Conditional deletion of the MHC class I-related receptor FcRn reveals the sites of IgG homeostasis in mice

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The MHC class I-related receptor FcRn regulates the levels and persistence of IgG in vivo. This receptor salvages IgG from lysosomal degradation within cells, and the binding properties of an IgG for FcRn correlate with in vivo half-life. FcRn is expressed at multiple different sites throughout adult life. However, the cell types and sites at which FcRn maintains IgG homeostasis are not well defined. Toward understanding the sites of FcRn function, we have generated a mouse strain in which this Fc receptor can be conditionally deleted. In combination with mice that express Cre recombinase under the control of the Tie2 promoter (Tie2-Cre), the effect of site-specific deletion of floxed FcRn in endothelial and hematopoietic cells on IgG persistence was analyzed. The pharmacokinetics and steady-state levels of IgG in Tie2-Cre mice that are homozygous for the floxed FcRn allele reveal a complete loss of FcRn function in regulating the half-lives of wild-type IgG. The primary sites for the maintenance of endogenous IgGs in mice are therefore endothelial and hematopoietic cells.

endothelial | half-life | hematopoietic | antibody

The regulation of IgG levels represents a fundamental aspect of humoral immunity. The MHC class I-related receptor FcRn plays a pivotal role in this process by transporting IgGs across and within cells in the transcytotic or recycling pathways, respectively (1–4). Cell biological studies indicate that this Fc receptor salvages bound IgG in endosomes from lysosomal degradation (5). Most naturally occurring IgGs bind to FcRn at acidic pH, but not at near-neutral pH (6, 7); consequently, uptake into cells before endosomal sorting is primarily dependent on fluid phase processes (8, 9). The binding properties of an IgG for FcRn directly impact uptake, efficiency of endosomal salvage, and release from cells, which in turn relates to the in vivo persistence of the antibody (10–14). Recent studies have also shown that FcRn can affect antigen presentation by regulating the intracellular trafficking and longevity of antigen in complex with IgG (9, 15). The properties of FcRn therefore not only offer opportunities for the engineering of therapeutic IgGs to modulate their in vivo half-lives, distribution, and transport across cellular barriers (16), but have implications for T cell-mediated responses (9, 15). Albumin also binds to FcRn at a site that is distinct to the region that interacts with IgG, and analyses in mice indicate that FcRn regulates the levels of this abundant serum protein (17, 18).

There are multiple candidate cell types and tissues that could be involved in controlling the concentration and persistence of IgG. For example, FcRn is present in endothelial, epithelial, and hematopoietic cells at diverse body sites throughout adult life (1, 4, 19–23). Although recent studies using bone marrow chimeras have shown that FcRn-expressing hematopoietic cells such as dendritic cells (DCs), monocytes, and macrophages play a role in regulating IgG half-lives, these analyses indicated that there are other contributing sites of FcRn expression (15, 24). The vascular location of endothelial cells combined with earlier in vivo distribution studies (20, 24) is suggestive of a role for these cells in IgG homeostasis. It is also possible that epithelial cells are involved in maintaining the levels of IgG following extravasation.

Site-specific ablation of FcRn expression would provide a direct approach to investigate the role of this Fc receptor in different cell types and at distinct sites throughout the body. To date, although mice in which the FcRn α-chain has been globally deleted have been described (25), mice in which FcRn can be conditionally deleted are not available. In the present study we have generated mice harboring floxed FcRn exons that can be selectively deleted in endothelial and bone marrow-derived cells by Cre recombinase expression under the control of the Tie2 promoter (26, 27). Tie2-Cre-mediated deletion of FcRn results in a complete loss of the regulation of the half-life of wild-type IgG and abnormally low serum IgG and albumin levels, whereas the persistence of IgA is not affected. In addition to DCs, monocytes, and macrophages (21), we show that FcRn is expressed in primary B cells in wild-type mice. Endothelial and hematopoietic cells that include DCs, monocytes, macrophages, and B cells are therefore the primary sites for the maintenance of IgG and albumin concentrations in vivo. The delineation of the in vivo sites of FcRn activity has direct relevance to understanding how this receptor contributes to multiple aspects of immunity and albumin homeostasis, and is also of importance for the effective delivery of antibody-based therapeutics.

Results

Generation of a Mouse Strain That Conditionally Expresses FcRn. The targeting strategy used to generate conditional FcRn knockout mice involved the flanking of exons 5–7 of FcRn by loxP sites (Fig. 1). Homologous ES cell recombinants were identified by Southern blotting using the approach shown in Fig. 1. Mice containing the loxP-flanked FcRn allele were backcrossed onto a C57BL/6 background and then intercrossed with Tie2-Cre mice (26) to generate floxed FcRn/Tie2-Cre heterozygotes. Heterozygotes were intercrossed to produce Cre-expressing mice that were homozygous for the floxed allele (Tie2-FcRn$^{flox/flox}$) in addition to littermate controls. Genotypes of these mice were determined using the PCR (described in *SI Text* and Fig. S1).

