Renal FcRn Reclaims Albumin but Facilitates Elimination of IgG

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ABSTRACT

The widely distributed neonatal Fc receptor (FcRn) contributes to maintaining serum levels of albumin and IgG in adults. In the kidney, FcRn is expressed on the podocytes and the brush border of the proximal tubular epithelium. Here, we evaluated the role of renal FcRn in albumin and IgG metabolism. Compared with wild-type controls, FcRn−/− mice had a lower t½ for albumin (28.7 versus 39.9 h) and IgG (29.5 versus 66.1 h). Renal loss of albumin could account for the former, suggested by the progressive development of hypoalbuminemia in wild-type mice transplanted with FcRn-deficient kidneys. Furthermore, serum albumin levels returned to normal in FcRn−/− recipients of wild-type kidneys after removing the native FcRn-deficient kidneys. In contrast, renal loss could not account for the enhanced elimination of IgG in FcRn−/− mice. These mice had minimal urinary excretion of native and labeled IgG, which increased to wild-type levels in FcRn−/− recipients of a single FcRn-sufficient kidney (t½ of IgG was 21.7 h). Taken together, these data suggest that renal FcRn reclaims albumin, thereby maintaining the serum concentration of albumin, but facilitates the loss of IgG from plasma protein pools.


The neonatal Fc receptor (FcRn) is a heterodimer consisting of a MHC class I-like heavy chain and β2-microglobulin light chain.1 It is so named because it transports maternal IgG across the placenta and the fetal small intestine to passively immunize the fetus.2,3 FcRn is also widely expressed in adults, including in vascular endothelium, liver, spleen, lung, and kidney;4–6 hence, its gene designation as IgG Fc receptor, alpha chain transporter (FcgRT).

FcRn binds to albumin and IgG with high affinity at acidic pH (<6.5) but not at physiologic pH (7.4).3,7,8 Notably, the interactions of FcRn with albumin are distinct from those with IgG.9 Informative studies by the Ward group using cultured endothelial cells have shown that FcRn acts on pinocytosed IgG, saving it from lysosomal degradation and instead recycling it intact into the medium.10 Consistent with its important role in albumin and IgG metabolism, mice with targeted mutations of FcRn or its β2-microglobulin partner have lower serum t½s for albumin and IgG compared with wild-type mice.11,12 Taken together, it is thought that vascular endothelial FcRn plays a critical role in conserving intravascular albumin and IgG in the whole animal.

The main function of the kidney is to filter plasma and subsequently retain the vast majority of that filtrate. The glomerular filtration barrier is made up of three components—the fenestrated endothelium, the glomerular basement membrane, and the podocyte slit diaphragm—together functioning as a size- and charge-selective barrier.13 The concept that this barrier prevents serum proteins such as albumin and IgG from appearing in any appreciable quantity in the urine has been called
into question with recent evidence for active tubular uptake of albumin. In the kidney, FcRn is expressed on the podocytes and the brush border of the proximal tubular epithelial cell (PTEC), with both sites clearly relevant to IgG metabolism. To further define the roles for renal and extrarenal FcRn in albumin and IgG metabolism, we used fluorescence-labeled proteins and the transplantation of kidneys between mice differing only by a targeted FcRn gene knockout.

RESULTS

Clearance of Albumin and IgG by Renal FcRn

The serum concentrations of Alexa-labeled albumin and IgG over time after intravenous injection were determined in wild-type and FcRn−/− mice. As expected, greater quantities of albumin and IgG remained intravascular from 24 to 96 h postinjection in wild-type mice compared with FcRn−/− animals (Figure 1, A and B). The second-phase τ/2 of albumin was 39.9 ± 2.2 h in wild-type mice and 28.7 ± 2.6 h in FcRn−/− mice (P = 0.071), whereas that of IgG was 66.1 ± 12.0 h in wild-type mice and 29.5 ± 3.9 h in FcRn−/− mice (P = 0.040). Interestingly, FcRn−/− mice had a nearly 3-fold lower volume of distribution (Vd) for IgG compared with wild-type animals, as evidenced by the higher serum quantities within 12 h of injection; extrapolated y-intercepts (time “zero”) were 19,442 ± 944 and 6432 ± 337 U/ml in FcRn−/− and wild-type mice, respectively (P = 0.042). Consistent with the quicker disappearance of IgG from the circulation, the curves in the two strains crossed early after injection (Figure 1B).

The fractional urinary excretion (FE) of albumin and IgG (relative to creatinine) 3 h after intravenous injection was considerably higher than subsequent values, which were fairly constant in a given animal. These suggested there was saturation initially followed by attainment of steady state. As such, data were divided into those 3 h and 24 to 96 h postinjection, which illustrated that FEalbumin was modestly higher in FcRn−/− mice compared with wild-type mice (Figure 1C), whereas FcRn−/− mice had markedly lower FEIgG compared with wild-type animals (Figure 1D). Thus, although urinary losses could account, at least in part, for the reduced τ/2 of albumin in FcRn−/− mice, this was not a viable explanation in the case of IgG.

