Contribution of FcRn binding to intestinal uptake of IgG in suckling rat pups and human FcRn-transgenic mice


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PERMEATION OF PROTEINS THROUGH the intestinal wall is typically limited to peptides of four amino acids or less (or molecules <800 Da) owing to the presence of tight junctions between epithelial cells (17). In the case of neonates, the intestinal mucosal barrier is not mature until after weaning (39) and allows for passage of growth factors (11) and immunoglobulin (IgG; allows for passage of growth factors (11) and immunoglobulin epithelial cells (17). In the case of neonates, the intestinal /H11021 800 Da) owing to the presence of tight junctions between neonatal Fc receptor (FcRn); however, the contribution of FcRn vs. FcRn-independent uptake to serum IgG levels had not been determined in either rat pups or human (h)FcRn-expressing mice (Tg276 and Tg32). In isoflurane-anesthetized rodents, serum levels were determined after regional intestinal delivery of human monoclonal antibodies (hIgG) with either wild-type (WT) Fc sequences or variants engineered for different FcRn binding affinities. Detection of full-length hIgG was by immunocytochemistry. High (μg/ml) serum levels of hIgG were detected after proximal intestinal delivery (0.1–10 mg/kg) in 2-wk-old rats. Human FcRn was visualized in epithelial cells of Tg276 mice, but low serum hIgG levels (<10 ng/ml) were obtained. In rat pups, intraintestinal hIgG1 WT administration resulted in dose-related and saturable uptake, whereas uptake of a low FcRn-binding affinity variant was nonsaturable. There were no differences in hIgG levels from systemic and hepatic portal vein serum samples, and intense hIgG immunostaining was noted in villi enterocytes and within lymphatic lacteal-like vessels. This study demonstrated that FcRn-mediated uptake in rat pups accounted for ~80% of serum hIgG levels and that IgG enters the circulation via the lymph and not the hepatic portal vein. The remaining uptake though the immature intestine is nonreceptor mediated. Intestinal epithelial cell hFcRn expression occurred in Tg276 mice, but receptor-mediated transport of IgG was not observed. The suckling rat pup intestine is a mechanistic model of FcRn-IgG-mediated transcytosis.

neonatal receptor; antibody; IgG; FcRn; gastrointestinal; transcytosis

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Intestinal FcRn function has only been assessed in the reverse direction; that is, from the systemic administration of IgG into the intestinal lumen in hFcRnTg/hβ2mTg transgenic mice (43). The function of FcRn in the uptake of monoclonal IgG antibodies from the intestines has not been explored, to our knowledge. Therefore, a second purpose of the present study was to assess the localization and functional activity of hFcRn in the intestines of commercially available mFcRn−/−hFcRn-expressing mice (Tg32 and Tg276).

MATERIALS AND METHODS

All animal studies were performed in accordance with the Federal Animal Welfare Act and were approved by the Institutional Animal Care and Use Committee at Centocor R&D (currently known as Biotechnology Center of Excellence, Janssen Pharmaceutical Companies of J&J). Male and female mFcRn−/−hFcRn-expressing mice (Tg32 and Tg276; licensed from The Jackson Laboratory, Bar Harbor, ME and bred at Ace Animals, Boyertown, PA), and 4- to 8-wk-old Sprague-Dawley rats (Ace Animals) were used in these studies. The strains of mice have been confirmed in-house for their extended circulating half-life of monoclonal antibodies after intravenous administration (31). Near-term, pregnant Sprague-Dawley rats obtained from Charles River Kingston (Stone Ridge, NY) gave birth at our facility and the litters were maintained with the dams until the study day at 15–17 days old (2-wk suckling) or 19–20 days old (3-wk suckling). Rodents were group housed in polycarbonate cages at an ambient temperature of 21–23°C with an automated 12:12-h light-dark cycle and access to water and a commercial rodent food ad libitum. Rats (4–8 wk old) and mice were fasted overnight with ad libitum access to water prior to treatment.

