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Localization of the site of the murine IgG1 molecule that is involved in binding to the murine intestinal Fc receptor*

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Site-directed mutagenesis of a recombinant Fc hinge fragment has recently been used to localize the site of the murine IgG1 molecule that is involved in the control of catabolism (the "catabolic site"). In the current study, the effects of these CH2 and CH3 domain mutations (Ile 253 to Ala 253, His 310 to Ala 310, Gln 311 to Asn 311, His 433 to Ala 433 and Asn 434 to Gln 434) on intestinal transfer of Fc hinge fragments in neonatal mice have been analyzed. Studies using direct transfer and competition assays demonstrate that the mutations affect the transmission from intestinal lumen into serum in a way that correlates closely with the effects of the mutations on pharmacokinetics. Binding studies of several of the Fc hinge fragments to isolated neonatal brush borders have been used to confirm the *in vivo* transmission data. These analyses have resulted in the localization of the binding site for the intestinal transfer receptor, FcRn, to specific residues of the murine Fc hinge fragment. These residues are located at the CH2-CH3 domain interface and overlap with both the catabolic site and staphylococcal protein A (SpA) binding site. The pH dependence of IgG1 or Fc fragment binding to FcRn is consistent with the localization of the FcRn interaction site to a region of the Fc that encompasses two histidine residues (His 310 and His 433). To assess whether one or two FcRn binding sites per Fc hinge are required for intestinal transfer, a hybrid Fc hinge fragment comprising a heterodimer of one Fc hinge with the wild-type IgG1 sequence and a mutant Fc hinge with a defective catabolic site (mutated at His 310, Gln 311, His 433 and Asn 434) has been analyzed in direct and competition transmission assays. The studies demonstrate that the Fc hybrid is transferred with significantly reduced efficiency compared to the wild type Fc hinge homodimer and indicate that the binding to FcRn, and possibly subsequent transfer, is enhanced by the presence of two FcRn binding sites per Fc hinge fragment.

1 Introduction

The transfer of passive immunity from mother to young in mice and rats occurs primarily after birth through a specific transport mechanism for IgG across the enterocytes of the proximal small intestine [1, 2]. This provides the newborn rodent with humoral immunity for the first few weeks of life. Brush border FcRn that are involved in the transcytosis bind to IgG with high affinity at the luminal pH of 6–6.5 and release the IgG at the pH of the serum (pH 7.4) [3, 4]. This pH dependence of IgG binding by FcRn has been demonstrated using both isolated neonatal rat brush borders [3, 5] and purified rat FcRn [4, 6].

FcRn is a heterodimer of two polypeptides of M_r 45–53 kDa and 14 kDa [6], and the genes encoding the heavy chain of mouse [7] and rat FcRn [8] have been isolated and share a high degree of homology. Consistent with the efficient

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Abbreviation: **SpA:** Staphyloccocal protein A

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transport of IgG in rodents up to days 16 to 20 after birth, these receptors are temporally expressed at high levels during this period [8]. FcRn is distinct from the poly Ig receptor [9] involved in IgA transcytosis, but interestingly, shares homology with the members of the class I major histocompatibility complex glycoproteins [8]. The FcRn heavy chain has three extracellular domains that are similar to the class I α1, α2 and α3 domains and is anchored to the membrane by a hydrophobic transmembrane region. Using both biochemical studies [8] and indirect methods involving analysis of transcytosis in a β2-microglobulin-deficient mouse [10], the 14-kDa component of the FcRn heterodimer has been shown to be β2-microglobulin. Rat FcRn has been expressed in soluble form [11] and binds to the IgG Fc fragment in a ratio of two FcRn to one Fc [12]. To date, the binding site for FcRn on the IgG Fc fragment has not been localized, but it has been shown that individual CH2 and CH3 fragments do not bind to FcRn [13], suggesting that regions in both CH2 and CH3 domains are required for the interaction.

