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MULTIPLE ROLES FOR THE MAJOR HISTOCOMPATIBILITY COMPLEX CLASS I–RELATED RECEPTOR FcRn

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■ **Abstract** Multiple functions have recently been identified for the neonatal Fc receptor FcRn. In addition, a human homolog of the rodent forms of FcRn has been identified and characterized. This major histocompatibility complex class I-related receptor plays a role in the passive delivery of immunoglobulin (Ig)Gs from mother to young and the regulation of serum IgG levels. In addition, FcRn expression in tissues such as liver, mammary gland, and adult intestine suggests that it may modulate IgG transport at these sites. These diverse functions are apparently brought about by the ability of FcRn to bind IgGs and transport them within and across cells. However, the molecular details as to how FcRn traffics within cells have yet to be fully understood, although in vitro systems have been developed for this purpose. The molecular nature of the FcRn-IgG interaction has been studied extensively and encompasses residues located at the CH2-CH3 domain interface of the Fc region of IgG. These Fc amino acids are highly conserved in rodents and man and interact with residues primarily located on the $\alpha 2$ domain of FcRn. Thus, it is now possible to engineer IgGs with altered affinities for FcRn, and this has relevance to the modulation of IgG serum half-life and maternofetal IgG transport for therapeutic applications.

INTRODUCTION

The neonatal Fc receptor FcRn was first identified in rodents as the receptor that transfers maternal gammaglobulins (IgGs) from mother to young via the neonatal intestine (1–6). However, more recent data have indicated that this receptor not only delivers IgGs across the maternofetal barrier during gestation (7–9), but is also responsible for the maintenance of serum IgG levels (10–13). How does FcRn carry out these apparently diverse functions? It appears that the ability of FcRn to transport IgGs in intact form both within and across cells is exploited in

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each of these activities (reviewed in 14). However, the molecular details as to how FcRn functions in distinct cellular environments have yet to be unraveled. Recent developments (15, 16) have resulted in model systems that could provide valuable tools for both cellular and molecular studies directed towards a better understanding of FcRn function. The role of FcRn as an IgG transporter offers novel routes towards the generation of therapeutics for use in situations in which IgG delivery is required (e.g. fetal immunization) or a modulation of IgG levels is desirable (e.g. autoimmunity). Thus, understanding how this protein functions has a multitude of potential applications in the treatment of human disease.

FcRn: THE GENES AND PROTEIN

The gene encoding rat FcRn was first isolated by Simister & Mostov in 1989 (17). Unexpectedly, FcRn was shown to comprise a heterodimer of β2-microglobulin and a 45- to 53-kDa protein, suggesting that the heavy chain might be a major histocompatibility complex (MHC) class I homolog. The cloning and sequencing of the heavy-chain gene confirmed this possibility (17) and expanded the function of this class of molecules beyond their known role in antigen presentation. All three extracellular and transmembrane domains of FcRn share homology with the corresponding regions of MHC class I molecules, with much less homology between the cytoplasmic domains (17). The divergence in the cytoplasmic regions is consistent with the different functional activities of the two types of proteins.

The X-ray crystallographic structure of the extracellular domains of FcRn confirmed that it is structurally similar to MHC class I molecules (18). Notably, the peptide groove that is occupied by peptide or, in some cases, glycolipid ligand in classical and nonclassical MHC class I molecules (19–22) is occluded in FcRn. This occlusion is primarily caused by the presence of proline at position 165 of the α2 domain helix, which introduces a kink. However, it is interesting that the introduction of a proline at the corresponding position in the MHC class I molecule H-2D^d does not affect peptide presentation to T cells (23), suggesting that additional structural features of FcRn may result in the closed groove.

More recently the genes encoding both mouse and human FcRn alpha chains have been isolated (24, 25). The rodent and human genes share homology, with mouse and rat FcRn being highly related and the human form more divergent. The identification of human FcRn in human syncytiotrophoblast (25–28) led to the suggestion that it plays a role in the maternofetal transfer of IgGs, which is discussed in more detail below. The isolation and characterization of human FcRn provided an important link between studies of FcRn in rodents and humans.

THE MOLECULAR DETAILS OF THE FcRn-IMMUNOGLOBULIN G INTERACTION

Mapping the Binding Site for FcRn on Immunoglobulin G

A combination of approaches has been used to localize the interaction site for FcRn on IgG. This has involved the analysis of mutated derivatives of human and mouse IgGs/Fc fragments in both in vivo and in vitro binding studies (9, 29–32). The in vivo studies include the analysis of the IgGs or Fc fragments using assays in which FcRn is known to play a role, namely transfer across the neonatal intestine, maternofetal transfer, and serum half-life determination (discussed further below). Activity in these assays almost invariably provides an indication of FcRn affinity (9, 29, 31). The studies have resulted in the identification of several conserved amino acids located at the CH2-CH3 domain interface that play a central role in the interaction of rat or mouse FcRn with human or mouse IgGs. Ile253 and His310 are key players in the interaction (9, 29–31). These residues are highly conserved across species (Table 1), and their location on the three-dimensional structure of human IgG1 (hIgG1)-derived Fc (33) is shown in Figure 1 (see color insert). His436 plays a minor but significant role in the mouse FcRn-IgG interaction (31). The lack of conservation of this amino acid across species (Table 1) is consistent with more limited involvement.

TABLE 1 Variations of IgG sequences in the region involved in the binding of FcRn

		Amino Acid Sequence at Position:														
		252	**253**	**254**	**255**	**256**	**257**	**307**	**308**	**309**	**310**	**311**	**433**	**434**	**435**	**436**
mouse	IgG1	Thr	Ile	Thr	Leu	Thr	Pro	Pro	Ile	Met	His	Gln	His	Asn	His	His
	IgG2a	Met	Ile	Ser	Leu	Thr	Pro	Pro	Ile	Gln	His	Gln	His	Asn	His	His
	IgG2b	Met	Ile	Ser	Leu	Thr	Pro	Pro	Ile	Gln	His	Gln	Lys	Asn	Tyr	Tyr
	IgG3	Met	Ile	Ser	Leu	Thr	Pro	Pro	Ile	Gln	His	Gln	His	Asn	His	His
rat	IgG1	Thr	Ile	Thr	Leu	Thr	Pro	Pro	Ile	Leu	His	Gln	His	Asn	His	His
	IgGa	Thr	Ile	Thr	Leu	Thr	Pro	Pro	Ile	Val	His	Arg	His	Asn	His	His
	IgG2b	Leu	Ile	Ser	Gln	Asn	Ala	Pro	Ile	Gln	His	Gln	His	Asn	His	His
	IgG2c	Met	Ile	Thr	Leu	Thr	Pro	His	Ile	Gln	His	Gln	His	Asn	His	His
human	IgG1	Met	Ile	Ser	Arg	Thr	Pro	Thr	Val	Leu	His	Gln	His	Asn	His	Tyr
	IgG2	Met	Ile	Ser	Arg	Thr	Pro	Thr	Val	Val	His	Gln	His	Asn	His	Tyr
	IgG3*	Met	Ile	Ser	Arg	Thr	Pro	Thr	Val	Leu	His	Gln	His	Asn	Arg	Phe
	IgG4	Met	Ile	Ser	Arg	Thr	Pro	Thr	Val	Leu	His	Gln	His	Asn	His	Tyr

*Allotype containing Arg instead of His at Position 435

In functional studies in mice, mutation of His433 to alanine in recombinant Fc fragments derived from both human and mouse IgG1 does not affect the activity of the protein (31, 32), although simultaneous mutation of His433 and Asn434 does have a moderate effect (29). In contrast, mutation of His435 to alanine results in a dramatic loss of function (31, 32). Thus, the effect of the double His433/Asn434 mutation is most likely caused by perturbation of the conformation of the critical residue His435. The spatial location of these amino acids is shown in Figure 1. A more recent study in our laboratory has indicated that the sequence difference of hIgG1 and hIgG3 (G3m,s$^-$,t$^-$ allotype) at position 435 (His in IgG1; Arg in G3m,s$^-$,t$^-$ allotype of IgG3) is responsible for the lower activity of IgG3 in FcRn-mediated functions, providing further support for a role for residue 435 in the FcRn interaction (32). In contrast to our in vivo and in vitro analyses, surface plasmon resonance analyses of chimeric human-mouse IgGs (containing the hIgG4 constant region) in which His435 was mutated to arginine detected no significant effect on the pH dependence of the FcRn-IgG interaction, and, in the same study, His433 was implicated in FcRn binding (30). There is therefore a discrepancy between the relative roles of His433 and His435 in the FcRn (rodent)-IgG interaction, and this is possibly a reflection of the different systems and approaches used. However, the available data unequivocally demonstrate the involvement of IgG histidines in FcRn binding, and Table 1 shows that these histidines are highly conserved in mouse, rat, and human. This provides an explanation for the strict pH dependence (binding at pH 6–6.5; very weak or undetectable binding at pH 7.2) of the FcRn-IgG (or Fc) interaction that is observed by using soluble recombinant FcRn in binding studies (30, 34, 35) and cell-binding assays (3, 4, 6, 36). This mechanism of achieving pH-dependent binding in ranges of pH that are physiologically relevant avoids the need to invoke conformational changes and could be exploited in the engineering of other proteins. Indeed, the crystallographic analysis of rat FcRn at pH 6.5 and pH 8 shows no major conformational differences between the two forms (37). By analogy, other proteins, such as the hemochromatosis protein HFE, bind to the transferrin receptor in a pH-dependent way with binding at slightly basic pH and release at acidic pH, that is, the reverse pH dependence to that seen with FcRn, and this is again mediated by histidines (38).

Further analyses of the activity of rat IgGs in FcRn-mediated functions in mice revealed a role for several additional residues in proximity to the CH2-CH3 domain interface (39). The putative involvement of these amino acids was probed by transplanting the rat IgG sequences (Table 1) onto the corresponding positions in the highly homologous mouse IgG1 Fc region. Analysis of the resulting recombinant Fc fragments demonstrated that amino acids at position 257 and, to a lesser extent, positions 307 and 309 play a role in the FcRn-Fc interaction. The location of residue 257 is shown in Figure 1. The study also excluded the involvement of residues 386 and 387, which are located in an exposed loop in the vicinity of the FcRn interaction site (40). The role of residues 257, 307, and 309 is less marked than that of Ile253, His310, and His435. However, sequence variations at 257,

307, and 309 can be used to explain the different affinities of rat IgGs for FcRn [affinities decreasing in the order IgG2a > IgG1 > IgG2c > IgG2b (39)]. This ranking of affinities is consistent with the binding activities of rat IgGs to isolated neonatal rat brush borders, with the exception of rat IgG2c, which was reported to have undetectable activity (41). It is possible that the discrepancies between the two studies (39, 41) are caused by functional differences between mouse and rat FcRn and/or variations in the sources of the ligands used.

Consistent with the functional data, the 6-Å resolution structure of the rat FcRn-Fc complex indicated that FcRn interacts with Fc residues located at the CH2-CH3 interface (40). Furthermore, fragment B of staphylococcal protein A (SpA), which, from the three-dimensional structure of an SpA (fragment B)-human Fc complex (33), is known to bind to amino acids at this interdomain interface, competes with IgG for binding to FcRn (42). Earlier studies indicated that blockade of the FcRn interaction site on rabbit IgG by SpA (or fragment B) resulted in a dramatic reduction in serum half-life (43), consistent with the more recent data in mice supporting a role for FcRn in serum IgG homeostasis (10–13).