Human monoclonal antibodies. Human monoclonal antibodies on an IgG1 isotype to anti-respiratory syncytial virus (RSV) were dosed by direct intestinal administration, except for M14 IgG, an anti-growth factor, which was also dosed by oral gavage. Anti-RSV was selected for generating Fc variants to prevent potential binding of endogenous ligand. Variants with increased FcRn binding affinity were generated by mutating asparagine at position 434 to alanine (42) or methionine at position 428 to leucine (45). To serve as a negative control, a variant with decreased binding affinity to FcRn was made by mutating the histidine at position 435 to alanine (12). For binding affinity determinations using the ProteOn XPR36 (7, 36), rat FcRn (rFcRn) was immobilized onto a GLC sensor chip (Bio-Rad, Hercules, CA) by use of standard amine coupling. Each surface was activated with SULFO-NHS/EDC for 240 s at 30 μl/min followed immediately by injection of rFcRn at 310 ng/ml in 10 mM sodium acetate (pH 4.5). This method resulted in coupling rFcRn at response levels of ~170 response units (RU) per channel (1 RU = 1 pg protein/mm²). Human IgGs were then tested in PBS-TE buffer (pH 6.0, 0.01% Tween-20, 3 mM EDTA) with 1 μM as the highest concentration of a fivefold dilution series, while leaving one analyte flow lane for buffer only. The five concentrations of each analyte were injected simultaneously at a flow rate of 60 μl/min for 1 min using running buffer at either pH 6.0 or pH 7.4. The processed response was determined by use of ProteOn bivalent analyte model software.

Gastrointestinal dosing and sample IgG analysis.

Fig. 1. A–C: serum levels obtained following intraduodenal dosing of 0.1–10.0 mg/kg hlG1 WT in 2- to 8-wk-old rats where only 2-wk-old rats (○) showed time- and dose-dependent increases in IgG1 wild-type (WT) serum levels. D: pups 19–20 days old (1.0 mg/kg hlG1) showed lower IgG1 N434A serum levels (●) with greater variability than the same dose given to 2-wk-old pups (shown in B) and not different from the serum levels of hlG1 H435A (○). Lower limit of detection = 0.1 ng/ml; n = 6/group. E: Western blot of intestinal mucosal scrapings showing intense bands reactive to anti-mouse neonatal Fc receptor (FcRn) from 2-wk-old but not 8-wk-old rats. Rat FcRn is expected to be a slightly higher molecular weight (Mol wt) than mouse FcRn.
surgical anesthesia was reached, a midline laparotomy was performed and the stomach was gently lifted to place a 20-gauge Angiocath or 27-gauge needle attached to a 1-ml syringe through the antral wall into the small intestine via the pyloric sphincter. For intraileal administration a 27-gauge needle was placed directly into the ileum ~1–2 cm proximal to the cecum. After bolus intestinal dosing of hIgG (0.1–10 mg/kg diluted to 10 ml/kg in PBS) the needle was removed and a small ligating clip was placed to prevent any leakage into the abdominal cavity. The abdominal tissue was approximated and temporarily closed with two to three 7-mm staples. Oral gavage was performed with a 24-gauge stainless steel feeding needle.

Blood (~200 μl) was collected at 5, 45, and 90 min from the retro-orbital venous plexus and at 90 min from the hepatic portal vein. The serum was separated and frozen at ~80°C until assayed. Small intestine segments (~1 cm) were opened longitudinally and placed flat between biopsy sponges in 10% neutral buffered formalin and embedded within 48 h in paraffin for immunohistochemistry. The remaining small intestine was then divided into thirds. Contents from proximal, mid, and distal segments were collected separately, and mucosal scrapings were added to individual 2-ml tubes containing 250 μl of rat or mouse serum with protease inhibitor (Roche Diagnostics, Indianapolis, IN), and centrifuged (3,500 rpm for 10 min). The supernatant was collected and stored at ~80°C until assayed.

