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Abnormally short serum half-lives of IgG in β2-microglobulin-deficient mice

The MHC class I-related receptor, FcRn, mediates the transfer of maternal gamma globulin (IgG) to young rodents, primarily via intestinal transcytosis, and this provides humoral immunity for the first few weeks after birth. In a previous study, the site of mouse IgG1 (mIgG1) with which FcRn interacts has been mapped using recombinant wild-type and mutated Fc-hinge fragments. The site encompasses residues at the CH2-CH3 domain interface of Fc (Ile253, His310, Gln311, His433 and Asn434) and the same amino acids are involved in regulating the pharmacokinetics of the Fc-hinge fragments. This suggests that in addition to its known function, FcRn might also play a role in IgG homeostasis. Consistent with this hypothesis, in this study, we demonstrate that FcRn α-chain mRNA is present not only in neonatal brush border but also in other tissues of adult animals (liver, lung, spleen and endothelial cells). In addition, analysis of the pharmacokinetics of mouse Ig/Fc-hinge fragments in genetically manipulated mice that are deficient in the expression of FcRn demonstrates that the β-phase half-lives are abnormally short. These findings suggest that FcRn is involved in IgG homeostasis.

1 Introduction

Despite the central role that gamma globulins (IgG) plays in immunity, little is known about the molecular mechanisms and dynamics by which remarkably constant IgG levels are maintained in the serum. Understanding the molecular processes responsible for IgG homeostasis is of relevance to the treatment of IgG deficiencies and the effective delivery of therapeutic antibodies. Site-directed mutagenesis has been used to map the amino acid residues of mouse IgG1 (mIgG1) that regulate IgG catabolism [1]. These Fc residues (Ile253, His310, Gln311, His433 and Asn434; EU numbering [2]) are located at the CH2-CH3 domain interface and are highly conserved in both human and rodent IgG [2]. Functional studies in neonatal mice indicate that the same amino acids are also involved in binding to the MHC class I homolog FcRn [3], and this is consistent with the X-ray crystallographic structure of a rat FcRn: Fc complex [4]. Rodent FcRn has been implicated in passive transfer of IgG from mother to young primarily via neonatal transcytosis [5, 6], and comprises a 45–50-kDa α-chain associated with β2-microglobulin (β2m; [7]). The effects of mutation of Ile253, His310, Gln311, His433 and Asn434 on the β-phase biological half-life of recombinant Fc-hinge fragments and on neonatal transcytosis correlate closely [3]. This suggests that FcRn, or a closely related protein, might be the as-yet unidentified Fc receptor that was originally suggested by Brambell and colleagues to be involved in regulating serum IgG levels [8]. Such Fc receptors were proposed to maintain IgG homeostasis by binding and releasing IgG back into the circulation and when IgG reaches saturating concentrations for the receptors, excess IgG is destined for degradation [8].

The catabolism of IgG is a diffuse process occurring not only in specific organs such as liver [9] and intestine [10], but also in tissues containing reticuloendothelial components such as spleen, skin and muscle [11]. Paradoxically, these cells may also bear the putative Fc receptors that recycle IgG [8]. The ubiquitous expression of rat FcRn [12] and a human FcRn homolog [13] outside the cells involved in materno-fetal/neonatal transfer of IgG would be consistent with a role in controlling IgG levels at sites throughout the body.

In the current study, the expression of FcRn in mouse tissues and cell lines has been analyzed using reverse transcriptase (RT)-PCR. FcRn α-chain mRNA is ubiquitously distributed in adult tissues, particularly those that are rich in endothelial cells. The pharmacokinetics of Fc-hinge fragments in genetically manipulated mice that lack FcRn expression [14, 15] due to disruption of the β2m gene (β2m−/− mice) have also been analyzed. The data support the involvement of FcRn in regulating IgG catabolism.

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Abbreviations: **β2m:** β2-microglobulin **CHAPS:** 3-[(3-cholamidopropyl)-dimethylammonio]-1-propane sulfonate **RT-PCR:** Reverse transcriptase-polymerase chain reaction **SVEC:** SV40-transformed endothelial cells **SR:** Synthesis rate **WT:** Wild-type

Key words: IgG catabolism / FcRn / β2-microglobulin-deficient mice / Recombinant Fc-hinge fragment

2 Materials and methods

2.1 Cell lines

The mouse B cell line BCL_1/3B3 [16] and T cell hybridoma 2B4 [17] used in this study were generous gifts of Profs. E. Vitetta and M. Davis, respectively. The two endothelial cell lines, mouse pulmonary capillary endothelial cells (B10, D2.PCE) and SV40-transformed endothelial cells [18] (SVEC) were derived from lungs of B10.-DBA/2 mice and C3H/HeJ mice, respectively. The B10, D2.PCE cell

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line was a generous gift of Prof. A. Curtis. The murine hepatoma line Hepa 1–6 was obtained from the ATCC (Bethesda, MD; 1830-CRL).

