Receptor-mediated Immunoglobulin G Transport Across Mucosal Barriers in Adult Life: Functional Expression of FcRn in the Mammalian Lung

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Abstract

Mucosal secretions of the human gastrointestinal, respiratory, and genital tracts contain the immunoglobulins (Ig)G and secretory IgA (sIgA) that function together in host defense. Exactly how IgG crosses epithelial barriers to function in mucosal immunity remains unknown. Here, we test the idea that the MHC class I–related Fc-receptor, FcRn, transports IgG across the mucosal surface of the human and mouse lung from lumen to serosa. We find that bronchial epithelial cells of the human, nonhuman primate, and mouse, express FcRn in adult-life, and demonstrate FcRn-dependent absorption of a bioactive Fc-fusion protein across the respiratory epithelium of the mouse in vivo. Thus, IgG, like dimeric IgA, can cross epithelial barriers by receptor-mediated transcytosis in adult animals. These data show that mucosal surfaces that express FcRn reabsorb IgG and explain a mechanism by which IgG may act in immune surveillance to retrieve lumenal antigens for processing in the lamina propria or systemically.

Key words: FcRn • transcytosis • IgG • epithelial cells • lung

Introduction

Host defense against infection at mucosal surfaces depends on humoral immunity (1). Both IgA and IgG contribute (2–5). In the human intestine, lung, and genitourinary tract, a single layer of columnar epithelial cells forms the protective barrier between host and environment (6). IgA crosses this barrier to function in lumenal secretions by binding the polymeric Ig receptor (pIgR). The pIgR mediates vesicular transport of dimeric IgA for secretion across the epithelial cell in a process termed transcytosis (7). Some mucosal secretions in humans also contain IgG, where it functions together with sIgA in host defense (4, 8). In humans, IgG concentrations predominate over sIgA in the lumen of the lower respiratory and female genital tracts (9, 10), and rectal secretions contain IgG in concentrations that may exceed 700 μg/ml (11). Systemically administered IgG protects against mucosal infection by respiratory syncytial virus in the human lung (12) and HIV in the monkey intestine and vagina (5, 13). Humans deficient in IgG exhibit an increased incidence and severity of mucosal and systemic infections, and in particular of infections caused by microbes that invade or colonize the respiratory tract. The mechanism, however, by which IgG may cross epithelial barriers to function in mucosal secretions remains unknown.

In the intestine of suckling rodents and in the human placenta, the MHC class I–related Fc-receptor for IgG, FcRn, mediates transport of IgG across epithelial barriers by transcytosis. Transepithelial transport by FcRn explains how humoral immunity transfers from mother to infant. After weaning, however, epithelial cells of the rodent intestine downregulate expression of FcRn to nearly undetectable levels, and adult rodents do not absorb orally administered IgG. In contrast, absorptive epithelial cells lining the

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intestine of humans continue to express FcRn in adult life (14), and when tested in vitro, human, canine, and rodent epithelial cells in culture that express FcRn exhibit FcRn-dependent transcytosis of IgG in both directions across the epithelial monolayer (15–17). Based on these data, we recently proposed that FcRn may function in the adult human to shuttle IgG or IgG-antigen complexes across epithelial barriers for immune surveillance, host defense, or both (15, 18).

We now report that bronchial epithelial cells of the adult human, nonhuman primate, and mouse express FcRn. To test for FcRn-dependent IgG transport at this site (by examining absorption from lumen to serosa), we prepared a fusion protein consisting of the hormone erythropoietin (Epo)* attached to the Fc fragment of murine IgG1 (Epo–Fc). Epo binds to erythroid progenitor cells in the bone marrow and promotes proliferation of the red cell lineage. The Fc-fragment binds specifically to FcRn. Thus, Epo–Fc acts as a tracer to measure FcRn-dependent transepithelial transport in vivo, assessed as a reticulocytosis 4 d after administration of the fusion protein to mucosal surfaces. The use of Epo as a bioactive marker for IgG transport confers a high degree of sensitivity to the experimental approach. Our data show that FcRn mediates the absorption of intact Epo–Fc across the lung of the adult mouse by receptor-mediated transcytosis. These results define a function for FcRn at mucosal surfaces in adult animals and explain how IgG may get reabsorbed from lumenal secretions to participate in mucosal immunity.

Materials and Methods

Preparation of Epo–Fc Fusion Proteins. cDNA for full-length mouse Epo was amplified by PCR from a plasmid provided by T.R. Lappin (Queens University, Belfast, UK; reference 19) using the forward primer 5′-ATCTAGCGCGCACTCCGCTCCCCCCACGCCTCATC and backward primer 5′-GATCATGTCGACCGCAGCGGCCCTGTCCCCTCTCCTGCAG to introduce a short COOH-terminal extension (GGSGGS). The Fc-fragment of mIgG1 containing hinge, CH2 and CH3 domains was cut from cDNA provided by Christine Ambrose (Biogen, Cambridge Center, Cambridge, MA). Both DNA fragments were ligated into the vector scFvExpress-sec (provided by Jasper zu Putlitz, Einrich Hewe University, Berlin, Germany; reference 20). Mutations were introduced into the resulting Epo-Fc construct by using site-directed mutagenesis (Stratagene).

