Net Absorption of IgG via FcRn-Mediated Transcytosis
across Rat Alveolar Epithelial Cell Monolayers

Running head: Net IgG Transcytosis across Alveolar Epithelium

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Various serum proteins are known to be present in alveolar lining fluid, although the transport mechanisms underlying their presence remain unclear. In this study, we characterized immunoglobulin G (IgG) transport across rat alveolar epithelial cell monolayers cultured on permeable supports. Unidirectional fluxes of biotin-labeled rat IgG (biot-rIgG) were measured in the apical-to-basolateral (ab) and opposite (ba) directions as functions of [rIgG] in upstream fluids at 37 and 4°C. Specificity of IgG transport was explored by measuring unidirectional biot-rIgG fluxes in the presence of excess Fc, Fab, F(ab′)2, or chicken Ig (IgY) in either apical or basolateral upstream fluid. Expression of the IgG receptor FcRn, and the effects of dexamethasone on FcRn expression and biot-rIgG fluxes, were determined. Results show that ab flux of biot-rIgG is about 5-fold greater than ba flux at an upstream concentration of 25 nM biot-rIgG at 37°C. Both ab and ba fluxes of rIgG saturate, resulting in net absorption with $K_t$ and $J_{max}$ of 7.1 nM and 1.3 fmol/cm$^2$/hr. At 4°C, both ab and ba fluxes significantly decrease, collapsing net absorption. The presence of excess unlabeled Fc (but not Fab, F(ab′)2, or IgY) in either apical or basolateral upstream fluid significantly reduces the respective unidirectional biot-rIgG fluxes. RT-PCR analyses demonstrate the expression of α- and β-subunits of rat FcRn in rat alveolar epithelial cell monolayers. Northern analyses further confirm the presence of α-subunit of rat FcRn mRNA with an estimated size of ~1.6-kb. Dexamethasone exposure for 72 hr decreases the steady-state level of mRNA for rat FcRn α-subunit and the ab (but not ba) flux of biot-rIgG. These data indicate that IgG transport across alveolar epithelium takes place via regulable FcRn-mediated transcytosis, which may play an important role in alveolar homeostasis in health and disease.
Keywords
receptor-mediated, saturable transcytosis, net IgG absorption, lung defense, pulmonary immune system

Abbreviations
biotinylated rat immunoglobulin G (biot-rIgG), biotinylated rat Fc (biot-rFc), potential difference (PD), transepithelial electrical resistance (Rt), rat alveolar epithelial cell monolayers (RAECM), apical-to-basolateral (ab), basolateral-to-apical (ba), immunoglobulin (Ig), chicken Ig (IgY), apparent half maximal concentration (Kt), maximal flux (Jmax).
INTRODUCTION

Alveolar epithelium lines the distal airspaces of the lung and provides high resistance to the leak of solutes and fluid from the surrounding interstitial and vascular spaces (18). Various serum proteins (e.g., albumin, transferrin, and immunoglobulin G) are known to be present in alveolar fluid lining distal airspaces, although the underlying transport mechanisms that account for their presence are not well delineated (10, 12, 19). Alveolar protein clearance is essential for resolution of both hydrostatic and (especially) high permeability pulmonary edema. Understanding the mechanisms of alveolar protein clearance may be useful in the management of patients with alveolar pulmonary edema and in providing new insights into trans-pulmonary delivery of exogenous protein drugs.

Proteins in the alveolar space may be cleared by endocytosis and degradation inside alveolar epithelial cells, by transcytosis across the alveolar epithelium, or by restricted diffusion through the epithelium (10, 12, 19, 20). The relative contributions of each of these three pathways to total clearance of proteins from the air spaces are not known. Previous reports suggest the possibility of transcellular mechanism(s) (e.g., receptor-mediated or adsorptive transcytosis (24) for transport of macromolecules across alveolar epithelium.

Protein transport studies utilizing intact lung are not ideal for inferring mechanistic information because of the anatomical complexity and inherent problems (e.g., series and parallel arrangement of biological barriers, presence of unstirred layers, and unknown distribution volumes and surface areas for solute transport) associated with interpretation of experimental data. In this regard, a simplified model of the alveolar epithelial barrier (primary cultured rat alveolar epithelial cell monolayers (16, 21)) has been utilized widely to
study transport mechanisms. The monolayers exhibit morphologic and phenotypic characteristics of in vivo type I pneumocytes (4), develop high barrier resistance (> 2,000Ω·cm²), and actively reabsorb Na⁺ (about 0.2 µEq/cm²/h) from apical fluid (17). Using this in vitro model, transport of dextrans (22) was shown to primarily occur paracellularly with little contribution by pinocytosis. Horseradish peroxidase (HRP) was shown to be transported transcellularly via non-specific fluid phase endocytosis (23), while enkephalin transport mediated via simple diffusion has been reported (35). Transport of HRP (conjugated to transferrin) was enhanced due to receptor-mediated transcytosis of transferrin (6, 36).

