Neonatal Fc Receptor: From Immunity to Therapeutics

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Neonatal Fc Receptor: From Immunity to Therapeutics

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Abstract The neonatal Fc receptor (FcRn), also known as
the Brambell receptor and encoded by *Fcgrt*, is a MHC
class I like molecule that functions to protect IgG and
albumin from catabolism, mediates transport of IgG across
epithelial cells, and is involved in antigen presentation by
professional antigen presenting cells. Its function is evident
in early life in the transport of IgG from mother to fetus and
neonate for passive immunity and later in the development
of adaptive immunity and other functions throughout life.
The unique ability of this receptor to prolong the half-life of
IgG and albumin has guided engineering of novel thera-
peutics. Here, we aim to summarize the basic understanding
of FcRn biology, its functions in various organs, and the
therapeutic design of antibody- and albumin-based thera-
peutics in light of their interactions with FcRn.

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Introduction

Decades before the identification of neonatal Fc receptor
(FcRn) as the receptor that transports IgG from mother to
offspring, early experiments revealed passive transfer of
immunity via gamma-globulin. Francis William Rogers
Brambell (1901–1970) was an Irish-born zoology professor
who made significant contributions to these observations
and subsequently hypothesized the existence of a receptor
that can mediate the transfer of maternal gamma-globulin
from mother to infant as well as the protection of gamma-
globulin from catabolism [1, 2]. Later studies by Richard
Rodewald's group provided biochemical evidence of IgG
transport at the neonatal rat intestinal epithelium with pH
binding dependency [3]. This finally led Neil Simister and
Keith Mostov to purify and sequence a Fc receptor from
the intestine of an 11-day-old rat (hence the name neonatal
Fc receptor) that was associated with beta (β)-2-micro-
globulin and exhibited structural similarity to MHC class I
molecules [4].

FcRn Structure and Ligand Binding Characteristics

Similar to MHC class I, FcRn structure consists of three
extracellular alpha (α) domains, a single pass transmem-
brane domain, and a short cytoplasmic tail of approximately
44 amino acids. Crystal structural resolution by Pam
Bjorkman's group, who had previously resolved the crystal
structure for MHC I, showed that the α1 and α2 domain

perch on top of the α3 domain and β2m. The two sites where peptide termini anchors in MHC class I are blocked in FcRn by an arginine side chain and another by a proline residue in the α2 helix region [5]. Two FcRn molecules bind to a single IgG with a 2:1 stoichiometry, and the binding occurs at pH 6.0–6.5 with minimal structural conformation change and not at pH 7.5 [5, 6]. The critical binding site at acid pH in the IgG Fc domain resides within a pair of histidines that occur in the α3 domain [7]. Rat FcRn and IgG binding studies have shown that the FcRn contact sites for IgG involve Aspartic acid 137, an N-glycan moiety within the α2 domain, and the N terminus of β2m [8].

The observation that staphylococcal protein A blocks FcRn and IgG binding helped to identify the CH2–CH3 domain interface as a critical site of FcRn–IgG interactions. Site-directed mutagenesis studies have proven that the critical binding sites in the Fc domain of IgG for FcRn are Histidine 310, Histidine 435, and Isoleucine 253 and to a lesser extent Histidine 433 and Tyrosine 436 [9, 10]. At acid pH, the protonated histidine residues on IgG form a salt bridge with the FcRn α2 domain at Glutamic acid 117, Glutamic acid 132, and/or Glutamic acid 135 and Aspartic acid 137.

Binding Site of Albumin

Although a putative albumin receptor was also hypothesized by Schultze and Heremans in 1966, it was not until 2003 when Clark Andersen's group demonstrated a biochemical association between FcRn and albumin [11, 12]. FcRn also protects albumin from catabolism and thus in humans the endogenous albumin half-life is approximately 19 days, while in rodents, it is 1.5–2.5 days. FcRn in the liver is the critical site that maintains albumin concentration homeostasis by producing the same quantity as is degraded systemically [13]. FcRn binds albumin with a 1:1 stoichiometry. Similar to the pH dependency of FcRn–IgG interactions, FcRn and albumin also bind together at acidic pH but not at neutral pH. The contact site of FcRn also involves a histidine residue (H166) within the α3 domain but located on the opposite structural face of FcRn from that which is involved in contacting IgG [14, 15]. The FcRn contact site on albumin resides somewhere within the last third of its three major structural domains. It is still not known whether a single FcRn can simultaneously bind and transport both IgG and albumin in physiologic conditions. Also, the crystal structure FcRn bound to albumin has yet to be resolved. Nonetheless, genetic deficiency of FcRn in genetically engineered animal models has clearly shown that such mice are both hypoalbuminemic and hypogammaglobulinemic [13, 16].