Protein Expression. Chinese hamster ovary (CHO) cells were transfected using Pfx-7 Lipid (Invitrogen) and selected for G418 resistance and subcloned. Recombinant proteins were purified from CHO cell supernatants by affinity chromatography using Protein G Sepharose 4 Fast Flow (Amersham Pharmacia Biotech). Large quantities were prepared by National Cell Culture Center.

Enzyme-Linked Immunosorbent Assay. Epo–Fc was measured using a standard sandwich ELISA with anti-Epo mAb for capture (expressed and purified from BF-11 cells obtained from American Type Culture Collection), and goat anti–mouse Fc-HRP conjugated Ab for detection (Sigma-Aldrich). For in vivo studies, Epo-Fc levels were quantitated in serum samples using an Epo ELISA (Quantikine; R&D Systems) and calibrated against standard curves of purified fusion protein.

Surface Plasmon Resonance. Studies were performed on a BIAcore 2000 (Biacore International AB) as described previously (21). Flow cells of CM5 sensor chips were coupled with Epo–Fc (444 RU), Epo-Fc/IHH (1283 RU) and Epo-Fc/LLG (2430 RU) using amine coupling. As a reference cell, one flow cell was treated with buffer only during the coupling cycle. Recombinant mouse FcRn (22) was injected at concentrations of 1,000 nM, 500 nM, 250 nM, 125 nM, and 62.5 nM at a flow rate of 10 μl/minute in PBS pH 6.0 containing 0.01% Tween 20. After each dissociation phase, residual FcRn was removed from the sensor chip by injection of PBS, pH 7.2, plus 0.01% Tween. Data was processed using either BIAevaluation 3.0 software or custom written software (provided by Raimund Ober). All data were zero adjusted and baseline subtracted before generating the sensorgrams presented in Fig. 1, C and D.

SDS-PAGE and Western Blot Analysis. Equal mass of recombinant proteins were analyzed by SDS-PAGE (10–20% gradient gels) under reducing and nonreducing conditions, before and after reaction with PNGase F (New England Biolabs) that cleaves NH_2-linked oligosaccharides and detected by Western blot using goat anti–mouse IgG-HRP for detection of Epo–Fc proteins. For detection of FcRn, we used an affinity purified rabbit anti-peptide Ab 176/190–1 (amino acids 176–190) specific to FcRn (gift from N. Simister, Brandeis University, Waltham, MA; reference 16) followed by anti–rabbit HRP. Lysates of intestinal epithelial cells, whole tissue, and epithelial cell lines were prepared as described previously (14, 15).

In Vivo Models. Female BALB/c mice 4–6 wk of age (average weight 17 g) and 10-d-old pups (average weight 7 g) from Taconic, and for Fig. 2 D μMT/μMT and control C57BL/6 mice from The Jackson Laboratory were maintained under pathogen-free conditions. Mice were anaesthetized with Isoflurane by inhalation and Epo–Fc fusion proteins were injected intraperitoneally, fed intragastrically using a ball-point needle (once, twice, or four times 12 h apart as indicated), or administered intranasally by instilling a total volume of 14 μl into the nostrils. Intragastric (i.g.) proteins were administered with 80–400 μg of soybean trypsin inhibitor in 100–500 μl carbonate buffer, pH 8.8, for mice 10-d or 4-wks-old, respectively. Proteins administered by nose were suspended in PBS alone. Normal mouse IgG and chicken IgY (both from Lampire Biological Laboratories) were included in the total volume where indicated. Mice were killed by CO_2 inhalation 8 h or 4 d later and whole blood was obtained by cardiac puncture. All animal studies were approved by the Institutional Review Board.

Flow Cytometric Analysis. Whole blood samples were added to ReticOne Reagent according to the manufacturer's instructions. Flow cytometry was performed with a Coulter Epics XL machine. Acquisition parameters were calibrated each time by Retic-Cal Biological Calibration and Retic-C Cell Control. 40,000 total events in the red blood cell gate were acquired and analyzed with ReticOne automated software for percentage of reticulocytes (all materials from Beckman Coulter).

Immunohistochemistry. For bright field microscopy, tissues obtained from adult mouse lung, adult human lung operative specimens, and adult cynomolgus macaque lung (Wyeth Genetics Institute) were immediately fixed in 4% paraformaldehyde and paraffin embedded. 5-μm sections were treated as described previously (23) with 5% urea for antigen retrieval, followed by affinity purified rabbit anti-peptide Ab 176/190–1 (amino acids 176–190) specific to FcRn (16) (1:200) or equimolar preim-

Abbreviations used in this paper: Epo, erythropoietin; i.g., intragastric.

mune serum as control, biotin-conjugated goat anti–rabbit IgG, tyramide-HRP amplification (Perkin Elmer), and counterstained with methyl green. Images were viewed with a Zeiss-Axiophot microscope equipped with a Spot digital camera (Diagnostic instruments) and captured in Adobe Photoshop (Adobe Systems Inc.).