The Conformational Dependence of the FcRn Interaction Site on Immunoglobulin G

It is interesting that analyses of chimeric IgGs comprising mouse variable regions linked to human constant-region domains indicate that amino acids distal to the CH2-CH3 domain interface may also be involved in regulating serum half-life in mice and, by extension, in binding to FcRn (44). It will be of interest to determine the affinities for FcRn of these recombinant IgGs in which the constant-region domains of IgG2 and IgG1 were shuffled with the corresponding regions of IgG3 and IgG4, respectively. The data obtained for these engineered IgGs led to the suggestion that the FcRn interaction can be affected by long-range conformational effects of amino acids that are distal to the FcRn footprint (44). In this respect, critical IgG residues for the FcRn interaction (9, 29–31) are located on three loops that are spatially close but distal in primary amino acid sequence (Figure 1). This suggests that they might be highly dependent on the conformation of the β strands that support them and also on the relative disposition of the CH2 and CH3 domains. Several observations are consistent with this explanation. (*a*) The effect of mutation (Pro to Ala) at position 257 of a recombinant mouse IgG1-derived Fc fragment on serum half-life is most likely from perturbation of the conformation of the loop encompassing Ile253 (39). This same sequence difference is probably responsible for the shorter serum half-life of rat IgG2b relative to rat IgG1/IgG2a in mice (39). (*b*) Mutation of Glu333 to Ala results in a significant decrease in serum persistence of a recombinant Fc fragment [mouse IgG1-derived (V Ghetie & ES Ward, unpublished observations)]. This amino acid is located in a β strand in the CH2 domain that is on the 'interior' of the CH2 domain and therefore not suitably positioned for direct interaction with FcRn (33). (*c*)

Removal of the hinge region by recombinant techniques from an Fc fragment results in a shorter serum half-life (45). The role of the hinge appears to be to constrain the CH2 domains such that the configuration of the FcRn interaction site at the CH2-CH3 domain interface is optimal, rather than through direct FcRn:hinge interactions. This suggestion was validated by replacing the wild-type hinge sequence with a synthetic hinge that has a distinct sequence but allows -S-S-linked homodimerization of the Fc (CH2-CH3) domain monomers. This engineered, disulfide-constrained Fc fragment has the same half-life as an Fc fragment containing the wild-type hinge sequence (45). In this context, the longer and more flexible hinge region of hIgG3 compared with hIgG1 (46) might account, in part at least, for the shorter serum half-lives of IgG3 hinge-containing chimeras compared with those containing IgG2-derived hinge sequences (44).

The data from these mutagenesis studies suggest that the FcRn interaction site on IgG or Fc is highly conformational dependent. This may make it a challenging task to mimick FcRn binding by an IgG-derived peptide, in contrast to the demonstrated ability of a CH2 domain-derived peptide to compete with IgGs for binding to C1q (47). However, the demonstration that an Fc region-derived peptide encompassing residues 308–317 blocks the binding of SpA to IgG (48) suggests that individual strands encompassing key FcRn interaction residues may be effective mimics.

Is There a Relationship Between the FcRn and FcγR Interaction Sites on Immunoglobulin G?

Mutagenesis studies from several different laboratories have indicated that residues in the lower hinge region of IgG are important for binding to FcγRI, FcγRII, and/or FcγRIII (49–55). However, each receptor sees this site with a slightly different footprint, and, in addition, there are two other regions that play a role in binding to FcγRI (Pro331) and FcγRII (Glu318) (50). These regions are spatially close to the lower hinge (33). The available data indicate that FcγR binding is also dependent on glycosylation of IgG (56–58) and that even minor alterations in carbohydrate structure can have an effect on this activity (59, 60). Thus, the FcRn interaction site appears to be distinct from the region of IgG involved in FcγR binding. Furthermore, at least for some species/isotypes, FcRn binding is not affected by the absence of CH2 domain glycosylation (29, 35).

It is interesting that the recent crystal structures of human FcγRIIa (61) and FcγRIIb (62) resulted in two interaction models for FcγRII-IgG complex formation that are fundamentally different. FcγRIIa was solved as a crystallographic dimer in which the binding regions of each monomer were brought into proximity to generate a single IgG interaction site, and the IgG-FcγRIIa interaction was postulated to involve the lower hinge region of IgG (61). This model is consistent with data from mutagenesis studies (reviewed in 54). In contrast, FcγRIIb was suggested to bind to IgG or Fc in the region encompassing the CH2-CH3 domain interface, with both sites on IgG occupied to form a symmetric 1:2 IgG:FcγRIIb

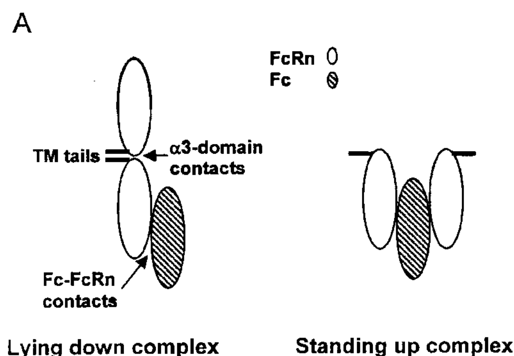
complex (62). The validity of the model proposed for FcγRIIa has been investigated by carrying out surface plasmon resonance-binding studies with recombinant, soluble human FcγRIIa or mouse FcγRI and mouse/hIgGs (M Hogarth, personal communication). For both FcγRs, binding to IgG was not inhibited by either SpA or recombinant soluble FcRn, providing clear evidence that the FcγR interaction site does not encompass the CH2-CH3 domain interface. Consistent with this are earlier observations that mutations of IgGs affecting FcγR binding do not alter the activity of the IgG in a function that is known to be mediated by FcRn, namely serum persistence (63).

Mapping the Interaction Site for Immunoglobulin G on FcRn

Although the X-ray crystallographic structure of rat Fc-FcRn complexes indicated the potential contacts for Fc on FcRn (40), the resolution was not sufficiently high to unequivocally define the amino acids involved. The binding site of mouse/rat IgG on rat FcRn has therefore been mapped using site-directed mutagenesis followed by analysis of the mutants by in vitro binding assays (42, 64). This has resulted in the identification of several α2 domain and one β2-microglobulin residue as playing a direct role in the interaction. The α2 domain residues (Glu117, Glu132, Trp133, Glu135, and Asp137) are reasonably well conserved across species (17, 24, 25), and their location on the rat FcRn structure (18) is shown in Figure 2 (see color insert). The acidic nature of four of these amino acids, together with the role of IgG histidines in binding, suggests that electrostatic forces may play a predominant role in mediating the Fc/IgG-FcRn interaction. Hydrophobic effects may also be involved, and it is plausible that Trp133 interacts with the conserved Ile253 of IgGs (64). Residue 1 (Ile) of β2-microgobulin, which is conserved in rats, mice, and humans (46), has also been shown to be involved in binding to IgG but to a lesser extent than the α2 domain residues (64). This amino acid has been proposed to contact IgG via hydrophobic interactions in the vicinity of residue 309 and/or 311. Alternatively, this N-terminal residue may play a role through more indirect effects on Glu117 in the α2 domain (64). The α2 domain residues that are involved in binding to IgG are located in close proximity on a region at the end of the α2 domain helix (18; Figure 2). Residues 84–86 of the α1 domain are spatially close to this region of FcRn, but, from mutagenesis studies, have been shown not to contact IgG (64). The majority of contacts for IgG on FcRn therefore entail amino acids in the α2 domain. Furthermore, the interaction of IgG with FcRn occurs in a mode distinct from that reported for T-cell receptor-peptide-MHC class I interactions (65, 66), in which the T-cell receptor footprint spans the surface of the two MHC helices and binds antigenic peptide in a diagonal orientation.

Much evidence from in vitro studies has supported a role for FcRn dimerization in high-afffinity binding to IgG, resulting in the formation of "lying-down" complexes comprising an FcRn dimer bound to one IgG/Fc molecule (42, 64, 67,

68; Figure 3). First, FcRn dimers mediated via α3 domain contacts have been observed in several different crystal forms of rat FcRn (18, 40). Second, mutation of Gly191, His250, His251, and a loop encompassing residues 219–224 (all α3 domain residues) results in a reduction in the affinity of FcRn for IgG (42, 64). The effects of these mutations have been interpreted to be caused by destabilization of FcRn dimerization and/or loss of contacts to an IgG molecule bound to an adjacent FcRn molecule in a lying-down complex (42, 64, 69). Third, immobilization of FcRn molecules on a biosensor chip favors the formation of the dimeric, high-affinity form of FcRn (68). In addition, oriented coupling of FcRn via exposed, engineered cysteines located at different sites indicates that the ori-



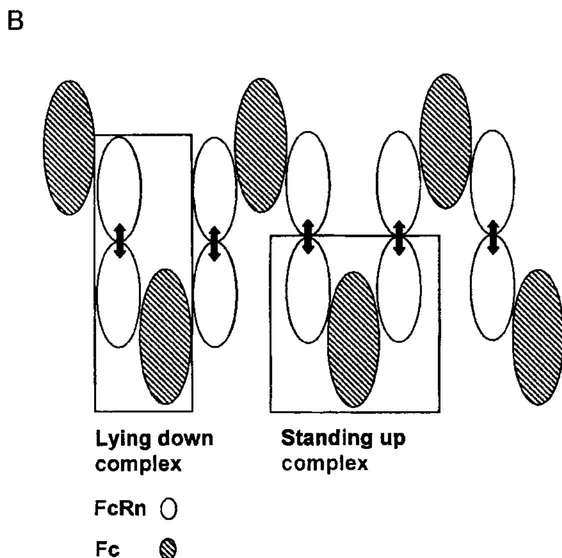


Figure 3 *A*. Schematic representation of "lying-down" and "standing-up" FcRn-Fc complexes (40). Both complexes comprise a stoichiometry of 1 Fc:2 FcRn molecules, but they show fundamental differences in the configuration of the constituent molecules. Lying-down complexes comprise Fc bound to an FcRn-FcRn dimer, with the dimer mediated primarily via α3 domain contacts and associated carbohydrate (37). In contrast, the standing-up complex comprises a symmetric FcRn-Fc-FcRn configuration. However, in the lying-down complex, additional contacts may be made between the Fc molecule and the secondary, that is, distal, FcRn molecule (64), but for simplicity these are not shown. *B*. Schematic representation of oligomeric ribbons (82) in which both standing-up and lying-down complexes coexist. For simplicity the transmembrane regions (TM) of FcRn are not shown. The *arrows* indicate the contacts that mediate FcRn dimerization.

entation of FcRn immobilization affects the extent of dimerization and, in turn, the affinity of its interaction with IgG (67).