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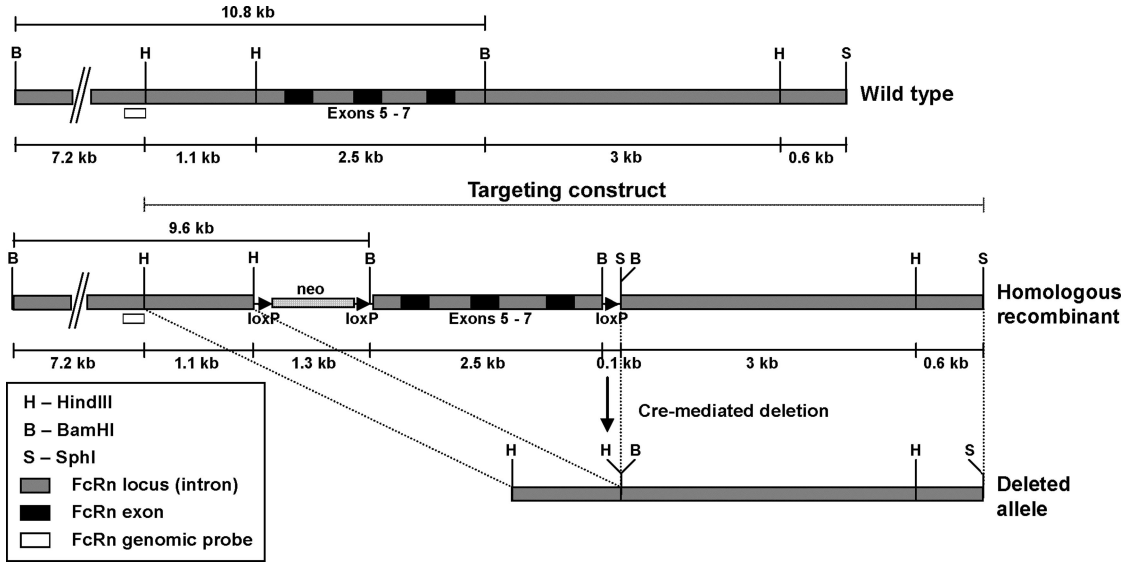

Fig. 1. Strategy to insert exons 5–7 of FcRn flanked by loxP sites by homologous recombination into the FcRn locus. Schematic representations of wild-type FcRn allele (36), FcRn allele following homologous recombination with targeting construct (indicated), and Cre-mediated deletion of exons 5–7 of FcRn are shown. Homologous ES recombinants were screened by Southern blotting of BamHI-digested DNA with the indicated FcRn genomic probe. For clarity, only BamHI, HindIII, and SphI sites are shown.

FcRn Is Not Expressed in Endothelial and Hematopoietic Cells of Tie2-FcRn$^{flox/flox}$ Mice. The expression of FcRn was analyzed in tissue sections derived from liver, spleen, and intestine (duodenum) of adult Tie2-FcRn$^{flox/flox}$ mice and their Tie2-FcRn$^{flox/+}$ littermate controls (Cre expressing, heterozygous for floxed allele). To assess endothelial expression of FcRn in the vasculature of spleens and livers, mice were injected intravenously with Alexa 647-labeled, mutated human IgG1 (MST-HN). In previous studies we have shown that this IgG, which is engineered to bind to mouse FcRn with high affinity in the pH range 6.0–7.4, can be used as a live cell stain for FcRn-expressing cells (8). This mutant accumulates in FcRn-expressing cells to relatively high levels because it is internalized by receptor (FcRn)-mediated uptake and is not released efficiently from FcRn during exocytic events at the plasma membrane. As controls for nonspecific binding or possible uptake by the classical FcγRs, we used Alexa 647-labeled, mutated human IgG1 (H435A) that, relative to wild-type human IgG1, has a $\approx 10^3$-fold reduced affinity ($K_D \approx 25\ \mu M$ for H435A vs. $K_D \approx 24$ nM for wild type) (7) for mouse FcRn at pH 6.0 and does not bind detectably at pH 7.4. This mutant retains binding to the classical FcγRs (28).

Serum samples of mice were harvested 30 or 90 min following injection of labeled MST-HN or H435A mutant to assess the levels of delivered protein (data not shown) to ensure that lack of staining under some conditions was not due to variations in injected dose. Subsequently, organs were excised and stained using an antibody specific for the endothelial marker endomucin. These analyses show that FcRn is not expressed in the splenic or liver endothelium of Tie2-FcRn$^{flox/flox}$ mice, whereas expression in Tie2-FcRn$^{flox/+}$ mice can be clearly detected (Fig. 2 *A* and *B*). The FcRn dependence of uptake into endothelial cells of Tie2-FcRn$^{flox/+}$ mice was shown by the background levels of signal when organs were analyzed from mice injected with labeled H435A (Fig. 2 *A* and *B*). For the analysis of FcRn expression in intestinal epithelial cells, a different approach was taken because such cells are not directly accessible to intravenously delivered proteins. Duodena were sectioned and stained with Alexa 647-labeled MST-HN and anti-E-cadherin. Importantly, and consistent with the expression pattern of Tie2-Cre reported earlier (26, 27), FcRn is expressed in epithelial cells of both Tie2-FcRn$^{flox/flox}$ and Tie2-FcRn$^{flox/+}$ controls. Background levels of staining were observed when Alexa 647-labeled H435A was used (Fig. 2*C*).