Three complementary approaches were utilized to extend upon these quantitative data and examine potential degradation of urinary albumin and IgG. Using trichloroacetic acid, Alexa 488 and Alexa 594 fluorescence intensities were consistently over 96% precipitable, support that Alexa probes remained protein-bound in sera and urines from these short-term experiments. Next, Sephacryl S-100 size-exclusion chromatography to separate urinary albumin was performed as described by the Comper laboratory. This showed albumin appeared as a single intact peak in urines from both mouse strains (Figure 1E). Albumin and IgG were also labeled with infrared dyes to allow direct visualization of their urinary forms after separation by SDS-PAGE. By this approach, albumin and IgG were also predominantly intact (Figure 1F). These data collectively showed that FcRn−/− mice excreted greater amounts of urinary albumin but less IgG than wild-type mice after systemic administration. Moreover, albumin and IgG appeared intact in urines, thereby excluding significant proteolysis.

Intrarenal Localization of Albumin and IgG

The fate of intravenously-injected, Alexa-labeled albumin and IgG in kidneys was determined quantitatively (Figure 2A) and qualitatively 5 to 30 min postinjection in wild-type (Figure 2, B, D, F, G, I, and J) and FcRn−/− mice (Figure 2, C, E, H, and K). In both strains, Alexa 594-albumin associated with PTECs by 5 min postintravenous injection (Figure 2, B and C). In wild-type kidneys, this declined rapidly along with the appearance of intracellular vesicular bodies containing Alexa 594-albumin (Figure 2J, arrows). In contrast, Alexa 594-albumin remained associated with the brush border of FcRn−/− PTECs, with lesser amounts reaching intracellular compartments (Figure 2K, arrows). Greater quantities of Alexa 488-IgG were in the kidneys of wild-type mice compared with FcRn−/− animals (Figure 2A). In both mouse strains, Alexa 488-IgG was associated with PTECs. In wild-type mice, this was in basolateral compartments largely distinct from Alexa 594-albumin (Figure 2, F and I), whereas it appeared to be associated together with Alexa 594-albumin in the brush border of FcRn−/− kidneys (Figure 2K, arrows). The representative photomicrographs shown in Figure 2 illustrate a variation in PTEC uptake of Alexa probes, which was highly consistent in all experiments but was also clearly not related to relative location within the kidney (i.e., cortical versus medullary). Alexa 488-IgG was also evident within wild-type (Figure 2J) but not FcRn−/− glomeruli (Figure 2K); in the former, this appeared to be mesangial and in association with podocyte cell bodies marked with anti-synaptopodin antibodies (Figure 2J, arrows).

Kidney Transplantation to Determine Roles for Renal and Extrarenal FcRn

To evaluate albumin and IgG handling by renal and extrarenal FcRn in greater detail, we transplanted kidneys between wild-type C57BL/6 mice and those in which the FcRn targeted mutation was carried forward in 13 backcrosses onto the C57BL/6 strain (Table 1). In all animals, a single native nephrectomy was performed at the time of transplantation; thus, in these mice, there were single functional native and transplanted kidneys. In studies to determine whether a FcRn-sufficient kidney could “rescue” a FcRn-deficient animal, a second native nephrectomy was performed after several weeks, thereby generating a FcRn−/− mouse with a single functional wild-type kidney. Evidence that the transplant protocol itself did not affect the function of FcRn-sufficient or deficient kidneys included normal renal function (Table 1) and absence of renal pathology in the transplanted kidneys at sacrifice; specifically, no kidney had glomerular or vascular pathology, and only 1 of the 18 transplanted kidneys had tubulointerstitial pathology (mild interstitial nephritis, scored as 1 of 4).
Albumin Metabolism after Renal Transplantation

As designed, the experimental protocol allowed us to examine the fate of native albumin over time after the various transplant combinations. In unmanipulated wild-type and \( \text{FcRn}\text{-}/\text{-}\) mice, serum albumin levels were 30.0 ± 2.1 and 26.9 ± 3.8 mg/ml, respectively (NS). Control experiments were performed in which a wild-type kidney was transplanted into a wild-type mouse, thereby leading to single functioning (\( \text{FcRn}\text{-sufficient}\)) native and transplanted kidneys. In these animals, serum albumin levels remained constant over time (Figure 3A, B).