The aim of this study was to identify the amino acid residues of the Fc that are involved in binding to FcRn and to analyze whether one site per Fc is sufficient to mediate binding and transfer. Previously, site-directed mutagenesis of a recombinant Fc hinge fragment has been used to identify the region (the "catabolic site") of the murine IgG1 molecule that is involved in controlling the clearance rate of this isotype in mice [14, 15]. The hypothesis of Brambell [16] suggested that the control of catabolism of IgG takes place

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by a mechanism similar to that of IgG transmission from mother to young [1], and both processes involve receptors that bind and protect IgG from degradation. The more recent identification and characterization of FcRn suggests that, although the mechanism of catabolism control and transmission might be shared, the receptors involved are distinct. The catabolic site overlaps the SpA binding site [14] and involves residues located at the CH2-CH3 domain interface, and these residues are highly conserved in human and murine IgG isotypes [17] and include two histidines (His 310 and His 433). In this study the effects of the catabolic site mutations on transcytosis have been analyzed. Using both *in vivo* transcytosis and *in vitro* binding assays, this analysis has resulted in the identification of the region of the murine IgG1 molecule that is involved in interacting with FcRn.

2 Materials and methods

2.1 Bacterial strains and plasmids

The bacterial strain and plasmids used to produce recombinant Fc hinge fragments for this study have been described previously [14, 15].

2.2 Expression and purification of Fc hinge fragments

Recombinant wild-type (WT) Fc hinge and mutant Fc hinge fragments were expressed and purified using Ni^{2+}-NTA-agarose [18], as described previously [14]. The mutants are described in Table 1. The Fc hybrid comprises a heterodimer of one WT Fc hinge polypeptide (tagged with a carboxy-terminal His6 peptide tag, [14]) associated with one HQ-310/HN-433 mutant Fc hinge polypeptide (tagged with a carboxy-terminal c-myc peptide tag, [19]) and was purified using Ni^{2+}-NTA-agarose and 9E10-Sepharose as described [15]. The SpA binding characteristics of the mutant Fc hinge fragments have been reported [14], and as expected from the knowledge of the SpA-IgG interaction [20], all mutants have reduced binding activity relative to the WT Fc hinge.

2.3 Murine IgG1 and papain digestion

The murine RFB4 antibody (obtained from Abbott Laboratories, Needham, MA) was used for these studies and recognizes human CD22 [21]. Fab and Fc fragments were obtained by papain digestion at pH 7 followed by purification using DEAE-Sepharose (Pharmacia) and SpA-Sepharose [22].

2.4 Radiolabeling of proteins

Murine IgG1 and recombinant Fc hinge fragments were labeled using $Na^{125}I$ (Amersham) and the Iodo-Gen procedure [23]. Free iodine was removed by two gel filtrations using Sephadex G-25 equilibrated in 50 mM phosphate buffer pH 5.9 containing 1 mg/ml BSA.

2.5 Transintestinal transfer

BALB/c neonates (10–14 days old, weight 5–9 g) were fasted for 2–5 h prior to force feeding (by intubation) with a 30-μl dose of protein solution in phosphate-buffered saline (pH 6) as described by Guyer and colleagues [13]. The protein solution contained either radiolabeled ligand (0.066–0.132 nmoles protein, 5×10^5 cpm) for direct transfer assays, or a mixture of ^{125}I-labeled IgG (0.066 nmoles protein, 10^6 cpm) with 132 nmoles of unlabeled ligand for inhibition assays. The unlabeled/labeled molar ratio of 2000 was used following preliminary experiments designed to determine the dose-dependency of the inhibition of the transmission of ^{125}I-labeled IgG by unlabeled IgG. Maximum inhibition was observed at this ratio. Animals were bled with 22.4-μl heparinized capillary glass tubes from the retro-orbital plexus at different times following feeding. Whole blood or plasma (isolated by centrifugation) was used for analyses. The radioactivity present in the blood/plasma, either before or after precipitation with 10 % trichloroacetic acid (TCA) was quantitated by gamma counting. The percentage of radioactivity transferred from gut to circulation was calculated for each mouse by using a blood volume equal to 7.2 % of body weight [13]. For all samples, more than 90 % of the radioactivity was precipitated by 10 % TCA, and therefore no correction for non-protein-bound radioactivity was made.

