH 6.0–7.4 [86,87] and can also bind with four functional sites per antibody (two in the variable domain and two in the Fc fragment). Based on previous studies, these differences are expected to result in distinct pharmacokinetic and pharmacodynamic behavior [18,82]. Specifically, variable domain-based agents may induce a more rapid inhibitory effect and have shorter intrinsic half-lives due to reduced exocytic release from FcRn-expressing cells. Interestingly, however, studies in non-human primates and humans have revealed that despite very rapid clearance, a prolonged reduction in serum IgG levels that extended beyond that based on the pharmacokinetic behavior of the inhibitor was observed [86,87]. This may be due, in part, to the assays used to assess remaining levels

of inhibitor: typically, serum levels are analyzed and do not yield insight into intracellular load. The rapid internalization of FcRn blockers into FcRn-expressing cells may result in higher intracellular versus circulating concentrations [33,86,87].

To date, the results from studies in non-human primates and clinical trials for several FcRn-based inhibitors indicate that they induce significant and sustained decreases in endogenous IgG levels in healthy volunteers and also have beneficial effects in the autoimmune disease myasthenia gravis [86,87] ([www.argenx.com](http://www.argenx.com)). Although such FcRn inhibitors have considerable potential in the clinic for the management of autoimmunity, they induce a global reduction in IgG levels. This has motivated the development of an approach to selectively deplete antibodies of a particular binding specificity, while not affecting the clearance of antibodies of distinct specificities, using engineered antigen-Fc fusions called Seldegs (for selective degradation of antigen-specific antibodies) [94] (Figure 4B). Seldegs comprise an antigen molecule fused to an engineered Fc fragment that, through knobs-into-holes mutations that are designed to insert protrusions and cavities in the Fc chains [95], heterodimerizes with a second Fc fragment with no antigen (Figure 4B). Heterodimer formation results in monomeric display, and the Fc fragment is engineered to bind to FcRn with increased affinity in the pH range 6.0–7.4. As such, Seldegs bind to, and are rapidly internalized by, surface-exposed FcRn followed by entry into the lysosomal pathway. Consequently, antibodies that bind to the antigen-Fc fusion protein are co-opted into this degradative pathway, resulting in their destruction. To date, Seldegs have been shown to specifically clear antigen-specific antibodies without affecting the levels of antibodies of irrelevant specificities. The mechanism of action of Seldegs requires only a subset of FcRn molecules to be targeted; consequently, relatively low doses that do not lower total IgG levels are sufficient for efficacy [94]. This contrasts with FcRn inhibitors that are used at higher doses to achieve quantitative binding to functional FcRn in the body, resulting in effective competition with endogenous IgG.

### Concluding Remarks and Future Directions

The identification of FcRn as a major player in the regulation of IgG pharmacokinetics has prompted several waves of antibody engineering to produce novel antibody-based platforms. Half-life extension technology has been implemented to result in longer-lived antibodies. The development of antibodies with prolonged *in vivo* persistence has been followed by technologies to inhibit/block the salvage function of FcRn and thereby reduce the levels of endogenous IgG. More recently, strategies to expedite the clearance of deleterious antigens and antigen-specific antibodies have been developed through acid-switching and/or sweeping approaches. The ongoing clinical trials implementing several of these strategies present an exciting era for FcRn-based therapeutics. Despite these developments, there are multiple outstanding questions (see Outstanding Questions) such as how does the molecular nature of FcRn–IgG and antigen–IgG interactions correlate with the dynamic behavior of IgG or antigen at the whole-body level? Furthermore, how do the FcRn interaction properties of different FcRn inhibitors affect their therapeutic efficacy in distinct disease settings? Addressing these questions using a combination of molecular and cellular approaches is expected to lead to important insight into these issues that, in turn, could result in improved therapeutics for human disease.

### Acknowledgments

We thank our current and past laboratory members and collaborators who have contributed to our FcRn-related studies. We apologize that, due to space limitations, we have not been able to cite many relevant publications. We are grateful to Sungyong You and JunHaeng Cho for providing expert assistance with the figures. This research was supported by grants from the National Institutes of Health (NIH) (R01 AI039167, R01 AR056478), Cancer Prevention Research Institute of Texas

### Outstanding Questions

What is the maximum increase in half-life that is achievable for an IgG?

How can we predict the optimal binding properties of acid-switched/sweeping antibodies for clearance of antigens that differ in the rates at which they are generated (biosynthesized), in their concentrations in the body, etc.?

How does the binding and subcellular trafficking behavior of an FcRn inhibitor affect its activity as a therapeutic to treat autoimmune disease?

(CPRIT) (RP110070, RP110441, RP140141, RP160051), National Multiple Sclerosis Society (RG 4308), MedImmune (Scientific Research Agreement), and argenx (Scientific Research Agreement) to E.S.W. and from the NIH (R01 GM071048, R01 GM085575) and CPRIT (RP110441) to R.J.O.

### Disclaimer Statement

E.S.W. is a (co)inventor on licensed patents related to half-life extension and Abdeg technology and has a financial interest in argenx. The licensed patents are owned by UT Southwestern Medical Center.

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