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FcRn: From Molecular Interactions to Regulation of IgG Pharmacokinetics and Functions

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Abstract The neonatal Fc receptor, FcRn, is related to MHC class I with respect to its structure and association with $\beta_2$microglobulin (β_2m). However, by contrast with MHC class I molecules, FcRn does not bind to peptides, but interacts with the Fc portion of IgGs and belongs to the Fc receptor family. Unlike the 'classical' Fc receptors, however, the primary functions of FcRn include salvage of IgG (and albumin) from lysosomal degradation through the recycling and transcytosis of IgG within cells. The characteristic feature of FcRn is pH-dependent binding to IgG, with relatively strong binding at acidic pH (<6.5) and negligible binding at physiological pH (7.3–7.4). FcRn is expressed in many different cell types, and endothelial and hematopoietic cells are the dominant cell types involved in IgG homeostasis in vivo. FcRn also delivers IgG across cellular barriers to sites of pathogen encounter and consequently plays a role in protection against infections, in addition to regulating renal filtration and immune complex-mediated antigen presentation. Further, FcRn has been targeted to develop both IgGs with extended half-lives and FcRn inhibitors that can lower endogenous antibody levels. These approaches have implications for the development of longer lived therapeutics and the removal of pathogenic or deleterious antibodies.

Abbreviations

APCs	Antigen presenting cells
BBB	Blood–brain barrier
CNS	Central nervous system
DCs	Dendritic cells

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ECs	Endothelial cells
FcRn	Neonatal Fc receptor
GBM	Glomerular basement membrane
HCs	Hematopoietic cells
HIV	Human immunodeficiency virus
HSV	Herpes simplex virus
ICs	Immune complexes
Ig	Immunoglobulin
IVIG	Intravenous immunoglobulin
KO	Knockout
LP	Lamina propria
mAbs	Monoclonal antibodies
MALT	Mucosa-associated lymphoid tissue
MHC	Major histocompatibility
MLNs	Mesenteric lymph nodes
Myo Vb	Motor myosin Vb
OVA	Ovalbumin
PCT	Proximal convoluted tubule
β_2m	$\beta_2$microglobulin
TC	Transport carrier
TLR	Toll-like receptor
WT	Wildtype

Contents

1 Introduction

The neonatal Fc receptor (FcRn), as the name indicates, was first described for its role in the transfer of IgG from mother's milk across the neonatal gut epithelial barrier into the neonatal bloodstream (Brambell 1970). It is also referred to as a

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major histocompatibility (MHC) class I-related receptor since it shares structural similarity with MHC class I (Simister and Mostov 1989). FcRn belongs to the class of Fc receptors that bind to immunoglobulin G (IgG). However, FcRn differs from other members (collectively referred as FcγRs) of this class in multiple ways: (1) FcRn is expressed in hematopoietic cells (HCs) as well as non-HCs (Borvak et al. 1998; Zhu et al. 2001; Akilesh et al. 2007; Perez-Montoyo et al. 2009), whereas FcγR expression is primarily confined to cells of hematopoietic origin (Nimmerjahn and Ravetch 2008; Hogarth and Pietersz 2012); (2) the cytoplasmic domain of FcRn lacks the ability to signal intracellularly (Kuo et al. 2009), whereas FcγRs (except human FcγRIIIB) or their subunit (γ chain) have immunoreceptor tyrosine-based activatory or inhibitory motifs (ITAMs or ITIMs) in their cytoplasmic domains, which can mediate intracellular signaling (Nimmerjahn and Ravetch 2008; Hogarth and Pietersz 2012); (3) the key function of FcRn involves recycling and transcytosis of IgG (Roopenian and Akilesh 2007; Ward and Ober 2009; Kuo et al. 2010), while FcγRs regulate the immune complex-mediated effector functions of innate immune cells (Nimmerjahn and Ravetch 2008; Hogarth and Pietersz 2012).

Two primary and very well-studied functions of FcRn include the regulation of IgG homeostasis and IgG transport across cellular barriers (Ward and Ober 2009). FcRn is expressed in many different cell types, some of which can be found in all organs of the body (Akilesh et al. 2007; Perez-Montoyo et al. 2009). As a result, the functions of FcRn are not localized to a single organ or cell type, an attribute required for regulating the homeostasis and transport of the ubiquitous immune molecule, IgG. FcRn also regulates the homeostasis of albumin (Chaudhury et al. 2003), although the binding site on FcRn is different for the two molecules (Andersen et al. 2006; Oganesyan et al. 2014) and hence they do not compete with each other for FcRn binding. Recently, FcRn has been shown to also play an important role in the regulation of renal filtration (Akilesh et al. 2008; Sarav et al. 2009) and antigen presentation (Qiao et al. 2008; Baker et al. 2011). In this review, we discuss data that elucidate the mechanisms through which FcRn performs these multiple functions. The well-defined role of antibodies in autoimmunity (Naparstek and Plotz 1993) and the emergence of IgG-based therapeutics (Chan and Carter 2010; Scott et al. 2012) have motivated the development of many FcRn-targeting therapies that have shown promise in preclinical studies. These studies will also be reviewed.

2 FcRn Biology

FcRn exists as a heterodimer of the MHC class I-like heavy chain and $\beta_2$microglobulin (β_2m), which are noncovalently associated (Simister and Mostov 1989). Association with β_2m is required for the expression and normal functioning of FcRn (Claypool et al. 2002). The MHC class I-like heavy chain includes glycosylated α1–3 domains, a transmembrane domain and a ~42 amino acid