Rat intestine mucosal scrapings were collected in lysis buffer (T-PER tissue extraction reagent, Thermo Scientific, with complete protease inhibitor cocktail) and homogenized for FcRn Western blot analysis. Reduced lysate samples (20 μl) containing 15–100 μg of protein were loaded per lane along with a recombiantly expressed mFcRn (50–100 ng produced in-house) on a 4–12% SDS-PAGE and blotted. The blot was stained with mouse anti-mFcRn polyclonal antibody (1 μg/ml, generated in-house), and then with goat anti-mouse IgG 680 (Licor 1:700) or goat anti-rabbit IgG (1:10,000). Similar SDS-PAGE conditions were used for detecting hFcRn in lanes loaded with lysates from Tg276 mouse intestinal mucosa (100 μg) and recombiantly expressed hFcRn followed by incubation using polyclonal rat anti-hFcRn (1 μg/ml).

To measure full-length hIgG1 in the serum and gastrointestinal tract samples a MesoScale Discovery electrochemiluminescent assay was developed with a sensitivity of 0.1–500 ng/ml. An anti-idiotypic antibody to anti-RSV or antigrowth factor was labeled with Sulfo-NHS-LC-Biotin and used as a capture antibody. A mouse or rat antibody to anti-RSV or antigrowth factor was labeled with Sulfo-NHS ester was used as the detection antibody. The luminescence readout from duplicate standard curves was used for back-calculation (ng/ml) of unknowns and controls using GraphPad Prism regression analysis (variable slope) with an assay sensitivity of 0.1–500 ng/ml. Serum and intestinal content levels of hIgG1 are reported as means ± SE (ng/ml). The total blood volume was estimated using a conversion factor of 0.6 ml/10 g body wt.

Immunohistochemistry. Paraffin sections (5-μm) on glass slides were dried at 60°C, dewaxed, and pretreated in the capillary gap in the upper chamber of a Black and Decker Steamer modified (23) as part of a steam heat-induced epitope recovery pretreatment system (SHIER2). These procedures were completely automated by using a TechMate 500 (BioTek Solutions). Primary antibodies used were rat anti-human FcRn polyclonal Ab (affinity-purified over an FcRn column, generated in-house) and rabbit anti-human IgG1 polyclonal (heavy and light chain; Epitomics, Burlingame, CA), as well as nonimmune control serum. After staining, slides were dehydrated, permanently sealed with glass coverslips and Permount, and examined under a microscope. Positive staining was indicated by the presence of a brown chromogen (3,3’-diaminobenzidin-horseradish peroxidase) reaction product with hematoxylin counterstaining (blue nuclear stain) to assess cell and tissue morphology. Semiquantitative staining scores (0 = absent, 1 = sparse staining, 2 = low intensity, 3 = moderate intensity, 4 = very intense) for the presence of anti-hIgG was performed by a histopathologist blinded to the treatments. Representative images were obtained with an Olympus Microfire digital camera (M/N S97809) attached to an Olympus BX60 microscope and displayed on a Dell flat panel monitor with the DPI set at 96 and the screen resolution set at 1,152 × 864 pixels. Images were brought into sharp focus by use of Koehler illumination with the substage condenser set to brightfield.