In addition, although the Tie2-Cre transgenic line that we used was originally reported to result in Cre recombinase expression only in endothelial cells (26), Tie2-Cre transgenes have in some cases been shown to also be active in hematopoietic cells (27). We therefore assessed whether FcRn was expressed in splenic DCs, monocytes, macrophages, and B cells of Tie2-FcRn$^{flox/flox}$ mice. In earlier studies, FcRn was shown to not be expressed in primary B cells and in vitro B cell-derived lines (15, 19, 21). However, our recent RT-PCR and functional analyses in H-2^u (B10.PL) mice indicate that this receptor is expressed in primary splenic B cells (9) and these cells were therefore analyzed in the present study. Splenocytes were incubated with Alexa 647-labeled MST-HN, and uptake was quantitated by flow cytometry (Fig. 3*A*). To assess the FcRn dependence of uptake, accumulation of MST-HN was compared with that of the H435A mutant (Fig. 3*A*), and assays were also carried out in the presence of the anti-FcγR (IIB/III) blocking antibody 2.4G2 (Fig. 3*B*). The analyses show that DCs, monocytes, macrophages, and B cells do not express FcRn in Tie2-FcRn$^{flox/flox}$ mice, whereas FcRn is present in these cells in Tie2-FcRn$^{flox/+}$ controls. Uptake of MST-HN protein in CD4$^+$ T cells that do not express FcRn and FcγRs (15, 19, 21, 29) was at background autofluorescence levels, analogous to those observed for the antigen-presenting cell (APC) subsets in Tie2-FcRn$^{flox/flox}$ mice (Fig. 3*A*).

Rapid Clearance of Wild-Type IgG in Tie2-FcRn$^{flox/flox}$ Mice. Having established that FcRn is not expressed in the endothelial and hematopoietic cells of Tie2-FcRn$^{flox/flox}$ mice, the impact of FcRn deficiency at these sites on the clearance rate of human IgG1 was investigated (Fig. 4 and Table 1). The (β-phase) half-life of IgG1 is 8.2 ± 1.6 h in Tie2-FcRn$^{flox/flox}$ mice vs. 169.8 ± 27.5 h in FcRn-sufficient Tie2-FcRn$^{flox/+}$ controls (Table 1). The short half-life in Tie2-FcRn$^{flox/flox}$ mice is also less than that of ≈24 h reported for human IgG1 in FcRn α-chain knockout mice (30). The half-life of human IgG1 in FcRn$^{flox/flox}$ mice (no Cre recombinase expression, homozygous for floxed FcRn) is 146.1 ± 43.9 h (n = 9 mice) and is not significantly