In this study, we explored the mechanisms of IgG transport across primary cultured rat alveolar epithelial cell monolayers. In order to test the hypothesis that IgG is transported via receptor-mediated transcytosis across alveolar epithelium, we investigated the effects of IgG concentration and temperature on fluxes of biotin-labeled IgG in the apical-to-basolateral (ab) and opposite (ba) directions. In addition we also investigated the effects of day in culture, apical fluid pH and dexamethasone to provide further insights into IgG transport across alveolar epithelium. Our results support the hypothesis that IgG translocation across the alveolar epithelial barrier occurs predominantly via transcellular saturable processes mediated by the neonatal Fc receptor, FcRn.
MATERIALS AND METHODS

Primary culture of rat alveolar epithelial cell monolayers (RAECM)

Type II pneumocytes isolated enzymatically from male Sprague-Dawley rats were further enriched by IgG panning and plated onto tissue culture-treated polycarbonate filters (0.45 µm pores, 12 mm diameter Transwells, Costar-Corning, Cambridge, MA) at 1.2 x 10^6 cells/cm² (2, 21). Cells were maintained for 48 hr at 37°C in a humidified atmosphere of 5% CO₂/95% air, using culture medium containing 10% newborn bovine serum in minimal defined serum-free medium (MDSF, a 1:1 mixture of DME/F12 medium (Sigma, St. Louis, MO)) supplemented with 1% nonessential amino acids, 2 mM glutamine, 100 U/mL penicillin, 100 µg/mL streptomycin, and 0.1% bovine serum albumin). Medium is changed at 48 hr and monolayers are maintained thereafter in MDSF unless otherwise noted. From day 3 onwards, confluent monolayers are populated with cells that have undergone transdifferentiation to exhibit morphologic (e.g., bulging nuclei with thin cytoplasmic extensions (4)) and phenotypic (e.g., reactivity towards an antibody recognizing a rat type I cell epitope (5)) features similar to those found in type I pneumocytes in vivo. Monolayers (n = 96) develop transepithelial electrical resistance (Rt) = 2.52 ± 0.03 kΩ cm² and transmonolayer potential difference (PD) = 11.3 ± 0.1 mV (apical negative) on days 3-4 in primary culture.

Measurement of unidirectional fluxes of biotinylated rat IgG (biot-rIgG)

For flux studies, both apical (0.5 mL) and basolateral (1.5 mL) fluids of RAECM cultivated on 12 mm Transwells were washed once with pre-warmed MDSF and allowed to equilibrate in a humidified incubator (5% CO₂ in air, 37°C) for 1 hr. Following equilibration and measurement of Rt and PD with a MilliCell ERS device (Millipore, Malborough, MA), unidirectional fluxes of IgG in the apical-to-basolateral (ab) direction were initiated by
removing 0.05 mL of the upstream fluid and immediately replacing it with 0.05 mL of 250 nM biotinylated rat IgG (biot-rIgG, Jackson Immunoresearch, West Grove, PA) at time \( t = 0 \), achieving a final concentration of 25 nM biot-rIgG in apical upstream fluid. At 3, 6 and 18 hr, samples (0.2 mL) were taken from basolateral downstream fluid. These samples were stored at -20°C until performance of enzyme-linked immunosorbent assay for biot-rIgG (see below). Transport in the basolateral-to-apical (ba) direction was similarly assessed, except that 0.15 mL of basolateral upstream fluid was replaced with 0.15 mL of 250 nM biot-rIgG and downstream samples (0.05 mL) were taken from apical fluid. Steady-state fluxes of biot-rIgG were estimated from the linear slope of the relationship between the amount (femtomoles, fmol) of biot-rIgG appearing in downstream fluid and time (hr). Apparent permeability, \( P_{\text{app}} \), was determined by normalizing flux against the initial concentration gradient of biot-rIgG (25 nM) and the monolayer surface area (1.13 cm\(^2\)). Monolayer PD and Rt were measured at the end of flux studies to monitor the integrity of the barrier, which showed no appreciable changes over the time periods studied.