Statistical Analysis. Statistical analysis was performed by ANOVA using StatView (SAS Institute Inc.). P values < 0.05 by multiple comparison procedures were considered significant.

Online Supplemental Material. Supplemental data available at http://www.jem.org/cgi/content/full/jem.20020400/DC1 include (a) detailed sequence primers used to introduce mutations into the Epo-Fc, (b) immunostaining and Western blots of adult and suckling mouse intestine for FcRn, (c) photograph of mouse lung showing distribution of delivery of intranasal Epo-Fc/methylene blue to the respiratory tract, and (d) dose response for reticulocytosis induced by Epo-Fc delivered intravenously to adult mice.

Results

Characterization of the Epo–Fc Construct. To test if FcRn transports IgG across mucosal barriers in vivo, we prepared recombinant proteins containing human Epo fused to the Fc fragment of mouse IgG1 including the hinge, CH2 and CH3 domains. To demonstrate specificity for FcRn, we also prepared fusion proteins containing one, two, or three inactivating point mutations in the FcRn-binding site of the Fc domain (Fig. 1 A, Epo–Fc/IHH identifies the triple mutant protein; reference 24), and fusion proteins containing three inactivating mutations in the FcγR1-binding site (Epo–Fc/LLG) (25, 26). FcγR1 represents the only other Fc-receptor known to efficiently bind and internalize monomeric IgG (27).

All fusion proteins formed disulfide-linked homodimers of glycosylated peptides that bound specifically to protein G indicating functional preservation of the Fc domain (Fig. 1 B, and unpublished data). In binding analyses using surface plasmon resonance (BIAcore), both immobilized Epo–Fc and Epo–Fc/LLG bound well to mouse FcRn, whereas the IHH mutant exhibited negligible binding (Fig. 1, C and D). When injected intraperitoneally into 10-d-old suckling mice, both Epo–Fc and the triple mutant Epo–Fc/IHH induced a nearly twofold increase in the reticulocyte fraction (from 22 ± 0.9 to 38 ± 1.1%; Fig. 1 E). These data show that both WT and mutant fusion proteins contain functional Fc- and Epo-domains.

Epo–Fc/IHH exhibited slightly lower potency relative to WT Epo–Fc in its ability to induce a reticulocytosis when administered intraperitoneally (Fig. 1 E). This could reflect an effect of the IHH mutation on the serum half-life of the mutant protein caused by the lack of FcRn binding. While adult rodents do not express FcRn in intestinal epithelial cells, FcRn remains functional in some cell types and acts to protect IgG from intracellular degradation by recycling IgG away from digestive organelles and back into the circulation. Such trafficking of internalized IgG by FcRn extends IgG half-life (28) and should prolong the half-life of the WT Epo–Fc fusion protein, but not of the IHH mutant that lacks the binding site for FcRn. Thus, in all in vivo studies described below, we addressed this variable by demonstrating receptor-mediated transport directly using excess IgG to block ligand-binding to FcRn, and by dou-