Figure 1. Role of FcRn to metabolize intravenously injected Alexa-labeled albumin and IgG. (A and C) Alexa 594-labeled albumin and (B and D) Alexa 488-labeled IgG were injected into wild-type (\( n = 3\)) and \( \text{FcRn}\text{-}/\text{-}\) mice (\( n = 4\)) for (A and B) measurements of sera \( t_{1/2}\) and (C and D) urinary excretion. For clearance data (A and B), logarithmically transformed data were used from individual animals (\( r > 0.940\), median 0.988) and were averaged to create the curves shown in the figures. All data as presented were normally distributed (Anderson–Darling tests, \( H_\alpha \geq 0.1\)) and are means with SEM values shown in C and D. +\( P < 0.005\) versus all other groups (ANOVA followed by Fisher’s pairwise comparisons). Urines from wild-type and \( \text{FcRn}\text{-}/\text{-}\) mice were also assessed by (E) Sephacryl S-100 HR size-exclusion chromatography and (F) infrared-imaged SDS-PAGE gels after injection of unlabeled albumin or IRDye-labeled albumin and IgG, respectively. In F, wild-type mouse urine before injection was used as control, the entire gels are shown (i.e., extending beyond the dye fronts), and the locations of labeled proteins, including IgG heavy (H) and light (L) chains and molecular weight markers are indicated.
E), supporting that the presence of a transplanted wild-type kidney, or the transplant protocol itself, did not alter albumin metabolism. FcRn−/− mice receiving a wild-type kidney had increasing albumin levels over time. This rose further to normal when the remaining FcRn-deficient kidney was removed in these mice (Figure 3A, ○) (P < 0.02), evidence to support the ability of an FcRn-bearing kidney to reclaim albumin. In contrast, the transplantation of a single FcRn-deficient kidney into a wild-type host resulted in progressively lower serum albumin levels over time (Figure 3A, ○). Furthermore, these animals developed anasarca, thus exhibiting a clinical feature of the nephrotic syndrome. The explanation for why FcRn−/− mice have relatively preserved serum albumin levels and lack any evidence for nephrotic syndrome includes increased hepatic albumin synthesis, presumably developing over time because the gene deficiency is present at conception in these mice.

Figure 2. Intrarenal appearance of intravenous-injected Alexa 594-albumin and Alexa 488-IgG. Renal Alexa 594-labeled albumin (red) and Alexa 488-IgG (green) were quantified (A) over time and visualized (B and C) 5, (D and E) 15, and (F through K) 30 min after intravenous injection into (C, E, H, and K) FcRn−/− and (B, D, F, G, I, J) wild-type mice. Stained blue in these photomicrographs is (F, J, and K) wheat germ agglutin or (l) the podocyte marker synaptopodin. The arrows depict Alexa 594-albumin in PTECs. (K) In FcRn−/− mice, albumin colocalized with Alexa 488-IgG, whereas in wild-type mice this was in distinct vesicular structures (J). Alexa 488-IgG was present within (F, arrowheads) basolateral aspects of tubules, (I and J) glomeruli of wild-type mice, and (I, arrowheads) in regions associated with synaptopodin-bearing podocytes. (A) Nonparametric data (Anderson–Darling tests, Ha ≥ 0.006) were derived as described in Concise Methods from one animal per time (4 to 12 measurements each) and presented as medians. The dashed line for data of IgG intensity in wild-type mice is shown simply to draw attention to the break in the y-axis. Differences between wild-type and FcRn−/− mice were determined using Kruskal–Wallis testing for albumin (P = 0.001) and IgG (P = 0.011). Original magnifications were 200× in B through H, 400× in J and K, and 600× in I.
Table 1. Transplant groups

<table>
<thead>
<tr>
<th>Group</th>
<th>Donor Kidney</th>
<th>Recipient Mouse</th>
<th>BUN (mg/dl)*</th>
<th>n</th>
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</thead>
<tbody>
<tr>
<td>WT → FcRn⁻/⁻</td>
<td>WT kidney</td>
<td>FcRn-deficient host</td>
<td>31.6 ± 3.0</td>
<td>8</td>
</tr>
<tr>
<td>FcRn⁻/⁻ → WT</td>
<td>FcRn-deficient kidney</td>
<td>WT host</td>
<td>27.3 ± 0.8</td>
<td>7</td>
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<tr>
<td>WT → WT</td>
<td>WT kidney</td>
<td>WT host</td>
<td>30.3 ± 3.2</td>
<td>3</td>
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</tbody>
</table>

All recipients had a single native nephrectomy performed at the time of transplantation. WT, wild type.

*aBUN values 4 wk after transplantation (mean ± SEM).

The second native FcRn-deficient kidney was removed 4 wk or later after transplantation.

Our transplant protocol evidently did not allow such compensation to occur in wild-type mice.

To further evaluate albumin metabolism in FcRn⁻/⁻ mice with a FcRn-sufficient wild-type kidney, we evaluated the t½ of intravenous-injected Alexa 594-albumin. Data from four individual FcRn⁻/⁻ mice with a transplanted wild-type kidney are shown (Figure 3B, gray symbols and lines), with average data from FcRn⁻/⁻ (red line) and control transplanted wild-type mice (blue line) as reference. These data show the t½ of albumin was normalized in FcRn⁻/⁻ mice containing a FcRn-sufficient wild-type kidney. To confirm that the transplant protocol did not affect urinary albumin metabolism, experiments evaluating urinary albumin by size-exclusion chromatography (i.e., comparable to those shown in Figure 1E) showed albumin appeared as a single peak (data not shown). Taken together, these results are entirely consistent with the premise FcRn in the kidney is important to salvage albumin, such that an FcRn-deficient kidney “leaks” albumin, whereas a wild-type kidney retains it.