2.2 Mice

β2m−/− ([15]; C57BL/6 × 129/Ola and C57BL/6 background; for the mixed background, 129/Ola mice have been backcrossed at least two-three times onto a C57BL/6 background and the colony maintained by littermate crosses) and β2m+/+ [(C57BL/6 × 129/Ola)F2] mice were from Jackson laboratories (Bar Harbor, ME). β2m+/+ (C57BL/6) mice were from the Southwestern Medical Center Animal Resources Center.

2.3 Reverse transcriptase (RT)-PCR analysis

Lung, liver, hepatocytes, spleen and yolk sac were isolated from adult BALB/c mice and neonatal brush border from 12-day-old BALB/c mice. For the isolation of hepatocytes, a method described previously for the isolation of rat hepatocytes was used [19]. This resulted in a population of cells that was greater than 95% hepatocytes. RNA was extracted from tissue/cell lines and cDNA synthesis primed with primer B (Fig. 1; complementary to bases 1075–1095 of the coding strand of the FcRn α-chain [20] with 13 bases appended to add a restriction site) using previously described methods [1]. Aliquots of the cDNA syntheses were used in the PCR with either primers A and B (Fig. 1) or B and C (Fig. 2). Primer A and C match bases 640–656 and 964–996 of the coding strand of the FcRn α-chain gene [20], respectively. Primer C has 12 bases appended to add a restriction site. The expected sizes of the RT-PCR products are 469 bp (primers A and B) and 157 bp (B and C).

As controls for the RT-PCR shown in Fig. 1, β-actin primers (bases 352–368 of the mouse β-actin coding sequence and complementary to bases 781–787 of the β-actin coding sequence) were used in cDNA synthesis and PCR. Southern blotting [21] was carried out using a ^{32}P-labeled *Sac* I-*Bst* EII fragment derived from the cloned FcRn α-chain (bases 688–1028) as probe.

2.4 Isolation and nucleotide sequencing of the complete FcRn α-chain gene from endothelial cells

The gene encoding the entire FcRn α-chain was isolated as two overlapping clones. Total RNA was isolated from endothelial cell lines and the gene segment encoding the extracellular domains (including leader peptide) was isolated by RT-PCR using primers that matched bases 1–24 and were complementary to bases 841–870 of the coding sequence of FcRn [20]. This gene, derived from D10, D2.PCE cells, has been used to construct a plasmid for the expression of soluble FcRn in insect cells [22]. The segment encoding bases 640–1095 (including the transmembrane region and cytoplasmic tail) was isolated using RT-PCR and primers A and B (see Sect. 2.3). PCR products were cloned into pGEM-T (Promega, Madison, WI) and then recloned as *Sph* I-*Sal* I fragments in both orientations into pUC118/pUC119. ssDNA was isolated from resulting clones and sequenced using the dideoxynucleotide method [23] and Sequenase (US Biochemicals, Cleveland, OH). For each fragment, several independent PCR isolates were analyzed.

2.5 Quantitative PCR

Essentially, the methodology of Scheuermann and Bauer was used [24]. A gene segment encoding the C-terminal region of FcRn plus the 3′-untranslated region [20] was isolated using the PCR and the primers 5′ TCT GGC TCC TCC GTG CT 3′ (matches bases 640–656 of FcRn coding sequence) and 5′ ATC ATC TAG A*TT TTT TTG TTG GGG CCA AAT TTA TG* 3′ (*Xba* I site is underlined, and the sequence complementary to the poly(A)$^+$ tail and the upstream region is italicized). The PCR product was then restricted with *Xba* I (encoded in the primer) and *Nco* I (internal sites in FcRn coding sequence and FcRn untranslated 3′ tail) to generate an *Nco* I fragment (bases 992–1199) and an *Nco* I-*Xba* I fragment (bases 1200–1285). These two fragments were used with a pUC119 derivative containing sequences encoding bases 640–1095 (Sect. 2.4) to assemble a gene encoding bases 640–1095 of the FcRn coding sequence plus 3′ untranslated region, *i.e.* bases 640–1285. This construct was restricted at a unique *Bst* EII site (bases 1021–1027 of FcRn) and a 100-bp filler fragment inserted prior to recloning into pSP64 (Promega). Poly(A)$^+$ RNA was synthesized using the Riboprobe system II (Promega) and poly(A)$^+$ RNA purified using oligo(dT) cellulose. This RNA (FcRn poly A) was used as an internal standard in the quantitative PCR.

