The Journal of Immunology

The MHC Class I-Like IgG Receptor Controls Perinatal IgG Transport, IgG Homeostasis, and Fate of IgG-Fc-Coupled Drugs

Derry C. Roopenian, Gregory J. Christianson, Thomas J. Sproule, Aaron C. Brown, Shreeram Akilesh, Nadja Jung, Stefka Petkova, Lia Avanessian, Eun Young Choi, Daniel J. Shaffer, Peter A. Eden and Clark L. Anderson

J Immunol 2003; 170:3528-3533; ;
doi: 10.4049/jimmunol.170.7.3528
http://www.jimmunol.org/content/170/7/3528

The Journal of Immunology is published twice each month by
The American Association of Immunologists, Inc.,
1451 Rockville Pike, Suite 650, Rockville, MD 20852

Print ISSN: 0022-1767 Online ISSN: 1550-6606.

The MHC Class I-Like IgG Receptor Controls Perinatal IgG Transport, IgG Homeostasis, and Fate of IgG-Fc-Coupled Drugs[1]

Derry C. Roopenian,[2]* Gregory J. Christianson,* Thomas J. Sproule,* Aaron C. Brown,* Shreeram Akilesh,* Nadja Jung,* Stefka Petkova,* Lia Avanessian,* Eun Young Choi,* Daniel J. Shaffer,* Peter A. Eden,* and Clark L. Anderson†

Abs of the IgG isotype are efficiently transported from mother to neonate and have an extended serum $t_{1/2}$ compared with Abs of other isotypes. Circumstantial evidence suggests that the MHC class I-related protein, the neonatal FcR (FcRn), is the FcR responsible for both in vivo functions. To understand the phenotypes imposed by FcRn, we produced and analyzed mice with a defective *FcRn* gene. The results provide direct evidence that perinatal IgG transport and protection of IgG from catabolism are mediated by FcRn, and that the latter function is key to IgG homeostasis, essential for generating a potent IgG response to foreign Ags, and the basis of enhanced efficacy of Fc-IgG-based therapeutics. FcRn is therefore a promising therapeutic target for enhancing protective humoral immunity, treating autoimmune disease, and improving drug efficacy. ***The Journal of Immunology*****, 2003, 170: 3528–3533.**

Immunoglobulin G Abs comprise the major class responsible for long-term humoral immunity. They can also act as pathological effectors of systemic autoimmune diseases such as systemic lupus erythematosus, organ-specific diseases such as rheumatoid arthritis, myastenia gravis, and spontaneous abortion in multiparous females. Two strategies have evolved to maximize the effectiveness of these Abs. The first is to ensure that IgG is efficiently transported from mother to neonate, thus engendering in newborn animals protective immunity. The second is to ensure that IgG produced as a consequence of antigenic exposure is available for long-term protective immunity. Similar to most other serum proteins, Abs of non-IgG subclasses have a relatively short $t_{1/2}$ (~1–2 days). In contrast, the $t_{1/2}$ of most IgG Abs is considerably longer (~6–8 days in mice (1) and 22–23 days in humans (2)), increasing the availability of sufficient specific IgG to resolve infection. This homeostatic mechanism is absolutely dependent on the Fc region of IgG (reviewed in Ref. 3). Exploiting this mechanism has additionally proven to be an important component of drug design in that the coupling of IgG Fc to therapeutic proteins considerably extends their pharmacokinetics (3).

Brambell (4) hypothesized many years ago that perinatal transport and IgG homeostasis were consistent with receptor-mediated events, and proposed that a similar receptor might be responsible for both functions. The best candidate for Brambell's common receptor is the so-called neonatal FcR (FcRn),[3] originally identified by Simister and colleagues (5, 6). FcRn is a distant member of the MHC class I protein family, most members of which act as ligands for T cell and NK receptors. However, FcRn has diverged evolutionarily to bind the hinge region of IgG Fc at a low pH (~6–6.5) (7). FcRn is highly expressed in the neonatal rodent gut and, more weakly, in adult tissues such as the vascular endothelium, where it is thought to perform its IgG protection function (8). Intracellularly, FcRn resides primarily in the early acidic endosomal vesicles, where it is thought to bind and then recycle endocytosed IgG normally destined for endosomal degradation (9–11).

Because a functional FcRn molecule is dependent on dimerization with β_2-microglobulin (β_2m), the obligate L chain of all class I proteins, β_2m-deficient mice have been used as a model for understanding the in vivo biology of FcRn. However, this model is encumbered by the many immunological and nonimmunological processes dependent on this common class I L chain, including the elaboration of CD8 T cells, natural T cells, conventional NK cells (reviewed in Ref. 12), the control of serum iron concentration (13), and neuronal plasticity (14). β_2m-deficient neonatal mice fail to take up maternal IgG (15), and their adult counterparts have an abnormally short IgG $t_{1/2}$ and reduced homeostatic IgG levels (16–18). Studies have variably reported that IgG responses to antigenic stimuli are reduced (16, 19) or increased (20, 21) in β_2m-deficient mice, and similarly variable effects have been reported in mouse models for autoimmune diseases with an Ab etiology (16, 22–26) (D. Roopenian, manuscript in preparation). The phenotypes imposed in vivo as a direct consequence of FcRn thus remain to be established.

Materials and Methods

Production of FcRn-deficient and human FcRn-transgenic mice

To produce a standard *FcRn* targeting vector, a 2.7-kb 129/J-derived genomic *Bam*HI fragment carrying sequences 5′ of the *FcRn* transcriptional start site was inserted upstream of PGK-Neo^r into a pGTN/28 targeting vector (New England Biolabs, Beverly, MA), and a 5.2-kb fragment starting at the *Apa*I site in exon 2 was inserted 3′. The 129/SvJ-derived ESV/J-1182 embryonic stem cells (Genome Systems, St. Louis, MO) were electroporated with the *Not*I-linearized targeting construct. DNAs from clones surviving G418 selection were digested with *Eco*RV for Southern blot screening for homologous recombination of the 5′ targeting vector arm and with *Eco*RV + *Hin*dIII for recombination of the 3′ arm. PCR amplicons based on DNA sequences immediately 5′ and 3′ of the targeting vector arm were then used as probes for Southern blotting, and identified

*The Jackson Laboratory, Bar Harbor, ME 04609; and †Ohio State University, Columbus, OH 43210

Received for publication October 30, 2002. Accepted for publication January 23, 2003.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

[1] This work was supported by the Alliance for Lupus Research and National Institutes of Health R01 DK56597 to D.C.R., and HD38764 and CA88053 to C.L.A.