More recent X-ray crystallographic studies have demonstrated that α3 domain-associated carbohydrate (at Asn225) interacts to form much of the contacts at the FcRn dimer interface via a "carbohydrate handshake" (37). This indicates that, depending on the expression host used to make recombinant FcRn, the type of carbohydrate could affect the stability of FcRn dimerization with consequent effects on affinity measurements of the Fc/IgG-FcRn interaction (37). However, neither the carbohydrate addition site nor Gly191 in the α3 domain is conserved in human FcRn (25), suggesting that, if this FcRn species forms an analogous dimer, then other molecular mechanisms must be involved.

The Stoichiometry of the Fc/Immunoglobulin G-FcRn Interaction

The presence of two potential binding sites for FcRn on IgG suggested that FcRn might bind to ligand in a symmetric 2:1 "standing up" complex (Figure 3A). For rat FcRn-Fc complexation, this possibility was supported by isothermal-titration calorimetry and column-binding assays (70). However, more recent studies have indicated that this symmetric complex may not always be formed (35). Although the need for two functional FcRn interaction sites on a recombinant Fc fragment was observed using in vivo studies in mice to assess FcRn function (71), the stoichiometry of the complex formed between recombinant mouse FcRn and mouse Fc was observed to be 1:1 (35). This apparent discrepancy prompted us to propose a model analogous to the situation described as "half-sites reactivity" in enzymology (14, 72, 73). In this model, we predicted that the FcRn interaction sites on IgG might not be equivalent and that, when binding occurs on one side of the Fc region, the affinity of the other site decreases. The segmental flexibility of IgG (74–76) would be consistent with such a model. This is also reminiscent of data obtained for the FcεRI-IgE interaction, in which a 1:1 complex is observed despite the presence of two potential interaction sites on IgE (77, 78). Furthermore, by fluorescence resonance energy transfer methods, evidence to support a bent configuration for IgE in solution has been obtained (76). In the same study (76), the flexibility of IgG was observed to be greater than that of IgE. Furthermore, for IgE the asymmetry is so extreme that it is not possible to detect the binding of a second FcεRI molecule to IgE.

An asymmetric model for the FcRn-IgG interaction has recently been supported by modeling studies (69) and equilibrium gel filtration (79) or sedimentation equilibrium analyses (80). The high degree of flexibility of IgG in solution (76) is consistent with the concept that FcRn binding induces asymmetry (69) rather than the asymmetry being preformed before FcRn docking, and Fc distortion after FcRn binding was suggested in the earlier crystallographic analysis of rat Fc with FcRn (40). It has also been reported that the stoichiometry of the IgG-FcRn interaction varies depending on the IgG species/isotype and glycosylation

state of the recombinant FcRn (37, 79). More generally, it is probable that the binding observed in solution with soluble (or biosensor chip-bound) FcRn might not be representative of the physiological situation when FcRn has both transmembrane regions and a cytoplasmic tail that might mediate direct or indirect interactions via adaptor proteins etc. Thus, although valuable data concerning the IgG-FcRn interaction have been obtained from in vitro binding studies with recombinant FcRn, the validity of extrapolating these findings to the in vivo situation may be questioned. It is clear, however, that in vitro binding affinity of an IgG or Fc for recombinant FcRn almost invariably correlates with the in vivo activity in FcRn-mediated functions (31, 81).

A model that incorporates both standing-up FcRn-IgG:FcRn complexes and lying-down IgG-FcRn dimer (through α3 domain contacts) complexes observed in the FcRn-Fc cocrystal has been proposed by Bjorkman and colleagues (82). This embraces data supporting the existence of both types of complex (40, 67, 68) and proposes the formation of oligomeric ribbons (Figure 3B). Such ribbons have been suggested to mediate membrane vesicularization by bridging adjacent membranes (83). Some in vitro data support the model (40, 67, 68), but to date the existence of oligomeric ribbons in vivo has not been proven owing to obvious technical limitations. However, this model explains the effects of mutations at both the FcRn-Fc interaction site and the FcRn-FcRn dimer interface (42, 64).

MULTIPLE FUNCTIONS FOR FcRn

FcRn: the Neonatal Fc Receptor

FcRn was first identified as the Fc receptor responsible for transferring maternal IgGs from mothers' milk across the intestinal epithelial cells of the neonatal gut of rodents (1–6). In rodents, this is the major route by which IgGs are transferred, whereas, in humans, essentially all IgGs are transferred prenatally across the placenta (see below). Early studies of rodent FcRn, using isolated rat brush border membranes, indicated that FcRn binds to IgG in a pH-dependent way, with binding at pH 6–6.5 and an undetectable interaction at pH 7.2 (3, 4, 6, 36). This result, combined with histochemical analyses, was used to build a picture of how FcRn functions as an IgG transporter (84). IgG binds to the luminal surface of the epithelial cells via FcRn and the FcRn-IgG complexes are taken up by receptor-mediated endocytosis. The complexes are then transcytosed across the cells and delivered via exocytosis at the basolateral surface of the cells. FcRn-IgG dissociation occurs at this site owing to the instability of the interaction at pH 7.4 (3, 4, 6, 36). This model has formed the basis of our current understanding of FcRn function, but variations on this theme need to be invoked when this receptor operates in different cellular environments (discussed below). Several observations indicate that FcRn is the only IgG transporter involved in the delivery of maternal IgGs from mothers' milk. (*a*) In β2-microglobulin-deficient (β2m −/−)

mice that do not express functional FcRn, maternal IgGs are not transferred (8, 85). (*b*) Analyses of mutated murine IgG1-derived Fc fragments in assays directed towards determining their ability to be transferred across the neonatal gut indicate a direct correlation between binding affinity for FcRn and the activity in inhibiting transfer of wild-type IgG1 (29, 31).

FcRn and the Maternofetal Transfer of Immunoglobulin Gs

The isolation and characterization of rat FcRn from the yolk sac endoderm led to the proposal by Roberts et al (7) that this Fc receptor is involved in the maternofetal transfer of IgGs. Quantitatively, this represents the minor route of IgG delivery from mother to young in rodents. The studies demonstrated that the FcRn-IgG interaction occurred in apical vesicles and not on the yolk sac cell surface (7). In addition, cell surface expression of FcRn could not be detected. This led to the suggestion that FcRn binding to IgG occurs only after nonspecific uptake via fluid-phase pinocytosis by yolk sac. Bound IgG is then transported across the cell and delivered to the basolateral surface, where it is released at the slightly basic pH. Binding in acidic endosomes is consistent with the known pH dependence of the FcRn-IgG interaction. There is therefore an important mechanistic difference between yolk sac and intestinal transfer. This difference may result in yolk sac transfer being less efficient (particularly at low IgG concentrations), because IgGs are not captured by cell surface receptors, but the difference is apparently a necessary tradeoff for the ability of FcRn to bind ligand in a strictly pH-dependent way. Interestingly, in vitro assays have shown that the neonatal intestine does not need to be bathed in an acidic medium on the apical surface to carry out transcytosis (86), indicating that, even for this tissue, receptor-mediated cell surface binding of IgG is not a prerequisite for uptake. By analogy with intestinal transfer, in neonates, maternofetal IgG transfer is ablated in β2m−/− mice (8, 85). Furthermore, the efficiency of transfer of different IgG isotypes or mutated Fc fragments in mice correlates with binding affinity for FcRn (9). FcRn therefore appears to be solely responsible for IgG transport.

Maternofetal transfer of IgGs in humans shows some specificity insofar as there is preferential transport of some isotypes over others (see below). In addition, the Fc region of IgG is known to be essential (87; reviewed in 88). The isolation of FcRn cDNA from human syncytiotrophoblast suggested that it is responsible for maternofetal transfer (25), consistent with the criteria that this transfer show some specificity and be mediated through the Fc region. In humans, the different organization of the fetal membranes results in IgG transfer across the syncytiotrophoblast of the chorioallantoic placenta rather than the yolk sac, and it is the major, if not sole, route of IgG delivery to offspring. Human FcRn has subsequently been identified at the protein level in syncytiotrophoblast (26–28) and in one study has been reported to be undetectable on the apical membrane (28). Regardless of whether it is on the apical surface, the consensus

appears to be that, in the apical region of the cells, the majority is located in vesicles that are most likely acidic endosomes. However, it is controversial whether human FcRn is expressed in placental endothelial cells (26–28). If not expressed in this location, how IgGs transfer across this cellular barrier to the fetal circulation remains an open question.

Colocalization studies indicate that FcRn associates with IgG in endosomes in syncytiotrophoblast cells (28). Thus, IgG-FcRn association most likely occurs after IgG uptake in acidic, apical endosomes in an analogous way to that proposed for rodent yolk sac. Consistent with this result, IgG uptake by syncytiotrophoblast is coincident with uptake of a fluid-phase marker (89). In this respect, other proteins such as placental alkaline phosphatase and annexin II have been suggested to play a role in mediating IgG uptake, but recent data argue against this (90; reviewed in 88). Furthermore, the expression of FcγRs in placenta (87, 91, 92) suggested that these may play a role in transferring IgGs. However, this is not supported by the selectivity of binding of these FcRs to different IgG isotypes. For example, FcγRIIb binds preferentially to IgG3 compared with IgG4 (54), but this preference does not impinge on the relative efficiency of maternofetal transfer of these isotypes (see below). The function of placental FcγR expression is most likely in the clearance of immune complexes, particularly on Hofbauer cells, in which all three forms are expressed (87, 91, 92; reviewed in 88).

The relative transfer efficiencies of hIgGs across the placenta remain a controversial issue, with some studies indicating poor transport of IgG2 (93–95) and others showing equivalent passage of all subclasses (96, 97). The discrepancies are most likely caused by the systems used and obvious ethical limitations on experimentation. The use of an in vitro perfused placental assay that gives a good indication of the ability of a protein/drug to cross the placenta during pregnancy indicated that the hierarchy of transfer is hIgG4 > hIgG1 > hIgG3 > hIgG2 (98). The development of in vitro systems to assess IgG transport across trophoblast cells (15, 99) should provide the opportunity to obtain quantitative data concerning FcRn-mediated transport, and this would have obvious practical implications. However, limitations of such cell systems may be that only one cell type is being investigated, in contrast to the situation during in vivo transfer, when several distinct cellular barriers must be traversed.

A Role for FcRn in Immunoglobulin G Homeostasis

Over 30 years ago, Brambell and colleagues proposed that the receptors mediating transfer of maternal IgGs might be related to the protective receptors that regulate serum IgG half-life (100, 101). These receptors were suggested to function by binding and protecting IgGs against lysosomal degradation; that is, they act as salvage receptors. Consistent with this hypothesis, analyses of the binding affinity for murine FcRn of mutated mouse IgG1-derived Fc fragments with reduced serum persistence indicated that the sites of IgG or Fc that binds to FcRn and

regulates serum half-life closely overlap (29, 31, 102). This result prompted us to analyze the serum half-lives of murine IgG1 and IgG1-derived Fc fragments in mice that are β2m deficient (β2m−/−) and, as a result, do not express functional MHC class I molecules or homologs including FcRn (10). The half-lives of these proteins were abnormally short in these mice (10), and these results have been independently confirmed by others (11, 12). In fact, in β2m−/− mice, the half-life of the wild-type Fc fragment is essentially the same as that of a mutated Fc with no detectable binding affinity for FcRn (10).