Total RNA was extracted from cell lines as described [1] or using RNeasy total RNA kits from Qiagen (Chatsworth, CA). cDNA synthesis reactions were carried out using 0.8–2.2 μg RNA (kept constant for each cell line) plus 10^5–10^8 FcRn poly(A) molecules (varied within this range) and the poly(A)$^+$ primer described above. Aliquots of the cDNA syntheses were used in duplicate PCR containing the following primers: 5′ ATC ACC ATG GCC GGT *AGG ATG CGC AGC GGT CTG CCA GCC* 3′ (italicized bases match bases 967–990 of the FcRn coding sequence) and ^{32}P-labeled 5′ ATC AGT CGA CCT T*GG AAG TGG GTG GAA AGG CAT T* 3′ (italicized bases are complementary to bases 1075–1095 of FcRn). One-tenth of each PCR was analyzed on 4% agarose gels, and bands corresponding to the PCR products were excised and cpm determined by gamma counting (products derived from FcRn poly(A) could be distinguished from those derived from authentic FcRn transcripts due to the 100-bp size difference). Incorporated cpm for standard and test samples were plotted against the amount of standard added and the point of intersection taken to correspond to equality of amounts of FcRn transcripts and FcRn poly(A) standard. As it was impossible to count single brush-border cells, quantitation was expressed as number of transcripts per μg total RNA.

2.6 Radiolabeling of immunoglobulins

mIgG1, recombinant Fc-hinge fragments and IgA were iodinated using Iodo-Gen (Pierce, Rockford, IL) as described [1].

2.7 Binding studies of recombinant Fc-hinge fragments

Confluent layers of SVEC cells were incubated with 0.26 or 0.4 μg/ml ^{125}I-labeled wild-type Fc-hinge or HQ-310/HN-433 mutant overnight (16–18 h) at 37 °C, washed with medium (complete RPMI + 10% FCS; Gibco, Grand Island, NY) and detached by incubation with 5 mM Na_2EDTA in 50 mM phosphate buffer pH 7.5 for 5 min. Cells were transferred and radio-activity per 10^7 cells determined. Cells were then pelleted and resuspended in 2 ml 2.5 mg/ml CHAPS, 0.1 M Tris-HCl pH 8.0 containing 0.3 mg/ml PMSF, 25 μg/ml pepstatin and 0.1 mg/ml aprotinin and incubated for 30 min at room temperature. The suspension was centrifuged at 12000 × g for 30 min and the amount of radioactivity in pellets and supernatants determined. Supernatant values were used to calculate the amount extracted per 10^7 cells.

2.8 Pharmacokinetic studies

Pharmacokinetics of iodinated mIgG1, recombinant Fc-hinge fragments and IgA were determined as described [1].

2.9 Determination of serum Ig concentrations

The concentration of serum Ig was determined using radial immunodiffusion and Bindarid kits (The Binding Site Ltd., Birmingham, GB). Precipitin ring diameters were measured electronically.

2.10 Determination of the synthesis rate of mouse IgG1

Synthesis rates (SR) of IgG1 were calculated from the β-phase half-lives (days) and serum concentrations (c, in mg/ml) using the equation [11]: SR (mg/day/mouse) $(2.77\ c)/T_{1/2}$. The constant 2.77 is calculated $(100 \times \ln 2 \times V)/(IV)$, where V = volume of blood (taken as 2 ml for all mice) and IV = percent intravascular IgG1 (taken as 50 % for all mice).