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cytoplasmic tail (Kuo et al. 2009). Crystallographic studies of a rat FcRn-rat Fc (IgG2a) complex revealed that the α2 domain residues (Glu117, Glu118, Glu132, Trp133, Glu135, and Asp137) and Ile1 of β_2m combined with the carbohydrate of rat FcRn interacts with residues (Ile253, His310, His435, and minor role for His436) at the CH2–CH3 interface of rat Fc (Martin et al. 2001). The role of His433 of the Fc region in these interactions is contentious (Raghavan et al. 1995; Medesan et al. 1997; Kim et al. 1999; Shields et al. 2001). The stoichiometry of the interaction between FcRn and Fc or IgG is 2:1, as shown by equilibrium gel filtration or sedimentation equilibrium assays (Sanchez et al. 1999; Schuck et al. 1999). The FcRn:Fc (or IgG):FcRn interaction is asymmetric, with different dissociation constants for the two binding sites (Schuck et al. 1999). This, combined with a recent three-dimensional structure of human FcRn bound to an engineered human Fc fragment (Oganesyan et al. 2014), indicate that occupancy of the 'first' site on IgG results in conformational changes that reduce the affinity of FcRn for the second site. Further, the FcRn-IgG interaction is highly pH-dependent, with relatively high affinity binding at acidic pH (<6.5) and no detectable binding at physiological pH (7.4) (Raghavan et al. 1993; Popov et al. 1996). Site-directed mutagenesis studies have shown that the pH-dependence is imparted by His310 and His435 of human Fc (Raghavan et al. 1995) (or His310, His435, and His436 of rodent Fc (Medesan et al. 1997)), which get protonated at acidic pH. These positively charged histidines can then form a salt bridge with the corresponding residues of the FcRn heavy chain (Martin et al. 2001). However, the crystal structure of the complex of human FcRn bound to an engineered human Fc fragment (M252Y/S254T/T256E) was recently solved, which indicates that His310 of human Fc is the most important histidine residue for pH-dependent binding (Oganesyan et al. 2014).

Studies using mutated versions of FcRn have localized the endocytosis and transcytosis signals within the cytoplasmic tail of FcRn, which include the conserved motifs tryptophan (Trp311) and dileucine (Leu322, Leu323) (Newton et al. 2005). A calmodulin-binding site in the membrane proximal region of human FcRn has also been identified that controls the transcytosis and half-life of FcRn in epithelial cells in a calcium-dependent manner (Dickinson et al. 2008). Also, rodent FcRn has three extracellular N-glycan moieties that are absent in human FcRn, which has only one N-linked glycan common to both human and rodent FcRn (Kuo et al. 2009). Interestingly, when human FcRn is rodentized in terms of N-glycan moieties, its steady-state distribution changes (from basolateral) to the apical membrane and its predominant direction of transcytosis (basolateral to apical) is reversed, resulting in the transport of IgG from the apical to basolateral side (Kuo et al. 2009).

Although recent data suggests a slightly different picture (see Sect. 3.1), in the past it was hypothesized that FcRn in vascular endothelial cells (ECs) is most important for recycling of IgG, since these cells form a large surface area that is in contact with the bloodstream. Therefore, FcRn trafficking with respect to IgG recycling has been extensively studied in ECs (Ober et al. 2004a, b; Prabhat et al. 2007; Gan et al. 2009). The recycling process has been characterized in human

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FcRn-Green Fluorescent Protein (GFP)-transfected human microvasculature ECs (HMEC-1), using live-cell fluorescence imaging (Ober et al. 2004b). In these studies, fluorescently labeled wildtype (WT) human IgG1 was used to trace the path of recycling IgG, and a mutated variant (H435A), which binds to FcRn with negligible affinity at both physiological and acidic pH, was used to track IgG that does not bind to FcRn. Based on the results from these and subsequent studies (Ober et al. 2004a; Prabhat et al. 2007; Gan et al. 2009, 2013), a model for FcRn recycling/transcytosis has been constructed, which can be summarized in three steps (Fig. 1): (1) Cells nonspecifically pinocytose extracellular fluid including IgG into adaptor protein containing pH domain, PTB domain, and leucine zipper motif 1 positive ($APPL1^{+}$) vesicular transport carriers (TCs), which then fuse with sorting endosomes. The acidic environment in these compartments facilitates IgG binding to FcRn. (2) FcRn-IgG complexes are sorted into recycling or transcytotic TCs. These TCs subsequently fuse with the plasma membrane, followed by the release of IgG into the serum or interstitial space due to the physiological (near-neutral) pH. (3) Meanwhile, the sorting endosomes mature to late endosomes, which deliver their luminal contents to lysosomes, resulting in the degradation of any IgG that failed to be recycled by FcRn.

FcRn-mediated transcytosis has also been extensively studied using Madin–Darby canine kidney (MDCK) cells (Claypool et al. 2004; Tesar et al. 2006), which form polarized monolayers when cultured in vitro, a property necessary for studying transcytosis. In human FcRn-transfected MDCK cells, FcRn localizes predominantly to apical intracellular compartments, with surface expression primarily on the basolateral side. Importantly, FcRn was demonstrated to transcytose IgG in both basolateral to apical and apical to basolateral directions, the latter being dominant (Claypool et al. 2004). What factors define whether IgG is recycled or transcytosed? Although this question has not been answered completely, studies have identified molecular effectors for these processes which include Rab *GTPases* and motor myosin Vb (Myo Vb). Rab *GTPases* are regulated by GTP-GDP exchange cycles, and in combination with soluble NSF attachment protein receptors (SNAREs) can regulate the merging of different organellar membranes (Somsel and Wandinger-Ness 2000; Miaczynska and Zerial 2002; Jahn et al. 2003). Also, when active, Rab *GTPases* can activate or recruit effector molecules such as kinases, phosphatases, motors, etc. Consequently, these proteins control multiple intracellular trafficking processes (Stenmark 2009; Agola et al. 2011). On the other hand, myosin motors are mechanical, enzymatic motors, which generate energy by hydrolyzing ATP to drive cargo along actin filaments (Hammer and Sellers 2012). Rab11 *GTPase* associates with FcRn during recycling in HMEC-1 cells (Ward et al. 2005), and regulates recycling in MDCK cells (Tzaban et al. 2009), whereas Myo Vb and Rab25 *GTPase* are involved in bidirectional transcytosis in MDCK cells (Tzaban et al. 2009).