2.6 Preparation of intestinal brush borders

Methodology as described by Wallace and Rees [5] for the isolation and purification of rat brush borders was used. Briefly, suckling BALB/c mice (10–14 days old) were killed and the proximal half of the jejunum removed into ice-cold 5 mM EDTA pH 7.4 containing 2 mM phenylmethylsulphonyl fluoride (Sigma) and 1 μg/ml pepstatin (Sigma). The mucosa was scraped off and the preparation resuspended by pipetting with a Pasteur pipetta until a homogeneous creamy yellow suspension was obtained. Hyaluronidase (Sigma) was added to a final concentration of 0.5 mg/ml and the mixture incubated at room temperature for 30 min with stirring. The suspension was forced three times through a 23-gauge syringe needle and centrifuged at $1000 \times g$ for 20 min at 4 °C. The pellet was resuspended in 20 % of the initial volume of 0.1 M NaCl/1 mM EDTA pH 7.4 containing 0.2 mg/ml deoxyribonuclease I (Sigma) and left at room temperature with stirring for 10 min. After centrifugation the pellet was washed once and resuspended in 50 mM MES (Sigma) buffer containing protease inhibitors as above plus 2 mg/ml bovine serum albumin at pH 5.9 (MES-BSA buffer). Preliminary experiments in which the pH was varied from 5 to 7.4 indicated that pH 5.9 was optimal for the specific binding of ^{125}I-labeled IgG1 to brush border preparations, and this was therefore used for subsequent studies.

2.7 Binding of IgG1 and IgG1-derived fragments to brush borders

2.7.1 Direct binding

Aliquots of 100 μl brush border suspension (containing 100–200 μg protein) were incubated with a 400-μl volume

Table 1. Fc hinge derivatives used in this study

Designation [Ref.]	Mutation
WT Fc hinge [14]	None
I-253 [14]	Ile 253 → Ala 253
HQ-310 [14]	His 310 → Ala 310 and Gln 311 → Asn 311
HN-433 [14]	His 433 → Ala 433 and Asn 434 → Gln 434
HQ-310/HN-433 [14]	HQ-310 and HN-433 within same Fc hinge
Fc-hybrid [15]	Hybrid comprising heterodimer of WT Fc hinge and HQ-310/HN-433 mutant

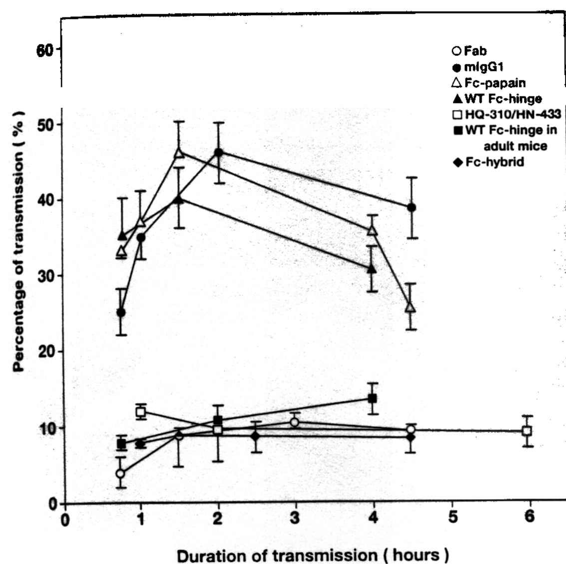

Figure 1. Intestinal transfer of murine IgG1, Fab, Fc-papain and recombinant Fc hinge fragments. The numbers of mice used for each experiment were 6 (Fab), 12 (mIgG1), 16 (Fc-papain), 31 (WT Fc hinge), 5 (HQ-310/HN-433), 5 (WT Fc hinge in adult mice) and 14 (Fc hybrid).

of MES-BSA containing increasing amounts of radioactive IgG1, Fab and Fc hinge fragments (Fc hinge fragments used in this study are described in Table 1). Following incubation for 2 h at 37 °C with occasional stirring, the suspension was centrifuged at 2000 × g for 10 min and washed three times with ice-cold MES-BSA buffer. The amount of protein non specifically bound to the brush borders was determined by measuring the amount of bound radioactivity after an additional washing with 50 mM phosphate buffer pH 7.4 (PBE), as IgG bound specifically to FcRn would be released at this pH [4, 6].