**Enzyme linked immunosorbent assay (ELISA)**

ELISA for IgG detection (7) was modified to measure the level of biot-rIgG in bathing fluids of RAECM used for flux studies. Streptavidin-coated 96 well plates (Boehringer Mannheim, Indianapolis, IN) were blocked for 1 hr with gentle shaking in the presence of freshly made 0.2 mL blocking solution (comprised of 0.5% (v/v) fish gelatin (Sigma, St. Louis, MO) in 0.05% (v/v) Tween-20, 1 mM ethylenediamine-tetraacetic acid di-sodium salt, and 0.05% (w/v) sodium azide). After the blocking solution was removed by flicking a 96 well plate, the thawed fluid samples (0.2 mL, which in the case of apical fluid samples required dilution with fresh MDSF) were added to each well and incubated for 1 hr at room temperature with shaking to allow binding of biot-rIgG to streptavidin. Background levels
were estimated using bathing fluids of monolayers that were treated similarly but without exposure to biot-rIgG. Some of the microplate wells contained serial dilutions of fresh biot-rIgG (ranging from 0.125 to 100 pM) dissolved in MDSF for generation of a standard curve.

The 96 well plate was washed 3 times for 5 min each with shaking, using 0.2 mL wash solution (comprised of 0.1 M phosphate-buffered saline, pH 7.4, supplemented with 0.05% Tween-20) per washing step per well. The washed plate was blocked again for 1 hr with shaking, followed by incubation at room temperature with 0.1 mL of 1 µg/mL primary antibody (goat anti-rat IgG, Jackson Immunoresearch) for 1 hr. The plate was then washed as above, and each well further incubated at room temperature with shaking in 0.1 mL of 2 µg/mL secondary antibody (donkey anti-goat IgG conjugated with horseradish peroxidase (HRP) (Jackson Immunoresearch)) for 1 hr. The plate was again washed as above and each well incubated for 15 min with shaking in 0.1 mL TMB substrate solution (KPL, Gaithersburg, MD) comprised of equal parts of 0.4 g/L 3,3′,5,5′-tetramethylbenzidine and 0.02% fresh H₂O₂ in citric acid buffer which was mixed immediately before usage. Absorbance of the blue color produced from the reaction of TMB and HRP was measured spectrophotometrically at 650 nm using a microplate reader (Labsystems, Franklin, MA).

Investigation of concentration dependency and competitive inhibition of IgG transport

In order to determine if IgG flux saturates with the upstream concentration of IgG, upstream concentrations were varied from 5 to 1000 nM. To determine if other immunoglobulin-related macromolecules compete with IgG transport, unlabeled molecules (e.g., Fc, Fab and F(ab’)₂ or chicken immunoglobulin (IgY)) in 100x molar excess (i.e., 2500 nM) were added to upstream fluid 30 min prior to the instillation of biot-rIgG (25 nM). After 3 hr, downstream appearance of biot-rIgG in the presence and absence of competing molecules
was measured. In a separate series of experiments, unidirectional fluxes of biotinylated rat Fc (biot-rFc) were measured (at 25 nM) for comparison with biot-rIgG fluxes at the same upstream concentration. The amount of biot-rFc was determined by ELISA, using the same procedure as for biot-rIgG estimation, except for usage of goat anti-rFc and rabbit anti-goat IgG conjugated with HRP as primary and secondary antibodies, respectively.

Effects of temperature, apical fluid pH, and day in culture on IgG transport

To determine the degree of cellular energy expenditure required for IgG transport, biot-rIgG fluxes (at 25 nM) were studied at 4°C. In a separate series of experiments, the effects of apical fluid pH (e.g., 5.5, 6.0, 6.5, 7.0, and 7.4) on biot-rIgG fluxes (with upstream concentrations of 25 nM) were determined, while keeping basolateral fluid pH at 7.4. For maintaining apical pH at different values, MDSF was supplemented with 15 mM 2-(N-morpholino)ethanesulfonic acid for pH 5.5 to 6.5 or N-(2-hydroxyethyl)piperazine-N’-(2-ethanesulfonic acid) for pH 7.0 or 7.4, respectively. In another series of experiments, the effects of day in culture on biot-rIgG transport rates (at 25 nM) were investigated utilizing monolayers maintained for 3, 6, 14, and 25 days.

Effects of glucocorticoids on IgG transport

The effects of glucocorticoid exposure on IgG transport were investigated. Dexamethasone (100 nM) was added at various time points in culture to both apical and basolateral bathing fluids and allowed to incubate for different time intervals (e.g., 12, 24, 48, and 72 hr). Unidirectional fluxes of biot-rIgG (at 25 nM) were measured as above.