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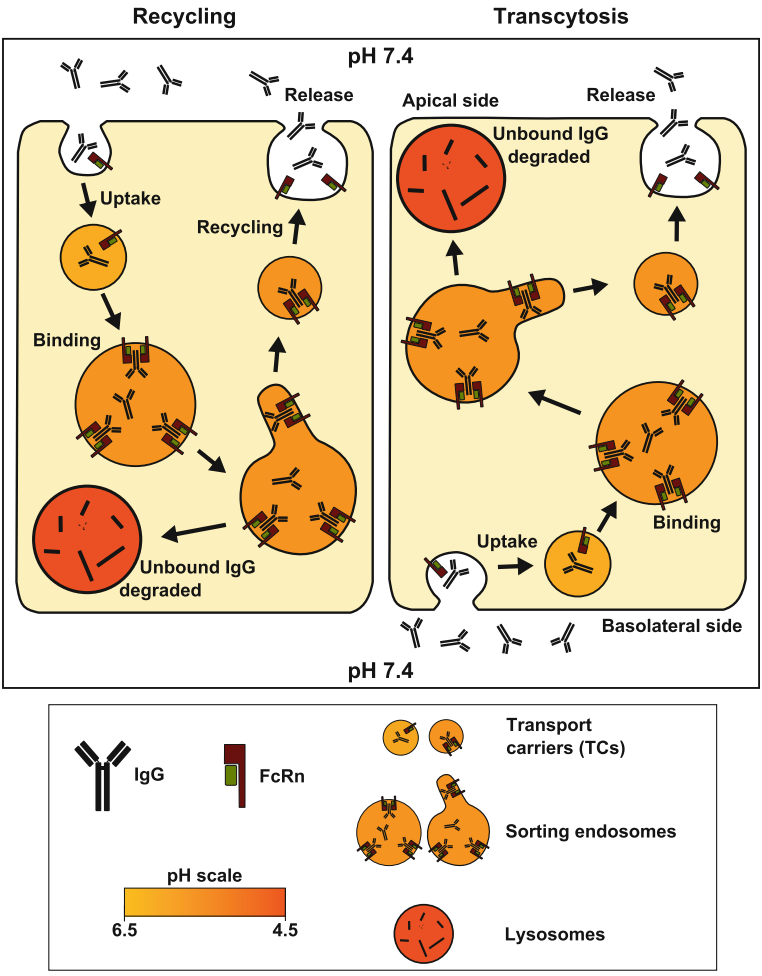

Fig. 1 FcRn-mediated recycling and transcytosis of IgG. Cells internalize IgG through fluid-phase pinocytosis into tubulovesicular TCs, which subsequently fuse with sorting endosomes. The acidic pH in these compartments favors the binding of IgG to FcRn. FcRn with bound IgG sorts into TCs, which either recycle or transcytose to the plasma membrane. The near-neutral pH on the plasma membrane results in the release of IgG from FcRn into the extracellular fluid

3 Functions of FcRn

3.1 IgG Homeostasis

IgG and albumin constitute ~80 % of total serum protein with mean concentrations as high as 10 and 40 mg/ml, respectively (Dati et al. 1996). The primary reason for the high abundance of these proteins is their extraordinarily long serum

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half-life. IgG has a serum half-life of ~22 days in humans (Spiegelberg and Fishkin 1972) and ~8 days in mice (Vieira and Rajewsky 1988; Ghetie et al. 1996). Multiple studies have convincingly shown that the extended half-life of IgG (and albumin) is FcRn-mediated. The first in vivo evidence for this came from studies using β_2m-deficient knockout (KO) mice, which do not express functional FcRn in addition to having other defects such as $CD8^+$ T cell deficiency. In these mice, IgG has an extremely short half-life (Ghetie et al. 1996; Israel et al. 1996; Junghans and Anderson 1996). Later, similar conclusions were obtained using FcRn KO mice (Roopenian et al. 2003), which are more specific tools than β_2m KO mice for studying FcRn biology. In addition, based on archived blood samples a study has identified two deceased humans (with familial hypercatabolic hypoproteinemia), who were analogous to β_2m KO mice, i.e., β_2m expression was almost completely inhibited in these patients (soluble β_2m levels in their serum were <1 % of normal) due to a point mutation in the leader peptide of their β_2m gene (Wani et al. 2006). IgG and albumin levels were abnormally low in their serum, also indicating a role for FcRn in humans in protecting IgG and albumin from catabolism.

As mentioned earlier, FcRn is expressed in many different cell types across the body. In adult humans, FcRn expression can be found in skin microvasculature, retinal, and placental ECs (Antohe et al. 2001; Ober et al. 2004b; Powner et al. 2014), monocytes, macrophages, dendritic cells (DCs) (Zhu et al. 2001), T and B lymphocytes (van Bilsen et al. 2010), keratinocytes (Cauza et al. 2005), hepatocytes (Andersen et al. 2012), epithelial cells of intestine (Israel et al. 1997; Dickinson et al. 1999), mammary gland (Cianga et al. 2003), kidney (Haymann et al. 2000), lung (Spiekermann et al. 2002), eye (Powner et al. 2014) and the female genital tract (Li et al. 2011). In adult mice, FcRn has been localized to vascular ECs of some, but not all organs (Akilesh et al. 2007), macrophages, DCs (Akilesh et al. 2007; Perez-Montoyo et al. 2009), B cells (Perez-Montoyo et al. 2009) and epithelial cells of kidney (Akilesh et al. 2008), alveolus (Spiekermann et al. 2002), intestine (Akilesh et al. 2007), choroid plexus (Akilesh et al. 2007), eye (Kim et al. 2008), and the female genital tract (Li et al. 2011). It is not clear in which cell types/organ FcRn is crucial for persistence of IgG (and albumin). Experiments using bone marrow chimeras of WT and FcRn KO mice revealed that FcRn in HCs and non-HCs contribute almost equally to IgG homeostasis (Akilesh et al. 2007; Kobayashi et al. 2009). Subsequent studies using Cre-loxp technology-based cell type-specific FcRn KO mice demonstrated that FcRn-expressing ECs and HCs are the major sites of IgG homeostasis (Perez-Montoyo et al. 2009).