2.7.2 Inhibition binding assay

Brush border suspensions of 100 μl were incubated with 400 μl of unlabeled ligand (amount of protein added ranging from 1.3 to 264 nmoles) in MES-BSA buffer. Following a 15 min incubation at 37 °C, 10 μl of radiolabeled mouse IgG1 (0.066 nmoles protein, 2×10^5 cpm) was added and the mixture incubated for a further 90 min at 37 °C with occasional stirring. The suspension was centrifuged at 2000 × g for 10 min and washed three times with ice-cold MES-BSA buffer as above. Inhibition of binding was expressed as the amount of labeled IgG1 bound in the presence of inhibitor relative to the amount bound in the absence of inhibitor.

3 Results

3.1 *In vivo* experiments

Intestinal transfer of radiolabeled IgG1 and Fc hinge fragments from the proximal intestine to the circulation was analyzed *in vivo* by feeding neonates with nanomolar quantities of the appropriate protein. Approximately 45 % of the radioactivity associated with the IgG1 molecule and Fc fragment produced by papain digestion (designated Fc-papain) could be detected in the plasma (Fig. 1). The WT Fc hinge fragment behaved similarly in these studies, with 40 % of the radioactivity being transferred into the circulation (Fig. 1). In contrast, for the HQ-310/HN-433 mutant and hybrid Fc hinge, only 10 % transfer was observed. This value is close to that of radiolabeled Fab fragment used as a negative control, and therefore probably represents transfer by a nonspecific mechanism. In adult mice, the same low level of transfer of the WT Fc hinge was observed, consistent with the report that FcRn is expressed only in the intestine of neonatal mice [7]. For the WT Fc hinge fragment and Fc-papain, the concentration of radiolabeled ligand reached a maximum after 1.5 h, whereas for the IgG1 molecule, the maximum occurred after 2 h (Fig. 1). This difference is possibly due to the increased molecular size of the IgG1 molecule relative to the Fc fragments. In contrast, the lower levels of mutant Fc hinge and Fc hybrid were observed to remain constant at the different time points at which plasma samples were analyzed. The observation that only about half of the IgG1, Fc-papain or WT Fc hinge are transferred from the intestine into the circulation is curious, but is similar to the observations of others in both rats [2] and mice [13]. A possible explanation may be that a proportion of the protein is partitioned away from the proximal intestine and as a consequence does not come into contact with FcRn as it passes down the gastrointestinal tract [13].

The HQ-310/HN-433 mutant is cleared from the serum at a much higher rate than the WT Fc hinge fragment [14], and it is therefore conceivable that the lower level of radioactivity in the serum of the neonatal mice might be due to more rapid clearance rather than lower transmission. To demonstrate that the mutant is deficient in transfer, experiments to assess the ability of the mutant Fc hinge fragments to inhibit transfer of radiolabeled IgG1 have therefore been carried out. The results from these experiments would be expected to be independent of clearance rate. In addition to the analysis of the inhibitory capacity of the WT Fc hinge and HQ-310/HN-433 mutant, other mutants and the Fc hybrid that have been previously shown to have altered pharmacokinetics have been used in these competition experiments [14, 15]. Consistent with the direct transfer data, the inhibition experiments show that the WT Fc hinge and Fc-papain have similar inhibitory capacities to unlabelled IgG1, whereas the mutant Fc hinge fragments and Fc hybrid inhibit transfer at much lower

Table 2. Inhibition of intestinal transmission of radiolabeled murine IgG1 by recombinant Fc hinge derivatives

Competitor (unlabeled)	Number of animals	Transmission[a)] (%)	% Inhibition of transmission
PBS	29	54.1 ± 9.7	–
IgG1	29	26.3 ± 5.7	51.4
Fc-papain	15	26.3 ± 7.1	62.5
WT	17	24.2 ± 4.3	55.3
HQ-310/Hn-433	9	51.2 ± 8.3	5.4
Fc-hybrid	5	42.5 ± 5.9	21.5
HQ-310	9	49.3 ± 7.3	8.9
I-253	9	43.2 ± 5.3	20.2
HN-433	8	30.1 ± 4.4	44.4
BSA	5	49.3 ± 5.1	8.9