[2] Address correspondence and reprint requests to Dr. Derry Roopenian, The Jackson Laboratory, 600 Main Street, Bar Harbor, ME 04609. E-mail address: dcr@jax.org

[3] Abbreviations used in this paper: FcRn, neonatal FcR; β_2m, β_2-microglobulin; CD40L, CD40 ligand; KLH, keyhole limpet hemocyanin; TNP, trinitrophenyl; GpR, Global Pattern Recognition; wt, wild type.

0022-1767/03/$02.00

embryonic stem cell clone 573 carried the predicted 5′ and 3′ restriction fragment. After genotypic confirmation, clone 573 was injected into C57BL/6J (B6) blastocysts and implanted into pseudopregnant B6 mice by The Jackson Laboratory Cell Biology and Microinjection Service. Chimeric mice and their germline transmitting progeny were typed by PCR using primers designed to distinguish the targeted from the wild-type (wt) FcRn alleles: FcRn o393 F-GGGATGCCACTGCCCTG, FcRn vs o394 R-CGAGCCTGAGATTGT-CAAGTGTATT, 248-bp wt allele; targeting vector o395 F-GGAATTC-CCAGTGAAGGGC vs o394, 378-bp targeted allele. Protein expression of both mouse FcRn and the human FcRn transgene (see below) was determined by Western blot analysis using an antiserum produced by immunizing FcRn-deficient mice with recombinant, bacterially expressed human FcRn in CFA, followed by boosts with the same protein in IFA. Protein extracts (25 μg) from neonatal small intestine were separated on a 4–20% gradient gel, transferred to polyvinylidene difluoride membrane, and incubated with antiserum against FcRn as a primary Ab and HRP-conjugated goat anti-mouse Ig (BD Biosciences, San Diego, CA) as a secondary Ab. β-actin was revealed using a rabbit anti-mouse β-actin antiserum (Sigma-Aldrich, St. Louis, MO) and a HRP-conjugated donkey anti-rabbit F(ab′)$_2$ (Amersham Biosciences, Piscataway, NJ) as a secondary Ab. Bands were visualized by ECL detection. To produce mice transgenically expressing a complete human *FcRn* gene, a 34-kb *Xho*I fragment was excised from a human-derived bacterial artificial chromosome (Genome Systems) and cloned into a SuperCos vector (Stratagene, La Jolla, CA). This fragment, which encompasses the complete ~11-kb *FcRn* gene plus ~10-kb 5′ and 3′, was microinjected into zygotes from C57BL/6J female mice, and implanted into pseudopregnant female mice. A chimeric, germline transmitting male founder was used to establish the C57BL/6J-human FcRn transgenic line 44. Expression of human FcRn was confirmed by RT-PCR (not shown) and by Western blot analysis. Unless otherwise indicated, mice ranged from 6 to 12 wk of age and were age and sex matched within each experiment. FcRn$^{-/-}$ mice used as indicated were either mixed 129:B6 stocks, congenic B6 background (generated after five backcross generations), or isogenic 129/J background stocks. FcRn$^{-/-}$ human FCRN transgenic mice were on a mixed 129:B6 genetic background.

Ig transfer and clearance

For maternal Ig transfer, a female FcRn$^{+/-}$ mouse was mated to a male FcRn$^{-/-}$ mouse. At 17 days of gestation, the pregnant female was injected i.v. with 500 μg IgG1 anti-trinitrophenyl (TNP) (mAb 1B7.11) tracer Ab. Sera from her 3-day-old pups were assayed for anti-TNP Ab activity by ELISA, and their liver DNA was used to determine their FcRn genotypes. Ig clearance in adult mice was determined as described (16). Briefly, 100 μg of mouse IgG1 anti-TNP, mouse IgA anti-TNP (mAb 2F.11.15), purified human IgG (GammaGuard; Baxter Healthcare, Deerfield, IL), or directly biotinylated mouse IgG1, IgG2a, IgG2b, and IgG3 (Sigma-Aldrich) tracer was injected into mice i.p., and sera prepared from retro-orbital plexus blood were assayed by ELISA. β-phase Ig tracer clearance was based on the amount of Ig retained normalized to that present 24 h after injection.

Serum Ig concentrations and flow cytometry

Total serum Ig and specific mouse isotypes were quantified as described (16, 22). Total Ig and isotype quantification was based on duplicate serum dilutions in which at least two dilutions hit the exponential curve of serially diluted Ig standards: Ig (Calbiochem, San Diego, CA), IgG1 (BD PharMingen, San Diego, CA), IgG2a (PharMingen), IgG2b (Southern Biotechnology, Birmingham, AL), IgG3 (Southern Biotechnology), IgM (BD PharMingen), and IgA (Southern Biotechnology). Data are expressed as equivalents of Ig compared with the Ab standards. For flow cytometry, splenocytes or bone marrow cells were isolated and incubated with propidium iodide and specific conjugated Abs at 4°C for 50 min in staining buffer (1× PBS with 0.1% BSA and 0.1% sodium azide), washed twice, and then analyzed with live cell gating using a FACSCalibur equipped with CellQuest software (BD Biosciences). Conjugated mAbs, CD4 CY3$^+$, CD8a PE, CD45R/B220 APC, CD45R/B220 Cy5, CD19 FITC, and CD138 APC, were obtained from BD Pharmingen.