The relative contribution of different cell types to IgG recycling depends on many factors, including the number of FcRn-expressing cells within each group, FcRn expression levels, the rate of pinocytic/phagocytic activity and the concentration of IgG in the respective microenvironments. Also, the relative contribution of cells might change during inflammation, since toll-like receptor (TLR) ligands and proinflammatory cytokines have been shown to modulate FcRn expression. In particular, CpG oligodeoxynucleotide (TLR9 ligand), *lipopolysaccharide* (TLR4 ligand), tumor necrosis factor (TNF)-α and interleukin-1β have been shown to

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upregulate FcRn expression in intestinal epithelial cells and/or monocytes (Liu et al. 2007b). By contrast, interferon-γ has been shown to downregulate FcRn expression in intestinal epithelial cells and monocytes (Liu et al. 2008). Determining the contribution of each cell type to IgG protection, and how this changes under inflammatory conditions, will aid in developing accurate pharmacokinetic-modeling tools required for optimizing the delivery of IgG-based therapeutics.

3.2 Transport of IgG Across Cellular Barriers

3.2.1 IgG Transfer from Mother to Fetus or Neonate

IgG is the only immunoglobulin subclass that is actively transported from mother to fetus/neonate. Although both mother-to-fetus and mother-to-neonate transfer of IgG can occur in rodents and humans, the former is dominant in humans while the latter plays a major role in rodents.

In mice, FcRn expression in the yolk sac mediates the materno fetal transfer of IgG (Medesan et al. 1996). However, at birth, the concentration of IgG in the serum of neonatal mice is only 20–30 % of that in adult mice (Appleby and Catty 1983) and hence, IgG transport during gestation in mice is considered to be of relatively low importance. In rodents, the transfer of passive immunity in the form of IgG primarily occurs postnatally (Appleby and Catty 1983). Upon ingestion of IgG-containing maternal milk, IgG, and other milk proteins reach the proximal small intestine (the stomach is less acidic in neonates). Acidic pH in the duodenum allows IgG to be selectively endocytosed by enterocytes in an FcRn-dependent fashion (Jones and Waldmann 1972; Rodewald and Abrahamson 1982; Rodewald and Kraehenbuhl 1984). Internalized IgG is then transcytosed across the cell to the basolateral membrane, where the physiological, near-neutral pH results in the release of IgG from FcRn into the intestinal tissue. IgG can subsequently transfer into the blood through the lymphatics. Coincidentally, in rodents, FcRn expression in enterocytes rapidly decreases at around weaning age (Martin et al. 1997; Akilesh et al. 2007).

In newborn infants, the concentration of IgG in the serum is at levels similar to those observed in mothers (Salimonu et al. 1978). This indicates that maternofetal transport of IgG (during the third trimester of pregnancy) is extremely efficient in humans. The transport is mediated by FcRn expressed in syncytiotrophoblasts (Leach et al. 1996; Simister et al. 1996; Firan et al. 2001), which constitute the continuous, multinucleate epithelium separating the mother from fetus. On the apical side, the brush border surface of syncytiotrophoblast is bathed in maternal blood, whilst the basolateral membrane faces fetal blood capillaries. In brief, the maternal serum containing IgG is pinocytosed into the endosomes of syncytiotrophoblasts, followed by IgG transcytosis to the fetal side (basolateral membrane), where the near-neutral pH enables IgG dissociation from FcRn.

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3.2.2 Transport of IgG to Sites of Pathogen Encounter and Immune Activation

The mucosal surfaces of the airways, urogenital tract, and intestine are the primary sites where a multicellular organism such as a mammalian species interacts with the environment. These surfaces employ multiple mechanisms to protect against invasion of pathogens or harmful agents, which include (McGhee and Fujihashi 2012): (1) a polarized epithelial cell barrier, (2) secretions (containing antimicrobial substances including IgA, IgG and IgD) toward the apical (environment) side of the epithelial cell layer and (3) mucosa-associated lymphoid tissue (MALT), positioned on the basolateral side (beneath) of the epithelial barrier. MALT is primarily composed of innate (DCs, macrophages, etc.) and adaptive (T and B cells) immune cells, however, the composition of MALT varies significantly at each mucosal surface. Importantly, $CD103^+$ DCs in the lamina propria (LP, part of MALT in the gut) extend processes through the epithelial cell barrier into the intestinal lumen and capture antigens. The DCs then carry the captured antigen to the mesenteric lymph nodes (MLNs) where they present antigenic peptides to T cells (Schulz et al. 2009). An analogous function of antigen sampling has been shown to be performed by FcRn in intestinal epithelial cells in mice (Yoshida et al. 2004). In this study, transgenic mice expressing human FcRn (under the control of endogenous human promoter) and human β_2m in the absence of endogenous mouse FcRn expression were used because, as mentioned earlier, intestinal epithelial cells in WT mice downregulate FcRn expression at around weaning age (Akilesh et al. 2007), whereas intestinal epithelial cells in adult humans continue to express FcRn (Israel et al. 1997; Dickinson et al. 1999).

In these human FcRn transgenic mice, intravenously delivered anti-ovalbumin (OVA) IgG reached the luminal fluid of the small intestine within a few hours, but such transport of anti-OVA IgG into small intestinal fluid was substantially lower in FcRn KO mice. Further, intragastrically administered IgG-OVA complexes were transported into the LP in human FcRn transgenic mice (but not in FcRn KO mice) and subsequently, OVA^+ DCs were detected in the MLNs. Notably, intravenous delivery of anti-OVA IgG and oral delivery of OVA lead to the expansion of OVA-specific $CD4^+$ T cells in the MLNs of human FcRn transgenic mice. A similar FcRn-mediated phenomenon was seen to occur in the nasal mucosa (Yoshida et al. 2004). These observations clearly establish two FcRn-dependent immune functions: (1) FcRn contributes to the humoral immune response at mucosal surfaces by transporting IgG from the basolateral side of the epithelial cell barrier to mucosal secretions on the apical side (site of antigen or pathogen encounter). This can explain how IgG reaches mucosal fluids of the nasal cavity ($\sim$300 μg/ml (Hanson and Brandzaeg 1980) and rectum ($\sim$800 μg/ml; (Kozlowski et al. 1997). (2) FcRn can mediate the delivery of antigen (in the form of immune complexes) from the mucosal surface to the corresponding MALT where T cells can be stimulated. These mucosal immune functions of FcRn have also been demonstrated in a mouse model of colitis induced by *Citrobacter rodentium* infection (Yoshida et al. 2006). Importantly, this study highlighted the importance

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of FcRn-mediated delivery of anti-pathogen IgG to the intestinal lumen, demonstrating that this antibody can prevent the attachment of *C. rodentium* to epithelial cells, an essential step in the initiation/progression of infection by this pathogen (Bry and Brenner 2004).