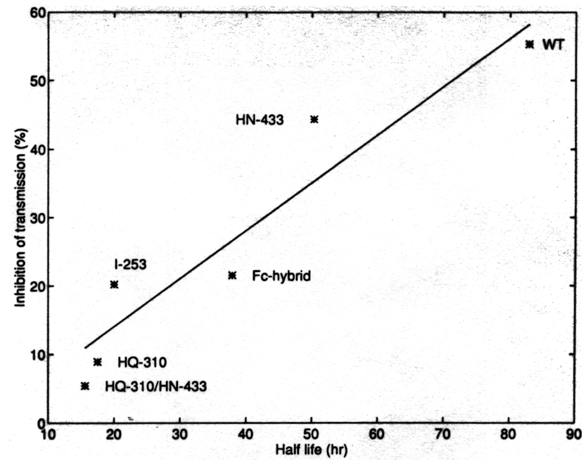

Figure 2. Correlation between β phase half life and inhibition of transfer for the recombinant WT and mutant Fc hinge fragments.

levels (Table 2). Mutations in the CH2 domain have a greater effect on the inhibition than those in the CH3 domain. These results indicate that the catabolic site, located at the interface of the CH2 and CH3 domain, overlaps with the site that is involved in intestinal transmission. Furthermore, the data for the Fc hybrid indicate that for an Fc fragment to effectively inhibit transfer it appears that two functional sites per fragment are necessary. There is an excellent correlation between the inhibitory capacity of a particular Fc hinge fragment and its half life (Fig. 2; Spearman coefficient = 1 with probability < 0.01) which suggests that the recognition sites involved in transmission and catabolism may be similar.

3.2 *In vitro* experiments

To confirm and extend the *in vivo* results, the binding of IgG1, WT Fc hinge and the HQ-310/HN-433 mutant to isolated neonatal brush border membranes have been analyzed in both direct and competition binding studies. Radiolabeled WT Fc hinge binds at approximately tenfold higher levels to brush borders than the HQ-310/HN-433 mutant, and the latter binds at only slightly higher levels than a murine Fab fragment produced by papain digestion that was used as a negative control to indicate the level of non-specific binding (Fig. 3). To extend these results, the ability of IgG1, WT Fc hinge and the HQ-310/HN-433 mutant to inhibit the binding of radiolabeled IgG1 was assessed in competition binding studies. The results show that at up to a 2000-molar excess the HQ-310/HN-433 mutant does not significantly inhibit the binding of IgG1, in contrast to the WT Fc hinge which inhibits in a similar way as the unlabeled IgG1 molecule (Fig. 4).

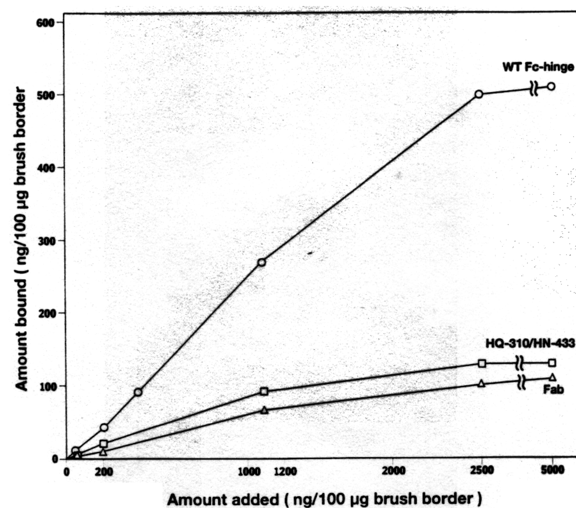

Figure 3. Binding of Fab, WT and HQ-310/HN-433 mutant Fc hinge fragments to isolated brush borders.

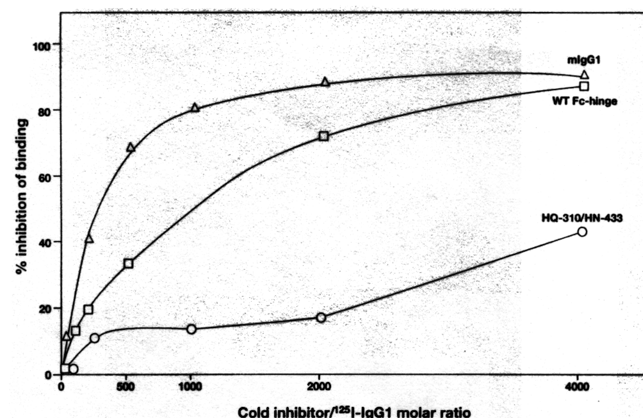

Figure 4. Inhibition of binding of ^{125}I-labeled IgG1 to isolated brush borders by unlabeled IgG1, WT and HQ-310/HN-433 mutant Fc hinge fragments.