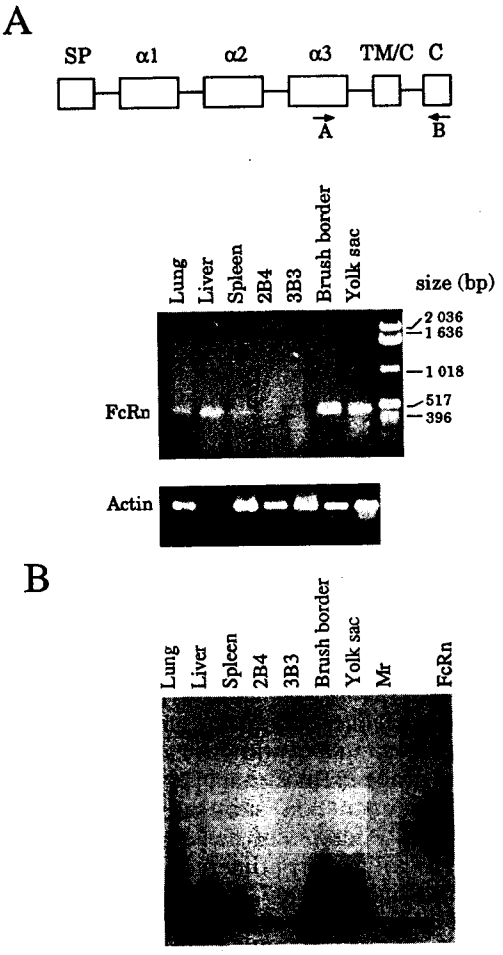

Figure 1. (A) RT-PCR analyses of expression of FcRn α chain. The genomic organization of the FcRn α-chain gene [20, 25] is shown, with exons represented by open boxes. SP = signal peptide, TM/C = transmembrane region/5′ cytoplasmic tail, C = 3′ cytoplasmic tail, and A, B (with arrows above) indicate the primers used in RT-PCR. (B) Southern blot of the upper gel shown in panel A. Mr, molecular weight standards; FcRn, 1 μg pGEM-T (Promega) derivative containing bases 650–1095 of the FcRn α-chain gene.

3 Results

3.1 FcRn α-chain mRNA expression

Recent data [3, 22] suggested that FcRn might be involved in regulating serum IgG levels. As the site of IgG catabolism has not yet been unequivocally determined [11], the expression of FcRn α-chain in a variety of mouse tissues and cell lines was analyzed using RT-PCR and primers derived from the FcRn α-chain gene [20] (Fig. 1). Southern hybridization was carried out to ensure that the RT-PCR were specific (Fig. 1). In addition to yolk sac and neonatal brush border, FcRn α-chain is expressed in lung, liver and spleen, but not at detectable levels in clonal lymphocyte populations represented by the B cell line BCL_1/3B3 [16] and T cell hybridoma 2B4 [17] (Fig. 1). Confirmation of the identity of the PCR products was obtained by nucleotide sequencing.

The ubiquitous expression of the FcRn α-chain suggested that it might be produced in the endothelial cells within these tissues. Thus, RT-PCR analyses were carried out using two mouse endothelial cell lines, B10, D2.PCE and SVEC [18], using primers specific for the FcRn α chain [20]. For both lines, expression of FcRn α-chain mRNA was observed (Fig. 2). Consistent with the work of others [20, 25], isolation and sequencing of the gene for the complete coding sequence of FcRn α chain from SVEC demonstrated it had the same sequence as that derived from C3H/HeJ mice [25]. This sequence differs from the mouse (FVB/N strain) FcRn gene originally described [20] at codons 26, 52, 212, 230, 244 and 299. The changes are silent with the exception of a G to A change at codon 52 (valine → methionine). The sequence of the FcRn α-chain gene from B10, D2.PCE cells differs from that of the FVB/N strain [20] at codons 52, 212, 230 and 244, and shares the same sequence as that of SVEC at these positions. The B10, D2.PCE gene therefore represents a polymorphic variant of FcRn α chain that has not been described previously. The liver has been suggested to be involved in IgG catabolism [9, 11], and therefore the expression of FcRn in this organ was analyzed further. Hepatocytes were isolated