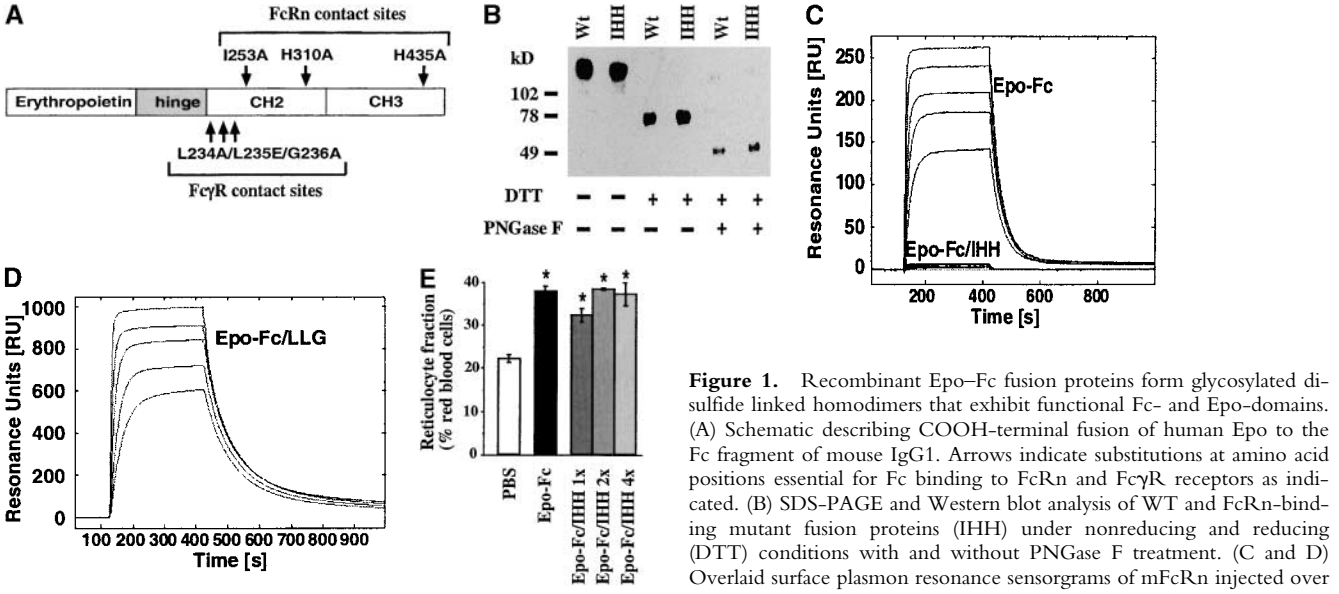

Figure 1. Recombinant Epo–Fc fusion proteins form glycosylated disulfide linked homodimers that exhibit functional Fc- and Epo-domains. (A) Schematic describing COOH-terminal fusion of human Epo to the Fc fragment of mouse IgG1. Arrows indicate substitutions at amino acid positions essential for Fc binding to FcRn and FcγR receptors as indicated. (B) SDS-PAGE and Western blot analysis of WT and FcRn-binding mutant fusion proteins (IHH) under nonreducing and reducing (DTT) conditions with and without PNGase F treatment. (C and D) Overlaid surface plasmon resonance sensorgrams of mFcRn injected over immobilized Epo–Fc, Epo-Fc/IHH, and Epo–Fc/LLG. mFcRn were injected at concentrations of 1,000 nM, 500 nM, 250 nM, 125 nM, and 62.5 nM at a flow rate of 10 μl/min (decreasing signal levels correspond to decreasing concentrations of mFcRn). Data show representative sensorgrams from duplicate injections, and were processed by zero adjustment and reference cell (blank flow cell) subtraction. Note that the different coupling densities of Epo–Fc (444RU) and Epo–Fc/LLG (2430 RU) result in different signal levels. (E) Reticulocyte fraction induced by intraperitoneal injection of Epo-Fc (0.5 μg/mouse, column 2, $n = 3$), Epo-Fc/IHH (0.5, 1, or 2 μg/mouse, columns 3, 4, and 5, $n = 3$ mice per group), or buffer alone (PBS, column 1, $n = 15$ mice from six independent experiments). Mean ± SEM. *$P < 0.05$ relative to PBS control.

ble-dosing the IHH mutant whenever possible, which abrogated the loss in potency of the IHH mutant as assessed by intraperitoneal injection (Fig. 1 E).

Functional Expression of FcRn in the Intestine. To demonstrate FcRn-dependent transport of Epo–Fc across the intestine, we used 10-d-old suckling and 4–6-wk-old adult BALB/c mice. Suckling mice express high levels of FcRn in absorptive epithelial cells lining the intestine and efficiently transport IgG across the intestinal barrier by FcRn-dependent transcytosis (29, 30). Adult mice do not. In suckling mice, i.g. administration of WT Epo–Fc induced a dose dependent increase in reticulocyte count (Fig. 2 A, apparent ED_{50} = 0.1 μg/animal, ~7 g body WT). I.g. administration of all fusion proteins containing amino acid substitutions in the FcRn binding site, except for the single alanine substitution at position I253, failed to induce a detectable increase in reticulocyte count, even when administered at twice the maximal dosage (Fig. 2 B). In contrast, the fusion protein Epo–Fc/LLG, containing inactivating mutations in the FcγR1-binding site, remained fully active (Fig. 2 B). Intraperitoneal injection of WT Epo–Fc or buffer alone provided positive and negative controls for these and all other in vivo experiments (Fig. 2, B and D, and studies described below).