Quantitative PCR analysis and 5′ RACE

Tissues were collected into RNALater (Ambion, Austin, TX), and total RNA was purified using the RNAqueous 4-PCR kit (Ambion) and DNase treated. Synthesis of cDNA from 5–10 μl total RNA was conducted using the Retroscript kit (Ambion). For FcRn expression analysis, PCR primers MuFcRn.5F, 5′-CCTCAAGACCCTGGAGAAGATATTAA-3′; MuFcRn.5R, 5′-CCGTGGGCACTGAGGAATTA-3′; and a TaqMan probe MuFcRn.5P, 5′-6FAM-CTGCTGGGCTGTGAACTGGCCTC-TAMRA-3′, were used to amplify and detect a 94-bp cDNA fragment spanning FcRn exons 3 and 4. Reactions (in triplicate) containing the equivalent of 0.5 μl cDNA in the presence of TaqMan PCR Master Mix were run in duplex with the TaqMan 18S Ribosomal RNA Control (Applied Biosystems, Foster City, CA), and the ABI Prism 7700 was used for real-time detection. Mean Ct were calculated; the FcRn expression levels were determined via the ddCt method (Applied Biosystems User Bulletin 2); and expression levels are represented relative to skeletal muscle. The ImmunoQuantArray customized gene expression platform, including the list of genes analyzed and the Global Pattern Recognition (GPR) expression analytical algorithm, has been described[4] (http://www.jax.org/research/roop/). The 5′ RACE of liver cDNA was performed using Ambion 5′ mRNA oligonucleotide adaptors and exon 5 FcRn outer and inner exon 4/5 junction reverse primers. No fragments were detected after 38 amplification cycles using the outer oligonucleotide primers, and only fragments with sizes inconsistent with the expected normal FcRn transcript size were detected with FcRn$^{-/-}$ mice cDNAs after the 38-cycle amplification using the nested primers. These fragments were cloned, sequenced, and proved to be from transcripts unrelated to FcRn.

Specific Ab responses after immunization and ELISPOT

Mice were immunized and challenged with 100 μg DNP-keyhole limpet hemocyanin (KLH) (Calbiochem, San Diego, CA), and sera from retro-orbital plexus blood were assayed for anti-DNP activity, as described (16). For ELISPOT, MultiScreen-HA plates (Millipore, Bedford, MA) were coated with 10 μg/ml of either DNP-KLH, goat anti-mouse IgG, or 100 μg/ml BSA in ELISA coating buffer at 4°C overnight. The plates were then washed three times with PBS and blocked with DMEM medium containing 5% FBS for 1 h at room temperature. Serial dilutions (starting at 1×10^6 cells/well) of spleen and bone marrow cells were added to the wells. The plates were incubated at 37°C with 5% CO_2 overnight and washed three times with PBS-Tween; Goat anti-mouseκ-alkaline phosphatase (0.5 μg/ml; Southern Biotechnology) was then added to each well. After a 1-h incubation at 37°C, the plates were washed three times with PBS-Tween, followed by PBS and chromogen substrate 5-bromo-4-chloro-3-indolyl phosphate/nitro blue tetrazolium (Sigma-Aldrich). The plates were then incubated in the presence of chromogen substrate at room temperature for 1 h, and the reaction was terminated by a water wash. Data are reported as the number of spots enumerated in the Ab-coated wells minus those obtained with the corresponding BSA-coated plates.

Costimulator blockade

B6-FcRn$^{-/-}$ and B6 wt control mice were injected i.p. on day −3 with PBS or 20 μg purified CTLA-4 Ig (27) and 20 μg anti-CD40 ligand (CD40L) IgG1 (28) in PBS (kindly provided by B. Soper, The Jackson Laboratory), grafted 1 day later with allogeneic 129/J tail skin, and injected with 12 μg purified CTLA-4 Ig and 12 μg anti-CD40L IgG1 3 days later. Graft survival was scored according to the method of Bailey and Usama (29), and analyzed by Kaplin-Meier Analysis (StatView; Abacus Concepts, Berkeley, CA).

Results

Production, verification, and overall health of FcRn-deficient mice

To address the role of FcRn in vivo, we used gene targeting to produce mice with a defective *FcRn* gene. Homologous recombination resulted in the insertion of a neomycin-resistance cassette and the deletion of a 1588-nt fragment that encompassed the promotor and extended through most of exon 2 to the *Apa*I site (Fig. 1, *A* and *B*). Quantitative PCR of cDNA from livers indicated that FcRn$^{-/-}$ mice produced a minimal signal (Fig. 1*C*), and extensive 5′ RACE analysis failed to detect any FcRn-specific transcript (Fig. 1*D* and data not shown). Western blot analysis of neonatal intestine extracts probed with an anti-FcRn antiserum failed to reveal any hybridized product (Fig. 1*E*).

Unlike β_2m-deficient mice, which are prone to perinatal lethality (D. Roopenian, unpublished data), FcRn$^{-/-}$ mice showed no evidence of a failure to thrive. Adult FcRn$^{-/-}$ and wt mice were indistinguishable based on weight and overt health. Histological analysis of liver, lungs, kidneys, and lymphoid organs of adult FcRn$^{-/-}$ mice was normal (data not shown).

[4] S. Akilesh, D. J. Shaffer, D. C. Roopenian. Customized molecular phenotyping by quantitative gene expression and pattern recognition analysis. *Submitted for publication.*

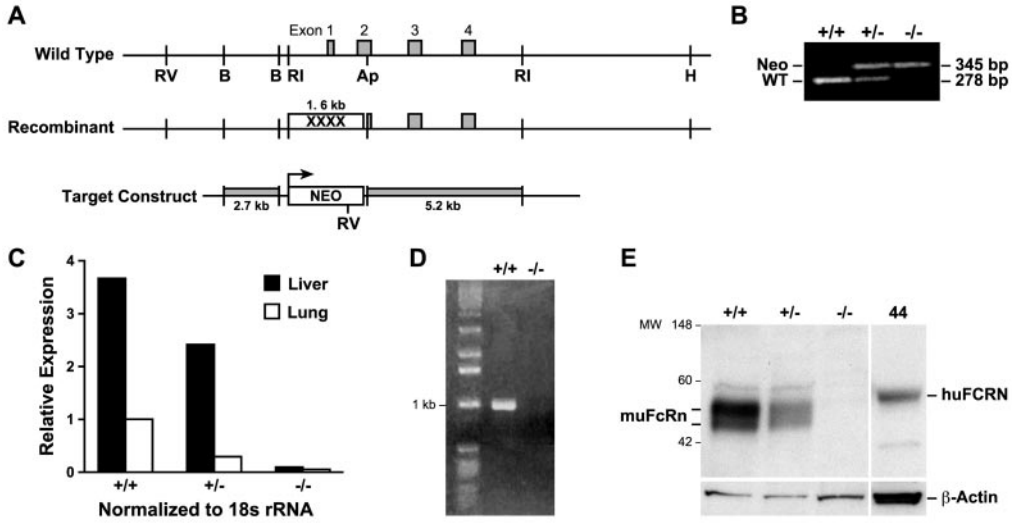

FIGURE 1. Production and verification of FcRn-deficient mice. *A*, FcRn targeting scheme (not to scale). *B*, PCR-based genotypic confirmation of targeted mice. *C*, Quantitative PCR of liver cDNA using PCR primers and a TAQman probe that revealed a cDNA fragment spanning FcRn exons 3 and 4. Values are reported relative to skeletal muscle. *D*, 5′ RACE of liver cDNA using a reverse FcRn primer originating in exon 3 yields the expected 920-bp fragment in wt mice. *E*, Western blot analysis of neonatal intestine extracts from FcRn-deficient and human FcRn transgenic mice revealed using anti-FcRn antisera or anti-β-actin antisera. *Lanes 1–3*, Extracts (25 μg/lane) from intestines of 10-day-old mice. *Lane 4*, Lung extract (25 μg/lane) from human FcRn-transgenic mouse line 44. Molecular weight markers are indicated in kDa.