RESULTS

The binding affinities (expressed as $K_D$) of hIgG1 WT and Fc variants to rFcRn at pH 6.0 were determined by surface plasmon resonance to be as follows: WT = 169 nM, N434A = 77 nM, M14 M428L = 2 nM, H435A > 1,000 nM. Each hIgG had a $K_D > 1,000$ nM to rFcRn at pH 7.4, as expected. Full-length hIgG IgG1 WT, dosed directly into the duodenum, was rapidly detected in the systemic circulation in 2-wk-old suckling rat pups, but not in 4- to 8-wk-old rats. In suckling rat pups, serum levels increased over time in a dose-related manner (0.1–10.0 mg/kg) after hIgG1 WT duodenal dosing (Fig. 1, A–C). In 19- to 20-day-old rat pups, a single 1 mg/kg dose of hIgG1 N434A variant (~2-fold higher affinity to FcRn than WT) resulted in ~15-fold lower hIgG serum levels at 90 min (166 ± 158 ng/ml; Fig. 1D) compared with 2-wk-old pups (3,152 ± 494 ng/ml; Fig. 1B), and serum levels were similar to
the low FcRn binding affinity variant hIgG1 H435A (90 ± 4 ng/ml). Thus just prior to weaning (~3 wk) there is a very rapid reduction in FcRn-dependent uptake of hIgG and all further experiments were performed on rat pups 12–16 days old (2 wk). Western blot assays for rFcRn from 2-wk-old suckling rat pup intestinal mucosa showed bands (~47 kDa) reactive to anti-mFcRn, at a similar molecular weight to recombinant mFcRn, but no rFcRn reactivity was noted in mucosa from 8-wk-old animals (Fig. 1E).

In 2-wk-old rat pups, the levels of hIgG1 in systemic and portal vein serum samples were similar and dose related at 90 min after duodenal dosing (Fig. 2A). Fractional uptake into systemic circulation was 40% of a 0.1 mg/kg dose of IgG1 WT, which was significantly higher than fractional uptake obtained with higher doses (1.0 mg/kg dose 19%, 10.0 mg/kg dose 14%) and consistent with a saturable mechanism (Fig. 2B). At 90 min after 10 mg/kg hIgG1 WT dosing, intense hIgG immunostaining was noted in villi of the proximal small intestine in suckling rat pups (Fig. 3A). Higher power magnification of hIgG immunoreactivity 2-wk-old rat pup intestine illustrates the intense staining in the lamina propria and a vascular-like staining pattern that appeared to be in the lymphatic lacteal-like vessels (Fig. 3B), but not in an adjacent section stained with control rabbit IgG (Fig. 3C). A 100-fold lower dose of IgG (0.1 mg/kg) resulted in detectable but less intense staining in the mucosa and, notably, there was a dramatic demarcation between enterocyte staining in the villus compared with absence of immunostaining in the crypts (Fig. 3D). Semiquantitative analysis of immunostaining confirmed intensely immunopositive enterocyte/brush border staining at both doses and more intense staining with the higher dose in the submucosal region and lamina propria (Table 1). The loss of detectable IgG immunostaining within the lamina propria at lower doses made it difficult to perform colocalization studies with a marker for lacteals. Therefore we have referred to this staining pattern as in lacteal-like vessels. In 8-wk-old rats dosed 10 mg/kg, hIgG

Table 1. Semiquantitative analysis of human IgG-immunoreactivity in the compartments of the proximal small intestine 90 min after dosing with hIgG1 into duodenum

<table>
<thead>
<tr>
<th>Dose</th>
<th>Enterocytes</th>
<th>Lamina Propria</th>
<th>Submucosa</th>
<th>Nerves</th>
</tr>
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<tbody>
<tr>
<td>0.1 mg/kg hIgG</td>
<td>3.3 ± 0.7</td>
<td>0.2 ± 0.2</td>
<td>0.7 ± 0.7</td>
<td>0.2 ± 0.2</td>
</tr>
<tr>
<td>10.0 mg/kg hIgG</td>
<td>4.0 ± 0.0</td>
<td>1.7 ± 0.7</td>
<td>2.8 ± 0.2*</td>
<td>1.7 ± 0.3</td>
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A dose-related effect on intensity of immunostaining was noted in different compartments with the submucosal staining attaining a level of significance.

*P < 0.05 by 2-way ANOVA and Bonferroni’s posttest comparison; n = 3/group. 0 = absent; 1 = sparse staining; 2 = low intensity; 3 = moderate intensity; 4 = very intense.
immunostaining was visualized in the luminal spaces between the villi (Fig. 3E) and occasional epithelial cell-like staining was noted (Fig. 3F) compared with the control serum (Fig. 3G); however, a 10-fold lower dose resulted in no detectable immunostaining (Fig. 3H).