The short serum half-lives of IgGs in β2m−/− mice are consistent with observations that, in these mice, the circulating IgG levels are abnormally low despite an apparently normal B-cell compartment (103). The rates of IgG synthesis in these mice are insignificantly different from those in wild-type mice (11). Although we reported differences in IgG synthetic rates between wild-type and β2m−/− mice when using the β phase half-life in the calculation (10), this difference becomes insignificant if the catabolic rate is used (V Ghetie & ES Ward, unpublished data). Consistent with the hypercatabolism that is observed in β2m−/− mice, mice crossed onto a β2m−/− background are also resistant to the IgG-mediated disease, bullous pemphigoid (104). Furthermore, mice crossed onto a β2m−/− background are resistant to the induction of systemic lupus erythematosus after idiotype (16/6Id) immunization (105). This was originally believed to be caused by the lack of classical MHC class I molecules in these mice, but an alternative explanation could be that these mice have abnormally low serum IgG levels. However, recent studies in systemic lupus erythematosus–susceptible mice in which the B cells are engineered to express only surface immunoglobulin have revealed an IgG-independent mechanism for renal and vascular disease (106).

In all studies to date, a good correlation between affinity for FcRn binding and serum half-life has been observed for Fc mutants or IgG variants (29, 31). Significantly, this correlation can be extended to engineered Fc fragments with higher affinity than their parent wild-type molecule. This was carried out by randomly mutating a recombinant murine Fc fragment at three residues located in proximity to the FcRn interaction site (81). These amino acids (Thr252, Thr254, and Thr256) were chosen because they are exposed and not highly conserved across species. The library of mutated fragments was expressed on the surface of bacteriophage and Fc fragments with higher affinity for recombinant mouse FcRn selected (81). One of these Fc fragments with mutations of Thr252, Thr254, and Thr256 to Leu, Ser, and Phe, respectively, had a significantly longer serum half-life than the wild-type Fc. This suggests that it should be possible to combine protein design with selection to generate therapeutic antibodies with increased serum persistence. In this context, a chimeric antibody (mouse variable domains linked to human constant regions) in which the constant region domains were shuffled to generate a hybrid IgG1/IgG4 molecule had a significantly longer half-life in mice than either of the parent IgG1 or IgG4 molecules (44). It will be of interest to unravel the molecular basis of this effect.

Where and How Does Immunoglobulin G Homeostasis Occur?

Much evidence supports the concept that FcRn is ubiquitously expressed in adult tissues (10, 25, 107), in addition to its earlier localization to neonatal gut and yolk sac/placenta. The expression of FcRn in almost every tissue analyzed led us and others to propose that the endothelial cells might be the site at which IgG homeostasis occurs (10, 108). This would be consistent with the close apposition of these cells to the bloodstream and earlier suggestions of Mariani & Strober (109) concerning the site of regulation of serum IgG levels. Distribution studies of murine IgG1, Fc fragments, and anti-FcRn antibodies indicate that the major sites of FcRn function in adult, nonpregnant mice are the skin and muscle, with a lesser involvement of liver and adipose (110). Furthermore, histochemical analyses of mouse muscle and liver indicate that FcRn is expressed in the endothelium of small arterioles and capillaries but cannot be detected in larger vessels such as the central vein and portal vasculature. Functional FcRn can also be isolated from a murine endothelial cell line (110). Interestingly, immunohistochemistry indicates that the steady-state distribution of FcRn in these cells is primarily intracellular, which is reminiscent of the data for rat FcRn expression in yolk sac (7). Analysis of FcRn expression in humans also indicates that it is expressed in endothelial cells (108), and this is consistent with earlier observations that human FcRn is widely expressed throughout adult tissues (25).

A model for how FcRn might function in its role as an IgG homeostat is shown in Figure 4. IgGs are taken up by endothelial cells by nonspecific pinocytosis and then enter acidic endosomes. If the IgGs bind to FcRn, they are salvaged from lysosomal degradation. Paradoxically, the endothelial cells are therefore involved in both the breakdown and salvaging of IgGs. The route by which FcRn takes in salvaging IgGs is far from clear; whether it acts as a recycling or transcytotic receptor or both in its role as the protective receptor is as yet unknown. By analogy with FcRn as an IgG homeostat, it is interesting that, when FcRn is apparently functioning as a transcytotic receptor during maternofetal transfer, significant amounts of IgGs are degraded (3, 111, 112). It is tempting to speculate that FcRn can act in recycling or transcytotic mode (or both), depending on the cell type in which it is expressed. The model (Figure 4) explains how FcRn acts as a homeostat that is finely tuned to regulate serum IgG levels. When IgG levels decrease, more FcRn is available for IgG binding so that an increased amount of IgG is salvaged. Conversely, if serum IgG levels rise, FcRn becomes saturated, and this results in an increase in the proportion of pinocytosed IgGs that are degraded.

A Possible Role for FcRn at Other Sites

In addition to its role in regulating serum IgG levels and transferring maternal IgGs, additional studies have indicated that the transporting function of FcRn is

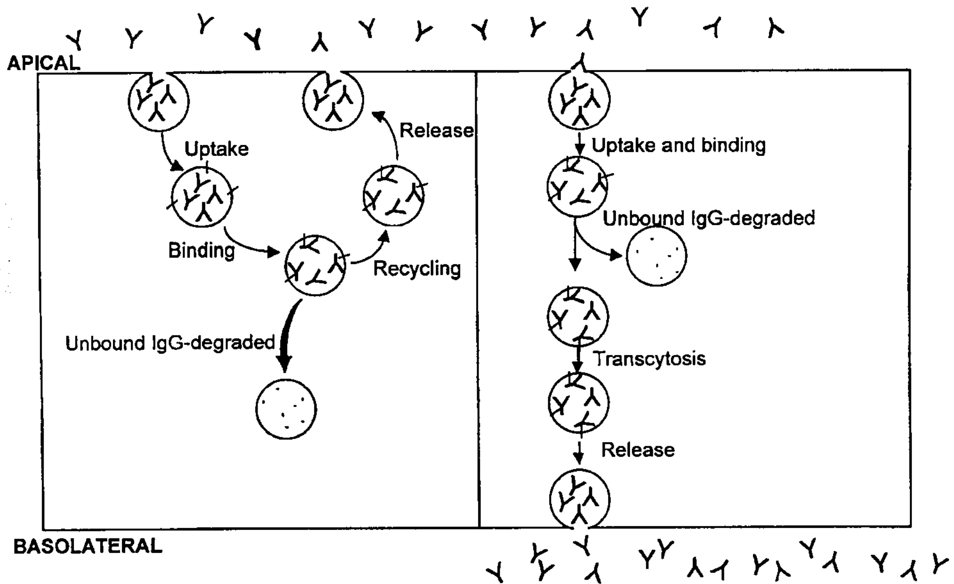


Figure 4 Models for the trafficking of FcRn in its role as an IgG homeostat in endothelial cells. IgGs are initially taken up by nonspecific pinocytosis because the pH of the bloodstream is not permissive for the IgG-FcRn interaction. Pinocytosed IgG molecules (*Y*) bind to free FcRn (−) in acidified endosomes. IgG that does not bind to FcRn is destined for degradation in lysosomes. The available evidence with in vitro cell lines indicates that FcRn can act as either a transcytotic or recycling receptor, and it is as yet unknown in which mode it operates during IgG homeostasis. However, if FcRn transcytoses IgG molecules across endothelial cells, then bidirectional transcytosis by necessity occurs for the IgG to be returned to the serum. Alternatively, IgG may traffic in one or both directions via transcellular channels (142), in addition to being transcytosed by an FcRn-mediated process. Surface expression of FcRn is not shown on this figure, because the available data indicate that the steady-state distribution in endothelial cells is primarily intracellular (110).

exploited for other purposes. For example, the detection of FcRn in human intestinal epithelial cells (113), and the bidirectional transcytosis of IgG across human intestinal T84 cell monolayers (113a) has led to several suggestions concerning its possible function. (*a*) It might be involved in the transfer of passive immunity via oral feeding of IgG. (*b*) FcRn expression at this site might act to detect pathogenic antibody in the lumen of the intestine. (*c*) By analogy with the transfer of immune complexes by the intestinal cells of the neonatal rodent, human FcRn might serve to deliver antigens to induce either immune activation or tolerance.

(*d*) By analogy with the cow (114), transfer of IgG into the gut via an FcRn-mediated process might play a role in the clearance of IgGs.

FcRn has also been identified in the adult rodent liver (110, 115; R Junghans, personal communication) and lactating mammary gland (116). For the liver, hepatocytic expression was suggested to play a role in delivering immune complexes from the canalicular space to the Kupffer cells and the bile (115). However, an inverse correlation between binding affinity for FcRn and transfer into bile has been observed for recombinant Fc fragments in mice (110). In addition, no significant differences were observed for serum-to-bile transport of IgGs in wild-type and β2m−/− deficient mice, and all IgG transport could be accounted for by passive transfer (Junghans, personal communication). These observations suggest that the function of FcRn in hepatocytes might be to act as a recycling receptor whereby it salvages IgGs back into the bloodstream. In contrast, lack of binding to FcRn appears to result in an Fc fragment transiting into the bile by an unknown mechanism (110).

By analogy with the activity of FcRn in liver, a similar function has been attributed to FcRn in the murine lactating mammary gland (116). For both Fc fragments and complete IgGs, an inverse correlation between delivery into milk and affinity for FcRn was observed. This unexpected observation led us to propose that this relationship might result in a balance of IgGs in the neonatal serum that reflects the composition in the mother's bloodstream. More specifically, for transfer of maternal IgGs into neonatal serum, an IgG has to pass through two barriers: the mammary gland and the neonatal gut. Censorship by FcRn at both barriers would result in dramatic decreases of the levels of IgG isotypes that bind relatively weakly to FcRn at the expense of higher-affinity binders. Hence, we envisage that the process of transfer may occur in two steps, the first (maternal blood to milk) at which an inverse correlation between affinity and transfer is observed and the second (gut lumen to neonatal blood) at which a direct correlation is observed (Figure 5). This hypothesis would suggest that FcRn operates primarily in recycling mode in the mammary gland and in transcytotic mode in the neonatal gut.

What relevance does this have to humans, in whom maternofetal transfer is the major, if not sole, route of passive IgG delivery? Interestingly, on day 2 postpartum, the levels of IgGs in colostrum range from ~30% (IgG1) to 2% (IgG4) of the levels in the maternal serum (117), suggesting that some maternal IgG transfer to the neonate may occur in the first few days of life. However, the levels of IgG in the colostrum/milk drop precipitously over the next few days post partum, resulting in IgG concentrations that are ~100-fold lower than in serum (117). This suggests that, if FcRn is expressed in the human lactating mammary gland, it may result in IgGs being recycled away from the milk to exclude this Ig class. In contrast, during colostrum production, it may be that FcRn is not as effective in this recycling function. Although ethical considerations make testing of this hypothesis difficult, it may be possible to address using in vitro cell lines.