FcRn Structural Comparisons Between Species

The rat and mouse FcRn peptide sequences are 98% identical and are encoded in chromosome 7, outside the MHC class I locus in chromosome 20 and 17, for rat and mouse, respectively. Human FcRn shares only 65% amino acid sequence similarity with rat FcRn. Human FcRn is encoded in chromosome 19, also outside of the MHC class I locus on chromosome 6. The molecular weight of human (h) FcRn is approximately 42–44 kDa with a single N-glycan moiety, as opposed to rat FcRn which has three additional N-glycans that account for its molecular weight of 48–52 kDa. This structural dissimilarity may explain the absence of hFcRn dimer formation, in the absence of IgG, in crystal structure studies. Perhaps it is the lack of the additional N-glycan moieties within the α2 domain that participate in the formation of dimer stability through a "carbohydrate handshake" as shown by X-ray crystallographic studies [17]. Another major difference between human and rodent FcRn involves the cross-species IgG binding. Rodent FcRn is known to be "promiscuous" in its ability to bind various species of IgG, while hFcRn can only bind to a limited species (human, rabbit, and bovine) [18]. "Murinizaton" by site-directed mutagenesis of hFcRn at residues 137 and 121–132 within the α2 domain was shown to allow binding to mouse IgG, thus helping to explain this cross-species IgG binding capability of rodent FcRn [19]. Although one of the N-glycans resides within the above-mutated site, it probably does not contribute to cross-species IgG binding, since "rodentization" of hFcRn by addition of N-glycans through site-directed mutagenesis failed to enhance binding to rodent IgG [20]. Although hFcRn cannot bind rodent IgG, recent findings have shown that hFcRn can bind rodent albumin, but rodent FcRn cannot bind human albumin [21]. Thus, the half-life of human albumin in rat is only 15 h [22].

FcRn has also been cloned from other species, such as cow, possum, sheep, water buffalo, and camel [23]. The major structural similarities between possum, sheep, and cow include a single N-glycan moiety and a cytoplasmic tail that is ten amino acids shorter than that of the rodent counterpart. To date, cross-species FcRn–β2m binding has been suggested to occur with bovine FcRn and mouse β2m when the bovine FcRn transgene was expressed in mice and was found to be functional [24].

Intracellular Trafficking of FcRn

β2m association with FcRn is required for proper folding of FcRn before exiting the endoplasmic reticulum (ER), and failure of such an association results in the formation of disulfide-bonded oligomers that accumulate in the ER [25].

Efficient intracellular trafficking, including IgG transport, also requires association with β2m [26]. Prior to binding with β2m, FcRn assembly involves association with calnexin and subsequently ERp57 which assist in the formation of the disulfide bond [27]. Calreticulin is also involved in the maturation of the FcRn–β2m complex in the ER [27].

FcRn expression in many different polarized epithelia in vitro systems that model the kidney, lung, placenta, and intestines has shown that FcRn expression endows upon the cell the ability to transcytose IgG bidirectionally [26, 28–31]. The uptake of IgG by FcRn requires an acid environment that can occur within the intestinal lumen especially during neonatal life or in acidified endosomes. In the neonatal rat proximal intestine, a luminal pH of 6.0–6.5 favors FcRn binding to IgG in milk for apical uptake and subsequent endocytosis and transcytosis to the contralateral basal surface where the pH of the circulation is approximately 7.5 which allows ligand dissociation. In the absence of an acidic pH, IgG uptake likely occurs by fluid-phase pinocytosis and subsequent binding to FcRn after acidification of the endosome. Whether FcRn undergoes recycling or transcytosis is still under active investigation. It is known that transcytosis involves actin motor myosin Vb and GTPase Rab25. Rab11a has also been found to be dispensable for transcytosis but regulates basolateral membrane recycling [32]. Exocytosis involves Rab11 which is important for endosomal fusion with the plasma membrane [33]. Recent electron microscopy of FcRn trafficking in rat intestine has revealed that both endocytic and exocytic processes are associated with clathrin [34].