Since the fractional uptake was similar after 1.0 and 10.0 mg/kg doses, the highest dose used in further studies was reduced to 3 mg/kg. After intraduodenal dosing (0.3, 1.0, and 3.0 mg/kg) to 2-wk-old rats, dose-related increases in serum levels of hIgG WT were significantly greater than the serum levels obtained with the low FcRn binding affinity variant, hIgG1 H435A, dosed to littermates (Table 2). In the case of hIgG1 WT, the fractional uptake in serum decreased as the dose increased; however, the fractional uptake of hIgG1 H435A was similar for all doses of this variant (Fig. 4A). The similar fractional uptake at all doses of hIgG1 H435A suggests that this uptake was FcRn-independent uptake and not receptor-mediated. When the data were converted to % serum levels of hIgG1 WT for each dose, between 8 and 18% of IgG1 WT serum levels could be considered to be due to FcRn-independent absorption (Table 2). The relative concentration of full-length hIgG1 in the proximal, mid, and distal thirds of the small intestine fluid indicated that the hIgG1 WT concentration was highest in the proximal and mid small intestine and related to the dose administered (Fig. 4B). This finding is consistent with the intense hIgG immunostaining in the proximal small intestine of these pups.

In the small intestine of C57 B/6 null mice (Fig. 5, A and B) and Tg32 hFcRn-expressing mice (Fig. 5, C and D) hFcRn immunoreactivity in tissues was nonspecific. However, in Tg276 hFcRn-expressing mice, hFcRn immunostaining in epithelial cells was noted throughout the proximal, mid, and distal small intestine. An example in the distal small intestine illustrates FcRn immunostaining in enterocytes of Tg276 hFcRn-expressing mice (Fig. 5E) that was markedly more intense than rat serum control staining (Fig. 5F). These results were confirmed by Western blot where a band was observed at 37–40 kDa in Tg276 small intestinal mucosa lysates that was absent in normal C57BL/6 mice (data not shown).

The functionality of the transporter in Tg276 mice was assessed after intragastric gavage and direct hIgG intestinal administration (Fig. 6). Irrespective of the region dosed, all groups had low serum levels (<10 ng/mL; Fig. 6, A and B). Since adult mice have mature gastrointestinal tracts with active proteolytic enzymes, the small intestinal hIgG levels were below quantification 90 min after gastric and duodenal dosing (Fig. 6A). Detectable levels of hIgG were noted in intestinal fluid after dosing into the distal small intestine, although serum levels were <2 ng/ml for both WT and non-FcRn binding variants in these mice (Fig. 6B).

**DISCUSSION**

By combining direct duodenal administration of hIgG WT and FcRn binding affinity variants with a method for detection of full-length hIgG monoclonal antibodies, the present study determined that FcRn-mediated IgG uptake from the neonatal intestine was saturable and accounts for >80% of detected hIgG levels in serum. The uptake of the remaining <20% of hIgG in serum, on the basis of data for a control IgG that lacks FcRn binding, was not saturable and presumably due to leakage of the immature intestinal barrier. Immunohistochemical localization of hIgG in enterocytes and lymphatic lacteal-like vessels is consistent with the preferential uptake of large molecules through the lymphatic system, and systemic and hepatic portal vein hIgG levels were similar. Mice (Tg276) genetically altered to express hFcRn (α-chain) had protein

Table 2. Serum levels of hIgG1 WT compared to hIgG1 H435A dosed to littermates at the same time