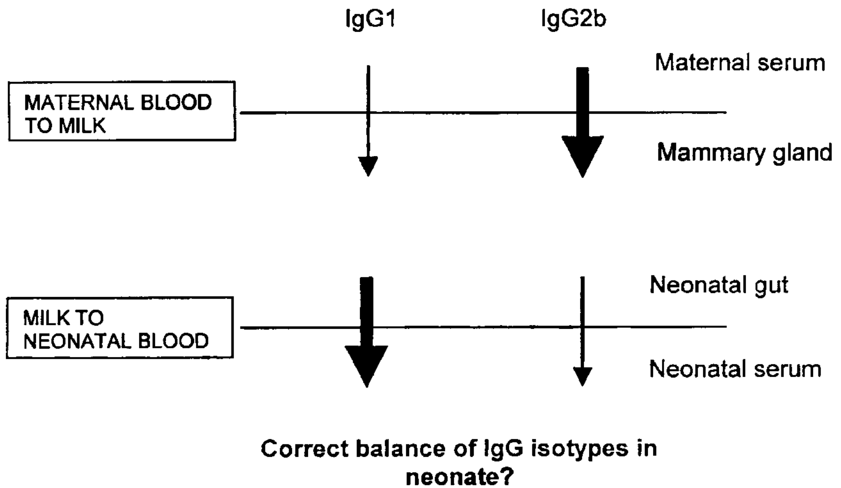


Figure 5 Schematic representation of a model for the transfer of IgGs from the maternal to neonatal bloodstream via mothers' milk, indicating the barriers that must be traversed and their relative transfer efficiencies. In mice, rat IgG1 accumulates in milk less efficiently than rat IgG2b (116), that is, in inverse correlation with affinity for FcRn (39). In contrast, the available data indicate that the transfer of an IgG molecule across the neonatal intestine correlates closely with its affinity for FcRn (29, 31). The outcome of this two-step transfer would be that the IgG subclass ratio in the neonatal serum would closely resemble that in the maternal blood.

ANALYSIS OF THE TRAFFICKING OF FcRn

The analysis of transcytosis of human IgGs across in vitro monolayers of ex vivo trophoblast cells has been reported (99). More recently this has been extended to the use of the BeWo choriocarcinoma cell line (15). For both systems, IgG transport occurs preferentially in the apical to basolateral direction, rather than the opposite direction. Although preferential binding of IgG to the apical surface of BeWo cells at pH 6.0 indicates that human FcRn is present on the cell membrane, this mode of binding is unlikely to be operative in vivo, where the cells are bathed at pH 7.4 [at which only nonspecific binding could be detected (15)]. The data from these analyses are consistent with the proposed model for transcytosis across the rat yolk sac (7) and also with IgG-human colocalization studies in isolated human syncytiotrophoblast (28). Thus, this in vitro system should be a valuable tool to study human FcRn function in the placental trophoblast.

To analyze the role of the cytoplasmic tail of FcRn in intracellular sorting, polarized Madin-Darby canine kidney (MDCK) cells have been transfected with FcγRIIb-rat FcRn cytoplasmic tail chimeras (16). Several types of constructs were made, but the most informative are the wild type, a variant in which the di-leucine

motif was mutated to di-alanine, and a truncated mutant with essentially no cytoplasmic tail. The di-leucine motif has been shown to mediate basolateral sorting and receptor internalization for many different receptors [e.g. FcγRII (118) and the CD3γ chain of the T-cell receptor (119)]. However, for rat FcRn the di-leucine motif appears to play no role in basolateral sorting, although truncation of almost all of the cytoplasmic tail resulted in reduced transport to the basolateral surface. This suggests that there may be an as yet unidentified motif involved in sorting of the FcγRII-FcRn chimera to the basolateral surface. In contrast to its lack of involvement in basolateral sorting, the di-leucine motif mediates efficient internalization of FcRn (16). It is interesting that receptor internalization was not completely ablated in the di-alanine variant, leading to the suggestion that a casein kinase II phosphorylation site in the cytoplasmic domain (N-terminal to the di-leucine motif) might also regulate the surface expression of rat FcRn. Such casein kinase II sites have been reported to play a role in receptor internalization in other systems (120). In contrast to the di-leucine motif, this site is not conserved in human FcRn (17, 25), suggesting that there may be subtle differences in FcRn trafficking across species.

In MDCK cell transfectants expressing the FcγRIIb-FcRn chimera, transcytosis occurred in both directions but was more efficient in the basolateral-to-apical direction than vice versa (16). Moreover, the apical surface was more active in recycling than the basolateral surface. However, these studies were carried out using anti-FcγRIIb Fab fragments, and whether these biases in transcytosis and recycling are seen with natural ligand remains to be analyzed. The MDCK system has provided much valuable information concerning the trafficking of other receptors (121, 122) and should be useful for the elucidation of the molecular mechanisms involved in FcRn function.

PROSPECTS: DOES FcRn HAVE RELEVANCE IN THE CLINIC?

The apparently diverse roles of FcRn are linked by the ability of FcRn to bind and traffic IgGs within and across cells. The observation that the catabolic rate of IgG is dependent on its plasma concentration (123), known as the concentration-catabolism phenomenon, was until recently without explanation. However, the identification of FcRn as the receptor that regulates serum IgG homeostasis gives a molecular explanation for this phenomenon. This leads to the question of how FcRn expression is regulated, because fluctuations in FcRn levels would in turn affect serum IgG concentrations. To date, little is known about this regulation, but the presence of motifs for two cytokine-inducible factors [nuclear factor (NF)–interleukin (IL)-6 and NF-1)] upstream of mouse FcRn (124) suggests that it may be upregulated during the acute phase of an immune response. It is tempting to suggest that dysregulation of FcRn expression may be involved in situations in

which hypercatabolism is observed, such as after burns (125) and in myotonic dystrophy (126). Furthermore, it is possible that some types of IgG deficiencies such as familial idiopathic hypercatabolism (127) may be caused by abnormalities in FcRn expression or function.

The role of FcRn as an IgG homeostat suggests that the modulation of FcRn function and/or expression might be an effective approach for the treatment of IgG-mediated disease. For example, in autoimmune diseases in which pathogenic IgGs are involved, blockade of FcRn function might be an effective treatment modality (128). Indeed, the use of intravenous immunoglobulin has been shown to be efficacious in such situations through a mechanism that involves the induction of hypercatabolism of endogenous IgGs (129). The synergism that is observed between intravenous immunoglobulin therapy and methylprednisone in the treatment of Guillain-Barre syndrome (130) is most likely caused by the ability of glucocorticoids to decrease FcRn expression at the transcriptional level (131). This explanation is also consistent with the earlier report that hydrocortisone treatment results in hypogammaglobulinemia (132). An alternative way of regulating FcRn function would be to generate novel FcRn ligands that block IgG binding or affect FcRn trafficking, and our expanding knowledge of FcRn at the molecular level may facilitate this task.

Understanding the molecular basis by which IgGs persist in the serum is of relevance to the engineering of improved antibodies for use in therapy (81, 133). For example, for the passive delivery of antitumor antibodies [e.g. anti-HER-2 (134) and anti-CD20 (135)], a longer half-life of the IgG could result in improved efficacy and the need for fewer doses. Alternatively, for use in imaging, it is desirable to have a short half-life and, although this can be achieved by using Fab fragments, it is now also possible to engineer complete antibodies with single amino acid substitutions [e.g. Ile253 to Ala (31, 32)], which would be predicted to have reduced serum persistence.

An additional FcRn function that could be exploited in the clinic is the passive delivery of therapeutic antibodies in maternofetal medicine. There are many situations in which this might be useful, but one may be in the specific delivery of antipathogen IgGs. In this respect, the engineering of IgGs with higher affinities for FcRn is attractive (81), because these would be expected to be transferred more efficiently. Although it has been reported that maternally derived IgGs can adversely affect the neonatal response to immunization (136), less is known about the effects of IgGs on T-cell responses. In fact, in vitro analyses have indicated that some IgGs can enhance (137, 138) or suppress (139, 140) T-cell responses to specific epitopes, most likely via effects on antigen processing. It is important that a recent study in mice has demonstrated that maternal IgG does not appear to have a detrimental effect on helper T-cell responses (141). Furthermore, early prime-boosting regimens can result in normal secondary responses even in the presence of passively transferred IgG (141). This may allay concerns about interference effects of maternally derived antibodies on subsequent vaccination of the

neonate, but has yet to be tested for the panoply of pathogens for which vaccines exist.

Finally, the expression of FcRn and its possible function at other sites such as the adult intestine (113) and liver (110, 115) (Junghans, personal communication) remain to be more fully investigated and could result in yet more roles being defined for this intriguing MHC class I relative.

Note Added in Proof

Recent studies by Martin and Bjorkman (143) have demonstrated that the "lying down" FcRn dimer-Fc complex proposed earlier by Bjorkman and colleagues (42, 64, 67, 68) does not exist in solution. However, this does not exclude the possibility that these complexes exist under physiological conditions when FcRn is membrane bound.

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LITERATURE CITED

1. Jones EA, Waldmann TA. 1972. The mechanism of intestinal uptake and transcellular transport of IgG in the neonatal rat. *J. Clin. Invest.* 51:2916–27
2. Rodewald R. 1973. Intestinal transport of antibodies in the newborn rat. *J. Cell Biol.* 58:189–211
3. Rodewald R. 1976. pH-dependent binding of immunoglobulins to intestinal cells of the neonatal rat. *J. Cell Biol.* 71:666–69
4. Rodewald R. 1980. Distribution of immunoglobulin G receptors in the small intestine of the young rat. *J. Cell Biol.* 85:18–32
5. Rodewald R, Abrahamson DR. 1982. Receptor-mediated transport of IgG across the intestinal epithelium of the neonatal rat. *Ciba Found. Symp.* 94:209–32
6. Wallace KH, Rees AR. 1980. Studies on the immunoglobulin-G Fc-fragment receptor from neonatal rat small intestine. *Biochem. J.* 188:9–16
7. Roberts DM, Guenthert M, Rodewald R. 1990. Isolation and characterization of the Fc receptor from the fetal yolk sac of the rat. *J. Cell Biol.* 111:1867–76
8. Zijlstra M, Bix M, Simister NE, Loring JM, Raulet DH, Jaenisch R. 1990. Beta