Regulation of intracellular trafficking in polarized epithelial cells has mostly been studied using kidney cell lines, such as MDCK or IMCD cells, which are grown as monolayers on the transwell system. As mentioned previously, β2m association is critical to effective function of FcRn [26]. The cytoplasmic tail has been demonstrated by several studies to play a critical role in basolateral membrane targeting, as well as endocytosis and transcytosis. hFcRn without a cytoplasmic tail or one that contains mutations in the calmodulin binding site results in redistribution of hFcRn from the basolateral to the apical surface and reduced IgG transport [35, 36]. rFcRn without the cytoplasmic tail or with a point mutation at the phosphorylation site serine-313 results in decreased apical-to-basolateral IgG transport [37]. Mutation of the tryptophan and dileucine motif in the cytoplasmic tail also results in increased apical membrane expression [38]. When the rFcRn cytoplasmic tail was attached to the FcγRII receptor, this mutant receptor could transcytose across MDCK cells in both directions [39]. The regulation of apical membrane targeting likely involves N-glycans. In a study where hFcRn was "rodentized" by expression of additional N-glycans to resemble that of rFcRn, the mutant hFcRn redistributed to the apical membrane, similar to that of wild-type rFcRn. The mutant hFcRn containing additional N-glycans also reversed the direction of predominant IgG transport to an apical-to-basolateral direction similar to that of wild-type rFcRn [20].

The pathway of FcRn trafficking is also determined by the valency of the ligand. In dendritic cells, FcRn bound to large multimeric immune complexes is routed to a LAMP1+ compartment while monomeric IgG is recycled back to the cell surface [40]. This is believed to facilitate antigen processing before antigen presentation in dendritic cells which are known to express FcRn [41]. In another study, wild-type Fc with two FcRn-binding sites was more efficiently transported and less likely to be destined for lysosomal degradation than mutant Fc with a single FcRn-binding site [42].

Regulation of FcRn Expression

Studies of the promoter regions (comparing neonatal intestinal epithelium and NIH 3T3 cells) have shown that different transcription factors can regulate the expression of FcRn at different developmental stages and tissue types consistent with the well-known developmental regulation of FcRn in rodent intestinal epithelia [43]. Upregulation of FcRn expression in intestinal epithelial cells, macrophage-like THP-1, and primary human monocytes can be induced by TNF-α and IL-1β, which are known to be elevated during inflammation [44]. Conversely, INF-γ was found to down-regulate FcRn expression [45].

FcRn expression in the neonatal intestinal epithelium of rat can also be subjected to hormonal control, and thus may explain the exceedingly high expression levels through early life. Cortisol and thyroxine can suppress FcRn expression in neonatal rat intestinal epithelium as well as inhibit IgG binding [46, 47]. Meanwhile, a different study showed that dexamethasone stimulation decreases FcRn mRNA in rat alveolar epithelial cells [31].

Intestinal Expression and Function of FcRn

FcRn expression in rat intestine is only detectable up to 19 days after birth, which coincides with weaning, and the level of expression decreases distal to the proximal duodenum. The acid pH at the proximal duodenum likely facilitates the uptake of IgG. Recent studies by Bjorkman and colleagues have tracked the intracellular pathways associated with individual nanogold-labeled Fc after uptake from the neonatal rat intestinal lumen and have shown the existence of a complex network of

tubules and vesicles that exit the basolateral membrane through clathrin-coated pits as important to intracellular pathway of transcytosis [34].

Although the expression of FcRn in the intestine of adult mice is nearly undetectable, induction has been observed upon intraperitoneal immunization with Cry1Ac protoxin from *Bacillus thurigiensis* but at levels less than that typically observed during neonatal life [48]. This is consistent with an increasing body of data on an important functional role of FcRn in intestinal epithelia during adult life (Table I).

In humans, FcRn is detected in both fetal and adult intestines and can mediate bidirectional transcytosis across the intestinal epithelium both in vitro and in vivo [28, 49–51]. FcRn in the intestine and other mucosal tissues therefore continues to play a significant role beyond the neonatal period, especially in immune surveillance and adaptive immunity. Using mice that were humanized to express human FcRn under the control of its endogenous promoter and β2m as transgenes within intestinal epithelial cells, FcRn at the intestinal epithelium was found to deliver IgG to the luminal surface where it bound to a cognate antigen before returning the complex back to the lamina propria for processing and presentation by dendritic cells to CD4$^+$ T cells [51]. In a murine model of intestinal infection using *Citrobacter rodentium*, which only infects intestinal epithelial cells and is dependent upon IgG for eradication, FcRn within the intestinal epithelium was shown to be essential in directing *C. rodentium*-associated antigens within the epithelium to regional lymph nodes for initiation of an adaptive immune response [52, 53]. In the absence of FcRn, *C. rodentium* elimination was slowed. These observations also help to explain the role of the high levels of antibacterial IgG that are observed within intestinal tissues and the lumen in inflammatory bowel disease (IBD). Using a murine model of colitis, antibacterial IgG was shown to drive colitis and was dependent upon FcRn expression in antigen presenting cells [54]. These studies thus show an important role for FcRn in mediating IgG-dependent anti-infective immunity and potentially autoimmunity in immune-mediated disorders such as IBD. Such observations have important implications for adaptive immunity in general.