<table>
<thead>
<tr>
<th>Serum concentration</th>
<th>Dose, mg/kg</th>
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<tr>
<td></td>
<td>0.3</td>
</tr>
<tr>
<td>hIgG1 WT (μg/ml)</td>
<td>2.1 ± 0.5</td>
</tr>
<tr>
<td>hIgG1 H435A (μg/ml)</td>
<td>0.3 ± 0.1*</td>
</tr>
<tr>
<td>hIgG1 WT (% WT total)</td>
<td>100 ± 22.7%</td>
</tr>
<tr>
<td>hIgG1 H435A (% WT total)</td>
<td>14.3 ± 4.2%</td>
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Data are shown as actual serum levels (μg/ml) and as percentage of IgG1 WT serum levels (n = 6/group). *P < 0.05 by 2-way ANOVA and Bonferroni’s posttest comparison with hIgG1 WT.
visualized throughout the intestinal epithelium, but receptor-mediated transport of IgG was not observed from the ileum despite confirmation of the presence of full-length hIgG in the intestinal fluid. Overall, these data provide several novel insights into intestinal FcRn that will be discussed in the context of the known literature.

The intestinal epithelium can actively take up macromolecules by both receptor-mediated and nonspecific pinocytosis, as well as permeation through the intestinal barrier, which is immature in neonates. A saturable process of IgG uptake via enterocytes in the neonatal rat intestine was demonstrated with radiolabeled IgGs in the 1970s (21), prior to the identification of FcRn. The present study confirms this finding and demonstrates that FcRn is the only receptor that actively transports IgG transport across the neonatal gut. By using immunoassay detection methods, a recent study showed that a single high oral dose (1 mg; ~500 mg/kg in a 2-wk-old rat pup) of mIgG resulted in ~30% fractional uptake at 6 h (28). Fractional uptake of 40% was obtained in the present study (0.1 mg/kg) at an earlier time (90 min) after intestinal delivery, with saturation occurring at hIgG doses >3 mg/kg. Therefore, the dose used by Martin and colleagues (28) was well above that which would be expected to be selective for receptor-mediated transport. Dosing below 10 mg/kg demonstrated that permeation accounts for <20% of IgG levels in serum. In suckling rat pups the endocytic system in intestinal epithelial cells is highly increased to facilitate the absorption of macromolecules and is subsequently reduced after weaning (2). In addition, rats are born after a short gestation and the development of gastrointestinal mucosal barriers may not be mature until after weaning (38, 39). However, our study provided no evidence of another receptor-mediated transport mechanism since the low FcRn binding variant hIgG uptake was nonsaturating over the range of doses tested.

The functionality of FcRn was reduced 15-fold in 19- to 20-day-old rats compared with 2-wk-old rats, which is consistent with markedly decreasing FcRn mRNA expression between 17 and 20 days (28). There also appears to be a time-dependent regulation of permeability in the maturing intestine. Since serum levels of hIgG1 WT were similar to the low FcRn binding variant hIgG uptake was nonsaturating over the range of doses tested.
but not before (25), and suggests a highly regulated time dependency of maturation. On the basis of this report, the pharmacology of FcRn binding affinity and dissociation rates were subsequently investigated in 2-wk-old rats (Cooper PR, Kliwinski C, Perkinson R, Ragwan E, Mabus JR, Dorai H, Giles-Komar J, Hornby PJ, unpublished observations).

The presence of hIgG in the proximal small intestine of suckling rat pups was consistent with the FcRn mRNA expression in rat pups, which is highest in the duodenum (20, 28). In addition, hIgG immunostaining was intense in brush border enterocytes of the villi with staining absent in cells toward the base of the crypts, where nonenterocyte epithelial cell lineages are located. Some investigators have suggested that FcRn is expressed on the apical surface of intestinal epithelial cells where the luminal pH of 6.0–6.5 in the duodenum would favor FcRn binding to IgG (34). On the basis of these observations, the relative contribution of surface-expressed FcRn on intestinal epithelium vs. pinocytosis with fluid phase FcRn binding in the endosomes was subsequently investigated in suckling rat pups (Cooper PR, Kliwinski C, Perkinson R, Ragwan E, Mabus JR, Dorai H, Giles-Komar J, Hornby PJ, unpublished observations).