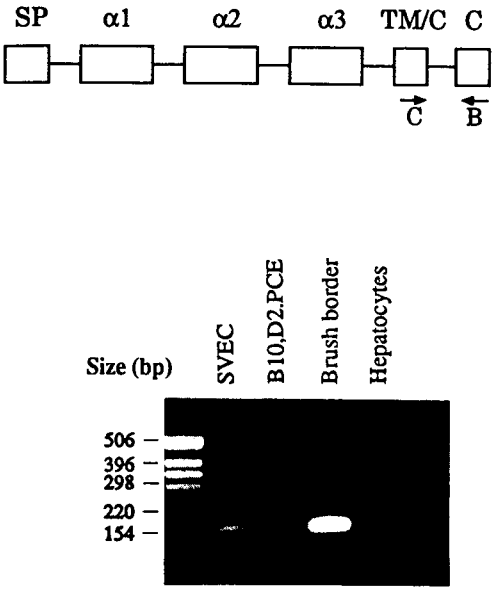

Figure 2. RT-PCR analysis of expression of FcRn α chain in endothelial cell lines and hepatocytes. C and B (with arrows above) indicate the primers used in RT-PCR. Cell lines B10, D2.PCE and SVEC are described in the text.

from adult mice and analyzed by RT-PCR, yielding a PCR product of the expected size (Fig. 2). As the isolated hepatocytes were not completely homogenous (less than 5% contamination with other cells such as Kupffer cells), RNA was extracted from the mouse hepatoma line Hepa 1–6 and analyzed by RT-PCR. This resulted in the isolation of a product of the expected size (not shown). The level of expression of FcRn in endothelial (SVEC, B10, D2.PCE) and Hepa 1–6 cell lines was studied by quantitative PCR: the level of FcRn α-chain expression is approximately 1000-fold lower in SVEC, B10, D2.PCE cells and Hepa 1–6 cells than in neonatal brush border.

3.2 Binding studies

Experiments were carried out to analyze the binding of wild-type (WT) and the HQ-310/HN-433 mutant Fc-hinge fragments to endothelial cells. The HQ-310/HN-433 mutant was used as it binds at background levels to isolated neonatal brush border [3] and at undetectable levels

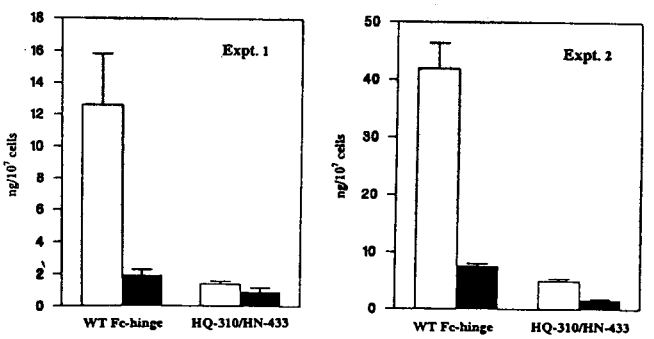

Figure 3. Binding of ^{125}I-labeled WT Fc-hinge and HQ-310/HN-433 mutant to SVEC. Open bars represent the amount bound to cells following washes, and filled-in bars represent the amount extracted from the cell pellet with 2.5 mg/ml CHAPS.

to recombinant soluble FcRn [22] due to mutation of His310, Gln311, His433, Asn434 to Ala310, Asn311, Ala433, Gln434. Binding studies with the endothelial cell line SVEC indicates that in two independent experiments, the WT Fc-hinge binds at much higher levels than the HQ-310/HN-433 mutant (Fig. 3). Furthermore, a higher proportion of the bound HQ-310/HN-433 is extracted with CHAPS than for the WT Fc-hinge fragment, although this was more marked in the first experiment (Fig. 3).

3.3 Pharmacokinetic analyses in β2m−/− mice

The above data support the concept that the ubiquitously expressed FcRn might regulate serum IgG levels. β2m−/− mice are known to be deficient in the expression of MHC class I molecules and FcRn [14, 15], and therefore provide a valuable tool to test this hypothesis. In earlier studies using BALB/c mice, the HQ-310/HN-433 mutant was found to have a significantly shorter β-phase half-life than the WT Fc-hinge fragment [1]. These two recombinant Fc-hinge fragments, in addition to mIgG1, were therefore radiolabeled and their pharmacokinetics compared in β2m−/− and β2m+/+ mice (C57BL/6 × 129/Ola and C57BL/6 backgrounds) (Fig. 4; Table 1). The β-phase half-lives of all three proteins in the two β2m−/− strains are not significantly different and are extremely short (Fig. 4; Table 1). In contrast, the β-phase half-lives of mIgG1 and WT Fc-hinge are substantially longer in β2m+/+ mice than that of the HQ-310/HN-433 mutant, consistent with our earlier observations in BALB/c mice [1]. Unexpectedly, the half-lives of mIgG1 and WT Fc-hinge are significantly longer in β2m+/+ (C57BL/6 × 129/Ola) than in β2m+/+ (C57BL/6) mice, and further analysis indicated that this is due to the abnormally low levels of serum IgG in these mice (see below).