Additional experiments were performed to determine potential effects of ischemia-reperfusion injury or uninephrectomy on albumin metabolism in FcRn⁻/⁻ mice. Within 3 h of reperfusion, urinary albumin was significantly increased in wild-type and FcRn⁻/⁻ mice (876 ± 166 and 887 ± 45 μg/mg creatinine, respectively), and returned to baseline values by day 7. With this, serum albumin levels did decrease to a mild extent, reaching 86.5 ± 3.7 and 86.6 ± 7.6% of baseline values in wild-type and FcRn⁻/⁻ mice, respectively, 1 to 2 wk after reperfusion. In FcRn⁻/⁻ mice subjected to uninephrectomy, urinary albumin did not change and serum albumin levels either remained stable or rose slightly, consistent with the premise that kidney FcRn is important in albumin metabolism.

IgG Metabolism after Renal Transplantation

Serum IgG levels were 10.9 ± 1.7 and 6.2 ± 1.2 mg/ml in a cohort of unmanipulated wild-type and FcRn⁻/⁻ mice (n = 7 to 8) (P = 0.038). Although mice remained housed in a barrier facility, the move from a specific pathogen-free facility and a systemic inflammatory response postsurgery were likely to account for a mild elevation in serum IgG in all transplant groups after surgery. Thus, IgG levels were 14.3 ± 1.8 and 16.6 ± 1.6 mg/ml in wild-type mice 4 wk after transplantation of a wild-type or FcRn⁻/⁻ kidney, respectively. Moreover, IgG levels in
**FCRN**−/− mice with a transplanted wild-type kidney were 11.9 ± 1.4 mg/ml 4 wk posttransplant and rose further to 13.0 ± 2.8 mg/ml 10 d after removal of the remaining native FcRn-deficient kidney.

To better understand the role for renal FcRn in IgG metabolism, the fate of intravenous-injected Alexa 488-IgG was studied in transplanted animals. Controls were wild-type mice into which wild-type kidneys were transplanted, which had comparable IgG clearance as wild-type mice (Figure 4A, averages shown as a blue line). Interestingly, the addition of a FcRn-deficient kidney to a wild-type host did not affect the t1/2 of IgG (Figure 4A, magenta symbols and lines for individual mice). In contrast, the already low t1/2 of IgG in FcRn−/− mice (Figure 4A, red line showing averages) was further reduced when they had a sole functioning wild-type kidney (Figure 4A, charcoal symbols and lines for individual mice) (i.e., 29.5 ± 3.9 to 21.7 ± 1.3 h).

Renal handling of Alexa 488-IgG was also determined in these studies by measuring FEIgG 48 h after intravenous injection (Figure 4B). Individual FEIgG measurements from wild-type mice receiving wild-type and FcRn−/− kidneys, and FcRn−/− mice with wild-type kidney transplants are shown, together with data from untransplanted FcRn−/− mice for comparison. For these studies, FcRn−/− mice with wild-type kidney transplants had both native FcRn-deficient kidneys removed before study. In an additional animal studied in which a single native kidney was left in place, FEIgG was 5.6% at 48 h. These data show the FEIgG depended strictly on the presence of a FcRn-sufficient kidney; in any circumstance in which there was at least one functional wild-type kidney, FEIgG was increased considerably beyond that evident in FcRn−/− mice, indicating IgG was excreted through FcRn-sufficient kidney(s).

**Localization of Albumin and IgG in Transplanted and Native Kidneys**

The data presented above suggested that kidneys can salvage albumin through PTEC FcRn; yet, renal FcRn also appears to serve as a conduit for IgG excretion, which is magnified when the host animal lacks FcRn in all other sites, including endothelia. Our transplant studies allowed us to examine the fates of Alexa-labeled albumin and IgG, which was particularly informative in those instances in which FcRn-sufficient and FcRn-deficient kidneys were present in wild-type and FcRn animals. Control studies included wild-type mice with wild-type kidney transplants, which showed findings in kidneys comparable to wild-type mice without transplantation (Figure 5, A and B).

As was true of kidneys in FcRn−/− mice that did not undergo transplantation, FcRn-deficient kidneys transplanted into wild-type mice (or native to transplanted FcRn−/− animals) had significant quantities of Alexa 594-albumin in PTEC brush borders 3h after intravenous injection (Figure 5C). Albumin was also present in PTEC brush borders of wild-type kidneys in wild-type (Figure 5, A and B) or FcRn−/− hosts (Figure 5, E and F), particularly in early postglomerular (i.e., S1) segments (Figure 5F, arrow). As observed previously, there was evidence for PTEC uptake in the wild-type kidneys (Figure 5B, arrow).