Expression and Function of FcRn in Mammary Gland

The mammary gland expresses various Ig receptors, including FcRn, pIgR, and CD23, for the transport of IgG, IgA, and IgE, respectively. In sheep, the major concentrations of Ig in colostrum in descending order are IgG1, IgA, IgE, IgM, and IgG2. In milk, the concentrations in descending order are IgA, IgM, IgE, IgG1, and IgG2 [55]. The concentration of IgG in human colostrum is 1 g/L,

while milk only contains 50 mg/L. On the other hand, the IgA concentration in human milk is 32 g/L.

FcRn has been found to be expressed in the mammary gland of human, mouse, cow, brushtail possum, sheep, swine, and camel [23]. In humans, FcRn is detected in the mammary gland endothelial cells rather than the epithelial cells [56]. In camels and water buffalo, immunohistochemistry has demonstrated labeling within the acini and ducts [57, 58]. FcRn is also detected in tumor tissues such as ductal, lobular, and medullary carcinoma, as well as metastatic epithelial cells in the lymph node [56]. It is also detected in the histiocytes residing within the interstitium in association with breast cancer.

It is believed that FcRn in the mammary gland likely recycles IgG (to retain IgG in circulation) rather than promoting transport from circulation to milk. In normal lactating mice, the concentration of IgG in milk is less than that of serum. In β2m-deficient mice, the concentration of IgG in milk was observed to be 20-fold less than that of serum [59]. Studies comparing transfer of Fc fragments and IgG with different affinities to FcRn revealed an inverse relationship between binding affinity and concentration in milk [60]. When a bovine FcRn transgene was expressed in the mammary gland of mouse, there was no increase in the milk of the infused mouse or bovine IgG which is consistent with this notion [24].

The expression levels of FcRn in the mammary gland can shift during lactation [61]. In cow and sheep, there appears to be a shift in the expression of FcRn to the apical membrane location after parturition [62, 63].

The clinical benefit of the long-observed transfer of passive immunity has recently been demonstrated using a murine model of asthma in which the transfer of maternal IgG1 from milk to the infant was shown to prevent allergen-specific airway disease [64–66]. Complete protection of offspring from antigen-specific allergic airway inflammation was observed.

Expression and Function of FcRn in Placenta

The transfer of passive immunity is predominantly postnatal in rodent but in utero during gestation in humans and rabbits [67]. In humans, maternal IgG in the fetal circulation increases from the early second trimester to term. IgG1 and IgG4 have found to be most efficiently transport, while IgG2 is the least. The transfer of IgG is believed to be due to FcRn in the human syncytiotrophoblast and the fetal intestine [49, 68]. In mouse, FcRn is expressed in the mouse yolk sac endoderm but not the chorioallantoic placenta, and it is likely the sole IgG transporter from mother to fetus [69]. Using BeWo cells (a human trophoblast-derived cell line that expresses FcRn)

Table I Functions of FcRn across a variety of tissues and associated therapeutic implications

Functions of FcRn	Tissue/cell type							Therapeutic implications
	Intestines	Mammary gland	Placenta	Lung	Hematopoietic cells	Kidney	Liver	
Bidirectional transcytosis of IgG	Postnatal maternal to fetal IgG transfer [28, 49–51]. Import of luminal antigens as immune complexes [51].	Transport of IgG into colostrum and milk [55]. Recycling of IgG back into maternal circulation [59].	Prenatal maternal to fetal IgG transfer [49, 67–69].	Transfer of IgG into pulmonary secretions [76].		Reabsorption of IgG from the glomerular basement membrane [88, 89].		Pulmonary or oral delivery of Fc fusion proteins to systemic circulation [79, 81]. Inhibition of trans-placental pathogenic antibody transport [73]. Treatment of illness linked to deposition of immune complexes [88, 89].
Catabolism protection of IgG					Protection of monomeric IgG from degradation [40]. Promotion of multimeric IgG degradation [40].		Protection of IgG from catabolism and biliary loss [94] (Kuo, unpublished)	Treatment of autoimmune disorders caused by pathogenic IgG [107, 110]. Engineered antibody-based therapy with prolonged half-life [107].
Catabolism protection of albumin						Reabsorption of albumin by proximal tubular epithelial cells [90].	Regulation of albumin homeostasis [13]. Prevent biliary loss of albumin (Kuo, unpublished).	Prolongation of the half-life of drugs conjugated to albumin [118].
Antigen presentation	Delivery of immune complexes to resident antigen presenting cells [51].				Promotion of MHC class II restricted antigen presentation [40]. Clearance of IgG-opsonized bacteria [86].			Eradication of IgG-opsonized intestinal pathogens [52]. Priming of immune responses against IgG-complexed antigen [54].