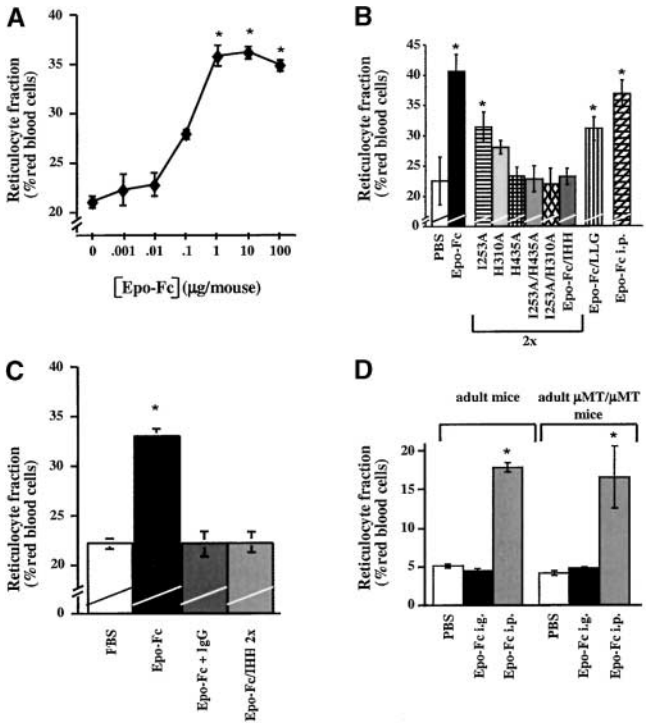

Figure 2. Absorption of Epo–Fc in the intestine of 10-d-old suckling mice depends on FcRn. (A) Dose dependent increase in reticulocyte fraction for Epo–Fc administered intragastrically. One of two representative independent experiments in 10-d-old mice, mean ± SD, n = 2 mice per dose. (B) Reticulocyte fractions induced by i.g. WT Epo–Fc (0.5 μg/mouse, column 2) and Epo–Fc variants containing the indicated mutation(s) in the FcRn- or FγR-binding sites. FcRn mutants were administered twice to account for predicted decrease in serum half-life as discussed in text. Orally administered PBS and intraperitoneally administered Epo–Fc provide negative and positive control. Mean ± SEM, n = 3 mice/group. (C) Reticulocyte fractions induced by i.g. WT Epo–Fc (0.5 μg/mouse) administered with 200-fold excess human IgG (column 3) or with buffer alone (column 2), by Epo–Fc/IHH (1 μg/mouse total, column 4), and by PBS alone (column 1). One of two representative independent experiments, mean ± SEM, n = 3 mice per group. (D) Reticulocyte fractions induced by i.g. Epo–Fc (100 μg/mouse, columns 2 and 5) in adult C57BL/6 (columns 1–3) or homozygous μMT/μMT mice (columns 4–6). Mean ± SEM, n = 3 mice/group. In all panels, *$P < 0.05$ above PBS-control baseline.

To demonstrate receptor-mediated transport directly, by a method independent of protein half-life, we examined Epo–Fc function in the presence and absence of competing ligand for FcRn. Competition with 200-fold excess IgG (100 μg/animal, representing <0.4% of total serum IgG) completely blocked Epo–Fc activity. Epo–Fc/IHH was completely inactive (Fig. 2 C). These studies indicate that absorption of Epo–Fc across the intestinal epithelial barrier of the suckling mouse depends on receptor-mediated transport, likely mediated by FcRn.

To confirm specificity for FcRn-dependent transport by a separate approach, we administered Epo–Fc to adult WT mice that exhibit undetectable levels of FcRn in epithelial cells of the intestine as assessed by immunohistochemistry (reference 31 and Supplemental Data). Even when administered at very high doses (100 μg/animal), i.g. Epo–Fc had no detectable effect on the reticulocyte count in adult animals (Fig. 2 D). Identical results were obtained using adult mice homozygous for the Igh-6^{tm1Cgn} mutation (μMT mice, reference 32). Homozygous μMT mice lack endogenous IgG, which may compete with Epo–Fc for binding to FcRn at the mucosal surface. Thus, adult mice do not express FcRn at detectable levels and do not display FcRn-dependent transepithelial transport in the intestine.

Functional Expression of FcRn in the Respiratory Tract. The high concentration of IgG in lumenal secretions of the adult human lung suggested to us that FcRn may function in IgG transport at this site. To test this idea, we examined the lungs of adult humans and nonhuman primates for expression of FcRn. Total cell lysates obtained from human and cynomologus macaque lung contained a ~45-kD glycosylated protein (~40-kD deglycosylated core) (Fig. 3 A) consistent with human FcRn as assessed by SDS-PAGE and Western blot using FcRn specific antibodies (15, 16). Total cell lysates prepared from two human bronchial epithelial cell lines also contained FcRn (Fig. 3 B). When visualized in paraffin sections by immunohistochemistry, FcRn localized predominantly to the apical region of bronchial epithelial cells lining the large and small airways of humans (Fig. 3 C, panel I) and macaques (Fig. 3 C, panel III), similar in intracellular distribution to that of FcRn expressed in epithelial cells lining the adult human intestine (14, 15). Alveolar tissue also exhibited staining for FcRn, but the weakly positive reaction product could not be assigned to epithelial or endothelial cells specifically (Fig. 3 C, panel II). The randomly scattered intense punctate staining observed for FcRn in alveolar sections likely represents alveolar macrophages that express FcRn at high levels in humans (33). These results indicate that bronchial epithelial cells lining the airways of both the adult human and