Reverse transcription-polymerase chain reaction (RT-PCR) to identify cDNA fragments of α- and β-subunits of rat FcRn gene
Total RNA from day 6 RAECM (grown on 24 mm tissue culture-treated Transwells) was extracted using RNeasy Mini Kit (Qiagen, Valencia, CA). The integrity of the extracted RNA was further evaluated by denaturing-agarose (1%) gel electrophoresis. Primer pair, 5’-CGGAGCTCAAGTTTCGATTC-3’ and 5’-GAAGCAGGCCACAAAAGAAG-3’, encompassing a 542-bp segment of the α-subunit of rat FcRn, was utilized for RT-PCR analysis. The Superscript One-Step RT-PCR kit with platinum® Taq (Gibco BRL, Rockville, MD) and the Mastercycler Gradient 5331 Thermal Cycler (Eppendorf-Brinkmann, Westbury, NY) were used. The RT-PCR procedure is comprised of one cycle of 50°C for 30 min followed by heating at 94°C for 2 min and subsequent 35 cycles of denaturation at 94°C for 15 seconds, annealing at 55°C for 30 seconds, extension at 72°C for 1 min, and one cycle of 72°C for 7 min. The volume of all the components added together was 50 µL and the final concentration of the primers was 0.4 µM. The same RT-PCR procedure was used for amplifying a 194-bp segment of rat β2-microglobulin (the β-subunit of rat FcRn), except for using primer pair of 5’-GTCTCAGTTCCACCCACCTC-3’ and 5’-TTTTGGGCTCCTTACAGGT-3’. Electrophoresis using the NuSieve 3:1 agarose (3%, BioWhittaker, Rockland, ME) was conducted to analyze the PCR amplification product. In one of the lanes, a DNA molecular weight ladder (Gibco BRL, Rockville, MD) was loaded to estimate the relative molecular size of RT-PCR products. GelStar stain (BioWhittaker) was used for DNA detection in a UV Transilluminator (Fotodyne, Hartland, WI) and photographed using a GelStar filter (BioWhittaker).

To further confirm the molecular sequence of the RT-PCR products obtained above, DNA bands of interest were excised from the agarose gel and the cDNA fragments extracted using the Ultrafree™ DA centrifugal filter device (Millipore, Bedford, MA). The extracted cDNA fragments were cloned using chemically competent E. coli that was supplied with the TOPO TA Cloning Kit (Invitrogen, Carlsbad, CA), and plasmid DNA was isolated using the
HiSpeed® plasmid purification kit (Qiagen). The plasmid was analyzed by restriction analysis using EcoR I digestion based on the vector map provided by the supplier. Molecular sequence of the DNA was identified by standard DNA sequencing methods.

Northern analysis

Total RNA was extracted from day 6 cell monolayers that were either exposed to dexamethasone (100 nM) for 72 hr as above or grown in MDSF (control). Five micrograms each of these RNA samples were fractionated on 1% denaturing-agarose gel and transferred to Nytran® Supercharge membrane (Schleicher & Schuell, Keene, NH) by the rapid downward transfer method using the Turboblotter™ Kit (Schleicher & Schuell, Keene, NH) by capillary action of the transfer solution of 3M NaCl in 0.3 M Na citrate (20x SSC, pH 7.0). The membrane was prehybridized for 2 hr at 55°C, followed by 4 hr hybridization at 55°C with the labeled cDNA (α-subunit of rat FcRn or rat GAPDH). The α-subunit of rat FcRn cDNA fragment obtained above was purified using the Microcon® PCR filter units (Millipore) and labeled using the North2South HRP labeling and detection kit (Pierce, Rockford, IL). Rat GAPDH cDNA fragment was generated using two primers (F: 5’GCCAAAAGGTCATCATCTC3’ and R: 5’CTCAGTGTAGCCCAGGATGC3’) with the same RT-PCR procedures used for α- and β-subunits of rat FcRn gene (except for the primers) and labeled with HRP.

Hybridized membranes were washed 3 times with 2x SSC at 55°C for 20 min each, followed by another 3 washes at room temperature with 1x SSC, and finally with 0.5x SSC, all for 20 min each. These wash solutions all contain 0.1% (w/v) SDS. Washed membranes were then incubated with a 1:1 mixture of the peroxidase substrate and luminol enhancer for 5 min. The membrane was exposed to X-ray film (Kodak, Rochester, NY) for up to 8 hr and processed for further analysis by densitometry (see below). For estimation of GAPDH level,
the membrane was stripped in boiling 0.1% (w/v) SDS for 5 min with gentle shaking, followed by another wash in boiling 0.1% (w/v) SDS with shaking. The membrane was cooled to room temperature and further rinsed in 3 M NaCl in 200 mM NaH$_2$PO$_4$ and 20 mM EDTA (pH 7.4) for 5 min. Reprobing of the membrane with HRP-labeled GAPDH cDNA fragment was then performed for the determination of the difference in RNA loading. Densitometric analysis was performed using NIH Image Analysis Software.