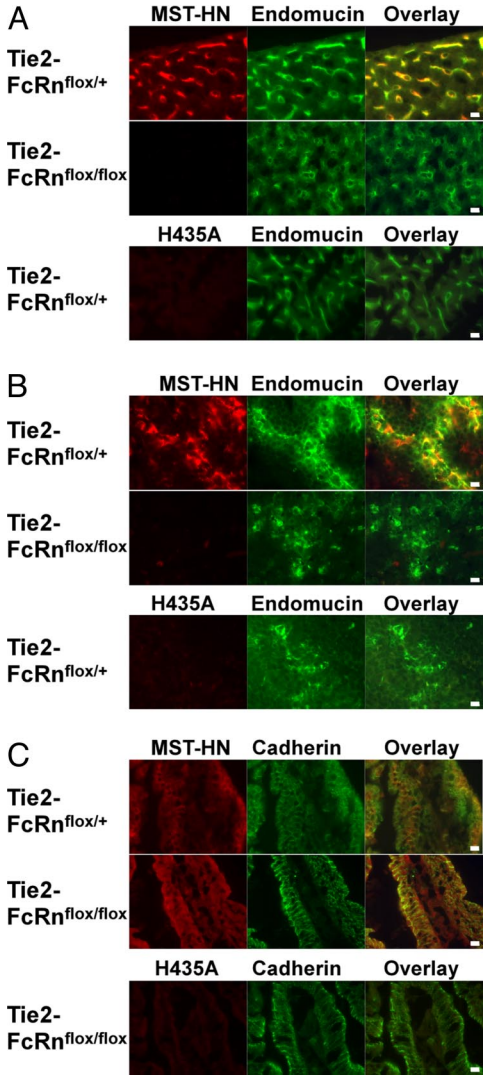

Fig. 2. FcRn is not expressed in the endothelium of Tie2-FcRn$^{flox/flox}$ mice, but can be detected in the duodenal epithelial cells. Liver (*A*) or spleen (*B*) sections from Tie2-FcRn$^{flox/+}$ or Tie2-FcRn$^{flox/flox}$ mice following i.v. injection with Alexa 647-labeled MST-HN or H435A mutants. Sections were incubated with anti-endomucin antibody followed by Alexa 488-labeled anti-rat IgG to detect endothelium. (*C*) Sections of duodena from Tie2-FcRn$^{flox/+}$ or Tie2-FcRn$^{flox/flox}$ mice. Sections were incubated with Alexa 647-labeled MST-HN (or H435A) and anti-E-cadherin followed by Alexa 488-labeled anti-rat IgG to detect FcRn and epithelial cells, respectively. For each organ, the same exposure times were used for Alexa 647-labeled IgGs. Data are representative of at least 2 independent experiments. (Scale bars: 10 μm.)

different from that in Tie2-FcRn$^{flox/+}$ mice. In addition, the clearance rate of human IgG1 in wild-type mice (i.e., no floxed alleles in the absence of Cre recombinase expression) is not significantly different from that in Tie2-FcRn$^{flox/+}$ mice (Table 1), indicating that deletion of one functional FcRn allele and/or Cre recombinase expression does not impact the persistence of IgG1. We also compared the half-lives of mouse IgA, which is not regulated by FcRn (19), in wild-type, Tie2-FcRn$^{flox/flox}$, and Tie2-FcRn$^{flox/+}$ mice. Importantly, there is no significant difference in IgA clearance rates between the 3 mouse strains, and the half-lives of wild-type IgG1 and IgA are similar in Tie2-FcRn$^{flox/flox}$ mice (Table 1). Collectively, the data show that the effect of FcRn deletion is specific for IgGs.

We reasoned that if FcRn expression at sites such as epithelium had an impact on the persistence of IgGs in Tie2-FcRn$^{flox/flox}$ mice, then the behavior of IgGs with different affinities for FcRn would differ in these mice. The half-lives of the H435A mutant in Tie2-FcRn$^{flox/flox}$ and Tie2-FcRn$^{flox/+}$ mice were therefore compared with those of wild-type IgG1 (Fig. 4 and Table 1). Both wild-type IgG1 and H435A show negligible binding to FcRn at near-neutral pH (8) but differ in their affinities at pH 6.0 by about 10^3-fold. Hence they are both dependent on fluid-phase uptake but differ at the level of endosomal sorting and recycling in FcRn-expressing cells (5, 8). The analyses show that the half-lives of wild-type human IgG1 and H435A are indistinguishable in Tie2-FcRn$^{flox/flox}$ mice (8.2 ± 1.6 h for wild-type IgG1, 9.2 ± 0.9 h for H435A). Consistent with earlier studies (28), the half-life of H435A is substantially shorter than that of wild-type IgG1 in FcRn-sufficient Tie2-FcRn$^{flox/+}$ mice (26.4 ± 8.1 vs. 169.8 ± 27.5 h; Table 1). The low residual affinity of H435A for FcRn results in a longer half-life in these FcRn-sufficient mice relative to Tie2-FcRn$^{flox/flox}$ mice (Table 1). Collectively, the data show that there is no detectable contribution of FcRn to the persistence of wild-type IgG1 in Tie2-FcRn$^{flox/flox}$ mice.

A Mutated IgG with Reduced pH-Dependent Binding to FcRn Shows Different in Vivo Behavior Relative to Wild-Type IgG1. The half-life of wild-type IgG1 in wild-type mice is governed by highly pH-dependent binding, which results in fluid-phase uptake into cells followed by endosomal sorting and recycling or transcytosis (5, 8). By contrast, in ref. 8 we described an engineered IgG ("Abdeg") that competitively inhibits FcRn function. Mutated IgGs such as MST-HN have high affinity for FcRn at near-neutral pH and therefore preferentially accumulate in FcRn-expressing cells due to receptor (FcRn)-mediated uptake and poor exocytic release (8, 9, 14) (Fig. 3). By contrast with wild-type IgGs that are recycled and released, IgGs of the Abdeg class accumulate in lysosomes following uptake into cells (9).