FcRn is the receptor required for perinatal IgG transport

To determine directly whether FcRn is responsible for perinatal transfer of maternal IgG, a female $FcRn^{+/-}$ mouse was mated to a male $FcRn^{-/-}$ mouse. In this way, the mother would be FcRn sufficient, while her progeny would be either $FcRn^{-/-}$ or wt. To track maternal IgG transmission, the pregnant female was injected i.v. at 17 days of gestation with anti-TNP IgG1. Her pups were then nursed on the same $FcRn^{+/-}$ mother. Her 3-day-old pups were genotyped for FcRn, and their sera were assayed for anti-TNP Ab activity by ELISA. Anti-TNP IgG1 was evident in sera from wt $FcRn^{+/-}$ neonates, but lowered a minimum of 190-fold in the $FcRn^{-/-}$ littermates (Fig. 2*A*). Although human neonates acquire their maternal IgG by transplacental routes, neonatal mice acquire most maternal IgG by transintestinal transport (30). Our results suggest that this FcRn-null allele functionally ablated perinatal IgG transport. However, they do not distinguish between the maternofetal vs transintestinal routes.

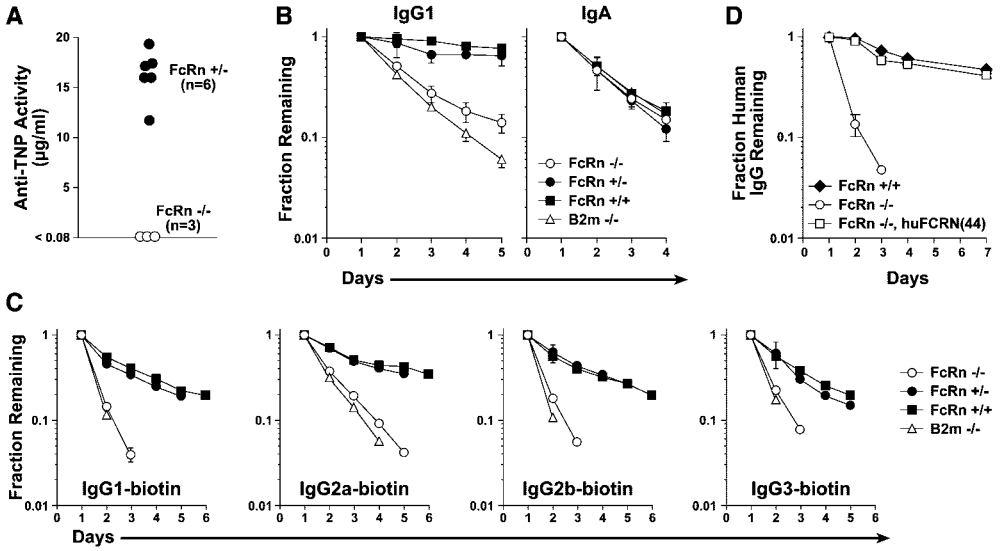

FIGURE 2. FcRn-deficient neonates are unable to absorb maternal IgG, and adult FcRn-deficient mice clear IgG rapidly. *A*, Anti-TNP IgG serum concentrations of 3-day-old pups born from an $FcRn^{+/-}$ mother who had been injected i.v. with IgG1 anti-TNP tracer Abs on day 17 of gestation. $-/-$ vs $+/-$, $p < 0.00001$ computed using the one-sided Student's *t* test. *B*, Mouse anti-TNP IgG1 mAb and IgA anti-TNP Abs were injected i.p. into groups of four to five 6- to 7-wk-old mice, and the concentration of retained IgG tracer from serially sampled sera was determined. Data are representative of four independent experiments and are shown as the mean $\pm$ SEM of the anti-TNP normalized to the day 1 values. In some cases, the SEM was smaller than the plot symbols. Values of *p* (two-sided *t* test) for $FcRn^{+/-}$ vs $FcRn^{-/-}$ on days 2–5, $p < 0.0001$; $\beta_2m^{-/-}$ vs $FcRn^{-/-}$, $p < 0.005$, day 5. *C*, $FcRn^{-/-}$ mice, $FcRn^{+/-}$ littermate controls (two to three per group) were injected i.p. with 25 μg directly biotinylated mouse IgG1, IgG2a, IgG2b, or IgG3. *D*, $FcRn^{-/-}$ human FcRn transgenic line 44, $FcRn^{+/+}$, and $FcRn^{-/-}$ mice were injected with i.p. purified human IgG tracer. Two to three mice/group were, and the data are representative of, three independent experiments. All mice except $\beta_2m^{-/-}$ cohorts were a mixed 129:B6 genetic background.

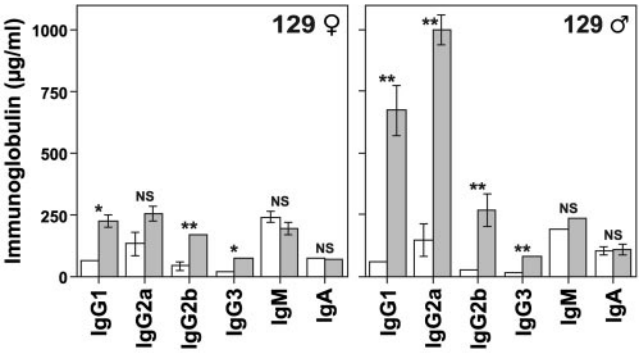

FIGURE 3. FcRn-deficient mice have low serum IgG. Sera from 10-wk-old 129/J FcRn$^{-/-}$ ($n = 5$) and FcRn$^{+/+}$ ($n = 5$) female and male mice were analyzed by ELISA for specific Ig isotypes. *, $p < 0.01$; **, $p < 0.001$; NS, $p > 0.05$. Values shown are the mean ± SEM.