and primary placental endothelial cells, bidirectional transport and recycling of IgG have been observed [29, 70].

Although FcγRIIb is expressed in the human placental villous endothelium and yolk sac vasculature and previously believed to also transport IgG across the villous endothelium, recent studies comparing FcγRIIb-deficient mice and wild-type mice have revealed that FcγRIIb does not mediate IgG transport in the mouse yolk sac [71].

Placental transfer of IgG from mother to fetus can also be used to transport therapeutic recombinant Fc fusion proteins in utero. When a β-glucuronidase (GUS)-Fc fusion protein was infused into the pregnant mouse in a murine model of GUS deficiency, the offsprings were found to have reduced manifestation of excessive lysosomal storage in the involved organs [72]. Conversely, potentially pathogenic antibodies may also be transferred via the placenta. In a murine model of fetal and neonatal immune thrombocytopenia (FNIT), where the transfer of pathogenic maternal antibody results in the destruction of fetal/neonate platelets, FcRn was found to be critical for this pathologic condition to occur [73]. The transport of the pathogenic IgG was likely facilitated by placental FcRn, since treatment with an anti-FcRn antibody or intravenous immunoglobulin (IVIg) was found to prevent FNIT. Other than pathogenic antibodies, teratogens or peptide inhibitors of FcRn can also block the transport of IgG in the placenta, leading to placental necrosis and/or disruption of embryo development [74]. However, such inhibitors may serve as a method of preventing transfer of pathogenic antibodies from the maternal circulation.

Expression and Function of FcRn in Lung

FcRn expression in the lung has been exploited as an endogenous vehicle for delivery of therapeutic Fc fusion proteins and in so doing revealed the presence of physiologic transport of IgG in adult lung by FcRn [75]. FcRn is expressed by bronchial epithelial cells of adult human, non-human primate, rat, mouse, and cow, wherein it can mediate transport of IgG. Consistent with this, significant quantities of IgG can be observed in pulmonary secretions [76]. FcRn is also detected at high levels in tracheal epithelial cells [77, 78]. This likely explains the efficacy of Fc fusion protein delivery when inhalation is directed to the upper airways in non-human primate experiments and human clinical trials [79, 80]. Consistent with this, mutation of the FcRn-binding site within the Fc domain of the chimerized IgG fusion disables such transport [81]. Although it is not detectable in alveolar cells of humans and non-human primates, rat alveolar epithelial cell monolayer studies have demonstrated expression of FcRn at this site that can mediate bidirectional

transport of IgG and with a predominant apical-to-basal direction [31, 77, 81].

Expression and Function of FcRn in Hematopoietic Cells

FcRn has been observed to be expressed in human monocyte, macrophage, dendritic cells, splenic B cells, and monocytic cell lines such as U937 and THP-1 [41, 82]. FcRn expression levels in these cell lines has been found to be increased by exposure to CpG, LPS, and TNF-α, and decreased with inhibition of NF-κB [44]. FcRn is also expressed in mouse hematopoietic cells, and within this cell type, it has been shown to be involved in IgG homeostasis together with endothelial cells [40, 41, 82, 83].

In dendritic cells, FcRn sorts multimeric IgG-containing immune complexes to intracellular compartments associated with antigen processing and ultimately degradation such that immune complexes exhibit diminished clearance in vivo in the absence of FcRn function [40, 84]. Consistent with this, FcRn in human and mouse dendritic cells plays a critical role in MHC class II-restricted antigen presentation [40]. FcRn in dendritic cells can also mediate MHC class I-restricted cross-presentation in the setting of chronic inflammation (K.B. and R.S.B. unpublished observations). Dendritic cells may also be involved in tolerance induction since oral administration of anti-CD3 antibody has been found to be effective in prevention and treatment of autoimmune diabetes and encephalomyelitis in mice [85]. In neutrophils, FcRn can facilitate the uptake of bacteria opsonized by antigen-specific IgG and deliver it to phagolysosomes [86].