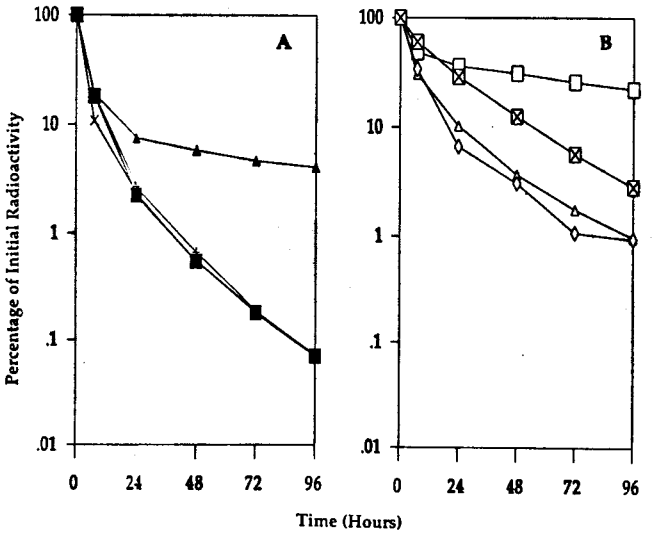

Figure 4. Catabolism of ^{125}I-labeled mIgG1, Fc-hinge fragments and IgA. (A) ▲, + = WT Fc-hinge and ■, X = HQ-310/HN-433 mutant in β2m+/+ (▲ and ■) and β2m−/− (+ and X) mice. (B) △, ◇ = IgA and □, ⊠ mIgG1 in β2m+/+ (△ and □) and β2m−/− (◇ and □) mice. For each protein, representative curves for one mouse from each group are shown. These data are for mice of the C57BL/6 background.

Table 1. β-phase half-lives of Ig and Fc-hinge fragments in β2m−/− and β2m+/+ mice

Strain of mice	β-phase half-life (hours) mIgG1	IgA	WT Fc[a)]	HQ-310/HN-433
β2m−/− (C57BL/6)	17.6 ± 0.7 (n = 5)[b)]	24.0 ± 0.8 (n = 3)	12.6 ± 0.7 (n = 4)	14.8 ± 0.6 (n = 3)
β2m−/− (mixed)[c)]	21.2 ± 0.9 (n = 3)	25.0 ± 2.2 (n = 4)	12.8 ± 1.5 (n = 5)	13.7 ± 1.7 (n = 6)
β2m+/+ (C57BL/6)	97.7 ± 5.6 (n = 3)	21.3 ± 0.7 (n = 3)	76.9 ± 3.6 (n = 4)	14.3 ± 0.5 (n = 4)
β2m+/+ (mixed)	199.5 ± 30.8 (n = 3)	27.0 ± 1.2 (n = 3)	154.3 ± 30.8 (n = 4)	15.2 ± 1.1 (n = 3)

a) Wild-type Fc-hinge fragment.
b) Numbers of mice used in parentheses.
c) C57BL/6 × 129/Ola background.

Table 2. Serum IgG, IgG1, IgA and IgM concentrations in β2m−/− and β2m+/+ mice

Strain of mice	Concentration (mg/ml) IgG	IgG1	IgA	IgM
β2m−/− (C57BL/6)	0.30 ± 0.05 (n = 9)[a)]	0.05 ± 0.01 (n = 9)	0.58 ± 0.16 (n = 9)	0.12 ± 0.02 (n = 9)
β2m−/− (mixed)[b)]	1.22 ± 0.35 (n = 19)	0.07 ± 0.03 (n = 19)	1.46 ± 0.68 (n = 19)	0.35 ± 0.10 (n = 19)
β2m+/+ (C57BL/6)	7.80 ± 1.22 (n = 14)	1.39 ± 0.33 (n = 14)	2.27 ± 0.48 (n = 14)	0.24 ± 0.11 (n = 14)
β2m+/+ (mixed)	1.52 ± 0.29 (n = 13)	0.25 ± 0.10 (n = 13)	ND[c)]	ND

a) Numbers of mice in parentheses.
b) C57BL/6 × 129/Ola background.
c) ND: not determined.