Wild-type kidneys transplanted into wild-type hosts had Alexa 488-IgG in the tubulointerstitium, basolateral aspects of tubules, and glomeruli (Figure 5, A and B); this pattern was accentuated in wild-type kidneys in a FcRn−/− host, particularly in interstitial spaces between glomeruli and tubules in sites of microvascular endothelium (Figure 5, E through G, arrowheads). As with native FcRn−/− kidneys, FcRn-deficient kidneys in a wild-type host had lesser amounts of IgG in tu-
bules (Figure 5C), and little within glomeruli (Figure 5D). Of note was the presence of apparent aggregates of Alexa 488-IgG in tubulointerstitium (Figure 5D, asterisk) and glomeruli (not shown). Taking all available data together, these support the concept that podocyte FcRn promotes IgG clearance through the glomerulus, which is partly reclaimed by the PTEC using its FcRn; when absent, IgG accumulates within renal sites. In addition to these sites involved in maintenance of the glomerular filtration barrier and urinary IgG, it also appears that renal vascular FcRn plays an important role in the kidney’s handling of IgG.

DISCUSSION

The results from these studies are consistent with past work by several investigators showing that FcRn is a key protein involved in systemic albumin and IgG metabolism.4,9,12,15,16,18–23 Yet we have extended these by transplanting kidneys between FcRn-sufficient and FcRn-deficient mice and monitoring sera, urines, and kidney tissue sites for fluorescence-labeled albumin and IgG. These results show FcRn differs in its roles inside and outside of the kidney, as well as in the handling of albumin and IgG. Thus, as predicted, FcRn within the kidneys is responsible for reclaiming albumin and restoring it to the systemic circulation.6 Yet, surprisingly, renal FcRn facilitates the loss of IgG from plasma protein pools. Furthermore, extrarenal FcRn, presumably that in endothelial cells, affects albumin and IgG in the systemic circulation and its accessibility to the kidney.

The quantity of glomerular filtrate is remarkable, approximating 50 plasma volumes daily. Nearly all of the water and many other filtered molecules must be actively returned to the circulation for survival. The glomerular filtration barrier has long been considered a passive size- and charge-selective sieve, capable of restricting IgG and albumin on the basis of size and charge, respectively.13,24 Yet, surprisingly, renal FcRn facilitates the loss of IgG from plasma protein pools. Furthermore, extrarenal FcRn, presumably that in endothelial cells, affects albumin and IgG in the systemic circulation and its accessibility to the kidney.

The normal glomerular ultrafiltrate contained substantially higher quantities of albumin than expected; this was reclaimed by normal but not diseased PTECs.14 Candi-
dates for albumin-binding proteins in the PTEC brush border include megalin and cubilin. From the brush border of the PTEC, albumin can enter a high capacity transcytotic pathway or a lower capacity pathway directed toward lysosomal delivery and degradation.

Our studies have confirmed and extended upon the studies of Anderson and colleagues relating to albumin metabolism in FcRn−/− mice, with the v5 for albumin in FcRn−/− mice 75% that of wild-type mice. The wild-type and FcRn−/− mice we studied were at steady state, with net production equaling that lost through metabolism. Reduced albumin levels in FcRn−/− mice have been shown to reflect the net of greater production and clearance. Although we did not directly study production, our data showing 28% greater clearance of albumin in FcRn−/− mice (i.e., measured albumin v5 values of 28.7 and 39.9 h, respectively) are consistent with previous data showing 32% greater clearance observed in FcRn−/− mice (in dimensionless units, not permitting direct comparisons). It should be noted that steady-state albumin levels we measured in wild-type and FcRn−/− mice were 30.0 and 26.9 mg/ml, respectively, which does differ in magnitude from previous data (respective values of 36.3 and 21.0 mg/ml), which we cannot explain.

Intrarenal handling of albumin was different between the two strains. In such a setting, there were greater quantities of albumin associated with PTEC brush borders in FcRn−/− mice, whereas wild-type mice appeared to have uptake of albumin into intracellular compartments followed by its disappearance. The Alexa 594 label did remain protein-bound in sera and urines, excluding metabolism to peptides; this was confirmed in short-term experiments by three complementary techniques. Beyond that, the precise fate of this intracellular albumin was undefined in our studies. Taking all data together, it does seem likely that a substantial portion was returned to the systemic circulation.

A key role for intrarenal FcRn in albumin metabolism was confirmed by transplanting kidneys between FcRn-sufficient and -deficient animals. The mild hypoalbuminemia present in FcRn−/− mice was partially corrected when there was an FcRn-sufficient kidney and fully restored when the second FcRn−/− kidney was removed. Moreover, placing a FcRn-deficient kidney in a wild-type host led to prompt development of hypoalbuminemia features of the nephrotic syndrome.