**Statistical analysis**

Data are presented as mean ± SEM. Unpaired Student’s $t$-tests were used to compare differences between two group means. For comparisons of multiple group means, one-way analysis of variance followed by Dunn’s multiple comparisons were used to contrast the difference(s). $p$<0.05 is considered to be statistically significant.
RESULTS

Figure 1 illustrates the linear relationships between amount of biot-rIgG transported into respective downstream fluids after 3, 6, and 18 hr when 25 nM biot-rIgG was present in upstream apical (ab) or basolateral (ba) fluid. Unidirectional fluxes of $1.90 \pm 0.20$ fmol/cm$^2$/hr and $0.43 \pm 0.07$ fmol/cm$^2$/hr can be estimated in the ab and ba directions, respectively, from steady-state rates of biot-rIgG appearing in downstream fluids. Asymmetric biot-rIgG fluxes indicate that biot-rIgG is preferentially absorbed across RAECM in the ab direction.

Both ab and ba transport of biot-rIgG saturate, with apparent half-maximal concentrations ($K_t$) of $16.0 \pm 0.3$ nM and $32.1 \pm 0.4$ nM in the ab and ba directions, respectively (Figure 2). Corresponding maximal fluxes ($J_{max}$) of $2.0 \pm 0.1$ fmol/cm$^2$/hr and $0.7 \pm 0.1$ fmol/cm$^2$/hr can be noted for the ab and ba fluxes, respectively, indicating involvement of receptor-mediated processes for transport of IgG across alveolar epithelial barrier. Net absorption of IgG in the ab direction across RAECM also saturates, with corresponding $K_t$ and $J_{max}$ of $7.1$ nM and $1.3$ fmol/cm$^2$/hr.

We investigated the effects of various IgG-related macromolecules on receptor-mediated transport of biot-rIgG, using 100x molar excess unlabeled macromolecules (i.e., 2500 nM) in upstream fluid that contained 25 nM biot-rIgG. As seen in Figure 3, the presence of unlabeled rFc, but not rFab, rF(ab')$_2$ or IgY, led to significant decreases (by ~80%) in unidirectional fluxes of biot-rIgG in both ab and ba directions. These data indicate that the Fc portion of the IgG molecule plays an important role in receptor-mediated IgG transport by competing with biot-rIgG binding to its cognate receptor. In support of this competition
afforded by Fc in IgG transport, no significant differences were observed for biot-rIgG vs biot-rFc fluxes in the $ab$ or $ba$ direction (Figure 4).

When IgG fluxes were studied as a function of day in culture, no significant changes over time were observed in either the $ab$ or $ba$ direction, maintaining the asymmetrical unidirectional fluxes of IgG and relatively constant net absorption (Figure 5). In contrast to this observation, Rt of RAECM declines steadily over time, decreasing by ~50% on day 25 compared to that on day 3. These data further indicate that restricted paracellular passive diffusion of IgG does not contribute appreciably to the observed unidirectional fluxes.

When experimental temperature was lowered from 37°C to 4°C, significant decreases in both $ab$ and $ba$ fluxes of biot-rIgG were observed (Figure 6). These drastic decreases in IgG fluxes occur in the presence of relatively small decreases in Rt (by ~16%) observed at the lower temperature. Apparent $Q_{10}$ for $ab$ and $ba$ transport of IgG can be estimated as 1.5 and 1.8, respectively. When pH in apical fluid was lowered below 7.4 (with constant pH in basolateral fluid), no significant changes in any of the unidirectional fluxes were observed (data not shown).

We determined if RAECM express the gene for an IgG receptor, FcRn, reported to be present in various epithelial/endothelial tissues using RT-PCR. As seen in Figures 7 and 8, 542-bp and 194-bp bands expected from the F- and R-primer sets for $\alpha$- and $\beta$-subunits of rat FcRn gene, respectively, were detected using RNA obtained from day 6 RAECM. When the gel-purified $\alpha$-subunit fragment of rat FcRn gene was ligated into the pCR II-TOPO vector and subsequently analyzed using EcoR I enzyme, an expected size of 560-bp for the fragment of the $\alpha$-subunit of rat FcRn gene was found. Moreover, sequencing of the purified plasmid using
the FcRn forward primer yielded 100% identical sequence to that reported for the \( \alpha \)-subunit of rat FcRn gene. With similar approaches, the sequence of the \( \beta \)-subunit of rat FcRn gene was also confirmed (data not shown). These data suggest that RAECM express both \( \alpha \)-and \( \beta \)-subunits of rat FcRn gene.