To ensure that β2m−/− mice do not have some generalized defect in the maintenance of Ig levels, the pharmacokinetics of murine IgA were also determined in both β2m−/− and β2m+/+ mice (Fig. 4; Table 1). There is no significant difference in clearance rates, and the IgA β-phase half-lives are typical of those observed in other β2m+/+ strains (Table 1 and [26, 27]).

3.4 Analysis of serum Ig levels

Determination of serum IgG levels in β2m−/− mice of both backgrounds indicated that they are abnormally low (Table 2), consistent with the observations of others [28, 29]. In addition, for β2m+/+ mice of the C57BL/6 × 129/Ola background, serum IgG levels are lower than those of C57BL/6 β2m+/+ mice. The concentrations of IgA and IgM have also been analyzed for both β2m+/+ and β2m−/− mice, and their levels are in the normal range [30]. Based on the pharmacokinetics and serum IgG1 concentrations, the rates of IgG1 synthesis can also be determined (Table 3): these data are discussed more fully below.

Table 3. Rates of IgG1 synthesis in β2m−/− and β2m+/+ mice

Strain of mice	Synthesis rate (mg/day/mouse)
β2m−/− (C57BL/6)	0.189
β2m−/− (mixed)[a)]	0.220
β2m+/+ (C57BL/6)	0.946
β2m+/+ (mixed)	0.083

a) C57BL/6 × 129/Ola background.

4 Discussion

In this study, the analysis of the pharmacokinetics of IgG1/Fc fragments in β2m−/− mice provides evidence in support of the concept that the β2m-dependent protein FcRn [14] might be involved in maintaining serum IgG levels. The implication of FcRn in this role is suggested by the ubiquitous expression of FcRn or its homolog in rats [12], man [13] and mice (Fig. 1) and, in addition, by the close overlap of the region of IgG involved in controlling neonatal transcytosis [3], IgG catabolism [1] and binding to recombinant FcRn [22].

RT-PCR analyses demonstrated that FcRn is expressed in liver, spleen and lung, but not in clonal B and T cell lines or hybridomas. Further analyses of expression in both mouse endothelial cell lines and hepatocytes indicated that FcRn is also expressed in these cell types. Quantitative PCR indicates that the level of expression in these cells is substantially lower than that in neonatal brush border, and this may account for the lack of detection of mouse FcRn α-chain mRNA in tissues other than neonata brush border and yolk sac that was previously reported using Northern blotting [20]. Direct binding studies with the endothelial cell line SVEC indicate that WT Fc-hinge binds at significantly higher levels, and more stably in the presence of CHAPS, than the HQ-310/HN-433 mutant. Our earlier observations demonstrating that the mutant Fc-hinge fragment binds at background levels to isolated neonatal brush border [3] and undetectably to recombinant FcRn [22] suggest that the differential binding is mediated by FcRn. The possibility that the differential binding is due to interaction with FcγRI, II or III, or both II and III, is made unlikely by reports which demonstrate that the interaction site of these receptors on Fc is distal to the CH2-CH3 domain

interface [31–33] and furthermore, that aglycosylated Fc fragments are impaired in binding to these receptors [34, 35]. The binding data therefore suggest that FcRn is functional in SVEC, and future experiments will be directed towards investigating this in the other cell types in which FcRn α-chain mRNA is expressed.

The functional significance of the expression of FcRn in both endothelial cells and hepatocytes suggests that either or both of these cell types might be involved in maintaining IgG homeostasis. In this respect, FcRn has been detected by immunoprecipitation from rat hepatocytes, and a role in mediating the trafficking of IgG into the biliary tract has recently been suggested to be of relevance for immunosurveillance at this site [36]. A distinct function for hepatocytic FcRn, however, might be that this protein sequesters bound IgG from delivery into the bile and only unbound (excess) IgG is delivered for catabolism in the biliary tract. This is consistent with data indicating that IgG is delivered via liver cells into the bile for breakdown in sheep [9], but the relative roles of different organs or tissues in the control of IgG catabolism clearly requires further investigation. Taken together with the earlier data of others (reviewed in [11, 37]), however, the findings in this study support the involvement of both the liver and the more diffusely located endothelial cells.