We compared the behavior of the MST-HN mutant in FcRn-sufficient and -deficient mice. Consistent with earlier analyses of Abdegs (12, 14), the half-life of MST-HN is shorter than that of wild-type IgG1 in FcRn-sufficient Tie2-FcRn$^{flox/+}$ mice (41.6 ± 7.0 vs. 169.8 ± 27.5 h; Table 1 and Fig. S2). In Tie2-FcRn$^{flox/flox}$ mice, MST-HN and wild-type IgG1 show similar clearance properties until 12 h postinjection (Fig. S2). Following this initial phase, the clearance rate of MST-HN in Tie2-FcRn$^{flox/flox}$ mice approaches that observed in Tie2-FcRn$^{flox/+}$ mice (Fig. S2). The high affinity of MST-HN for FcRn at near-neutral pH most likely enables accumulation in a subset of FcRn-expressing cells in Tie2-FcRn$^{flox/flox}$ mice, resulting in a β-phase half-life (26.9 ± 8.8 h) that approaches that in Tie2-FcRn$^{flox/+}$ mice. However, this cell subset does not contribute detectably to the homeostasis of IgGs such as wild-type IgG1, which bind with marked pH dependence to FcRn and consequently do not selectively target FcRn-sufficient cells in vivo (8, 9).

Reduced Serum IgG and Albumin Levels in Tie2-FcRn$^{flox/flox}$ Mice. We also compared the serum IgG levels in Tie2-FcRn$^{flox/flox}$ mice with their FcRn-sufficient Tie2-FcRn$^{flox/+}$ littermate controls (Table 1). Consistent with the increased clearance rate of IgG in Tie2-FcRn$^{flox/flox}$ mice, the serum IgG levels were approximately 4-fold lower than those in Tie2-FcRn$^{flox/+}$ or wild-type mice. In addition, serum albumin levels in Tie2-FcRn$^{flox/flox}$ mice were approximately 2-fold lower than in FcRn-sufficient controls (Table 1), indicating that FcRn expression in endothelial and hematopoietic cells regulates both IgG and albumin homeostasis.

Discussion

The MHC class I-related receptor FcRn is expressed at a diverse array of sites in adult tissues (1, 4, 19–23), raising the question as to which cell types contribute to the maintenance of IgG levels in vivo. This question is of fundamental importance for effective immunity and also has implications for the engineering of