Unlike most mucosal surfaces where IgA is found in higher concentrations than other immunoglobulin subclasses (Woof and Mestecky 2005), in the human female genital tract IgG is the predominant immunoglobulin subclass (Johansson and Lycke 2003). With respect to this, a recent study has shown that bidirectional transcytosis of IgG can be carried out by FcRn expressed by female genital tract epithelial cells of humans (in vitro) and the female genital tract of mice (in vivo) (Li et al. 2011). Also, this study showed that intraperitoneal-delivery of anti-herpes simplex virus-2 (HSV-2) IgG conferred higher protection against vaginal infection of HSV-2 in WT mice than in FcRn KO mice. In order to account for the higher rate of IgG catabolism in FcRn KO mice, a 1.4 to 2.8-fold greater amount of anti-HSV-2 IgG was used in the KO mice. The lower level of protection observed in FcRn KO mice was attributed to an absence of FcRn-mediated transfer of IgG to the genital mucosal surface. However, improved mouse models lacking FcRn expression specifically in epithelial cells (such a model would be expected to have normal IgG catabolism) would be valuable tools to determine the role of FcRn-mediated IgG transcytosis in vaginal infections.

Interestingly, another recent study has indicated that FcRn can aid the transfer of human immunodeficiency virus (HIV)-1 across the epithelial cell barrier of genital mucosa (Gupta et al. 2013). In this in vitro study, the acidic pH on the apical side (as is the case for cervicovaginal secretions/fluid) enhanced FcRn-mediated transcytosis of HIV-1 (in complex with anti-virus IgG) across the epithelial cell barrier, releasing viable virus toward the basolateral side. Although this FcRn-mediated process can enhance viral entry into the genital tissue, IgG-coated viral particles will be primarily taken up by FcγR-expressing cells (primarily professional antigen presenting cells (APCs)) in the MALT, where they could induce subsequent T cell activation. However, it remains to be determined whether FcRn can contribute to viral dissemination or clearance during this process.

3.3 Maintenance and Regulation of Renal Filtration

Blood is filtered in nephrons, the functional units of kidneys, to form urine. Nephrons are made up of different kinds of tubules, each performing a different function (Fig. 2a). The head portion of the nephron, called the glomerular capsule, performs filtration, and the following proximal convoluted tubule (PCT) performs reabsorption of salt, water, glucose, albumin, etc. Blood, destined for filtration flows into glomerular capillaries (enclosed by the glomerular capsule), where filtration occurs, and the resultant filtrate flows into the lumen of the glomerular capsule. For filtration to occur, the plasma has to pass through three layers of filters (Fig. 2b) with increasing size selectivity (Fox 2011). The first filtration barrier is

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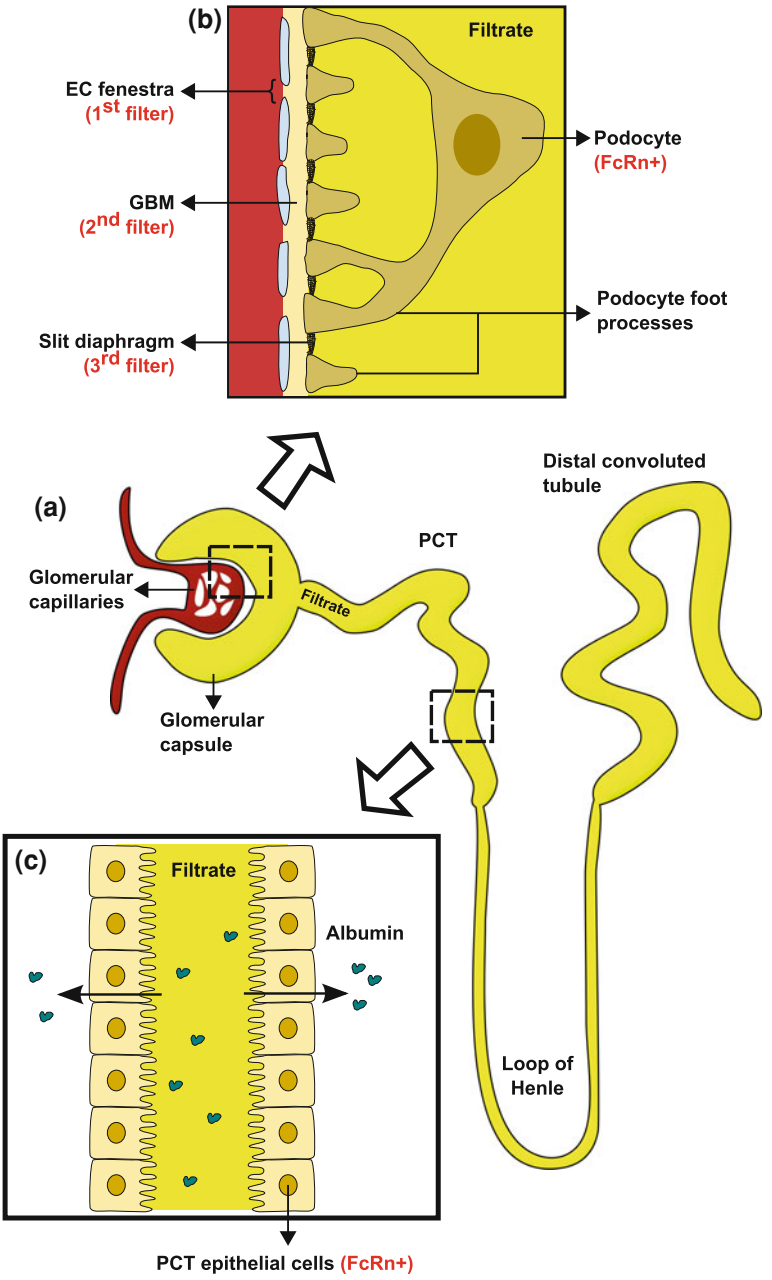