We determined the effects of dexamethasone (100 nM) on IgG transport across RAECM by measuring unidirectional fluxes and mRNA levels for FcRn. As seen in Figure 9, for up to 24 hr following exposure to dexamethasone, biotinylated rIgG flux in the \( ab \) direction measured with 25 nM biot-rIgG in apical upstream fluid, did not change. After 48 and 72 hr of exposure, \( \sim \)50% and 62% decreases in the \( ab \) flux of IgG were observed. By contrast, \( ba \) flux of IgG was not affected by dexamethasone for up to 72 hr period of exposure. These data indicate that dexamethasone exposure of RAECM differentially regulates IgG transport in a time-dependent manner.

Using the cDNA fragments for \( \alpha \)-subunit of rat FcRn gene obtained from RT-PCR as above, northern analysis was performed to determine the mRNA expression level of rat FcRn. As seen in the Figure 10, HRP-labeled cDNA fragment of \( \alpha \)-subunit of rat FcRn gene detected a signal at \( \sim \)1.6-kb for the RNA purified from day 6 RAECM. Another signal was also detected at \( \sim \)3.1-kb (data not shown), similar to the pattern of FcRn mRNA expression previously reported in other tissues. These data confirm expression of the FcRn gene in RAECM.

We also determined the mRNA levels for RAECM exposed to either 100 nM dexamethasone for 72 hr starting from day 3. As seen in Figure 10, northern analysis of RNA extracted from these monolayers showed that dexamethasone exposure led to \( \sim \)41% decrease in mRNA level for the \( \alpha \)-subunit of rat FcRn gene in comparison to control. On the other
hand, mRNA levels for an internal control, GAPDH, did not appreciably change (data not shown). These data indicate that dexamethasone downregulates the mRNA level of the rat FcRn gene.
DISCUSSION

In this study, we demonstrate that IgG transport across primary cultured rat alveolar epithelial cell monolayers occurs via a regulable saturable (i.e., receptor-mediated) process, yielding net absorption. The saturable process appears to favor interaction of the Fc region with the cognate IgG receptor, showing no influence by other regions of IgG (including Fab or F(ab’)2). Glucocorticoids downregulate IgG transport in the ab direction only, significantly decreasing expression of the FcRn mRNA. These data are consistent with the hypothesis that net absorption of IgG across alveolar epithelium occurs via regulable transcytosis mediated by FcRn.

Saturation of IgG flux with increasing upstream [IgG] occurs via IgG binding to its cognate receptors (i.e., a carrier-mediated process). Evidence for the presence of such receptors includes inhibition by excess unlabeled Fc fragment but not by other molecules (Fab, F(ab’)2, and IgY) of IgG transport in both the ab and ba directions, the presence of both α- and β-subunits of rat FcRn gene transcripts by RT-PCR analyses, and confirmation by northern analyses of the expression of mRNA for α-subunit of rat FcRn. It has been shown that FcRn lacks binding affinity for IgY (7). Moreover, other Fc receptors (e.g., FcγRI or FcRγII) have not been reported to be expressed in any epithelium to date. The similarity of the transport rates of biot-rIgG and biot-rFc, in both the ab and ba directions, provides further evidence for FcRn mediating saturable IgG transport across alveolar epithelium.

The invariant fluxes of rIgG across monolayers in either direction at different days in culture (up to 25 days) indicate that rates of IgG transport do not change as days in culture increase (suggesting maintenance of FcRn levels) and that passive paracellular leak of IgG
does not play a major role in IgG transport (since transepithelial electrical resistance of day 25 cell monolayers is only about 50% of that for cell monolayers at day 3). Since the Stokes radius of IgG is about 5.5 nm, close to the equivalent pore radius of RAECM (22), passive paracellular diffusion of IgG would be expected to be small compared to transcellular transport. We also found that alveolar epithelial IgG transport is strongly dependent on ambient temperature, with $Q_{10}$ values comparable to those we reported for alveolar epithelial albumin transcytosis (20), providing further evidence for non-diffusional IgG transport.