FcRn is responsible for extending the serum IgG $t_{1/2}$ *and for maintaining normal IgG levels*

To determine whether FcRn is responsible for the extended $t_{1/2}$ of IgG, adult FcRn$^{-/-}$ and FcRn$^{-/+}$, as well as β_2m-deficient mice, were injected with tracer anti-TNP IgG1 and IgA Abs, and serial blood samples were tested for the amount of tracer remaining. In FcRn wt mice, the $t_{1/2}$ of the IgG1 tracer (9 days) was predictably greater in comparison with the IgA tracer (1.4 days) (Fig. 2*B*). In contrast, the IgG1 $t_{1/2}$ was considerably shortened in both FcRn$^{-/-}$ (1.4 days) and β_2m$^{-/-}$ mice (1.0 days). The small difference in FcRn$^{-/-}$ vs β_2m$^{-/-}$ $t_{1/2}$ was significant ($p = 0.004$ at day 5), and was observed in multiple experiments. FcRn protected all mouse IgG isotypes, because FcRn$^{-/-}$ and β_2m$^{-/-}$ mice demonstrated rapid clearance of biotinylated mouse IgG1, IgG2a, IgG2b, and IgG3 in comparison with wt mice (Fig. 2*C*). Moreover, human IgG was cleared rapidly in FcRn$^{-/-}$ mice, but it was protected in FcRn$^{-/-}$ mice onto which a human FcRn transgene had been crossed (Fig. 2*D*). These results indicated that both mouse and human FcRn play an important role in extending the $t_{1/2}$ of IgG.

To determine the effects of FcRn deficiency on maintaining homeostatic Ab levels, serum Ig concentrations were determined in FcRn$^{-/-}$ and wt mice on an isogenic 129/J background. FcRn$^{-/-}$ mice had reduced concentrations of all IgG isotypes compared with their wt sex-matched counterparts, but IgM and IgA concentrations did not differ significantly from wt controls (Fig. 3). These results indicated that FcRn plays an important role in IgG homeostasis.

An FcRn deficiency does not alter transcriptional and cellular immunological profiles

To determine whether an FcRn deficiency affects the normal immunological status, FcRn-deficient and wt mice were subjected to a battery of phenotypic tests. We first tested whether an FcRn deficiency affects the transcriptional profile of immunologically important genes. RNA samples isolated from spleens and lungs of three replicate 129 mice isogenic for FcRn$^{-/-}$ and three 129-FcRn wt controls were analyzed using an ImmunoQuantArray, a quantitative PCR-based method that allows one to measure simultaneously the expression of 95 genes selected because they play significant roles in immune homeostasis and function.[4] Of the genes surveyed, only FcRn showed a significant difference by the global pattern recognition analytical algorithm (Table I). Thus, FcRn deficiency leads to negligible alterations of the transcriptional immunological phenotype.

Table I. *Transcription profiles*[a]

Tissue	Gene	GPR Score[a]	Up/Down vs wt	*p* Value[b]
Spleen	*FcRn*	0.71	Down	$p < 0.004$
	Remaining 94 genes	<0.07	NA	NA
Lung	*FcRn*	1.0	Down	$p < 0.004$
	Remaining 94 genes	<0.07	NA	NA

[a] Scores of the 95 gene ImmunoQuantArray analyzed by GPR software based on RNA from three 129-FcRn$^{-/-}$ and three 129-FcRn wt control mice. A GPR score of ≥0.4 (indicating that the gene has changed compared with 40% of the 95 genes analyzed) is regarded as highly significant (see text footnote 4) (http://www.jax.org/research/roop/).
[b] Significance by Bootstrap Analysis. NA, The remaining 94 genes did not achieve a sufficient GPR score to warrant Bootstrap Analysis.

Splenic total leukocyte numbers and leukocyte subsets, including plasma cell numbers, of spleens and bone marrow cells of FcRn$^{-/-}$ and wt mice were indistinguishable by multiparameter flow cytometric analysis (Table II and data not shown). ELISPOT analysis of spleen and bone marrow cells failed to suggest an increase in total or anti-TNP-specific IgG-secreting plasma cells after immunization (Table III). Spleen and lymph node cells from FcRn$^{-/-}$ mice showed normal in vitro proliferative responses to the T cell mitogens, Con A, and anti-CD3 mAbs. Recall proliferative responses to OVA and hen egg lysozyme were indistinguishable from their wt controls (data not shown). Similarly, FcRn$^{-/-}$ and wt spleen cells showed superimposable B cell proliferative responses to LPS and anti-IgM + anti-CD40 Abs (data not shown). Thus, despite lowered homeostatic serum IgG levels, FcRn deficiency does not lead to aberrations in the transcriptional status, cellular composition, or in vitro function of peripheral lymphoid cells.

FcRn is required for a robust IgG Ab response

To determine the consequences of an FcRn deficiency on a specific humoral immune response, FcRn$^{-/-}$ and wt C57BL/6 (B6) and 129 background mice were immunized and then challenged 3 wk later with DNP-KLH, and their serum anti-DNP-specific Ig titers were determined. FcRn deficiency on B6 background mice reduced substantially the specific anti-DNP response regardless of IgG Ig isotype (Fig. 4*A*). This decrease was not a consequence of numbers of plasma cells because TNP-KLH-immunized FcRn$^{-/-}$ and wt mice showed similar

Table II. *Flow cytometric analysis*[a]

Strain (no. of mice)	Tissue	CD4 T Cells	CD8 T Cells	B Cells	Plasma Cells
B6-FcRn wt (5)	Spleen	14.3 ± 0.7	10.1 ± 0.6	54.8 ± 1.6	0.5 ± 0.0
	Bone marrow	ND	ND	17.3 ± 0.5	0.27 ± 0.0
B6-FcRn$^{-/-}$ (4)	Spleen	13.9 ± 1.2	9 ± 0.6	59.1 ± 1.6	0.4 ± 0.1
	Bone marrow	ND	ND	16.4 ± 0.4	0.24 ± 0.0
129-FcRn wt (3)	Spleen	24.1 ± 1.3	7.7 ± 1.2	57.0 ± 0.8	ND
129-FcRn$^{-/-}$ (3)	Spleen	22.7 ± 1.5	8.1 ± 0.7	59.1 ± 1.2	ND

[a] CD4 T cells (CD4$^-$, B220$^-$, CD8$^+$); CD8 T cells (CD4$^-$, B220$^-$, CD8$^+$); B cells (CD19$^+$, B220$^+$); plasma cells (B220$^-$, CD138$^+$). ND, Not done. Mean % ± SEM is shown.