The pharmacokinetic data demonstrate that mIgG1 or WT Fc-hinge have abnormally short serum half-lives in β2m−/− mice. These serum half-lives are not due to some generalized defect in the maintenance of serum Ig levels, as the serum half-life of IgA is the same in both β2m+/+ and β2m−/− mice. Many studies have indicated that there is an inverse correlation between serum IgG concentrations and half-lives of IgG: this is called the concentration-catabolism phenomenon [26, 37]. The rapid elimination of mIgG1/WT Fc-hinge might therefore be due to abnormally high levels of endogenous serum IgG in β2m−/− mice. This is clearly not the case, however, as serum IgG levels are abnormally low in β2m−/− mice of both backgrounds, and these low serum IgG concentrations are consistent with the observations of others [28, 29]. In contrast, the serum IgA and IgM concentrations, which are regulated by a mechanism distinct from that involved in IgG homeostasis [38], are in the normal range.

To date, the role that IgG breakdown rates might have in mediating the low serum IgG concentrations in β2m−/− mice has not been investigated. It has previously been suggested that in normal mice, maternal IgG stimulates endogenous immunoglobulin synthesis, and lack of maternal transfer in β2m−/− mice accounts for the low IgG levels [29]. However, the data in this study show that although β2m−/− mice of both backgrounds have lower IgG1 synthesis rates, an additional cause of the low serum IgG levels is an increase in catabolic rates. The situation for mice of the C57BL/6 × 129/Ola background is made more complex by the observation that even for β2m+/+ animals of this background, IgG and IgG1 levels are abnormally low. This is due to a synthesis rate that, unexpectedly, is lower than that of β2m−/− mice of this mixed background. Thus, independently of the presence or absence of neonatal transfer of IgG in mice of the mixed background, the IgG1 synthesis rate is abnormally low and the reasons for this require further investigation. As a consequence of the low serum IgG concentrations in β2m+/+ mice of this background, and consistent with the concentration-catabolism phenomenon [26, 37], the half-lives of mIgG1 and WT Fc-hinge are significantly longer in this strain than in β2m+/+ C57BL/6 mice.

Our observations in β2m−/− mice are consistent with a model whereby β2m-dependent Fc receptors, which in normal mice regulate serum IgG levels, are either absent or dysfunctional in β2m−/− mice. However, alternative explanations cannot be excluded, particularly if loss of β2m is more pleiotropic than the currently available data indicate. This is made improbable by the presence of an apparently normal $CD4^+$ $CD8^-$ subset [14, 15] and the ability of B cells to mount T cell-dependent antibody responses [28, 39] in β2m−/− mice. Other possibilities, such as either a deficiency in IgG-producing precursor cells or the absence of factors or cytokines produced by $CD8^+$ cells resulting in the low serum IgG levels, are excluded by the observations that β2m−/− mice have normal numbers of $B220^+$/sIgM cells [28] and, in Lyt2 knockout mice, lack of $CD8^+$ cells does not result in reduced IgG levels [40]. In addition, the possibility that a β2m-dependent protein similar to FcRn, rather than FcRn itself, is involved in IgG homeostasis is made unlikely by Southern blotting data indicating that in mice, FcRn has no close homolog [25]. However, it is conceivable that an unrelated, as-yet unidentified β2m-dependent protein that binds to Fc or IgG at the same site as FcRn plays a role in maintaining serum IgG levels.

If FcRn is involved in maintaining IgG homeostasis, then how might this be achieved? The ability of FcRn to bind and mediate the traffic of IgG across neonatal intestinal and yolk sac cells suggests a mechanism by which FcRn in other tissues might protect IgG against degradation by binding and recirculating it into the serum. Constant levels of FcRn expression would explain IgG homeostasis despite variable IgG production by B cells, since once FcRn is saturated, excess IgG would be destined for degradation following endocytotic uptake [8]. Concerning the site of FcRn-IgG complex formation, the pH dependence of this interaction [5, 6, 41] suggests that for the maintenance of serum IgG levels, FcRn would bind to IgG following uptake by fluid-phase endocytosis into intracellular acidic compartments. This is in contrast to the FcRn-Fc interaction that occurs in the slightly acidic medium at the apical cell surface of jejunal epithelial cells during transcytosis across the neonatal intestine [42], but data in support of a similar mechanism for the materno-fetal transfer of IgG in both humans [43] and rats [44] has been reported.

In summary, the findings suggest a new role for FcRn that is distinct from previously assigned functions [5, 6], and this has relevance to understanding the molecular mechanisms of IgG homeostasis. The sequence similarities between rodent FcRn and a recently identified human FcR [13] suggest that it will be possible to extend our observations to humans. This has obvious implications for the therapy of IgG-related immunodeficiencies.

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