Consistent with the functional data for receptor-mediated transport of IgG, we demonstrated that both $\alpha$- and $\beta$-subunits of FcRn (epithelial type specific receptors that recognize IgG) are expressed in primary cultured pneumocytes. The mRNA (~1.6-kb) transcript we found for $\alpha$-subunit of rat FcRn is in concordance with that previously reported for rat FcRn mRNA of ~1.6-kb (29, 30) and hFcRn transcript of ~1.5-kb (7, 31, 32). A larger transcript of ~3.1-kb was also detected by northern analysis (data not shown), which may be due to hybridization with an unidentified primary mRNA transcript of FcRn or with a partially spliced transcript. A similarly sized transcript for rat FcRn was also noted by Simister and Mostov (29, 30) in addition to the regular 1.6-kb transcript. $\beta_2$-microglobulin (the $\beta$-subunit of FcRn not covalently bound to $\alpha$-subunit) is also expressed in primary cultured alveolar epithelial cells, providing additional evidence for the functional presence of both subunits of rat FcRn, since it was reported that IgG transport in endothelium is disrupted when $\beta_2$-microglobulin is knocked out (15).

The delayed effect of dexamethasone (100 nM) on IgG transport in the $ab$ direction only after 48 and 72 hr may be attributed to transcriptional or post-transcriptional effects on
FcRn gene expression. We do not currently know how the differential effects of dexamethasone on IgG transport occur. Dexamethasone-induced reduction in the steady-state level for FcRn mRNA (by about 40%) is expected to significantly decrease total cellular FcRn protein, which may decrease the more rapid ab transport of IgG without affecting the slower ba transport of IgG. In other tissues, it has also been reported that mechanisms of ab transport of IgG are different from those in the ba direction (25, 26). Decreased absorption of IgG and FcRn expression in the presence of dexamethasone is in accord with a previous report in newborn rats showing disappearance of Fc receptors from enterocytes of the proximal small intestine after dexamethasone injection (11). Moreover, when young rats are exposed to corticosterone (5 mg ip for 3 days), IgG transport from the lumen of the small intestine to blood decreased markedly (27).

Studies of IgG transport in other tissues have suggested involvement of the neonatal Fc receptor (FcRn). Small intestine of neonatal rat (30), adult human intestine (14), human placenta (34), fetal yolk sac of rats and mice (28), adult rat hepatocytes (1), adult human kidney (13), mouse endothelial cells (3) and mouse lung epithelial cells (33) all exhibit FcRn expression. Asymmetric transport of IgG across alveolar epithelium found in this study (~3-fold greater ab flux over ba flux) at saturating concentrations of IgG > 50 nM is remarkably pronounced. For comparison, an in vitro model of rat inner medullary collecting duct (IMCD) cell line transfected with rat FcRn cDNA has been reported to exhibit net absorption of iodinated human Fc (26), and absorptive IgG transport has been noted for a trophoblast cell model, BeWo cell line, although the net absorption rates are lower than that found in alveolar epithelial cell monolayers (8, 9). IgG transport in other tissues, however, is not always absorptive. For example, an in vitro intestinal crypt T84 epithelial cell model exhibits ~3.6-fold greater ba transport of IgG in comparison to ab transport when studied with an upstream
IgG concentration of 60 nM (7). These data suggest that rates, direction-dependence, and associated mechanisms of IgG transport are tissue- and/or cell-specific.

In summary, we have demonstrated that IgG transport across primary cultured rat alveolar epithelial cell monolayers takes place via a saturable (i.e., receptor-mediated) process, yielding net absorption. Interaction of IgG with its cognate receptor (FcRn) is mediated by the Fc region only. Alveolar epithelial IgG transport is regulated by glucocorticoids, where only apical-to-basolateral transport of IgG is decreased along with diminished expression of FcRn. We conclude that net absorption of IgG across alveolar epithelium occurs via regulable transcytosis mediated by FcRn. We suggest that net IgG absorption across alveolar epithelium may play important roles in alveolar homeostasis and mucosal defense of the distal respiratory epithelial tract.
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FIGURE LEGENDS

Figure 1. Cumulative biot-rIgG transported across day 6 RAECM as a function of time. The upstream concentration of biot-rIgG is 25 nM. $ab$ = apical-to-basolateral direction and $ba$ = basolateral-to-apical direction. Data represent mean ± SEM (n = 6).

Figure 2. Unidirectional fluxes of biot-rIgG across day 6 RAECM as a function of [rIgG] in upstream fluid. Apparent half-maximal concentration (Kt) and maximal flux (Jmax) of 16 nM and 2.0 fmol/cm$^2$/hr for $ab$ ($J_{ab}$) direction and 32 nM and 0.7 fmol/cm$^2$/hr for $ba$ ($J_{ba}$) direction can be noted for the respective saturable processes responsible for IgG transport. As a result, net IgG transport (i.e., the difference in unidirectional fluxes at each concentration studied between $ab$ and $ba$ directions) in the $ab$ direction also saturates with Kt of 7 nM and Jmax of 1.3 fmol/cm$^2$/hr. Data represent mean ± SEM (n = 6).