Table III. *ELISPOT analysis after TNP-KLH immunization*[a]

Strain (no. mice)	Tissue	IgG/10^6 Cells	Anti-TNP/10^6 Cells
B6-FcRn wt (3)	Spleen	875 ± 540	334 ± 104
	Bone marrow	234 ± 45	73 ± 9
B6-FcRn$^{-/-}$ (3)	Spleen	1,694 ± 259	359 ± 29
	Bone marrow	354 ± 44	134 ± 37

[a] Spleen and bone marrow cells from mice immunized with TNP-KLH + CFA, challenged on days 0 and 14 with TNP-KLH + CFA, and harvested on day 22. Mean values ± SEM are shown.

numbers of anti-IgG- and anti-TNP-secreting cells by ELISPOT analysis (Table III). On the 129/J background mice, the anti-TNP response was heightened overall, and an FcRn deficiency resulted in a reduction of all anti-DNP IgG isotypes, but it was more episodic compared with B6 background mice (Fig. 4*B*). Moreover, following immunization, the titers of anti-TNP IgG Abs in 129-FcRn wt mice dropped at an unexpectedly rapid rate given functional FcRn-mediated protection. A more sustained lowering in FcRn$^{-/-}$ mice was observed in total IgG concentrations (Fig. 4*C*, *lower right panel*). FcRn protection is known to be saturable at high serum IgG concentrations (half-maximal concentration ~5 mg/ml) (31). Immunization of 129 background mice may have induced sufficient hypergammaglobulinemia to exceed the FcRn saturation capacity, thus resulting in an attenuation of FcRn-mediated protection. Taken together, the data suggest that FcRn plays an important role in controlling the concentrations of serum IgG Abs in response to specific antigenic stimuli provided that the concentrations do not exceed the FcRn saturation threshold.

FcRn is required to achieve efficacy of IgG-Fc-conjugated therapeutics

It is well established that the covalent addition of an IgG-derived Fc moiety prolongs drug $t_{1/2}$ (3). However, whether this extension is solely dependent on FcRn as compared with other FcR is less clear. To determine directly whether FcRn is the molecule responsible for enhancing the pharmacological efficacy of IgG-coupled therapeutics, we addressed whether mice lacking FcRn were refractory to the immunosuppressive effects of a costimulatory blockade including CTLA-4 Ig and anti-CD40L (both of which possess IgG Fc moieties). B6-FcRn$^{-/-}$ and wt controls were treated with a cocktail of CTLA-4 Ig and anti-CD40L IgG1, and grafted with allogeneic 129/J tail skin (Fig. 5). The rate of allograft rejection by FcRn-deficient mice was retarded considerably compared with that of FcRn wt controls, and similar to that observed in mice that received PBS rather than the costimulatory blockade.

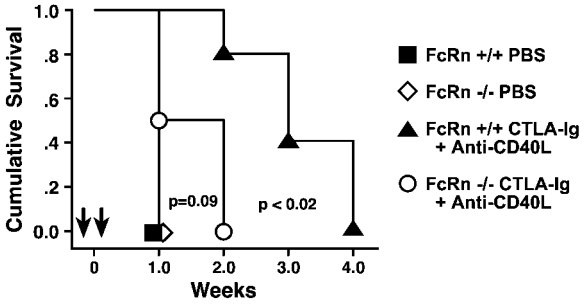

FIGURE 5. FcRn is required for efficacy of IgG-Fc-conjugated therapeutics. Groups of four or five mice were injected i.p. on days −1 and +3 with CTLA-4 Ig and rat anti-CD40L IgG1, and grafted on day 0 with allogeneic 129/J tail skin. Syngeneic allografts were retained throughout the course of the experiments (not shown).

These results indicated that FcRn is the FcR responsible for the enhanced pharmacological efficacy of IgG-Fc-conjugated drugs.

Discussion

The results reported in this work provide direct evidence that FcRn is Brambell's hypothesized receptor responsible for both perinatal IgG transport and IgG homeostatic functions. FcRn-deficient mice recapitulated the defective neonatal transport phenotype originally reported in β_2m-deficient mice (15). Moreover, FcRn-deficient mice very nearly matched the IgG homeostasis phenotype first observed in β_2m-deficient mice (16–18, 32). However, in multiple experiments, these mice do show a slightly increased IgG serum $t_{1/2}$ compared with β_2m-deficient mice. We were unable to detect any FcRn transcription by 5′ RACE or translated product by Western blot analysis (Fig. 1*E*), arguing against a leaky mutation. It is thus possible that other β_2m-dependent proteins act weakly to stabilize IgG. Nevertheless, the results prove unequivocally that FcRn is a critical receptor for both perinatal IgG transport and for extending the $t_{1/2}$ of IgG Abs in adults.

Until now, the only in vivo model to assess the function of FcRn was using mice deficient in the β_2m common L chain. It has therefore been difficult to dissociate the biology imposed by FcRn from that of many other β_2m-dependent class I molecules. The FcRn H chain-deficient mice reported in this work make it possible to understand the role of FcRn in vivo. The absence of FcRn did not lead to significant aberrations in the induction of T or B cell responses. However, it diminished the ability to retain normal concentrations of serum IgG. Moreover, the serum concentrations of IgG Abs available after immunization were considerably diminished, while the number of IgG Ab-secreting plasma cells was unchanged. Although we cannot formally exclude the possibility

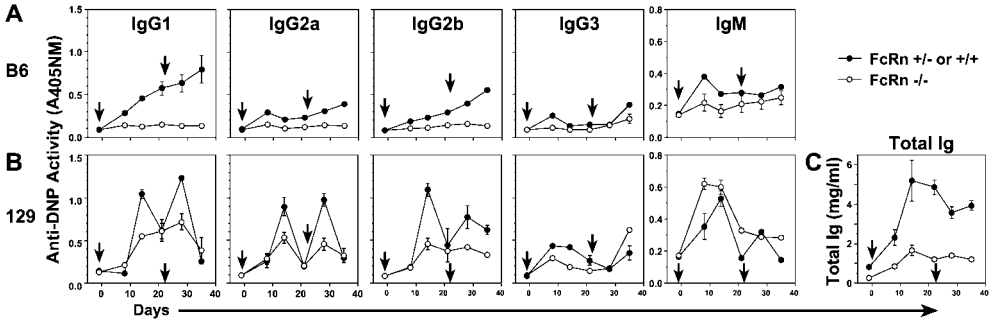

FIGURE 4. FcRn is required for a robust humoral response. Groups of four to five B6 background (*A*) and 129 background (*B*) FcRn$^{-/-}$ and wt FcRn$^{+/-}$ or FcRn$^{+/+}$ mice were immunized i.p. with DNP-KLH in CFA and challenged 21 days later with DNP-KLH in IFA. Serially sampled sera were analyzed for DNP-specific (*A*, *B*) and total Ig (*C*, 129 only) by ELISA. Values shown are the mean ± SEM.