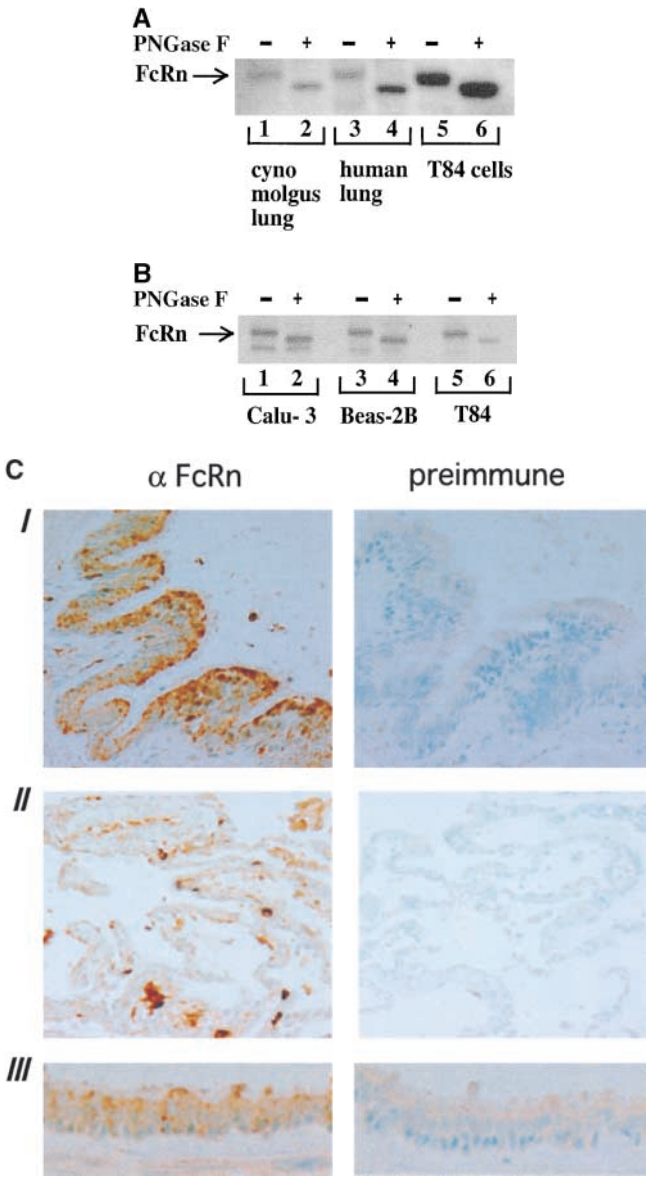

Figure 3. Expression of FcRn in bronchial epithelium of human and cynomolgus macaque. (A) SDS-PAGE and Western blot analysis for FcRn in total tissue lysates (170 μg/lane) of adult cynomolgus macaque lung (lanes 1 and 2), adult human lung (lanes 3 and 4), and cultured human intestinal T84 cells as positive control (lanes 5 and 6). Lanes 2, 4, and 6 represent samples treated with PGNase F. (B) SDS-PAGE and Western blot for FcRn in total cell lysates of Calu-3 (lanes 1 and 2), BEAS-2B (lanes 3 and 4), and T84 cell lines as positive control (lanes 5 and 6), with and without PNGase F treatment. Representation of 2 independent experiments. (C) Immunohistochemical staining for FcRn in paraffin sections of adult human bronchus (I) and alveolae (II) and bronchus of adult cynomolgus macaque (III) using a-FcRn specific (left) and nonspecific preimmune antibody as control (right). Original magnification: 20×, representative of three independent experiments.

nonhuman primate, and possibly alveolar epithelial cells, express FcRn.

To demonstrate FcRn function at this site, we examined the mouse lung. As in the human and nonhuman primate, total tissue lysates obtained from the lungs of adult mice contained a glycosylated 49-kD band consistent with FcRn

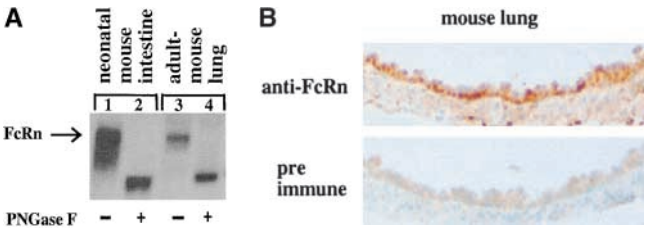

Figure 4. Expression of FcRn in bronchial epithelium of adult mice. (A) SDS-PAGE and Western blot analysis for FcRn in total tissue lysates (50 μg/lane) of adult mouse lung (lanes 3 and 4), and neonatal mouse intestine (lanes 1 and 2) as positive control. Lanes 2 and 4 represent samples treated with PGNase F. The deglycosylation product is consistent with the hydrolysis of predicted four glycosylation sites in mouse FcRn in contrast to the one glycosylation site in human FcRn as seen in Fig. 3 A. (B) Immunohistochemical staining for FcRn in paraffin sections of adult mouse bronchus using anti-FcRn specific (top) and nonspecific preimmune Ab control (bottom).

as assessed by SDS-PAGE and Western blot analysis (Fig. 4 A). Bronchial epithelial cells stained positive for FcRn as assessed by immunohistochemistry. A low level of background staining, presumed to be nonspecific based on our functional studies (see below and Fig. 5), was also observed in the lamina propria of the mouse using this Ab (Fig. 4 B).