Expression and Function of FcRn in Kidney

Consistent with studies of kidney cell lines, bidirectional transport of IgG in primary human renal proximal tubular epithelium had been observed [87]. FcRn is expressed in the podocyte and proximal tubule, but not the mesangial cell, parietal epithelial cells, interstitium, medulla, or distal tubular cells [88, 89]. FcRn in podocytes reabsorb IgG from the glomerular basement membrane which prevents deposition of immune complexes that might lead to glomerular diseases. This conclusion stems from the observation that FcRn-deficient mice exhibit accumulation of IgG in the glomerular basement membrane and delayed IgG clearance from the kidney. Excess IgG filtered by glomeruli can also be reabsorbed downstream by FcRn in the proximal tubules.

In a different study in which kidney transplants were performed between FcRn-deficient and wild-type mice,

FcRn in the kidney was found to salvage albumin from urinary loss but promotes IgG secretion [90]. Through IgG and albumin localization, FcRn was shown to be expressed in the podocytes, the proximal tubular epithelial cells, and the vascular endothelium of the kidney. It was proposed that albumin was filtered through the glomerulus and later reabsorbed at the level of the proximal tubular epithelial cell. Similarly, it was proposed that IgG is reabsorbed by the podocyte and that FcRn within the renal vascular endothelium removes IgG from the circulation into the interstitium for subsequent uptake by proximal tubular epithelial cells for transcytosis back to the urine to provide immunity against infection. Both studies thus concur that FcRn within podocytes functions to reabsorb IgG, but FcRn in the renal system outside of the glomeruli may be more complex in the need to balance immunity and salvage protein. Adding to the complexity, other proposed albumin-binding proteins at the proximal tubular epithelial cell-associated brush border, such as megalin and cubilin, may also be involved in albumin regulation in the urinary system [91].

Expression and Function of FcRn in Liver

FcRn expression in the liver had been demonstrated at both canalicular and sinusoidal membranes, and it is functional in IgG binding [92, 93]. The liver is the site of albumin synthesis, and FcRn in the liver regulates albumin homeostasis [13]. Previous attempts to understand the role of FcRn function in the liver using β2m-deficient mice and radio-isotope-labeled IgG and albumin failed to demonstrate transcellular transport through the hepatocyte and supported a role for FcRn in the liver for the protection of IgG from catabolism [94]. Surprisingly, the same study also did not show differences in the infused serum isotope-labeled albumin levels between β2m-deficient and wild-type mice raising concerns over the data presented. However, this may be a result of oversaturation of the infused albumin or inadvertent structural changes caused by isotope labeling.

Other than protection from catabolism, FcRn in the liver can prevent IgG and albumin loss through the biliary system. Our unpublished data has shown that the bile of FcRn-deficient mice exhibits elevated levels of endogenous IgG and albumin compared to that of the wild-type counterparts. Our in vitro studies using monolayer cells grown on a transwell system have shown that FcRn can also mediate bidirectional transport of albumin across polarized epithelia (MDCK II cells) as well as basolateral recycling. This suggests that FcRn salvages IgG and albumin from loss in the bile, either through basal membrane recycling and/or transport of IgG and albumin from the canalicular-to-sinusoidal membrane.

Expression and Function of FcRn in Brain

FcRn expression in the brain has been found to be located within the capillary endothelium and choroid plexus epithelium using confocal microscopy [95]. FcRn, along with other immune regulated genes, is also found to be upregulated in brain tumors, such as pilocytic astrocytomas, grade II astrocytomas, and oligodendrogliomas [96]. However, the role of FcRn in these tumors is still unclear.

FcRn at the blood brain barrier (BBB) is hypothesized to mediate the efflux of IgG from brain to blood in a process of "reverse transcytosis". Murine models of Alzheimer's disease have shown that FcRn within the BBB is involved in the removal of amyloid beta-peptide specific IgG complexes from the brain of older mice, and thus plays a role in Alzheimer's disease immunotherapy [97]. However, a recent study did not find FcRn as the major factor for the low exposure of IgG in the brain [98]. Therefore, the function of FcRn in the BBB and the brain will require further investigation.

Expression and Function of FcRn in Eye

FcRn in the eye has been localized to various ocular tissues, including corneal epithelium and endothelium, lens epithelium, retinal blood vessel, conjunctiva lymphatic vessel, nonpigmented ciliary epithelium, ciliary blood vessel, iris blood vessel, and optic nerve vessel. FcRn was not detected in the retinal pigment epithelium and the choroid [99]. Murine studies of intravitreal injection of IgG suggest that ocular FcRn removes IgG across the blood–retina barrier into the systemic circulation similar to the process of reverse transcytosis described for the BBB [100]. Whether ocular FcRn is also involved in the common ophthalmic manifestations associated with various autoimmune diseases still requires investigation.