2-microglobulin deficient mice lack CD4–8+ cytolytic T cells. *Nature* 344:742–46
9. Medesan C, Radu C, Kim JK, Ghetie V, Ward ES. 1996. Localization of the site of the IgG molecule that regulates maternofetal transmission in mice. *Eur. J. Immunol.* 26:2533–36
10. Ghetie V, Hubbard JG, Kim JK, Tsen MF, Lee Y, Ward ES. 1996. Abnormally short serum half-lives of IgG in beta 2-microglobulin-deficient mice. *Eur. J. Immunol.* 26:690–96
11. Junghans RP, Anderson CL. 1996. The protection receptor for IgG catabolism is the beta2-microglobulin-containing neonatal intestinal transport receptor. *Proc. Natl. Acad. Sci. USA* 93:5512–16
12. Israel EJ, Wilsker DF, Hayes KC, Schoenfeld D, Simister NE. 1996. Increased clearance of IgG in mice that lack beta 2-microglobulin: possible protective role of FcRn. *Immunology* 89:573–78
13. Christianson GJ, Brooks W, Vekasi S, Manolfi EA, Niles J, Roopenian SL, Roths JB, Rothlein R, Roopenian DC. 1997. Beta 2-microglobulin-deficient mice are protected from hypergammaglobulinemia and have defective antibody responses because of increased IgG catabolism. *J. Immunol.* 159:4781–92
14. Ghetie V, Ward ES. 1997. FcRn: the MHC class I-related receptor that is more than an IgG transporter. *Immunol. Today* 18:592–98
15. Ellinger I, Schwab M, Stefanescu A, Hunziker W, Fuchs R. 1999. IgG transport across trophoblast-derived BeWo cells: a model system to study IgG transport in the placenta. *Eur. J. Immunol.* 29:733–44
16. Stefaner I, Praetor A, Hunziker W. 1999. Nonvectorial surface transport, endocytosis via a Di-leucine-based motif, and bidirectional transcytosis of chimera encoding the cytosolic tail of rat FcRn expressed in Madin-Darby canine kidney cells. *J. Biol. Chem.* 274:8998–9005
17. Simister NE, Mostov KE. 1989. An Fc receptor structurally related to MHC class I antigens. *Nature* 337:184–87
18. Burmeister WP, Gastinel LN, Simister NE, Blum ML, Bjorkman PJ. 1994. Crystal structure at 2.2 Å resolution of the MHC-related neonatal Fc receptor. *Nature* 372:336–43
19. Bjorkman PJ, Saper MA, Samraoui B, Bennett WS, Strominger JL, Wiley DC. 1987. Structure of the human class I histocompatibility antigen, HLA-A2. *Nature* 329:506–12
20. Bjorkman PJ, Saper MA, Samraoui B, Bennett WS, Strominger JL, Wiley DC. 1987. The foreign antigen binding site and T cell recognition regions of class I histocompatibility antigens. *Nature* 329: 512–18
21. Zeng Z, Castano AR, Segelke BW, Stura EA, Peterson PA, Wilson IA. 1997. Crystal structure of mouse CD1: an MHC-like fold with a large hydrophobic binding groove. *Science* 277:339–45
22. Joyce S, Woods AS, Yewdell JW, Bennink JR, De Silva AD, Boesteanu A, Balk SP, Cotter RJ, Brutkiewicz RR. 1998. Natural ligand of mouse CD1d1: cellular glycosylphosphatidylinositol. *Science* 279:1541–44
23. Plaksin D, Polakova K, Mage MG, Margulies DH. 1997. Rigidification of the alpha2 helix of an MHC class I molecule by a valine to proline mutation in position 165 does not prevent peptide- specific antigen presentation. *J. Immunol.* 159:4408–14
24. Ahouse JJ, Hagerman CL, Mittal P, Gilbert DJ, Copeland NG, Jenkins NA, Simister NE. 1993. Mouse MHC class I-like Fc receptor encoded outside the MHC. *J. Immunol.* 151:6076–88
25. Story CM, Mikulska JE, Simister NE. 1994. A major histocompatibility complex class I-like Fc receptor cloned from human placenta: possible role in transfer

of immunoglobulin G from mother to fetus. *J. Exp. Med.* 180:2377–81
26. Simister NE, Story CM, Chen HL, Hunt JS. 1996. An IgG-transporting Fc receptor expressed in the syncytiotrophoblast of human placenta. *Eur. J. Immunol.* 26:1527–31
27. Leach JL, Sedmak DD, Osborne JM, Rahill B, Lairmore MD, Anderson CL. 1996. Isolation from human placenta of the IgG transporter, FcRn, and localization to the syncytiotrophoblast: implications for maternal-fetal antibody transport. *J. Immunol.* 157:3317–22
28. Kristoffersen EK, Matre R. 1996. Colocalization of the neonatal Fc gamma receptor and IgG in human placental term syncytiotrophoblasts. *Eur. J. Immunol.* 26:1668–71
29. Kim JK, Tsen MF, Ghetie V, Ward ES. 1994. Localization of the site of the murine IgG1 molecule that is involved in binding to the murine intestinal Fc receptor. *Eur. J. Immunol.* 24:2429–34
30. Raghavan M, Bonagura VR, Morrison SL, Bjorkman PJ. 1995. Analysis of the pH dependence of the neonatal Fc receptor/immunoglobulin G interaction using antibody and receptor variants. *Biochemistry* 34:14649–57
31. Medesan C, Matesoi D, Radu C, Ghetie V, Ward ES. 1997. Delineation of the amino acid residues involved in transcytosis and catabolism of mouse IgG1. *J. Immunol.* 158:2211–17
32. Kim J-K, Firan M, Radu CG, Kim C-H, Ghetie V, Ward ES. 1999. Mapping the site on human IgG for binding of the MHC class I related receptor, FcRn. *Eur. J. Immunol.* 29:2819–25
33. Deisenhofer J. 1981. Crystallographic refinement and atomic models of a human Fc fragment and its complex with fragment B of protein A from Staphylococcus aureus at 2.9- and 2.8-Å resolution. *Biochemistry* 20:2361–70
34. Gastinel L, Simister NE, Bjorkman PJ. 1992. Expression and crystalization of a soluble and functional form of an Fc receptor related to class I histocompatibility molecules. *Proc. Natl. Acad. Sci. USA* 89:638–42
35. Popov S, Hubbard JG, Kim J, Ober B, Ghetie V, Ward ES. 1996. The stoichiometry and affinity of the interaction of murine Fc fragments with the MHC class I-related receptor, FcRn. *Mol. Immunol.* 33:521–30
36. Guyer RL, Koshland ME, Knopf PM. 1976. Immunoglobulin binding by mouse intestinal epithelial cell receptors. *J. Immunol.* 117:587–93
37. Vaughn DE, Bjorkman PJ. 1998. Structural basis of pH-dependent antibody binding by the neonatal Fc receptor. *Structure* 6:63–73
38. Lebron JA, Bennett MJ, Vaughn DE, Chirino AJ, Snow PM, Mintier GA, Feder JN, Bjorkman PJ. 1998. Crystal structure of the hemochromatosis protein HFE and characterization of its interaction with transferrin receptor. *Cell* 93:111–23
39. Medesan C, Cianga P, Mummert M, Stanescu D, Ghetie V, Ward ES. 1998. Comparative studies of rat IgG to further delineate the Fc:FcRn interaction site. *Eur. J. Immunol.* 28:2092–100
40. Burmeister WP, Huber AH, Bjorkman PJ. 1994. Crystal structure of the complex of rat neonatal Fc receptor with Fc. *Nature* 372:379–83
41. Hobbs SM, Jackson LE, Peppard JV. 1987. Binding of subclasses of rat immunoglobulin G to detergent-isolated Fc receptor from neonatal rat intestine. *J. Biol. Chem.* 262:8041–46
42. Raghavan M, Chen MY, Gastinel LN, Bjorkman PJ. 1994. Investigation of the interaction between the class I MHC-related Fc receptor and its immunoglobulin G ligand. *Immunity* 1:303–15
43. Dima S, Medesan C, Mota G, Moraru I, Sjoquist J, Ghetie V. 1983. Effect of protein A and its fragment B on the catabolic

and Fc receptor sites of IgG. *Eur. J. Immunol.* 13:605–14

44. Zuckier LS, Chang CJ, Scharff MD, Morrison SL. 1998. Chimeric human-mouse IgG antibodies with shuffled constant region exons demonstrate that multiple domains contribute to in vivo half-life. *Cancer Res.* 58:3905–8
45. Kim JK, Tsen MF, Ghetie V, Ward ES. 1995. Evidence that the hinge region plays a role in maintaining serum levels of the murine IgG1 molecule. *Mol. Immunol.* 32:467–75
46. Kabat EA, Wu TT, Perry HM, Gottesman KS, Foeller C. 1991. *Sequences of Proteins of Immunological Interest.* Washington, DC: U.S. Dep. Health Hum. Serv. 5th ed.
47. Duncan AR, Winter G. 1988. The binding site for C1q on IgG. *Nature* 332:738–40
48. Frick IM, Wikstrom M, Forsen S, Drakenberg T, Gomi H, Sjobring U, Bjorck L. 1992. Convergent evolution among immunoglobulin G-binding bacterial proteins. *Proc. Natl. Acad. Sci. USA* 89:8532–36
49. Duncan AR, Woof JM, Partridge LJ, Burton DR, Winter G. 1988. Localization of the binding site for the human high-affinity Fc receptor on IgG. *Nature* 332:563–64
50. Canfield SM, Morrison SL. 1991. The binding affinity of human IgG for its high affinity Fc receptor is determined by multiple amino acids in the CH2 domain and is modulated by the hinge region. *J. Exp. Med.* 173:1483–91
51. Lund J, Pound JD, Jones PT, Duncan AR, Bentley T, Goodall M, Levine BA, Jefferis R, Winter G. 1992. Multiple binding sites on the CH2 domain of IgG for mouse Fc gamma R11. *Mol. Immunol.* 29:53–59
52. Lund J, Winter G, Jones PT, Pound JD, Tanaka T, Walker MR, Artymiuk PJ, Arata Y, Burton DR, Jefferis R. 1991. Human Fc gamma RI and Fc gamma RII interact with distinct but overlapping sites on human IgG. *J. Immunol.* 147:2657–62
53. Hogarth PM, Ierino FL, Hulett MD. 1994. Characterization of FcR Ig-binding sites and epitope mapping. *Immunomethods* 4:17–24
54. Hulett MD, Hogarth PM. 1994. Molecular basis of Fc receptor function. *Adv. Immunol.* 57:1–127
55. Gavin AL, Barnes N, Dijstelbloem HM, Hogarth PM. 1998. Identification of the mouse IgG3 receptor: implications for antibody effector function at the interface between innate and adaptive immunity. *J. Immunol.* 160:20–23
56. Tao MH, Morrison SL. 1989. Studies of aglycosylated chimeric mouse-human IgG: role of carbohydrate in the structure and effector functions mediated by the human IgG constant region. *J. Immunol.* 143:2595–601
57. Tsuchiya N, Endo T, Matsuta K, Yoshinoya S, Aikawa T, Kosuge E, Takeuchi F, Miyamoto T, Kobata A. 1989. Effects of galactose depletion from oligosaccharide chains on immunological activities of human IgG. *J. Rheumatol.* 16:285–90
58. Walker MR, Lund J, Thompson KM, Jefferis R. 1989. Aglycosylation of human IgG1 and IgG3 monoclonal antibodies can eliminate recognition by human cells expressing Fc gamma RI and/or Fc gamma RII receptors. *Biochem. J.* 259:347–53
59. Wright A, Morrison SL. 1994. Effect of altered CH2-associated carbohydrate structure on the functional properties and in vivo fate of chimeric mouse-human immunoglobulin G1. *J. Exp. Med.* 180:1087–96
60. Lund J, Takahashi N, Pound JD, Goodall M, Jefferis R. 1996. Multiple interactions of IgG with its core oligosaccharide can modulate recognition by complement and human Fc gamma receptor I and