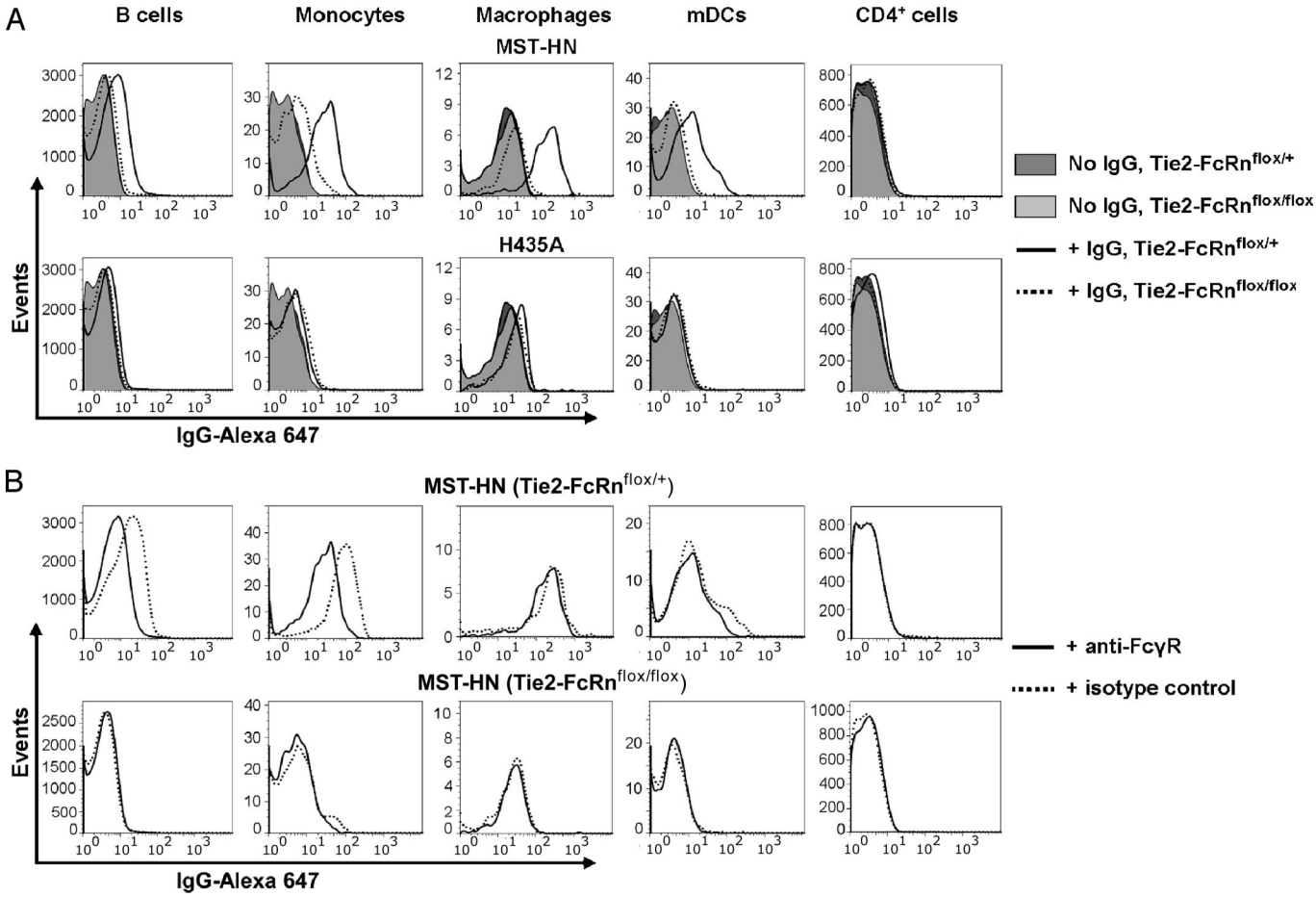

Fig. 3. Splenic DCs, monocytes, macrophages, and B cells in Tie2-FcRn$^{flox/flox}$ mice do not express FcRn. (*A*) Splenocytes were isolated and incubated with anti-FcγRIIB/III (2.4G2) antibody at 4 °C followed by Alexa 647-labeled MST-HN or H435A mutant at 37 °C to assess FcRn-mediated uptake. (*B*) Splenocytes were treated with MST-HN as in (*A*), except that data for cells treated with isotype control antibody are also shown. Data are representative of at least 4 independent experiments.

therapeutic or diagnostic IgGs with modulated in vivo half-lives. In the present study we have generated mice harboring a "floxed" FcRn allele to investigate the impact of Cre recombinase-mediated deletion of FcRn in endothelial and hematopoietic cells, such as DCs, monocytes, macrophages, and B cells, on the regulation of IgG persistence. In combination with analyses involving bone marrow transfers from FcRn-deficient mice (15, 24), our studies show that endothelial and hematopoietic cells are the primary cell types responsible for controlling the clearance rate of wild-type IgG, with no detectable contribution from other sites, such as epithelium.

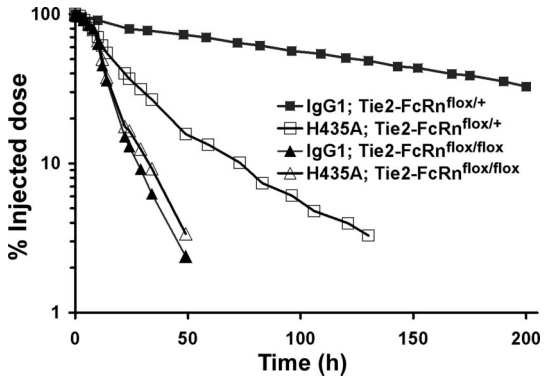

Fig. 4. IgG1 has a short half-life in Tie2-FcRn$^{flox/flox}$ mice that is the same as the half-life of a mutated IgG1 that binds with very low affinity to FcRn. Tie2-FcRn$^{flox/flox}$ and Tie2-FcRn$^{flox/+}$ mice were injected with ^{125}I-labeled human wild-type IgG1 or H435A mutant, and radioactivity levels assessed at the indicated times. Data shown represents means of 5 mice (Tie2-FcRn$^{flox/flox}$, IgG1), 4 mice (Tie2-FcRn$^{flox/+}$, IgG1), 6 mice (Tie2-FcRn$^{flox/+}$, H435A mutant), and 7 mice (Tie2-FcRn$^{flox/flox}$, H435A mutant). Data are compiled from at least 2 independent experiments and are derived from a subset of the total numbers of mice shown in Table 1.

Due to the absence of an appropriate mouse strain in which FcRn can be site-specifically deleted, the contribution of the endothelium to IgG homeostasis has to date not been analyzed. Recent studies using bone marrow transfers have shown that hematopoietic cells partially contribute to the regulation of the half-life of IgG (15, 24). Here we show a complete loss of function in the regulation of persistence of wild-type IgG1 in mice lacking FcRn expression in endothelial and hematopoietic cells (Tie2-FcRn$^{flox/flox}$ mice). In combination with the reduced steady-state levels of serum IgG in Tie2-FcRn$^{flox/flox}$ mice, our findings therefore underscore the important contribution of endothelial cells to the maintenance of IgG levels. Earlier studies have shown that FcRn is expressed in small arterioles and capillaries but not in larger vessels such as the portal vasculature and central vein, with skin, muscle, and, to a lesser extent, liver and adipose tissue being the major sites (20). This is consistent with recent analyses of FcRn expression in mice, in which this receptor was found to be highly expressed in the vasculature of skin, muscle, and liver (24). In addition, the high levels of

Table 1. β-phase half-lives of IgGs and serum levels of IgG and albumin in FcRn-sufficient and -deficient mice