that an FcRn deficiency decreases the amount of IgG secreted by each plasma cell, it is far more likely that the diminished IgG levels are a consequence of excessive IgG clearance. In any case, FcRn plays a pivotal role in maximizing the IgG Abs available for humoral effector mechanisms of immunity. Thus, situations in which β_2m-deficient mice are unable to clear viruses efficiently (reviewed in Ref. 12) and are less able to engender protective immunity after immunization (33) could at least in part be explained by their concurrent deficiency in FcRn-mediated protection. A similar explanation can be extended for findings that a β_2m deficiency ameliorates humoral pathology associated with systemic lupus erythematosus-like autoimmune diseases and bullous pemphigoid (16, 22, 23, 26, 34, 35). Thus, therapeutics designed to specifically block FcRn could prevent Ab-mediated effector mechanisms normally leading to autoimmune disorders.

This ability of FcRn to conserve IgG has important implications for pharmacological design. Although it is well established that therapeutic IgG Abs and proteins designed to include the Fc region of IgG show improved serum persistence, our studies are the first to show the critical involvement of FcRn to achieve therapeutic efficacy of such molecules. Previous work has shown that the serum persistence of mouse IgG molecules can be shortened or extended by altering hinge region amino acids thought to affect FcRn interaction sites, suggesting that engineering to enhance the FcRn-Fc interaction could lead to more efficacious drugs (reviewed in Ref. 36). Because mouse and human FcRn show differing isotype and species affinity (37) (D. Roopenian, unpublished data), mice deficient in mouse FcRn and transgenic for human FcRn are especially suited for such therapeutic development.

FcRn is a remarkable example of the functional plasticity of class I family proteins. Through minimal structural changes, FcRn has acquired a specialized Fc-binding function (38), resulting in IgG transport and stabilization. The role of IgG Abs in infectious and autoimmune diseases is unresolved. Models in which B cells are depleted, ablated, or developmentally compromised do not directly address this issue because B cells are important for cognate Ag presentation functions as well as acting as precursors for plasma cells. The discrete IgG-lowering phenotype of FcRn-deficient mice should be useful in addressing this central issue.

Note added in proof. We have recently found that FcRn extends the $T_{1/2}$ of albumin (39).

Acknowledgments

We thank Alexander Chervonsky and Elizabeth Simpson for critical review, Lori Goodwin and Theodore Duffy for excellent technical assistance, and Robin Wesselschmidt from Genome Systems for assistance in gene targeting.