Fig. 2 FcRn-mediated functions in the kidney. **a** Schematic structure of nephron. **b** Plasma from glomerular capillaries passes through three different filters before flowing into the lumen of the glomerular capsule. During this process, IgG and albumin accumulate at the GBM or slit diaphragm and IgG (and possibly albumin) is cleared by FcRn in podocytes. **c** The filtrate that forms in the glomerular capsule contains significant amounts of albumin and flows into the lumen of the PCT, where FcRn in epithelial cells mediates transcytosis of albumin from the filtrate into the interstitial space in the kidney

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formed by fenestrated ECs of glomerular capillaries. These fenestrae are large but charged, which may prevent bulky proteins from crossing the barrier. The second barrier is formed by the glomerular basement membrane (GBM), which has small and charged pores and lies immediately below the glomerular capillaries. Underneath the GBM lie specialized epithelial cells called podocytes, which have long extensions (foot processes) that wrap around the GBM. The foot processes interdigitate forming narrow slits, and are bridged by extracellular structures, referred to as slit diaphragms (Pavenstadt et al. 2003). The foot processes of podocytes along with associated slit diaphragms constitute the third filtration barrier. The pore size of the slit diaphragm is equal to or less than the size of albumin (Wartiovaara et al. 2004).

Considering the fact that ~180 L of glomerular filtrate is generated per day, it is very likely that albumin and IgG (which constitute ~80 % of serum proteins) accumulate at the GBM and/or slit diaphragm, resulting in the clogging of these biological filters. Hence, it has been hypothesized that a mechanism is in place to clear the filters of these accumulated proteins. In this context, a study has shown that FcRn in podocytes functions to remove accumulated IgG at the GBM (Akilesh et al. 2008). The role of renal FcRn in this process was confirmed primarily based on the observation that age-dependent glomerular accumulation of IgG is higher in FcRn KO mice by comparison with WT mice, despite the fact that serum IgG levels are significantly lower in FcRn KO mice. Based on the pattern of IgG accumulation observed in the glomerulus, podocytes were suggested to be the primary cells that clear the accumulated IgG. Also, the study shows that the protein-elimination function of podocytes is saturable. This finding might explain how immune complex deposition occurs in the kidneys of systemic lupus erythematosus (SLE) patients, which leads to nephritis.

The glomerular filtrate flowing into the PCT contains significant amounts of albumin, most of which is reclaimed by PCT epithelial cells (Russo et al. 2007). Importantly, these epithelial cells express high levels of FcRn (Akilesh et al. 2007). It has now become clear that FcRn in PCT cells is responsible for retrieval of albumin (Fig. 2c). The role of FcRn in this process is primarily based on two observations (Sarav et al. 2009). First, FcRn KO mice excrete more albumin in urine than WT mice. Second, in FcRn KO mice that were transplanted with one WT kidney (after nephrectomy of one native kidney) serum albumin levels increased, whereas WT mice transplanted with a KO kidney developed hypoalbuminemia. Also, based on the localization of exogenously added, labeled albumin in the kidneys of unmanipulated mice and transplant chimeras, it was suggested that albumin is reclaimed by the epithelial cells of the PCT. In this context, FcRn performs bidirectional transcytosis in human proximal tubular epithelial cells (Kobayashi et al. 2002). Hence, it is logical to assume that albumin reclaimed by the cells of the PCT would be transcytosed into the interstitium of kidneys, followed by drainage of albumin into the lymphatics and entry into the circulation. In addition, in the same study (Sarav et al. 2009), experiments using kidney transplant chimeras showed that renal FcRn aids elimination of IgG from plasma into urine. However, the mechanism through which IgG elimination occurs is unclear.

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3.4 Possible Role in Clearing IgG from Immune-Privileged Sites

Some sites in the body are considered immune-privileged because immune surveillance at these sites is limited or absent. These sites include the central nervous system (CNS), eye, fetus/placenta, and testis. Complex blood–tissue barriers exist at these sites that limit or restrict the entry of immune cells and molecules from the blood into the tissue. In the CNS, one such barrier is the blood–brain barrier (BBB), which is formed by ECs that line the cerebral microvessels, basal lamina, and astrocytic endfeet (Abbott et al. 2006). Adjacent ECs of the BBB are connected through tight junctions, which only allow the passage of small hydrophobic molecules. IgG is large and hydrophilic in nature and hence its entry through the BBB is highly restricted. The concentration of IgG in a tissue relative to plasma is 1:500 for brain and 1:10 for most nonleaky tissues (Wang et al. 2008).

FcRn is expressed by BBB ECs in both mice (Akilesh et al. 2007) and rats (Schlachetzki et al. 2002). The presence or absence of FcRn in human BBB ECs has not been reported. However, we have observed FcRn expression in the human BBB endothelial cell line hCMEC/D3 (Sripad Ram, Raimund Ober, E. Sally Ward, unpublished). In rats, one study has shown that intracerebrally injected IgG is rapidly effluxed out of the CNS into the blood (Zhang and Pardridge 2001). It was also shown that this efflux or reverse transcytosis of labeled IgG can be blocked by intracerebral injection of excess unlabeled IgG, indicating a role for an Fc receptor in this process. Another recent study in rats has confirmed that FcRn mediates efflux of IgG from brain to blood (Cooper et al. 2013). In this study, 24 h following intracranial injection of two mutant IgGs, N434A (similar to WT IgG except that it has increased binding to FcRn at pH 6) and H435A (has negligible binding to FcRn at pH 6 and 7.4), N434A levels in the brain decreased, whereas H435A levels remained almost unchanged in comparison to their levels at 5 min postinjection.

In mice, data exist to both support (Deane et al. 2005) and refute (Garg and Balthasar 2009; Abuqayyas and Balthasar 2013) the role of FcRn in mediating IgG efflux from brain. In one study that supports such a role, centrally delivered anti-Aβ IgG and anti-Aβ IgG-Aβ complexes were transported out of the brain, and this was blocked by simultaneous delivery of anti-FcRn IgG or the use of FcRn KO mice (Deane et al. 2005). By contrast, a study has shown that the brain to blood exposure ratio of intravenously delivered IgG is similar in WT and FcRn KO mice (Abuqayyas and Balthasar 2013). Additional work is required to unambiguously determine the role of FcRn in IgG transport across the BBB. Further, FcRn is expressed by (ECs) of retinal vasculature, and may play a role in excluding IgG from the eye across the blood–retinal barrier (Powner et al. 2014).