Overall, it does seem likely that the modest increase in FEalbumin we observed here in FcRn−/− mice is relevant and accountable for the development of hypoalbuminemia with time. PTEC FcRn may not bind urinary albumin directly but appears necessary to protect it from being lost in the urine and/or taken up and degraded by the PTEC. The data from the Comper group do indicate the albumin reclamation processes in the kidney operates at or near saturation. Our data in wild-type and FcRn−/− mice at steady state given a bolus of albumin (as Alexa-labeled albumin) are consistent with this concept; within hours of administration, the ability to reclaim albumin is overcome, leading to the high values of FEalbumin in both groups. This fits well with the concept that glomerular filtered albumin is much higher than previously thought and that albumin reclamation occurs in the proximal tubules, as supported, for instance, in data from protein overload models. The intrarenal appearance of Alexa-labeled albumin was also different between the two groups, with very little observed within 5 min in wild-type tubules. Later on, there was evidence for vesicular trafficking of albumin within both groups, which we feel is consistent with the low-capacity/high-affinity metabolic pathway. FEalbumin quickly comes back to much lower levels, presumably as the amount of albumin once again reaching the tubules declines to that able to be handled. Taken together with the data of others, we feel our results are consistent with an important role for renal tubular FcRn in the high-capacity/low-affinity reclamation pathway.

Our experimental design also allowed comparison between animals bearing a FcRn-sufficient and a FcRn-deficient kidney. There were remarkable differences in serum albumin levels depending on whether the host was a wild-type or FcRn−/− mouse; wild-type mice with FcRn-deficient kidneys had progressive hypoalbuminemia, whereas FcRn−/− mice with FcRn-sufficient kidneys increased albumin levels. This undoubtedly reflects the intricacies of albumin metabolism in the FcRn−/− mouse, including greater hepatic synthesis and altered endothelial cell handling.

Cultured human PTECs have a capacity for bidirectional IgG transport; in vivo, basolateral to apical transport of IgG would increase the urinary appearance of IgG. That podocytes use their FcRn6,35 to clear the glomerular filtration barrier was recently shown by Akilesh and Shaw in their studies of FcRn−/− mice in which injected heterologous IgG accumulated over time in the subepithelial space. Adding complexity to IgG clearance is its propensity to form latticed immune complexes containing antigen and complement components. This is clearly relevant in disease states such as postinfectious GN36 and membranous nephropathy,37 in which there is the development of IgG aggregates large enough to be visible histologically. Complement receptor 1 in human podocytes (and complement factor H in rodents)38,39 has the potential to interact with C3b in immune complexes. In addition to FcRn and complement receptor 1, the podocyte has other features of an immunologically active cell, including CD80 (B7-1),40 CD2-associated protein,41 and the ability to present antigen in vitro.42 Whether the podocyte, with its repertoire of complement and Fc receptors, actually serves as an antigen-presenting cell in vivo comparable to dendritic cells,43,44 or whether it is involved in a multistep process of antigen presentation ultimately relying upon parenchymal dendritic cells (for which there is ample supply in the kidneys45), as true for FcRn-bearing intestinal epithelial cells,18 is an exciting prospect to work out in future studies.

Renal FcRn is responsible for urinary clearance of IgG as shown by the very low FEIgG in FcRn−/− mice, which was markedly increased by providing a single transplanted wild-type kidney. Clearance through the podocyte is supported cir-
cumstantially by the presence of Alexa 488-labeled IgG in glo-
meruli (and near podocytes) in wild-type but not FcRn−/− mice. It does seem unlikely transport through glomeruli ac-
counts for the large differences in renal clearance of IgG be-
tween FcRn-deficient and -sufficient strains. In wild-type kid-
neys, there was considerable IgG in the tubulointerstitium,
supporting vasa recta endothelium transported IgG. As with
podocyte-trafficked IgG, it is intriguing to speculate that the
intrarenal dendritic cell network could also survey IgG (in im-
mune complexes) to potentially generate an immune response.
The subsequent fate of IgG could include retrieval through
lymphatics and transport by PTECs in a basolateral to apical
route. Overall, the latter is favored by our data showing local-
ization of IgG in PTEC basolateral sites, as well as the urinary
disposition of IgG.

Endothelial cells are believed to nonspecifically take up
albumin and IgG from the circulation. Subsequently, FcRn
protects both from a catabolic fate, and instead they are
recycled to the circulation or transcytosed into the intersti-
tial space.4,10 The differences in IgG Vd between FcRn+/+
and wild-type mice approximates the differences between
plasma and extracellular fluid volumes, supporting that the
FcRn in endothelium does promote IgG transcytosis to the
interstitium. This is further supported by examining the fate
of IgG in FcRn−/− mice with a wild-type kidney, in which
there was appearance of sizable quantities of IgG and an
extremely low tvs. This can be explained by a combination
of IgG restricted to the intravascular space outside of the kid-
ney because of a lack of endothelial FcRn, and a conduit for
its urinary excretion in the normal FcRn-containing kidney.
It is important to add that in this circumstance, the renal
lymphatics were no longer intact because of the transplan-
tation protocol, so recycling through renal lymph was not
possible. On balance, the low tvs in FcRn−/− mice with or
without a transplanted wild-type kidney also must include
its loss outside the kidney, for which endothelial cell catab-
olism is the likeliest candidate.4,10,18