influence the synthesis of its oligosaccharide chains. *J. Immunol.* 157:4963–69
61. Maxwell KF, Powell MS, Hulett MD, Barton PA, McKenzie IF, Garrett TP, Hogarth PM. 1999. Crystal structure of the human leukocyte Fc receptor, Fc gammaRIIa. *Nat. Struct. Biol.* 6:437–42
62. Sondermann P, Huber R, Jacob U. 1999. Crystal structure of the soluble form of the human Fc gamma-receptor IIb: a new member of the immunoglobulin superfamily at 1.7 Å resolution. *EMBO J.* 18:1095–103
63. Wawrzynczak EJ, Denham S, Parnell GD, Cumber AJ, Jones PT, Winter G. 1992. Recombinant mouse monoclonal antibodies with single amino acid substitutions affecting Clq and high affinity Fc receptor binding have identical serum half-lives in the BALB/c mouse. *Mol. Immunol.* 29:221–27
64. Vaughn DE, Milburn CM, Penny DM, Martin WL, Johnson JL, Bjorkman PJ. 1997. Identification of critical IgG binding epitopes on the neonatal Fc receptor. *J. Mol. Biol.* 274:597–607
65. Garcia KC, Degano M, Stanfield RL, Brunmark A, Jackson MR, Peterson PA, Teyton L, Wilson IA. 1996. An alphabeta T cell receptor structure at 2.5 Å and its orientation in the TCR-MHC complex. *Science* 274:209–19
66. Garboczi DN, Ghosh P, Utz U, Fan QR, Biddison WE, Wiley DC. 1996. Structure of the complex between human T-cell receptor, viral peptide and HLA-A2. *Nature* 384:134–41
67. Raghavan M, Wang Y, Bjorkman PJ. 1995. Effects of receptor dimerization on the interaction between the class I major histocompatibility complex-related Fc receptor and IgG. *Proc. Natl. Acad. Sci. USA* 92:11200–4
68. Vaughn DE, Bjorkman PJ. 1997. High-affinity binding of the neonatal Fc receptor to its IgG ligand requires receptor immobilization. *Biochemistry* 36:9374–80
69. Weng Z, Gulukota K, Vaughn DE, Bjorkman PJ, DeLisi C. 1998. Computational determination of the structure of rat Fc bound to the neonatal Fc receptor. *J. Mol. Biol.* 282:217–25
70. Huber AH, Kelley RF, Gastinel LN, Bjorkman PJ. 1993. Crystallization and stoichiometry of binding of a complex between a rat intestinal Fc receptor and Fc. *J. Mol. Biol.* 230:1077–83
71. Kim JK, Tsen MF, Ghetie V, Ward ES. 1994. Catabolism of the murine IgG1 molecule: evidence that both CH2-CH3 domain interfaces are required for persistence of IgG1 in the circulation of mice. *Scand. J. Immunol.* 40:457–65
72. Fersht AR. 1975. Demonstration of two active sites on a monomeric aminoacyl-tRNA synthetase: possible roles of negative cooperativity and half-of-the-sites reactivity in oligomeric enzymes. *Biochemistry* 14:5–12
73. Bosshard HR, Koch LE, Hartley BS. 1975. Aminoacyl-tRNA synthetases from Bacillus stearothermophilus: asymmetry of substrate binding to tyrosyl-tRNA synthetase. *Eur. J. Biochem.* 53:493–98
74. Nezlin R. 1990. Internal movements in immunoglobulin molecules. *Adv. Immunol.* 48:1–40
75. Burton DR. 1990. Antibody: the flexible adaptor molecule. *Trends Biochem. Sci.* 15:64–69
76. Zheng Y, Shopes B, Holowka D, Baird B. 1992. Dynamic conformations compared for IgE and IgG1 in solution and bound to receptors. *Biochemistry* 31:7446–56
77. Keown MB, Ghirlando R, Young RJ, Beavil AJ, Owens RJ, Perkins SJ, Sutton BJ, Gould HJ. 1995. Hydrodynamic studies of a complex between the Fc fragment of human IgE and a soluble fragment of the Fc epsilon RI alpha chain. *Proc. Natl. Acad. Sci. USA* 92:1841–45
78. Robertson MW. 1993. Phage and Escherichia coli expression of the human high

affinity immunoglobulin E receptor alpha-subunit ectodomain: domain localization of the IgE-binding site. *J. Biol. Chem.* 268:12736–43
79. Sanchez LM, Penny DM, Bjorkman PJ. 1999. Stoichiometry of the interaction between the MHC-related Fc receptor and its Fc ligand. *Biochemistry* 38:9471–76.
80. Schuck P, Radu CG, Ward ES. 1999. Sedimentation equilibrium analysis of recombinant mouse FcRn with murine IgG1. *Mol. Immunol.* 36:1117–25
81. Ghetie V, Popov S, Borvak J, Radu C, Matesoi D, Medesan C, Ober RJ, Ward ES. 1997. Increasing the serum persistence of an IgG fragment by random mutagenesis. *Nat. Biotechnol.* 15:637–40
82. Raghavan M, Bjorkman PJ. 1996. Fc receptors and their interactions with immunoglobulins. *Annu. Rev. Cell Dev. Biol.* 12:181–220
83. Bjorkman PJ. 1998. *Antibodies: Molecular, Cellular and Clinical Aspects.* Cancer Res. Inst. (Abstr.), April 1998
84. Rodewald R, Kraehenbuhl JP. 1984. Receptor-mediated transport of IgG. *J. Cell Biol.* 99:159s–64s
85. Israel EJ, Patel VK, Taylor SF, Marshak-Rothstein A, Simister NE. 1995. Requirement for a beta 2-microglobulin-associated Fc receptor for acquisition of maternal IgG by fetal and neonatal mice. *J. Immunol.* 154:6246–51
86. Benlounes N, Chedid R, Thuillier F, Desjeux JF, Rousselet F, Heyman M. 1995. Intestinal transport and processing of immunoglobulin G in the neonatal and adult rat. *Biol. Neonatè* 67:254–63
87. Kristoffersen EK, Ulvestad E, Vedeler CA, Matre R. 1990. Fc gamma receptor heterogeneity in the human placenta. *Scand. J. Immunol.* 32:561–64
88. Simister NE, Story CM. 1997. Human placental Fc receptors and the transmission of antibodies from mother to fetus. *J. Reprod. Immunol.* 37:1–23
89. Leach L, Eaton BM, Firth JA, Contractor SF. 1991. Immunocytochemical and labelled tracer approaches to uptake and intracellular routing of immunoglobulin-G (IgG) in the human placenta. *Histochem. J.* 23:444–49
90. Stefaner I, Stefanescu A, Hunziker W, Fuchs R. 1997. Expression of placental alkaline phosphatase does not correlate with IgG binding, internalization and transcytosis. *Biochem. J.* 327:585–92
91. Kameda T, Koyama M, Matsuzaki N, Taniguchi T, Saji F, Tanizawa O. 1991. Localization of three subtypes of Fc gamma receptors in human placenta by immunohistochemical analysis. *Placenta* 12:15–26
92. Sedmak DD, Davis DH, Singh U, van de Winkel JG, Anderson CL. 1991. Expression of IgG Fc receptor antigens in placenta and on endothelial cells in humans. An immunohistochemical study. *Am. J. Pathol.* 138:175–81
93. Wang AC, Faulk WP, Stuckey MA, Fudenberg HH. 1970. Chemical differences of adult, fetal and hypogammaglobulinemic IgG immunoglobulins. *Immunochemistry* 7:703–8
94. Hay FC, Hull MG, Torrigiani G. 1971. The transfer of human IgG subclasses from mother to foetus. *Clin. Exp. Immunol.* 9:355–58
95. Malek A, Sager R, Schneider H. 1994. Maternal-fetal transport of immunoglobulin G and its subclasses during the third trimester of human pregnancy. *Am. J. Reprod. Immunol.* 32:8–14
96. Sidiripoulos D, Hermann U, Morell A, Muratt G, Barundun S. 1986. Transplacental passage of intravenous immunoglobulin in the last trimester of pregnancy. *J. Pedriatr.* 109:505–8
97. Smith CIE, Hammarstrom L. 1985. Intravenous immunoglobulin in pregnancy. *Obstet. Gynecol.* 66:39S–40S
98. Malek A, Sager R, Sakher A, Schneider H. 1995. Transport of immunoglobulin G and its subclasses across the in vitro per-

fused human placenta. *Am. J. Obstet. Gynecol.* 173:760–67

99. Sooranna SR Contractor SF. 1991. Vectorial transcytosis of immunoglobulin G by human term trophoblast cells in culture. *Exp. Cell Res.* 192:41–45
100. Brambell FWR, Hemmings WA, Morris IG. 1964. A theoretical model of gammaglobulin catabolism. *Nature* 203: 1352–55
101. Brambell FWR. 1970. *The Transmission of Passive Immunity from Mother to Young.* Amsterdam: North Holland
102. Kim JK, Tsen MF, Ghetie V, Ward ES. 1994. Identifying amino acid residues that influence plasma clearance of murine IgG1 fragments by site-directed mutagenesis. *Eur. J. Immunol.* 24:542–48
103. Spriggs MK, Koller BH, Sato T, Morrissey PJ, Fanslow WC, Smithies O, Voice RF, Widmer MB, Maliszewski CR. 1992. Beta 2-microglobulin-, CD8+ T-cell-deficient mice survive inoculation with high doses of vaccinia virus and exhibit altered IgG responses. *Proc. Natl. Acad. Sci. USA* 89:6070–74
104. Liu Z, Roopenian DC, Zhou X, Christianson GJ, Diaz LA, Sedmak DD, Anderson CL. 1997. Beta2-microglobulin-deficient mice are resistant to bullous pemphigoid. *J. Exp. Med.* 186:777–83
105. Mozes E, Kohn LD, Hakim F, Singer DS. 1993. Resistance of MHC class I-deficient mice to experimental systemic lupus erythematosus. *Science* 261:91–93
106. Chan OT, Hannum LG, Haberman AM, Madaio MP, Shlomchik MJ. 1999. A novel mouse with B cells but lacking serum antibody reveals an antibody-independent role for B cells in murine lupus. *J. Exp. Med.* 189:1639–48
107. Simister NE, Mostov KE. 1989. Cloning and expression of the neonatal rat intestinal Fc receptor, a major histocompatibility complex class I antigen homolog. *Cold Spring Harb. Symp. Quant. Biol.* 54(1):571–80
108. Junghans RP. 1997. Finally! The Brambell receptor (FcRB). Mediator of transmission of immunity and protection from catabolism for IgG. *Immunol. Res.* 16:29–57
109. Mariani G, Strober W. 1990. *Immunoglobulin Metabolism, Fc Receptors, and the Action of Antibodies,* ed. H Metzger, pp. 94–177. Washington, DC: Am. Soc. Microbiol.
110. Borvak J, Richardson J, Medesan C, Antohe F, Radu C, Simionescu M, Ghetie V, Ward ES. 1998. Functional expression of the MHC class I-related receptor, FcRn, in endothelial cells of mice. *Int. Immunol.* 10:1289–98
111. Gitlin D, Kumate J, Urrusti J, Morales C. 1964. The selectivity of the human placenta in the transfer of plasma proteins from mother to fetus. *J. Clin. Invest.* 43:1938–51
112. Contractor SF, Eaton BM, Stannard PJ. 1983. Uptake and fate of exogenous immunoglobulin G in the perfused human placenta. *J. Reprod. Immunol.* 5:265–73
113. Israel EJ, Taylor S, Wu Z, Mizoguchi E, Blumberg RS, Bhan A, Simister NE. 1997. Expression of the neonatal Fc receptor, FcRn, on human intestinal epithelial cells. *Immunology* 92:69–74