Epo–Fc administered intranasally to adult BALB/c mice induced a dose-dependent increase in reticulocyte count (Fig. 5 A, apparent ED_{50} ~5–10 μg/animal, ~17 g body WT). Intranasal administration delivered the fusion proteins most intensely to proximal regions of the bronchial tree, though some fusion proteins also reached the distal lung as assessed by visual inspection using methylene blue as tracer (see Supplemental Data).

When delivered at 10 μg/animal, both Epo–Fc and the FcγRI-binding mutant Epo–Fc/LLG induced a near maximal reticulocytosis representing absorption of 1.2–1.5 μg Epo–Fc, or ~12–15% of the administered dose as assessed by calibration against Epo–Fc injected intravenously (Fig. 5 B, and Supplemental Data). The FcRn-binding mutant, Epo–Fc/IHH, induced a much smaller increase in reticulocyte count that differed marginally from baseline (Fig. 5 B). To demonstrate absorption of Epo–Fc directly, we measured serum levels of Epo–Fc and Epo–Fc/IHH by ELISA against human Epo at a time of maximal absorption, 8 h after intranasal administration (Fig. 5 C). Adult mouse lung exhibited dose-dependent absorption of Epo–Fc that displayed at least fivefold greater efficiency when compared with Epo–Fc/IHH. Serum levels at this time point depend far less on the rate of IgG catabolism. Thus, these data confirm specificity for FcRn. Nonetheless, at high doses, Epo–Fc/IHH was absorbed at detectable levels and induced a marginal reticulocytosis, indicating the possibility of less efficient absorption by FcRn-independent mechanisms. Such nonspecific transport of macromolecules may occur across the alveoli of rodents (34).

Thus, to directly demonstrate receptor-mediated transport of Epo–Fc across the respiratory epithelial barrier of the mouse lung, and specificity for FcRn, we used mouse IgG

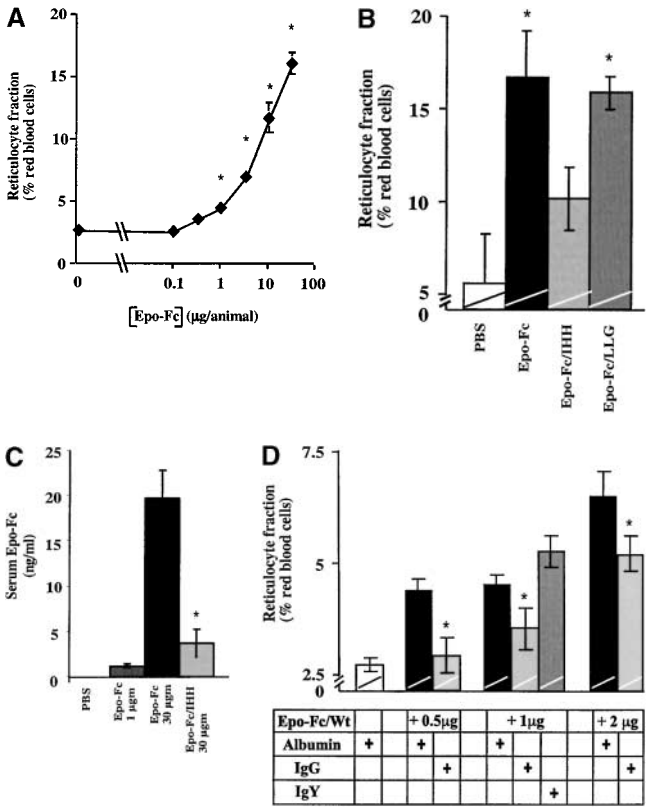

Figure 5. FcRn-specific absorption of Epo–Fc across the epithelial cell barrier of the adult mouse lung in vivo. (A) Dose dependent increase in reticulocyte count for Epo–Fc administered intranasally. Mean ± SEM, six mice per group. *$P < 0.05$ versus baseline. Maximal response in adult mice ~17%, see Fig. 2 D. (B) Reticulocyte fractions induced by intranasal administration of fusion proteins containing functional FcRn-binding sites, WT Epo–Fc (column 2) and Epo–Fc/LLG (column 4), by Epo–Fc/IHH that lacks an FcRn-binding site (column 3), and by PBS alone (column 1). All fusion proteins were administered at 10 μg/mouse. Mean ± SEM. $n = 5$ for all groups except $n = 2$ for PBS control. *$P < 0.05$ versus baseline. (C) Absorption of Epo–Fc and Epo–Fc/IHH as assessed directly by ELISA of serum obtained 8 h after nasal administration of fusion proteins at the indicated doses. (D) Reticulocyte fractions induced by intranasal WT Epo–Fc (2 μg, 1 μg, or 0.5 μg/mouse) administered together with 650-, 1,300-, or 2,600-fold excess mouse IgG (g/g, 1.3 mg total dose, light gray bar) 1,300-fold excess chicken IgY (1.3 mg total dose, dark gray bar) or nonspecific protein albumin (1.3 mg total dose, black bars) as competing ligands, and by PBS alone (white bar). Mean ± SEM from two independent experiments *$P < 0.05$ relative to Epo–Fc plus albumin control. $n = 12$ mice per group for doses of Epo–Fc 1 μg/mouse and PBS control. $n = 6$ mice/group for all other doses of Epo–Fc. In the absence of IgG block, absorption of 0.09–0.24 μg of Epo–Fc, or ~10–18% of the administered dose was detected as assessed by calibration against Epo–Fc injected intravenously (see Supplemental Data).