4 Discussion

In this study, the transport of a recombinant WT Fc hinge fragment and mutant derivatives across the intestinal epithelial cells of neonatal mice has been analyzed. The recombinant aglycosylated WT Fc hinge is transcytosed with kinetics and in an amount similar to the transcytosis of a glycosylated Fc fragment produced by papain digestion, confirming earlier observations that aglycosylation does

not affect transcytosis either *in vivo* or *in vitro* [24]. Taken together with the *in vivo* transmission data, the *in vitro* binding experiments using WT and mutant Fc hinge fragments show that the site of the IgG1 molecule that is involved in binding to FcRn is located at the CH2-CH3 domain interface and overlaps with both the SpA binding site [20] and the catabolic site [14]. The region encompasses Ile 253, His 310, Gln 311, His 433 and Asn 434. Significantly, mutations of the Fc hinge fragment that have been shown previously to affect catabolism [14] also affect transfer to an extent that correlates closely with the effect on pharmacokinetics.

The data obtained *in vivo* has been confirmed by *in vitro* studies in which the binding of IgG1, WT Fc hinge and the HQ-310/HN-433 mutant to isolated neonatal brush borders has been analyzed. These experiments show unequivocally that the mutations at the CH2-CH3 domain interface affect transfer at the first step in transcytosis, namely binding to FcRn. These binding data are also consistent with the *in vivo* competition transfer experiments indicating that the mutant Fc hinge fragments are deficient in inhibiting the transcytosis of the murine IgG1 molecule. The FcRn binding site of IgG1 is therefore located at the CH2-CH3 domain interface and is distinct from the lower hinge region that is involved in binding of human IgG to the "classical" FcγRI, II and III [25–28].

The WT Fc hinge fragment is transcytosed in a similar way to an Fc fragment derived by proteolysis from a glycosylated IgG1 molecule. This suggests that the conclusions concerning the location of the FcRn binding site drawn from experiments using aglycosylated Fc hinge fragments can be extended to glycosylated IgG1. Furthermore, as murine and rat IgG subclasses can cross-inhibit intestinal transfer of each other [29, 30], this suggests that the region of the IgG molecule that is involved in transcytosis may be shared by all subclasses and across species. Consistent with this, the amino acid residues that have been demonstrated to be important for the binding of murine IgG1 to FcRn in this study are highly conserved in murine isotypes and IgG isotypes of other species [17]. It is also conceivable that the same region of the IgG molecule is recognized by FcRn on yolk sac and placental trophoblast membranes.

An alternative explanation for the lack of transmission of the Fc hinge mutants might be that the mutants are more susceptible to proteolysis. The mutations at the CH2-CH3 domain interface are in a region of the IgG1 molecule that is sensitive to attack by proteases such as trypsin or pepsin, and it could therefore be argued that the mutations in this region might alter the susceptibility to cleavage by these enzymes. This possibility was explored by analyzing the digestion of the WT Fc hinge and HQ-310/HN-433 mutant by trypsin and pepsin. No difference in protease sensitivity following analysis by SDS-PAGE of the degradation products after various incubation times was observed (data not shown). These observations, together with the fact that before weaning young rodents have lower proteolytic activity in the proximal jejunum [31], indicate that the difference in the transmission of WT Fc hinge *versus* the HQ-310/HN-433 mutant is not a result of differential proteolytic sensitivity.

The analysis of the Fc hybrid (heterodimer Fc hinge comprising WT and mutant Fc hinge proteins) in transfer studies indicates that it is transcytosed in similar amounts as the HQ-310/HN-433 mutant and inhibits transfer of the radiolabeled IgG1 molecule at a level that is similar to the inhibitory capacity of the I-253 mutant. It could be argued that the presence of the c-myc peptide (11 amino acids, [19]) at the C terminus of the mutant Fc hinge polypeptide in the hybrid may have an adverse effect on transmission by affecting the structure of the Fc hinge fragment. To eliminate this possibility, a hybrid Fc hinge fragment was also made which comprised WT and mutant (HQ-310/HN-433) polypeptides both with His6 peptides. This hybrid Fc hinge had the same rate of transmission as the Fc-hybrid prepared by tagging the two constituent polypeptides differently. Bivalency therefore appears to be necessary for binding to FcRn and/or transfer, and this is consistent with a study in which it was shown that two recombinant FcRn molecules are bound by one rat Fc molecule [12]. In addition, dimeric IgA has been reported to be transcytosed by the poly Ig receptor with much higher efficiency than monomeric IgA [32–34], but this may be a reflection of the involvement of the J chain [32] rather than the need for multivalency for binding and/or transport.