113a. Dickinson BL, Badizadegan K, Wu Z, Ahouse X, Simister NE, Blumberg RS, Lencer WI. 1999. Bidirectional FcRn-dependent IgG transport in a polarized human intestinal epithelial cell line. *J. Clin. Invest.* 104:903–11

114. Besser TE, McGuire TC, Gay CC, Pritchett LC. 1988. Transfer of functional immunoglobulin G (IgG) antibody into the gastrointestinal tract accounts for IgG clearance in calves. *J. Virol.* 62:2234–37
115. Blumberg RS, Koss T, Story CM, Barisani D, Polischuk J, Lipin A, Pablo L, Green R, Simister NE. 1995. A major histocompatibility complex class I-related Fc receptor for IgG on rat hepatocytes. *J. Clin. Invest.* 95:2397–402

116. Cianga P, Medesan C, Richardson J, Ghetie V, Ward ES. 1999. Identification and function of neonatal Fc receptor in mammary gland of lactating mice. *Eur. J. Immunol.* 29:2515–23
117. Mehta PD, Isaacs CE, Coyle PK. 1991. Immunoglobulin G subclasses in human colostrum and milk. In *Immunology of Milk and the Neonate,* ed. J Mestecky, C Blair, PL Ogra, pp. 223–26. New York and London: Plenum
118. Hunziker W, Fumey C. 1994. A di-leucine motif mediates endocytosis and basolateral sorting of macrophage IgG Fc receptors in MDCK cells. *EMBO J.* 13:2963–67
119. Letourneur F, Klausner RD. 1992. A novel di-leucine motif and a tyrosine-based motif independently mediate lysosomal targeting and endocytosis of CD3 chains. *Cell* 69:1143–57
120. Simmen T, Nobile M, Bonifacino JS, Hunziker W. 1999. Basolateral sorting of furin in MDCK cells requires a phenylalanine-isoleucine motif together with an acidic amino acid cluster. *Mol. Cell Biol.* 19:3136–44
121. Mostov KE, Deitcher DL. 1986. Polymeric immunoglobulin receptor expressed in MDCK cells transcytoses IgA. *Cell* 46:613–21
122. Hunziker W, Mellman I. 1989. Expression of macrophage-lymphocyte Fc receptors in Madin-Darby canine kidney cells: polarity and transcytosis differ for isoforms with or without coated pit localization domains. *J. Cell Biol.* 109:3291–302
123. Sell S, Fahey JL. 1964. Relationship between gamma-globulin metabolism and low serum gamma-globulin in germfree mice. *J. Immunol.* 93:81–87
124. Kandil E, Noguchi M, Ishibashi T, Kasahara M. 1995. Structural and phylogenetic analysis of the MHC class I-like Fc receptor gene. *J. Immunol.* 154:5907–18
125. Hansborough JR, Miller LN, Field TO, Gadd NA. 1988. High dose intravenous immunoglobulin therapy in burn patients. pharmacokinetics and effects on microbial opsonization and phagocytosis. *Pediatr. Infect. Dis.* 7:S49–S46
126. Wochner RD, Drews G, Strober W, Waldmann TA. 1966. Accelerated breakdown of immunoglobulin G (IgG) in myotonic dystrophy: a hereditary error of immunoglobulin catabolism. *J. Clin. Invest.* 45:321–29
127. Waldmann TA, Miller EJ, Terry WD. 1968. Hypercatabolism of IgG and albumin: a new familial disorder. *Clin. Res.* 16:45
128. Yu Z, Lennon VA. 1999. Mechanism of intravenous immune globulin therapy in antibody-mediated autoimmune diseases. *N. Engl. J. Med.* 340:227–28
129. Masson PL. 1993. Elimination of infectious antigens and increase of IgG catabolism as possible modes of action of IVIg. *J. Autoimmun.* 6:683–89
130. Geha RS, Rosen FS. 1996. Intravenous immune globulin therapy. In *Therapeutic Immunology,* ed. KF Austen, SJ Burakoff, FS Rosen, TB Strom, pp. 280–96. Cambridge, MA: Blackwell Science
131. Martin MG, Wu SV, Walsh JH. 1993. Hormonal control of intestinal Fc receptor gene expression and immunoglobulin transport in suckling rats. *J. Clin. Invest.* 91:2844–49
132. Levy AL, Waldmann TA. 1970. The effect of hydrocortisone on immunoglobulin metabolism. *J. Clin. Invest.* 49: 1679–84
133. Riechmann L, Holliger P. 1997. Antibodies live long and prosper. *Nat. Biotechnol.* 15:617
134. Baselga JD, Tripathy D, Mendelsohn J, Baughman S, Benz CC, Dantis L, Sklarin NT, Seidman AD, Hudis CA, Moore J, Rosen PP, Twaddell T, Henderson IC, Norton L. 1996. Phase II study of weekly intravenous recombinant humanized anti-p185HER2 monoclonal Ab in patients with HER2/*neu*-overexpressing

metastatic breast cancer. *J. Clin. Oncol.* 14:737–44
135. Berinstein NL, Grillo-Lopez AJ, White CA, Bence-Bruckler I, Maloney D, Czuczman M, Green D, Rosenberg J, McLaughlin P, Shen D. 1998. Association of serum Rituximab (IDEC-C2B8) concentration and anti-tumor response in the treatment of recurrent low-grade or follicular non-Hodgkin's lymphoma. *Ann. Oncol.* 9:995–1001
136. Uhr JW, Baumann J. 1961. Antibody formation: I. The suppression of antibody formation by passively administered antibody. *J. Exp. Med.* 113:395–97
137. Celis E, Zurawski VRJ, Chang TW. 1984. Regulation of T-cell function by antibodies: enhancement of the response of human T-cell clones to hepatitis B surface antigen by antigen-specific monoclonal antibodies. *Proc. Natl. Acad. Sci. USA* 81:6846–50
138. Schalke BC, Klinkert WE, Wekerle H, Dwyer DS. 1985. Enhanced activation of a T cell line specific for acetylcholine receptor (AChR) by using anti-AChR monoclonal antibodies plus receptors. *J. Immunol.* 134:3643–48
139. Jemmerson R, Johnson JG, Burrell E, Taylor PS, Jenkins MK. 1991. A monoclonal antibody specific for a cytochrome c T cell stimulatory peptide inhibits T cell responses and affects the way the peptide associates with antigen-presenting cells. *Eur. J. Immunol.* 21:143–51
140. Watts C, Lanzavecchia A. 1993. Suppressive effect of antibody on processing of T cell epitopes. *J. Exp. Med.* 178:1459–63
141. Siegrist CA, Barrios C, Martinez X, Brandt C, Berney M, Cordova M, Kovarik J, Lambert PH. 1998. Influence of maternal antibodies on vaccine responses: inhibition of antibody but not T cell responses allows successful early prime-boost strategies in mice. *Eur. J. Immunol.* 28:4138–48
142. Simionescu M, Simionescu N. 1991. Endothelial transport of macromolecules: transcytosis and endocytosis. *Cell Biol. Rev.* 25:1–78
143. Martin WL, Bjorkman PJ. 1999. Characterization of the 2:1 complex between the class I MHC related Fc receptor and its Fc ligand in solution. *Biochemistry* 38:12639–47

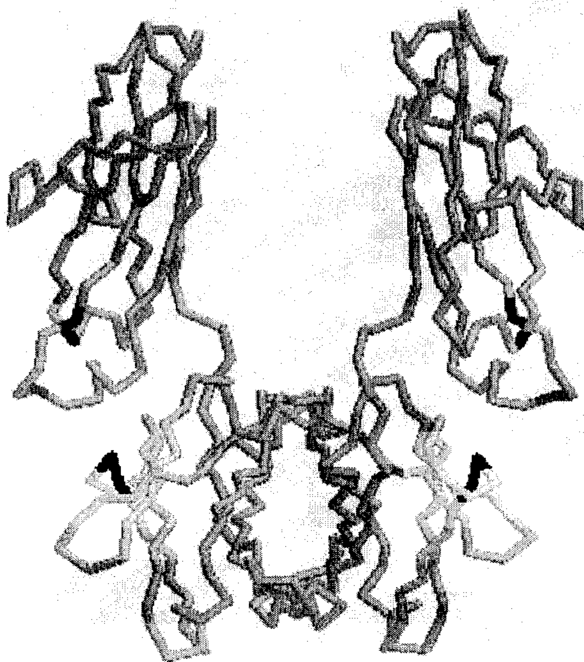

Figure 1 Alpha-carbon trace of the Fc region of human IgG1 (33). Residues shown to play a role in the FcRn:IgG interaction using both binding and in vivo assays (29-32, 39) are indicated (Ile253, green; Pro257, red; His310, blue; His435-Tyr436, black; note that the role of Tyr436 in human IgG1 has not been investigated, but His436 in mouse IgG1 is involved). Residues 307 and 309 play a less significant role (39) and are therefore not highlighted. The figure was drawn using RASMOL (Roger Sayle, Bioinformatics Research Institute, University of Edinburgh, Edinburgh, U.K.).

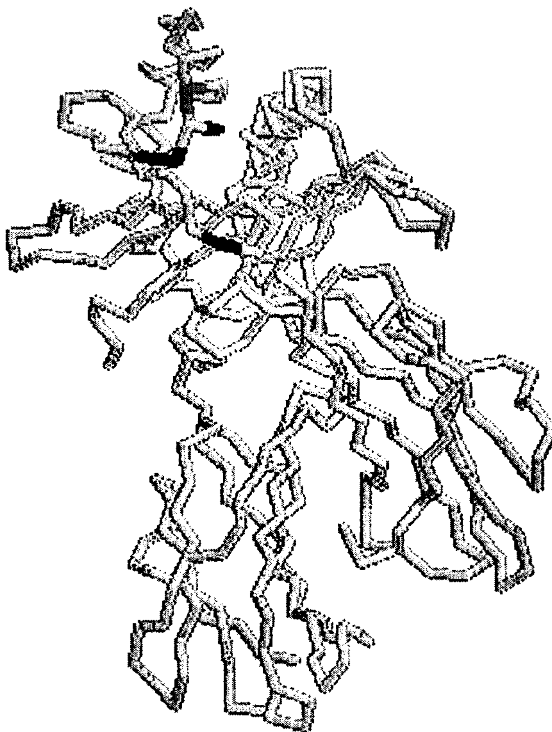

Figure 2 Alpha-carbon trace of rat FcRn with α1 and α2 domain helices perpendicular to the page (18). Indicated are the α2 domain residues shown by site-directed mutagenesis studies (64) to be involved in binding to rodent IgGs (Glu117, blue; Glu132-Trp133, red; Glu135, black; Asp137, green). The figure was drawn with RASMOL (Roger Sayle, Bioinformatics Research Institute, University of Edinburgh, Edinburgh, U.K.).