In summary, we have shown a role for renal FcRn in albu-
min and IgG metabolism. The locations of FcRn in the kidney
include in podocytes, PTECs, and vascular endothelia, each
appearing to have individualized functions. Albumin is filtered
at the glomerulus and reabsorbed by the PTEC. FcRn in the
latter is necessary for reclaiming albumin, such that in its ab-
scence, there is progressive loss of albumin through catabolism
and/or excretion in the urine. Podocyte FcRn facilitates clear-
ance of IgG that has traversed the glomerular filtration barrier,
whereas renal vascular endothelial FcRn removes IgG from the
circulation into the interstitium. Once there, the fate of IgG
includes uptake by the PTEC at its basolateral site, followed by
transcytosis into the urine. It appears the endothelial cell bed
keeps albumin within the intravascular space and moves IgG
transcellularly to access extracellular spaces. These functions
are logical because they maintain oncotic and other intravas-
cular functions of albumin while providing IgG to underlying
tissue as necessary for immunity to infectious agents.

CONCISE METHODS

Mice
FcRn−/− mice (Fcgr1tm1iders)41 were obtained from Jackson Laborato-
ries (Bar Harbor, ME). Mice were backcrossed onto the C57BL/6
background for 13 generations or more. Mice used in these studies
were derived from intercrosses between FcRn−/− mice. Age-matched
wild-type C57BL/6J control mice were also purchased from Jackson
Laboratories. Initial experimentation to determine albumin and IgG
clearances and renal localizations utilized normal wild-type and
FcRn−/− mice (n = 30). All work with mice was performed under the
auspices and approval of the University of Chicago Animal Care and
Use Committee.

Renal Transplantation
In these studies kidney transplantation was performed in mice to
evaluate renal and extrarenal effects of FcRn (Table 1), comparable
to our past studies with TNF receptor 1,46 Toll-like receptor 4,47
complement receptor 1-related gene/protein y,48 and complement
factor H.38 Male mice between 8 to 10 wk of age were used either as
a kidney donor or recipient. Blood and urine were collected from
all animals before transplant surgery to establish baseline values.
Donor mice were anesthetized, and the donor left kidney was re-
moved with artery, vein, and ureter attached and was preserved in
cold saline on ice. The recipient was then anesthetized, and the left
kidney was excised. Renal transplantation was performed with
end-to-side anastomoses of the donor renal vein, artery, and ureter
to the recipient inferior vena cava, aorta, and bladder, respectively.
Total cold ischemic time of the donor kidney ranged between 45
and 60 min. Blood and urine were collected weekly posttransplan-
tation. At 4 wk posttransplantation, FcRn−/− mice with wild-type
kidneys underwent nephrectomy of the right native kidney, such
that they relied solely on the transplanted FcRn-sufficient kidney
to provide renal function. At the time of sacrifice, native and trans-
planted kidneys were processed for histopathological analyses and
scored by a renal pathologist (A.C.) for GN, glomerulosclerosis,
interstitial nephritis, tubular atrophy, and arteritis, each on a scale
from 0 to 4.

In separate experiments to determine whether reperfusion injury
and/or the presence of a solitary kidney could affect albumin metab-
olism, wild-type and FcRn−/− mice (n = 3 each) had the left renal
pedicle clamped for 30 min or underwent left uninephrectomy.
Thereafter, serum albumin levels were measured biweekly.

Measurements of Albumin and IgG Clearances
Purified mouse albumin (Innovative Research, Novi, MI) and total
IgG (Lampire Biologic Laboratories, Pipersville, PA) were respec-
tively labeled with Alexa 594 and 488 fluorescence probes (Molec-
ular Probes, Invitrogen, Carlsbad, CA) or with IRDyes 800CW and
700 DX (LI-COR Biosciences, Lincoln, NE) following the manu-
facturer’s instructions. Labeled proteins were extensively dialyzed
extensively against PBS (pH 7.2) to remove unbound probe and
were more than 99% precipitable with trichloroacetic acid.
Mice were given a single intravenous dose of 2-mg Alexa 594-albumin and 0.5 mg of Alexa 488-IgG (or respective IRDye Infrared dyes). Blood and urine samples were collected 3, 12, and 24 h after injection and daily thereafter. All samples were collected on ice and stored at −80°F and limited to one freeze-thaw cycle. The quantities of fluorescence-labeled albumin and IgG were determined spectrophuorometrically (Synergy HT Multi-Mode Microplate Reader, Biotek, Winooski, VT). Excitation and emission wavelengths were 590 and 617 nm for Alexa 594-albumin, and 494 and 519 nm for Alexa 488-IgG. To minimize variation, sera and urines from a given animal were measured in the same microtiter plate at the same time. For each animal, serum and urine obtained before injection were used to determine background fluorescence, which was subtracted from all subsequent values to derive specific fluorescence units per milliliter serum or urine. These were used to calculate logarithmically transformed clearance curves as well as FE values. For experiments using IRDye-labeled proteins, urine was collected within 1 h of injection and separated by SDS-PAGE under reducing and nonreducing conditions, followed by direct imaging of the gel (Olympus Infrared Imaging System, LI-COR Biosciences). Urines collected before injection were used as controls.