Expression and Function of FcRn in Skin

FcRn has been found to be expressed in human epidermal keratinocytes, melanocytes, hair follicles, and sebaceous glands [101, 102]. The specific function in these cells has yet to be studied. In rodent models of bullous pemphigoid, pemphigus foliaceus, and pemphigus vulgaris, the disease was ameliorated in FcRn-deficient mice or following administration of high-dose IgG [103]. The therapeutic effect likely reflects increased overall degradation of pathogenic IgG antibodies. Whether FcRn in the skin also directly contributes to the development of autoimmune skin blistering diseases is still unknown.

Disease Associations with FcRn

Although animal models have shown that FcRn is involved in colitis, autoimmune skin blistering diseases, and transfer of immunity in asthma, to date, a specific human disease that is attributable to an "FcRn disorder" has yet to be found. A single report of FcRn deficiency was observed in two siblings of a consanguineous marriage who were recorded to have significantly decreased levels of IgG and albumin levels in the blood [104]. Using archived samples, an analysis revealed normal FcRn DNA sequence but a single nucleotide transversion at the signal sequence of the β2m gene that mutated a conserved alanine to proline. The resulting β2m and HLA concentration decreased to <1% of normal. The only reported human population genetic study so far involved 404 Han Chinese subjects with and without lupus nephritis. Analysis of the variable number of tandem repeat polymorphisms within the FcRn gene promoter did not show any differences [105].

Therapeutic Implications of FcRn Function

Engineering IgG and albumin conjugates for prolonged or decreased half-life has been the cornerstone of many types of therapeutic design. The transcytosis function of FcRn in mucosal surfaces and placenta can also allow for a novel mode of drug delivery. Optimizing the interactions between IgG and FcRn is crucial in maximizing therapeutic efficacy while avoiding potential toxic effects.

IgG-Based Therapeutics

Recombinant therapeutics genetically engineered to contain IgG fragments with the CH2–CH3 domain can have significantly prolonged half-life due to protection through catabolism by FcRn binding. This also has the benefit of decreasing the frequency of administration. Methods to increase IgG binding to FcRn have been performed by mutating IgG at various amino acid residues: Threonine 250, Methione 252, Serine 254, Threonine 256, Threonine 307, Glutamic acid 380, Methione 428, Histidine 433, and Asparagine 434 [106]. However, increased binding affinity does not appear to be proportional to the half-life extension. For example, when comparing variants of Herceptin (ERBB2-specific human IgG1 by Genentech) antibody with a threefold increase in FcRn binding at acidic pH and another variant with a 12-fold increased binding at acidic pH and also enhanced binding at neutral pH, both antibodies exhibited similar half-lives when tested in a humanized FcRn transgenic mouse model [107]. It has been hypothesized that increasing binding at

neutral pH prevents IgG release and may instead promote degradation.

Therapeutics can also be engineered to have decreased binding wherein short half-life is desirable to avoid toxic adverse effects. For example, radiographic diagnostic imaging and toxin-conjugated products (i.e., for cancer treatment) would not require prolonged circulation in the bloodstream [108, 109].

Elimination of pathogenic antibodies in autoimmune diseases can be achieved by plasmapheresis, corticosteroid administration, or B cell depletion. An alternative method of elimination involves direct inhibition of FcRn and its interactions with IgG to promote catabolism of native pathogenic antibodies. This has been traditionally achieved by administration of IVIg, which is believed to compete with FcRn binding, thus resulting in accelerated degradation of endogenous pathogenic IgG antibodies left unbound to FcRn. Treatment with IVIg in murine models of myasthenia gravis, bullous pemphigoid, idiopathic thrombocytopenia, and autoimmune arthritis confirmed the benefit of increasing pathogenic IgG degradation as a means to block the immune-mediated disorder, and it appears to be independent of FcγRIIb. In a similar manner, so-called "AbDeg" (antibodies that promote degradation of IgG) which are designed to bind with high affinity to FcRn have been shown to out-compete native IgG to allow for accelerated catabolism [107].