Depending on size and composition, molecules can enter the hepatic portal circulation or the lymphatic system via villous capillaries and lacteals, respectively. Once a molecule enters the interstitial space, size and hydrostatic pressure determine that molecules >20 kDa are favorably absorbed into the lymphatic capillaries, which lack tight junctions (24), and drain into the thoracic duct, which empties directly into the superior vena cava. The intensity of IgG immunostaining in lymphatic lacteal-like vessels suggested that IgG preferentially enters the lymph. Smaller proteins, such as endogenously released GLP-1 (27), as well as lipophilic drugs (14) are transported via intestinal lymphatics. Further support of this lymphatic pathway for IgG uptake comes from the finding that hepatic portal vein and systemic serum levels were similar after duodenal administration. Therefore, it is unlikely that FcRn expressed on rat hepatocytes (6) would rapidly sequester intestinally absorbed hIgG through a “first-pass” effect in the liver.

The transgenic mice used in this study were homozygous for a null mutation of FcRn α-chain that expresses a human FCGRT. These mice have an increased hIgG circulating half-life consistent with the increased expression of hFcRn (31). The major difference in strains is that Tg276 mice have a chicken β-actin promoter and Tg32 mice have a native human promoter. Intestinal epithelial FcRn of adult mice is nearly undetectable, although detectable luminal IgG complexes to orally administered antigen have been noted (43) and intraperitoneal immunization upregulates FcRn, but at levels lower than during neonatal life (40). In Tg276 mice, dosing of hIgG intragastrically into the duodenum and ileum resulted in very low (<10 ng/ml) serum levels. Given the similarity of IgG uptake in neonatal rodents, our results confirm conclusions based on lack of uptake of IgG in mice that lack either the 2-microglobulin subunit of FcRn or its α-chain (17). In contrast to the reduced activity of intestinal proteolytic enzymes in rat pups (30), adult Tg276 mice have active intestinal enzymes that could reduce the amount of intact IgG available for intestinal FcRn-mediated uptake. This was shown not to be the case when IgG was dosed into the distal small intestine, where full-length hIgG was noted in intestinal fluid 90 min later, but serum levels remained low irrespective of the hIgG FcRn binding affinity of the variants. Together, these data indicate that hFcRn expression is not functional in the intestines of these transgenic mice. The reason for this is not clear although one possibility is the association of rodent β2-microglobulin (4) and human FcRn subunit. Enhanced uptake of IgG in hFcRn-expressing Madin-Darby canine kidney (MDCK) cells occurs when human β2-microglobulin (8) is coexpressed compared with hFcRn alone (32). Therefore, the lack of functionality of hFcRn in Tg276 mice intestines may be due to faulty subunit formation of “hybrid” hFcRn with mouse β2-microglobulin, although functional hybrid activity has been shown in vivo where IgG circulating half-life is increased (31).

**Perspective.** Suckling rat pups have very rapid receptor-mediated uptake of monoclonal antibodies that is saturating, accounts for >80% of serum levels, and declines rapidly prior to weaning. The high fractional uptake of hIgG following
intraduodenal administration confirms the activity of FcRn in oral IgG absorption in suckling rats. However, because of the immature intestinal tract, enhanced endocytic system, and deficient intestinal mucosal barriers, IgG uptake in rats pups would not be expected to be predictive of “oral IgG bioavailability” in adult primates where FcRn is expressed in intestinal epithelial cell throughout life (10, 19). FcRn is also expressed in bronchial epithelial cells, and in nonhuman primates an inhaled Fc-fusion protein resulted in therapeutically meaningful levels in systemic circulation (5). Therefore, an adult model of hFcRn uptake by the intestine is highly desirable to determine whether the attainable serum levels are therapeutically relevant. Unfortunately, Tg276 transgenic mice that express human FcRn in the intestinal mucosa did not functionally transport hIgG and the sucking rat pup remains the mechanistic in vivo model for FcRn-mediated IgG uptake from the small intestine into the systemic circulation.

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DISCLOSURES

All authors were Johnson & Johnson employees and have no conflict of interest to declare.

AUTHOR CONTRIBUTIONS


REFERENCES