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3.5 Role in Antigen Presentation

Professional APCs (DCs, macrophages, and B cells) can present antigens to $CD8^+$ and $CD4^+$ T cells in the context of MHC class I and MHC class II, respectively. In general, intracellular antigens are proteasomally processed and presented on MHC class I molecules, and extracellularly derived antigens are processed in lysosomes and presented on MHC class II molecules (Neefjes et al. 2011). Under some circumstances, extracellular antigens are processed by proteasomes or within phagosomes and presented on MHC class I molecules. This type of antigen presentation can only be carried out by DCs (Kurts et al. 2010) and possibly macrophages (Houde et al. 2003; Asano et al. 2011) and is referred to as cross-presentation.

Importantly, all professional APCs in both mice and humans express FcRn (Zhu et al. 2001; Perez-Montoyo et al. 2009; van Bilsen et al. 2010). Professional APCs, except B cells, also express activating FcγRs, which in the presence of IgG-based immune complexes (ICs) mediate activation of APCs (Nimmerjahn and Ravetch 2008; Hogarth and Pietersz 2012; Guilliams et al. 2014). Further, antigens in the form of ICs are more efficiently internalized (through activating FcγRs) by APCs than soluble antigens and hence lead to more efficient T cell activation. With respect to this, a role similar to that played by FcγRs has been shown to be performed by FcRn (Qiao et al. 2008; Kobayashi et al. 2009). In one such study (Qiao et al. 2008), multimeric OVA ICs containing either WT IgG or a mutated IgG (IHH, no binding to FcRn at physiological and acidic pH, but no change in binding to FcγRs) were used in mouse $CD4^+$ T cell proliferation assays in the presence of either WT or FcRn KO DCs. In these assays, the proliferation of OVA-specific $CD4^+$ T cells decreased when DCs lacked FcRn or when ICs comprising IHH antibodies were used by comparison with that observed using WT DCs or ICs containing WT antibodies, respectively. These observations indicate a role for FcRn in IC-mediated antigen presentation. Similar observations were made using human cells, and also when in vitro-loaded (with ICs containing WT or IHH antibodies) WT or FcRn KO DCs were injected into WT mice. Based on the observed trafficking patterns of ICs and FcRn, it was demonstrated that FcRn rapidly transports WT ICs to lysosomes, leading to enhanced antigen presentation and T cell proliferation. In the assays described above, it is possible that some ICs would presumably cross-link FcγRs, leading to DC activation and cytokine secretion, which in turn would upregulate MHC class II and the associated invariant chain (Simmons et al. 2012; Guilliams et al. 2014). Invariant chain has been shown to also associate with FcRn and target it to late endosomes or lysosomal compartments (Ye et al. 2008). Hence, the invariant chain might have a role to play in diverting FcRn-bound ICs to lysosomes in APCs.

Recently, FcRn has also been shown to play a role in the cross-presentation of IC-derived antigens (Baker et al. 2011). In this study, mouse DCs pulsed with ICs comprising WT or IHH antibodies complexed with OVA (similar to those described above) were injected into WT mice that had also received labeled OVA-

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specific $CD8^+$ T cells. The antigen in this case is exogenous and hence $CD8^+$ T cells will only be stimulated if the antigen is cross-presented. The proliferation of $CD8^+$ T cells was found to be many fold higher when WT IgG ICs were used in comparison to the proliferation observed with IHH IgG ICs, highlighting the importance of FcRn in IC-mediated cross-presentation. Interestingly, only $CD8^-CD11b^+$ DCs, but not $CD8^+CD11b^-$ DCs (shown to be the major mediators of cross-presentation of soluble and tumor antigens (Hildner et al. 2008)) were able to efficiently cross-present IC-derived antigen to $CD8^+$ T cells. Using IgG-opsonized, OVA-containing beads (IC-beads), it was also shown that the $FcRn^+$ phagosomes formed upon WT IgG IC-bead internalization by DCs had many features that facilitated cross-presentation by comparison with phagosomes formed by IHH IgG IC-beads. The features included lower pH, persistence of antigen in the phagosomes and enrichment of components of the cross-presentation machinery such as the transporter associated with antigen processing 1 (TAP1) and MHC class I. Finally, the authors suggest that ICs are internalized by DCs in an FcγR-dependent fashion, followed by the transfer of ICs from FcγRs to FcRn in acidic, endosomal compartments followed by cross-presentation. Taken together, FcRn is indicated to be important for the presentation of IC-derived antigen to both $CD4^+$ and $CD8^+$ T cells.

4 FcRn-Targeted Therapies

Monoclonal antibodies (mAbs), due to their specificity and long half-lives, are considered to be one of the most effective and safe therapies for many diseases. Currently, there are almost 350 mAbs that are either in early development or Food and Drug Administration (FDA)-approved for the treatment of inflammatory disorders, cancers, infectious diseases, and solid organ transplant rejection (Mahmud et al. 2010; Reichert 2013). As mentioned in the previous sections, FcRn functions to regulate the levels and many functional activities of IgGs. As a result, many therapies (mostly IgG-based) have been developed that target FcRn, and have shown promise in treating animal models of autoimmune diseases and cancer. FcRn-targeting therapies can be broadly classified into two distinct categories: (1) mAbs with extended half-life, which will have applications in any disease where mAbs can be used therapeutically and (2) agents that deplete endogenous antibodies, which will have applications in antibody-mediated pathologies and other situations in which antibody clearance is indicated.