and the chicken Ig IgY in competition studies. Chicken IgY exhibits similar structure and function to mammalian IgG but does not bind FcRn (35). Consequently, when applied in excess, IgG should block FcRn-dependent absorption, but IgY should not. These studies showed the expected result. Mouse IgG in 650- to 2,600-fold excess (mg/mg), representing <5% of total serum IgG, inhibited each of three separate doses of Epo–Fc administered intranasally (Fig. 5 D). In contrast, competition with equal molar excess IgY or BSA had no effect. These data indicate that the mouse lung exhibits receptor-mediated absorption of Epo–Fc that displays sensitivity to competition with IgG but not IgY, a feature characteristic of FcRn.

Discussion

Our results show that the MHC class I–related Fc-receptor for IgG, FcRn, functions to transport IgG across epithelial barriers in adult life (15, 18). We find expression of FcRn in bronchial epithelial cells of the adult human, nonhuman primate, and mouse lung, and demonstrate FcRn-dependent absorption of a bioactive Fc-fusion protein across the respiratory epithelium of the adult mouse in vivo.

The idea that IgG may cross epithelial barriers by receptor-mediated transcytosis in humans and other adult animals represents a novel concept in mucosal immunology (15, 18). While abundant studies define the function of FcRn as a transporting receptor for absorption of IgG across the intestine in suckling rodents, the receptor disappears from the rodent intestine after weaning at 21 d of age. The few studies that directly examined absorption of IgG across the intestine of the human indicated absorption of only a small fraction (1% or less) of orally administered IgG (36), though these results may be confounded by intralumenal digestion of the IgG tracer. Most studies on the mechanism of IgG secretion suggested nonspecific transudation of serum proteins (1). On the other hand, two studies on mucosal secretions obtained from adult humans showed a distinct distribution of IgG isotypes when compared with IgG obtained from serum of the same individuals (37, 38). These data provide evidence for a specific mechanism of IgG transport across epithelial barriers. With the recent molecular identification of human FcRn (39), its detection in epithelial cells of the adult human intestine (14), and definition of FcRn function as a trafficking receptor for the bidirectional transcytosis of IgG in polarized epithelial cell lines in vitro (15, 16, 18), other explanations for IgG transport across mucosal epithelial barriers can now be entertained.

In this study, we find that FcRn acts in vivo as a trafficking receptor to transport IgG across mucosal epithelial barriers of the adult mouse lung. Several lines of evidence support this conclusion. First, only IgG, and not albumin or chicken IgY, blocked absorption of Epo–Fc. Such specificity for competition of protein transport represents the fundamental characteristic of a receptor-mediated process, and a feature characteristic of FcRn function (35, 40). Neither absorption by passive diffusion through intercellular tight junctions, nor endocytosis by fluid phase into the epithelial cell, can explain these results. We also found that inactivating mutations in the FcγR1-binding site had no effect on Epo–Fc activity. Thus, absorption of Epo–Fc cannot depend on binding FcγR1 receptors, the only other Fc-receptor that exhibits high affinity binding for monomeric IgG (26). Finally, our studies using the Epo–Fc/IHH mutant that cannot bind FcRn, even though potentially confounded by a predicted effect on protein half-life, imply specificity for FcRn: a conclusion confirmed by our studies

using IgY, which are independent of catabolic effect. These data indicate that IgG, like dimeric IgA, can cross epithelial barriers of adult animals by receptor-mediated transcytosis. Unlike IgA, however, FcRn moves IgG across epithelial barriers in the opposite direction (if not both) to get reabsorbed from lumenal secretions.

Thus, the results of these studies explain how IgG, or IgG–antigen complexes (41), or both, are reabsorbed across mucosal surfaces to function in immune surveillance and host defense. While it is well known that IgG binding to FcRn displays sensitivity to pH (35), the vectorial transport of IgG across epithelial barriers by FcRn does not depend on transepithelial pH gradients established at epithelial surfaces (42). As such, we predict that FcRn will function in IgG transport at any mucosal surface that expresses FcRn and propose that FcRn will mediate a steady-state and dynamic distribution of IgG across these barriers by reabsorbing IgG, with or without associated antigen, from lumen secretions. IgG and sIgA exhibit different functional characteristics, and by binding lumenal antigen, IgG may complement sIgA in immunoregulatory function at these sites. We also find that FcRn transports Epo–Fc across epithelial barriers as a fully folded and functional protein. These results define new technology to exploit FcRn clinically. Here, FcRn offers an endogenous mucosal receptor to absorb Fc-fusion proteins or vaccine antigens across epithelial surfaces that otherwise represent impermeable barriers to macromolecules.

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