References

1. Vieira, P., and K. Rajewsky. 1988. The half-lives of serum immunoglobulins in adult mice. *Eur. J. Immunol. 18:313.*
2. Spiegelberg, H. L., and B. G. Fishkin. 1972. The catabolism of human G immunoglobulins of different heavy chain subclasses. III. The catabolism of heavy chain disease proteins and of Fc fragments of myeloma proteins. *Clin. Exp. Immunol. 10:599.*
3. Ghetie, V., and E. S. Ward. 2000. Multiple roles for the major histocompatibility complex class I-related receptor FcRn. *Annu. Rev. Immunol. 18:739.*
4. Brambell, F. W. 1966. The transmission of immunity from mother to young and the catabolism of immunoglobulins. *Lancet 2:1087.*
5. Ahouse, J. J., C. L. Hagerman, P. Mittal, D. J. Gilbert, N. G. Copeland, N. A. Jenkins, and N. E. Simister. 1993. Mouse MHC class I-like Fc receptor encoded outside the MHC. *J. Immunol. 151:6076.*
6. Simister, N. E., and K. E. Mostov. 1989. An Fc receptor structurally related to MHC class I antigens. *Nature 337:184.*
7. Raghavan, M., L. N. Gastinel, and P. J. Bjorkman. 1993. The class I major histocompatibility complex related Fc receptor shows pH-dependent stability differences correlating with immunoglobulin binding and release. *Biochemistry 32:8654.*
8. Borvak, J., J. Richardson, C. Medesan, F. Antohe, C. Radu, M. Simionescu, V. Ghetie, and E. S. Ward. 1998. Functional expression of the MHC class I-related receptor, FcRn, in endothelial cells of mice. *Int. Immunol. 10:1289.*
9. McCarthy, K. M., Y. Yoong, and N. E. Simister. 2000. Bidirectional transcytosis of IgG by the rat neonatal Fc receptor expressed in a rat kidney cell line: a system to study protein transport across epithelia. *J. Cell Sci. 113:1277.*
10. Praetor, A., I. Ellinger, and W. Hunziker. 1999. Intracellular traffic of the MHC class I-like IgG Fc receptor, FcRn, expressed in epithelial MDCK cells. *J. Cell Sci. 112:2291.*
11. Ramalingam, T. S., S. A. Detmer, W. L. Martin, and P. J. Bjorkman. 2002. IgG transcytosis and recycling by FcRn expressed in MDCK cells reveals ligand-induced redistribution. *EMBO J. 21:590.*
12. Raulet, D. H. 1994. MHC class 1-deficient mice. *Adv. Immunol. 55:381.*
13. Santos, M., M. W. Schilham, L. H. Rademakers, J. J. Marx, M. de Sousa, and H. Clevers. 1996. Defective iron homeostasis in β_2-microglobulin knockout mice recapitulates hereditary hemochromatosis in man. *J. Exp. Med. 184:1975.*
14. Huh, G. S., L. M. Boulanger, H. Du, P. A. Riquelme, T. M. Brotz, and C. J. Shatz. 2000. Functional requirement for class I MHC in CNS development and plasticity. *Science 290:2155.*
15. Israel, E. J., V. K. Patel, S. F. Taylor, A. Marshak-Rothstein, and N. E. Simister. 1995. Requirement for a β_2-microglobulin-associated Fc receptor for acquisition of maternal IgG by fetal and neonatal mice. *J. Immunol. 154:6246.*
16. Christianson, G. J., W. Brooks, S. Vekasi, E. A. Manolfi, J. Niles, S. L. Roopenian, J. B. Roths, R. Rothlein, and D. C. Roopenian. 1997. β_2-microglobulin-deficient mice are protected from hypergammaglobulinemia and have defective antibody responses because of increased IgG catabolism. *J. Immunol. 159:4781.*
17. Ghetie, V., J. G. Hubbard, J. K. Kim, M. F. Tsen, Y. Lee, and E. S. Ward. 1996. Abnormally short serum half-lives of IgG in β_2-microglobulin-deficient mice. *Eur. J. Immunol. 26:690.*
18. Junghans, R. P., and C. L. Anderson. 1996. The protection receptor for IgG catabolism is the β_2-microglobulin-containing neonatal intestinal transport receptor. *Proc. Natl. Acad. Sci. USA 93:5512.*
19. Spriggs, M. K., B. H. Koller, T. Sato, P. J. Morrissey, W. C. Fanslow, O. Smithies, R. F. Voice, and C. R. Maliszewski. 1992. β_2-microglobulin-, $CD8^+$ T-cell-deficient mice survive inoculation with high doses of vaccinia virus and exhibit altered IgG responses. *Proc. Natl. Acad. Sci. USA 89:6070.*
20. Lehmann-Grube, F., J. Lohler, O. Utermohlen, and C. Gegin. 1993. Antiviral immune responses of lymphocytic choriomeningitis virus-infected mice lacking $CD8^+$ T lymphocytes because of disruption of the β_2-microglobulin gene. *J. Virol. 67:332.*
21. Noble, A., Z. S. Zhao, and H. Cantor. 1998. Suppression of immune responses by CD8 cells. II. Qa-1 on activated B cells stimulates CD8 cell suppression of T helper 2 responses. *J. Immunol. 160:566.*
22. Christianson, G. J., R. L. Blankenberg, T. M. Duffy, D. Panka, A. M. Marshak-Rothstein, J. B. Roths, and D. C. Roopenian. 1996. β_2 microglobulin dependence of lupus-like autoimmune syndrome of MRL-*lpr* mice. *J. Immunol. 176:4933.*
23. Maldonado, M. A., R. A. Eisenberg, E. Roper, P. L. Cohen, and B. L. Kotzin. 1995. Greatly reduced lymphoproliferation in *lpr* mice laking major histocompatibility complex class I. *J. Exp. Med. 181:641.*
24. Ohtelki, T., M. Iwamoto, S. Izui, and H. R. MacDonald. 1995. Reduced development of $CD4^-8^-B220^+$ T cells but normal autoantibody production in mice lacking major histocompatibility complex class I molecules. *Eur. J. Immunol. 25:37.*
25. Shenoy, M., R. Kaul, E. Goluszko, C. David, and P. Christadoss. 1994. Effect of MHC class I and CD8 cell deficiency on experimental autoimmune myasthenia gravis pathogenesis. *J. Immunol. 153:5330.*
26. Chan, O. T., V. Paliwal, J. M. McNiff, S. H. Park, A. Bendelac, and M. J. Shlomchik. 2001. Deficiency in β_2-microglobulin, but not CD1, accelerates spontaneous lupus skin disease while inhibiting nephritis in MRL-Fas(*lpr*) mice: an example of disease regulation at the organ level. *J. Immunol. 167:2985.*
27. Linsley, P. S., W. Brady, M. Urnes, L. S. Grosmaire, N. K. Damle, and J. A. Ledbetter. 1991. CTLA-4 is a second receptor for the B cell activation antigen B7. *J. Exp. Med. 174:561.*
28. Larsen, C. P., D. Z. Alexander, D. Hollenbaugh, E. T. Elwood, S. C. Ritchie, A. Aruffo, R. Hendrix, and T. C. Pearson. 1996. CD40-gp39 interactions play a critical role during allograft rejection: suppression of allograft rejection by blockade of the CD40-gp39 pathway. *Transplantation 61:4.*
29. Bailey, D. W., and B. Usama. 1960. A rapid method of grafting skin on tails of mice. *Transpl. Bull. 7:424.*
30. Appleby, P., and D. Catty. 1983. Transmission of immunoglobulin to fetal and neonatal mice. *J. Reprod. Immunol. 5:203.*
31. Junghans, R. P. 1997. IgG biosynthesis: no "immunoregulatory feedback." *Blood 90:3815.*
32. Israel, E. J., D. F. Wilsker, K. C. Hayes, D. Schoenfeld, and N. E. Simister. 1996. Increased clearance of IgG in mice that lack β_2-microglobulin: possible protective role of FcRn. *Immunology 89:573.*
33. Ghiasi, H., D. C. Roopenian, S. Slanina, S. Cai, A. B. Nesburn, and S. L. Wechsler. 1997. The importance of MHC-I and MHC-II responses in vaccine efficacy against lethal herpes simplex virus type 1 challenge. *Immunology 91:430.*
34. Mixter, P. F., J. Q. Russell, F. H. Durie, and R. C. Budd. 1995. Decreased $CD4^-CD8^-$ $\alpha\beta$ TCR^+ cells in *lpr/lpr* mice lacking β_2-microglobulin. *J. Immunol. 154:2063.*
35. Liu, Z., D. C. Roopenian, X. Zhou, G. J. Christianson, L. A. Diaz, D. D. Sedmak, and C. L. Anderson. 1997. β_2-microglobulin-deficient mice are resistant to bullous pemphigoid. *J. Exp. Med. 186:777.*
36. Ghetie, V., and E. S. Ward. 1997. FcRn: the MHC class I-related receptor that is more than an IgG transporter. *Immunol. Today 18:592.*
37. Ober, R. J., C. G. Radu, V. Ghetie, and E. S. Ward. 2001. Differences in promiscuity for antibody-FcRn interactions across species: implications for therapeutic antibodies. *Int. Immunol. 13:1551.*
38. Raghavan, M., and P. J. Bjorkman. 1996. Fc receptors and their interactions with immunoglobulins. *Annu. Rev. Cell. Dev. Biol. 12:181.*
39. Chadbury, C., S. Mehanz, J. M. Robinson, W. L. Hayton D. K. Pearl, D. C. Roopenian, and C. L. Anderson. The MHC-related Fc receptor (FcRn) binds albumin and extends its life span. *J. Exp. Med. In press.*