Figure 3. Effects of excess unlabeled macromolecules on biot-rIgG fluxes across day 6 RAECM in the apical-to-basolateral ($J_{ab}$) and in the basolateral-to-apical ($J_{ba}$) directions. Biot-rIgG concentration in upstream fluid was 25 nM and respective unlabeled macromolecules were present at 100x molar excess (i.e., 2500 nM). * and # indicate significant differences compared to control in the $ab$ and $ba$ directions, respectively. Data represent mean ± SEM (n = 6).

Figure 4. Comparison of biot-rIgG flux with biot-rFc flux across day 6 RAECM. Upstream concentrations of biot-rIgG and biot-rFc were the same at 25 nM. No significant differences between respective unidirectional fluxes of IgG and Fc in the $ab$ ($J_{ab}$) or $ba$ ($J_{ba}$) directions were found. Data represent mean ± SEM (n = 6).
**Figure 5.** Effects of days in culture on biot-rlgG flux across RAECM. Upstream concentration of biot-rlgG was the same at 25 nM for each day studied. No significant differences were noted in fluxes in \( ab \) (\( J_{ab} \)) or \( ba \) (\( J_{ba} \)) directions as functions of culture day. Data represent mean ± SEM (\( n = 6-7 \)).

**Figure 6.** Effects of temperature on biot-rlgG fluxes across day 6 RAECM. Upstream concentration of biot-rlgG was 25 nM. Significant decrements in fluxes in both \( ab \) (\( J_{ab} \)) and \( ba \) (\( J_{ba} \)) directions were noted when experimental temperature was lowered from 37 to 4°C. * and # indicate significant differences compared to the flux at 37°C in the \( ab \) and \( ba \) directions, respectively. Data represent mean ± SEM (\( n = 6 \)).

**Figure 7.** RT-PCR products for \( \alpha \)-subunit of rat FcRn gene in day 6 RAECM.

- Lane 1: 1000 bp DNA ladder
- Lane 2: negative control (Taq polymerase only; no reverse transcription)
- Lane 3: RT-PCR product showing a signal at 542-bp

**Figure 8.** RT-PCR products for \( \beta \)-subunit of rat FcRn gene, \( \beta 2 \)-microglobulin, in day 6 RAECM.

- Lane 1: 1000 bp DNA ladder
- Lane 2: RT-PCR product showing a signal at 194-bp

**Figure 9.** Effects of dexamethasone (100 nM) on IgG fluxes. Dexamethasone inhibits \( ab \) flux of biot-rlgG after 48 and 72 hr only, while not affecting \( ba \) flux across RAECM. Upstream concentration of biot-rlgG was 25 nM. * indicates significant differences from IgG flux estimated in the \( ab \) direction at 0 hr of dexamethasone exposure. Data represent mean ± SEM (\( n = 6-7 \)).
Figure 10. Northern analysis of total RNA (5 µg) extracted from day 6 RAECM using a 542-bp fragment of α-subunit of rat FcRn cDNA as a probe.

Lane 1: dexamethasone-treated (from day 3 through day 6) monolayers

Lane 2: untreated (control) day 6 monolayers
Figure 1.
Figure 2.

[Graph showing IgG flux vs. Upstream [IgG] (µM) with two markers, Jab and Jba.]
Figure 3.

Flux (fmole/cm²/hr)

Comparison of flux for different competing molecules:

- Control
- Fc
- Fab
- F(ab')₂
- IgY

Graph showing flux values for each molecule, comparing Jab and Jba.
Figure 4.

Unidirectional flux (fmole/cm²/hr)

- IgG
- Fc

Jab
Jba
Figure 5.

![Graph showing IgG flux vs. culture age](image-url)

- **Jab**
- **Jba**
Figure 6.

IgG flux (fmole/cm²/hr) vs. Temperature (37°C and 4°C).

- **Jab**
- **Jba**

* and # indicate statistical significance at 37°C and 4°C, respectively.
Figure 7.
Figure 8.
Figure 9.

![Graph showing IgG flux (fmole/cm²/hr) and Dexamethasone exposure (hr). The graph compares IgG flux for Jab and Jba under different exposure times, with significant differences indicated by asterisks.]
Figure 10.

Northern analysis

- Dexamethasone-treated (d3-d6) monolayers
- Control monolayers on d6

1.6 kb