	β-phase half-life, h					
	Wild-type IgG1	H435A	MST-HN	IgA	IgG, mg/ml	Albumin, mg/ml
Tie2-FcRn$^{flox/+}$	169.8 ± 27.5 (n = 11)[a]	26.4 ± 8.1 (n = 9)[b]	41.6 ± 7.0 (n = 4)[b]	14.7 ± 3.4 (n = 4)[a]	1.66 ± 0.4 (n = 8)[a]	33.9 ± 5.2 (n = 6)[a]
Tie2-FcRn$^{flox/flox}$	8.2 ± 1.6 (n = 7)[b,c]	9.2 ± 0.9 (n = 7)[b,c]	26.9 ± 8.8 (n = 6)[b]	11.6 ± 0.7 (n = 4)[a]	0.46 ± 0.1 (n = 8)[b]	19.0 ± 3.4 (n = 7)[b]
Wild type	217.8 ± 33.3 (n = 8)[a]	ND	ND	11.7 ± 1.4 (n = 4)[a]	1.74 ± 0.3 (n = 9)[a]	34.0 ± 3.3 (n = 6)[a]

[a]Not significantly different within each Ig group, IgG level group, or albumin level group. ND, not determined.
[b]Significantly different within each Ig group, IgG level group, or albumin level group.
[c]β-phase half-lives of wild-type IgG1 and H435A mutant in Tie2-FcRn$^{flox/flox}$ mice were not significantly different.

expression of FcRn in splenic microvessels (ref. 24 and this study) suggest that the spleen is an important site of IgG homeostasis.

By contrast with DCs, monocytes, and macrophages, earlier studies showed that FcRn is not expressed in cultivated B-cell lines or primary B cells isolated ex vivo (15, 19, 21, 24). In the present study we have directly compared the uptake of a high-affinity ligand for FcRn (MST-HN mutant of human IgG1) in FcRn-sufficient and -deficient B cells. Consistent with our recent analyses using wild-type B10.PL mice (9), FcRn is present in primary B cells of C57BL/6 mice, albeit at low functional levels. The role of FcRn in these cells is currently unknown, but analogous to observations with DCs (15), this receptor could contribute to antigen presentation. In addition, given the relative abundance of B cells in the body, FcRn in this APC subset might contribute to IgG homeostasis. In this context, the expression of FcRn in the major classes of professional APCs combined with recent studies of the T-cell stimulatory activity of antibody-antigen complexes or Fc fusion proteins (9, 15) indicate that this Fc receptor serves a dual role in regulating both the intracellular trafficking and longevity of antigen in complex with IgGs.

Wild-type IgG1 does not bind detectably to FcRn at near-neutral pH and is therefore primarily dependent on fluid-phase uptake for entry into cells (8). We recently described a class of engineered IgGs, such as the MST-HN mutant, that have high affinity for FcRn at near-neutral pH and efficiently accumulate in FcRn-expressing cells due to receptor-mediated uptake and poor exocytic release (8, 9, 14). MST-HN has different clearance properties relative to wild-type IgG1 in Tie2-FcRn$^{flox/flox}$ mice. This can be explained by the ability of this mutant to target FcRn-expressing cells (of nonhematopoietic/nonendothelial origin) in these mice. Although we cannot exclude the possibility that FcRn-expressing cells in Tie2-FcRn$^{flox/flox}$ mice also contribute to the persistence of wild-type IgG1, our observations indicate that the cell subset targeted by MST-HN does not significantly impact IgG1 homeostasis.

Under some circumstances, epithelial expression of FcRn can affect IgG persistence. For example, overexpression of FcRn in mammary gland epithelium of mice results in elevated levels of serum IgG (31). However, the data in the current study indicate that under normal, steady-state conditions, epithelial function of FcRn does not contribute to the homeostasis of IgGs that exhibit pH-dependent binding to FcRn. It is possible that under conditions leading to the extravasation of IgG, such as inflammation (32), the exposure of IgGs to epithelial surfaces might result in an impact of FcRn at these sites on whole-body IgG levels.

A large body of evidence indicates that in addition to its role in IgG homeostasis, FcRn also regulates serum albumin levels (17, 18). Our analyses show that ablation of FcRn expression in endothelial and hematopoietic cells results in an approximate 2-fold decrease in serum albumin. This decrease is not as marked as the 4-fold reduction seen for IgG, and similar observations were made in mice in which the FcRn α-chain is globally deleted (17). In this context, the half-life of albumin in wild-type mice is considerably shorter than that of IgG (17), suggesting that FcRn is a more effective salvage receptor for IgG than for albumin. Consequently, site-specific deletion of FcRn would be expected to have a lower impact on the steady-state levels of albumin relative to IgG. In addition, albumin biosynthesis is upregulated during hypoalbuminemia (33), whereas this is not observed for IgG in mice that are, for example, deficient in FcRn due to deletion of β2-microglobulin (4, 34).

In conclusion, FcRn is a receptor that is expressed in a large variety of cell types and has been shown to have a multitude of functions (1, 4, 19–23). An analysis of the relevance of the specific expression site for a particular function has been impeded by the lack of a conditional mouse model. Here, the generation and analysis of mice that conditionally express FcRn has allowed us to determine that endothelial and hematopoietic cells are the primary contributors to the homeostasis of wild-type IgG in vivo. Knowledge of the cell types involved in FcRn-mediated activities interfaces both humoral and cellular immunity, and also impacts the use of antibody engineering to optimize the delivery of therapeutic IgGs.