The region of FcRn which interacts with IgG has not yet been identified, although it has been suggested that stability differences of this receptor as the pH is varied from 6 to 8 may account for FcRn binding to IgG at pH 6.0–6.5 and release at pH 7.5 [35]. The structure of FcRn has also been modeled on the basis of the sequence similarities that it shares with structurally solved class I MHC molecules [35]. This allowed the identification of surface accessible histidine residues, and the pKa values in the range of 6–7 of the side chains of histidine suggested that these residues might be involved in interacting with IgG. However, mutagenesis of His 168, 250 and 251 of a recombinant soluble form of FcRn did not affect binding to IgG [35]. Our data clearly show that histidines are involved in the interaction of IgG with FcRn, but that these histidines are located in the IgG molecule, not in FcRn. These data, taken together with those of Raghavan and colleagues [35], indicate that the pH-induced binding and release of IgG during intestinal transfer could be brought about by both direct interactions involving histidine residues of IgG and more indirect effects involving regions of the FcRn molecule distinct from the binding site.

The coincidence of the SpA [20] and FcRn binding sites at the CH2-CH3 domain interface raises questions as to the extent of overlap. In this respect, the pH dependency of FcRn and SpA binding to the IgG1 Fc fragment are different [36, 37]. IgG1 or IgG1-derived fragments bind to SpA at neutral or basic pH (7–9) and dissociate from it at acidic pH (less than 5), whereas for FcRn, binding occurs at acidic pH (6–6.5) and dissociation at pH 7.5–8. This suggests that although some residues of the Fc are involved in interacting with both SpA [20] and FcRn, the nature of the interactions in terms of the numbers of residues and surface areas involved probably differ for the two ligands. The extent and nature of the overlap awaits the determination of the X-ray crystallographic structure of FcRn complexed with Fc [12].

The close correlation between the effects of the mutations of the Fc hinge fragments on intestinal transfer and pharmacokinetics is consistent with the earlier hypothesis of

Brambell [1, 16]. The theoretical model of Brambell suggested that IgG transcytosis across the placenta and intestine and the control of IgG catabolism involves cell-bound receptors. In the case of the control of IgG catabolism, these receptors were postulated to bind and protect the circulating IgG from degradation and to release them back into the circulation [16]. In contrast to intestinal transcytosis for which FcRn has been shown to be involved [4, 6], the Fc receptors and cell types involved in catabolism control have yet to be identified. Furthermore, if by analogy with intestinal transcytosis the protective receptors involved in the control of catabolism bind to IgG in a pH-dependent way as suggested by the involvement of histidine residues, it is tempting to speculate that binding of IgG to these receptors does not occur on the surface of the cells involved in catabolism, but in an intracellular acidic compartment. The internalized IgG could then be recycled and released at the cell surface. Evidence that supports such a model for the catabolism of IgG comes from a study in which rat yolk sac FcRn receptors were found to be localized in endosomal vesicles of the apical and basolateral cytoplasm and not at the luminal surface of the cells [38]. Clearly, the elucidation of the mechanism involved in controlling serum levels of IgGs awaits the characterization of Fc receptors that might be involved.

In conclusion, the site on the murine IgG1 molecule that interacts with FcRn has been localized to residues at the CH2-CH3 domain interface and overlaps with both the SpA binding site and the catabolic control site. In addition, two FcRn binding sites per IgG1 molecule appear to be necessary for efficient intestinal transfer. The results provide experimental support for the Brambell hypothesis [1, 16], in which it was proposed that the control of IgG catabolism and intestinal transcytosis occur by a related mechanism involving Fc receptors that bind and transport IgG and simultaneously sequester them from degradation. The identification of the FcRn binding site on IgG could be of value in analyzing the molecular mechanisms by which intestinal transcytosis occurs.

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