Other methods of interrupting FcRn and IgG interactions include small molecule or peptide inhibitors. Peptide inhibitors of FcRn–IgG interactions (e.g., Syntonix, SYN1436) have been found to be effective in reduction of IgG in cynomolgus monkeys by 80% without affecting the concentration of albumin [110]. Dimerization of the peptide increases the activity 200-fold compared to that of monomers [111, 112]. Although inhibition of β2m function with specific antibody can also reduce FcRn function, there may be unwanted side effects of inhibiting other β2m-associated molecules, such as MHC class I, CD1, and HFE [113].

Since FcRn is known to transport IgG across mucosal epithelia, Fc-conjugated therapeutics have been shown to reach the circulation from the lumen via transcytosis across the intestinal or pulmonary mucosa. Various proteins, such as erythropoietin (Epo), follicle-stimulating hormone, interferon-alpha and -beta, have been conjugated to the Fc region of human IgG1 to facilitate such drug delivery upon inhalation through the upper airway where FcRn resides in humans and non-human primates [81]. Fc fusion "monomers", which consist of Fc dimers with a monomeric therapeutic protein that replaces the Fab region, are found to have improved transport efficiency and longer half-life than Fc fusion dimers [81]. Fc fusion proteins of molecular weight ≥100 kDa can be administered in liquid aerosols wherein they are able to cross the pulmonary epithelium by

transcytosis within the upper/central airways to reach the circulation in an FcRn-dependent pathway [79]. When examined using an Epo-Fc monomer, the inhalation route results in 30–60% bioavailability in non-human primates, which is similar to that of subcutaneous injection of the standard erythropoietin protein.

Published studies have used newborn rats to demonstrate intestinal transport of FSH-Fc fusion proteins together with a long half-life of circulation that approaches approximately 60 h [114]. So far, the use of intestinal mucosa for the transport of Fc fusion proteins has yet to be rigorously investigated.

Given the utility of such IgG-based therapeutics, simpler strategies have been sought for the generation of such fusion proteins. The development of cows that contain a human IgG transgene to produce human IgG suggests that such strategies can represent an alternative way to create large quantities of Fc-based therapeutics protein generation [23].

Albumin-Based Therapeutics

The use of albumin to extend the half-life of therapeutic proteins began prior to the knowledge that FcRn is critical to albumin homeostasis. Three decades ago, chemical conjugation of "high molecular weight carriers", such as albumin to methotrexate was found to be effective in half-life extension, for example [115]. The clinical efficacy of albumin–methotrexate in subsequent treatment of renal carcinomas and rheumatoid arthritis was also attributed to the increased uptake of albumin as an energy source in the setting of inflammation and malignancy [116, 117].

Recombinant technology has now allowed for fusion of albumin to either the carboxy or amino terminus of a therapeutic protein of interest. Some examples include interferon-alpha 2b (Albinterferon), granulocyte colony stimulating factor (Albugranin), growth hormone (Albutropin), insulin (albudin), CD4, hirudin, and IgG fragments themselves [118]. Albinterferon (used for the treatment of chronic hepatitis C), for example, can be administered less frequently and with similar clinical efficacy as interferon conjugated to polyethylene glycol [119]. Since both FcRn and hepatitis C virus resides in the hepatocyte, it is unknown whether albumin conjugation also provides the additional benefit of targeting the liver. Furthermore, it is unknown whether its potential side effects may be also be related to FcRn expression in other organs such as the lung.

Albumin-based nanoparticles, such as Abrexane (albumin-encapsulated Paclitaxel), used for the treatment of metastatic breast cancer, were developed as a delivery vehicle to avoid chemical solvents like Cremophor. It is unknown how this interacts with FcRn and whether the side effects are associated with FcRn biology.

Other recent novel therapeutic designs have used indirect methods to bind to albumin. Insulin detemir (Levemir, Novo Nordisk) is an insulin analogue with a lysine residue bound to a fatty acid (myristic acid) which will bind to albumin in blood and later dissociate from the complex. Drug Affinity Complex (DAC™) is a design that couples medications to a maleimide group, which will bind specifically and irreversible to albumin [120]. The albumin-binding domain from *Streptococcus* strain G148 protein G has also been fused to a divalent anti-HER2 affibody and anti-CEA single-chain diabodies to extend half-life [121, 122].

Conclusion

Our understanding of antibody transport and immunity has expanded dramatically since the experimental era of Brambell subsequent to the cloning and crystallographic resolution of FcRn structure and the generation of experimental tools such as Fc-deficient mice. We have clarified the observations and predictions made by Brambell decades ago. As summarized in this review, these insights have lead and are rapidly leading to variety of enhanced therapeutics and therapeutic approaches which co-opt these new biological principles.

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