Materials and Methods

Generation of the Targeting Construct. Genomic DNA clones encoding mouse FcRn were isolated by screening a λ2001 library of strain 129-derived E14 TG2a ES cell DNA (35) using FcRn-specific probes. These clones were used to generate the targeting construct shown in Fig. 1. In brief, a fragment encoding a loxP site was cloned at the 3′ end of a genomic 2.5-kb HindIII-BamHI fragment encoding exons 5–7 of FcRn (36). A 3.6-kb BamHI-SphI fragment encoding the 3′ genomic sequences of the FcRn locus were then cloned downstream of this loxP site. Subsequently, a floxed neomycin gene (37) was inserted 5′ to the HindIII-BamHI genomic (exons 5–7) fragment. This was followed by insertion of an additional 1.1-kb HindIII fragment of genomic DNA that, in the wild-type allele, is located at the 5′ end of exon 5. Finally, the insert of this construct was combined as a SfiI-PvuI fragment with a 2-kb NotI-PvuI fragment encoding the thymidine kinase gene (37) in a 3-way ligation with SfiI-NotI-digested pHEN1 (38). All cloning was carried out using standard methods of molecular biology, and (intermediate) constructs were verified by PCR and sequencing.

Generation of Mice Harboring a Floxed FcRn Allele. ES cells derived from 129SV mice were cultured and transfected with linearized (using SfiI) targeting vector as in ref. 39. Forty-eight hours following transfection, cells were selected using 380 μg/ml G418. Genomic DNA was extracted from selected clones, digested with BamHI, and analyzed by Southern blotting using a ≈250-bp SalI-HindIII fragment that corresponds to the flanking DNA that is immediately 5′ to the genomic insert of the targeting construct. Clones with the homologously recombined floxed allele were identified by the presence of a hybridizing 9.6-kb BamHI fragment (10.8 kb in wild-type allele; Fig. 1). The presence of the loxP site at the 3′ end of exons 5–7 was confirmed using the PCR and primers 4 and 5 described in *SI Text*. ES cells from a homologously recombined clone were injected into blastocysts of CB20 mice. Tail DNA of offspring of fur-colored chimeras bred with C57BL/6 mice was analyzed for germline transmission using Southern blotting (Fig. 1). Mice were subsequently bred onto a C57BL/6 background for 5–7 generations and typed by PCR for the presence of the floxed allele using the primers shown in *SI Text*. Mice harboring the floxed allele were crossed with Tie2-Cre mice on a C57BL/6 background (26) (generously provided by Dr. M. Yanagisawa, University of Texas Southwestern Medical Center) and offspring intercrossed to generate Tie2-FcRn$^{flox/flox}$ mice and their littermate controls. Mice were typed for the presence of Tie2-Cre, floxed FcRn, deleted FcRn, or wild-type FcRn alleles using the PCR and the primers shown in *SI Text*. For use in experiments, mice were

6–10 weeks old. All mouse experiments were carried out with the approval of Institutional Animal Care and Use Committees.

Antibodies. Mouse IgA (Cappel™) was purchased from MP Biomedicals. The humanized anti-hen egg lysozyme antibody HuLys10 (human IgG1; cell line generously provided by Dr. J. Foote, Arrowsmith Technologies, Seattle, WA), a mutated derivative (MST-HN; Met-252 to Tyr, Ser-254 to Thr, Thr-256 to Glu, His-433 to Lys, and Asn-434 to Phe) that binds to FcRn with increased affinity at pH 6 and 7.2 (8) and a mutated derivative (His-435 to alanine, H435A) that has very low affinity for mouse FcRn, were purified as described (28, 40). Binding properties of the antibodies were confirmed using surface plasmon resonance. Antibodies were labeled with Alexa Fluor 647 (Alexa 647) carboxylic acid (succimidyl ester; Invitrogen) using methods as in ref. 5. Degrees of labeling of the MST-HN and H435A mutants were 5.14 and 5.42 mol fluorophore/mol IgG, respectively.

Surface Plasmon Resonance. Surface plasmon resonance experiments were carried out as described in *SI Text*.

Immunohistochemistry and Flow Cytometry. Immunohistochemical and flow cytometry analyses were carried out as described in *SI Text*.

Analyses of Pharmacokinetics, Serum IgG, and Albumin Levels. Methods for the pharmacokinetic analyses and determination of serum IgG and albumin levels are described in *SI Text*.

Statistics. Statistical analyses were carried out using ANOVA or Student's *t* test in the statistics toolbox of MATLAB (Mathworks). For ANOVA, paired comparisons between different mouse groups were conducted using the Tukey-Kramer test at a 95% confidence level. *P* values of less than 0.05 for Student's *t* test were taken to be significant.

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