Additional experiments were performed with urines collected for 3 h after injection of unlabeled albumin. These were diluted to 0.3 ml with PBS and separated over a 1.6 × 60 cm (120 ml) Sephaeryl S-100 HR column (GE Healthcare Life Sciences, Amersham). Absorbance at 280 nm was measured continuously and expressed relative to elution volume (Vₑ) divided by total column volume, Vₑ.

**Measurements from Serum and Urine**

Serum and urinary concentrations of albumin and total IgG were measured by ELISA (Bethyl Laboratories, Montgomery, TX). Serum and urinary creatinine were measured using a commercially obtained kit according to manufacturer’s instructions (Stanbio Laboratory, Boerne, TX). FE of albumin and IgG were calculated from concentrations of albumin, IgG, and creatinine from serum (S) and urine (U) samples obtained contemporaneously, using the following formula:

\[
FE_{\text{albumin}} = \frac{\ln(U_{\text{albumin}}) - \ln(S_{\text{albumin}})}{\ln(U_{\text{creatinine}}) - \ln(S_{\text{creatinine}})}
\]

\[
FE_{\text{IgG}} = \frac{\ln(U_{\text{IgG}}) - \ln(S_{\text{IgG}})}{\ln(U_{\text{creatinine}}) - \ln(S_{\text{creatinine}})}
\]

**Renal Localization of Albumin and IgG**

In some animals, a single intravenous dose of Alexa 594-albumin and Alexa 488-IgG was given as for clearance studies; these were followed at varying time intervals by euthanasia and tissue harvesting. Initial studies were performed in wild-type and FcRn−/− mice to determine appearances in kidney after intravenous injection of Alexa-labeled proteins. Tissues were snap-frozen in ice-cold 2-methylbutane. Cryostat sections (4 μm) were fixed in 4% paraformaldehyde in PBS (pH 7.4) and permeabilized with 0.2% Triton X-100. Sections were additionally stained with Alexa 350-conjugated wheat germ agglutinin (Molecular Probes, Invitrogen, Carlsbad, CA) to identify glomeruli and proximal tubules, or goat anti-synaptopodin (Santa Cruz Biotechnology, Santa Cruz, CA) followed by Alexa 350-labeled donkey anti-goat IgG (Molecular Probes) to visualize podocytes. All procedures were performed at 4°C with each step taking less than 5 min to minimize loss of tissue-bound fluorescence probes. Tissues that were being directly compared were processed contemporaneously. Images were acquired using an Olympus IX-81 spinning disk confocal microscope.

To determine image intensities within given fields of interest, we used Image J software (National Institutes of Health, Bethesda, MD) and a macro essentially as described by the Wright Cell Imaging Facility (Toronto, ON, Canada: http://www.uhnres.utoronto.ca/facilities/wcif). The steps used to measure image intensities included (1) pseudoflat field correction, in which the image was divided by a blurred version of itself to reveal the illumination pattern; (2) background correction, using the “rolling ball” algorithm to remove uneven background from images; (3) setting of a uniform threshold for all images to distinguish background from true positive staining; and (4) measurement of an integrated density by summing all pixel intensities above this threshold. To minimize any potential biases, a different individual scored the acquired images, which were saved as coded grayscale files. The first observer was masked to origin of the slides whereas the second was unaware of the code.

**Statistical Analysis**

Data were analyzed using Minitab statistical software (College Park, MD). Data sets were first analyzed with the Anderson–Darling normality test and considered parametric with H₀ > 0.05. Parametric data are presented as mean ± SEM. Comparisons between nontransplanted wild-type and FcRn−/− mice were performed by two-sample t test, whereas those among transplanted groups were first compared by one-way ANOVA. If these were significantly different between groups (P < 0.05), comparisons were made by Fisher’s pairwise testing. Nonparametric data sets were analyzed with the Kruskal–Wallis Test.

For clearance data, the equation \( y = 10^{a_2 - a_1 \ln(x)} \) was used, in which x and y were time after injection and fluorescence intensity, respectively. For each animal, \( a_2 \) and \( a_1 \) were calculated from log-transformed data using the least-squares method. Curves from individual animals had r values of 0.91 or more (median 0.987, mean 0.979, n = 33). Individual \( a_2 \) and \( a_1 \) values were averaged to generate clearance curves of albumin and IgG in the different groups.

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**DISCLOSURES**

None.
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