During the last decade or so, a significant component of Fc-engineering efforts has focused on developing IgG mutants that vary in their binding to FcRn and have enhanced in vivo half-life, with an aim to boost the efficacy and/or reduce the dosing frequency of IgG-based therapies. The first report demonstrating that Fc engineering can be used to generate IgGs with increased in vivo persistence came from a study in which a mutated mouse IgG1 Fc (T252L/T254S/T256F) was produced using random mutagenesis and phage display. This mutated Fc fragment

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has increased binding to mouse FcRn at acidic pH, but negligible binding at physiological pH, resulting in an extended half-life in mice by comparison with WT mouse IgG1-derived Fc (Ghetie et al. 1997). Subsequently, many engineered human IgGs have been developed with increased in vivo half-life, as validated in nonhuman primates (Hinton et al. 2004, 2006; Dall'Acqua et al. 2006; Yeung et al. 2009). Among these mutants, YTE (human IgG1—M252Y/S254T/T256E), exhibits ~4 fold increase in half-life relative to WT human IgG1 in nonhuman primates, which is the longest half-life extension reported to date (Dall'Acqua et al. 2006). Another mutant, HN (human IgG1—H433K/N434F), with increased pH-dependent binding to (human) FcRn has been shown to be more active than WT human IgG1 in FcRn-mediated transcytosis across the ex vivo human placenta (Vaccaro et al. 2006). Also, a recent study has shown that IgG with enhanced half-life has increased antitumor activity than WT IgG in tumor xenograft studies in mice (Zalevsky et al. 2010). Finally, based on the in vivo half-lives of various IgG mutants that were Fc-engineered with respect to their FcRn binding, it is clear that while an increase in IgG affinity toward FcRn at acidic pH is important, retention of low affinity at physiological pH is equally important to allow exocytic release from cells (Prabhat et al. 2007) and consequent persistence of an IgG (Dall'Acqua et al. 2002; Vaccaro et al. 2006; Yeung et al. 2009).

Autoantibodies lead to pathology in autoimmune diseases such as SLE, neuromyelitis optica, myasthenia gravis, and multiple sclerosis (Sherer et al. 2004; Conti-Fine et al. 2006; Jarius and Wildemann 2010; Popescu and Lucchinetti 2012). Also, antibodies can mediate rejection of organ allografts (Colvin and Smith 2005). Currently, approved treatments for depleting antibodies in such diseases, in a nonspecific manner, include plasmapheresis and high dose intravenous immunoglobulin (IVIG) (Orange et al. 2006; Winters 2012). Both these treatment modalities may lead to side effects or complications, but more importantly, the cost of these treatments is high (Heatwole et al. 2011; Winters et al. 2011). Hence, efforts have been undertaken to develop alternatives. IVIG lowers endogenous or pathogenic antibody levels only when used in high doses, which is essential for saturating FcRn (Hansen and Balthasar 2002; Li et al. 2005). Alternatively, FcRn can be saturated or blocked using low doses of agents that bind to FcRn with very high affinity. In the case of half-life extension, retention of low affinity towards FcRn at physiological pH limits the extent to which the affinity at acidic pH can be increased (Ward and Ober 2009; Yeung et al. 2009). Such a limitation is not relevant to the generation of effective FcRn blockers, and in fact, high affinity binding to FcRn at physiological pH is desirable in this case since it will enable the engineered antibody to be efficiently endocytosed by FcRn-mediated uptake into cells (Vaccaro et al. 2005; Prabhat et al. 2007). This in turn will result in increased competition with endogenous antibodies with respect to FcRn binding. One such Fc-engineered antibody is MST-HN (M252Y/S254T/T256E/H433K/N434F). Antibodies of this class have been shown to rapidly decrease endogenous antibody levels in mice and are called Abdegs (for antibodies that enhance IgG degradation) (Vaccaro et al. 2005). In a serum transfer model of arthritis in mice, Abdegs were able to reduce swelling and inflammation in the

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joints in both therapeutic and prophylactic disease settings (Patel et al. 2011). Importantly, by comparison with Abdegs, 25–50 times higher amounts of IVIG were required to achieve similar therapeutic effects. Recently, Abdegs were also shown to ameliorate disease in a passive model of antibody-mediated experimental autoimmune encephalomyelitis by mediating both the rapid clearance and reducing the accumulation of encephalitogenic antibodies in the CNS (Challa et al. 2013).

Antibodies that bind to FcRn through their variable domains have also been developed that can block FcRn-mediated recycling of IgGs. Anti-rat (4C9) and anti-human (DVN24) antibodies specific for FcRn were shown to reduce the levels of exogenously administered tracer antibody in rats and human FcRn transgenic mice, respectively (Getman and Balthasar 2005; Christianson et al. 2012). Similarly, another anti-rat FcRn IgG, 1G3, was shown to reduce pathogenic antibody levels and disease symptoms in both passive and active models of myasthenia gravis in rats (Liu et al. 2007a). On the downside, antibody-based, FcRn blockers have short in vivo half-lives due to strong binding to FcRn at physiological pH, which results in increased accumulation in FcRn-expressing cells and reduced exocytic release (Dall'Acqua et al. 2002; Vaccaro et al. 2006; Liu et al. 2007a; Perez-Montoyo et al. 2009). Peptide-based FcRn blockers have also been developed. In particular, SYN1436, a dimer of an FcRn-binding peptide was able to significantly reduce the levels of exogenously added human IgG in human FcRn transgenic mice and endogenous antibody in nonhuman primates (Mezo et al. 2008). These peptide-based agents would be expected to exhibit an in vivo half-life that is lower than that of antibody-based FcRn blockers, primarily due to renal-mediated clearance. As a result, PEGylation has been employed to improve the in vivo pharmacokinetics and efficacy of such peptide-based FcRn blockers (Mezo et al. 2011).

5 Concluding Remarks

It is clear that in addition to playing a role in the homeostasis of IgG and albumin, FcRn mediates IgG transport to inaccessible sites (fetus, neonate, or mucosal surfaces) and possibly excludes IgG from immune-privileged sites. This knowledge offers opportunities for engineering antibodies for modulation of the intrinsic half-life and transport of the antibody itself or, through FcRn inhibition, altering the dynamics and levels of endogenous antibodies. Further, FcRn regulates kidney filtration of its ligands and contributes to antigen presentation to both $CD4^+$ and $CD8^+$ T cells. Although functions for FcRn at multiple different sites have been identified, the role of FcRn in other specialized cells such as hepatocytes and keratinocytes remains poorly defined.

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