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Immunology

The protection receptor for IgG catabolism is the β_2-microglobulin-containing neonatal intestinal transport receptor

(Brambell receptor/Fc receptor/IgG survival/recycling/differential catabolism)

R. P. JUNGHANS*† AND C. L. ANDERSON‡

*Biotherapeutics Development Lab, Department of Medicine, Harvard Medical School, New England Deaconess Hospital, Boston, MA 02215; and ‡Departments of Internal Medicine, Molecular Genetics, and Medical Biochemistry, The Ohio State University, Columbus, OH 43210

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ABSTRACT **More than 30 years ago, Brambell published the hypothesis bearing his name [Brambell, F. W. R., Hemmings, W. A. & Morris, I. G. (1964) *Nature (London)* 203, 1352–1355] that remains as the cornerstone for thinking on IgG catabolism. To explain the long survival of IgG relative to other plasma proteins and its pattern of increased fractional catabolism with high concentrations of IgG, Brambell postulated specific IgG "protection receptors" (FcRp) that would bind IgG in pinocytic vacuoles and redirect its transport to the circulation; when the FcRp was saturated, the excess unbound IgG then would pass to unrestricted lysosomal catabolism. Brambell subsequently postulated the neonatal gut transport receptor (FcRn) and showed its similar saturable character. FcRn was recently cloned but FcRp has not been identified. Using a genetic knockout that disrupts the FcRn and intestinal IgG transport, we show that this lesion also disrupts the IgG protection receptor, supporting the identity of these two receptors. IgG catabolism was 10-fold faster and IgG levels were correspondingly lower in mutant than in wild-type mice, whereas IgA was the same between groups, demonstrating the specific effects on the IgG system. Disruption of the FcRp in the mutant mice was also shown to abrogate the classical pattern of decreased IgG survival with higher IgG concentration. Finally, studies in normal mice with monomeric antigen-antibody complexes showed differential catabolism in which antigen dissociates in the endosome and passes to the lysosome, whereas the associated antibody is returned to circulation; in mutant mice, differential catabolism was lost and the whole complex cleared at the same accelerated rate as albumin, showing the central role of the FcRp to the differential catabolism mechanism. Thus, the same receptor protein that mediates the function of the FcRn transiently in the neonate is shown to have its functionally dominant expression as the FcRp throughout life, resolving a longstanding mystery of the identity of the receptor for the protection of IgG. This result also identifies an important new member of the class of recycling surface receptors and enables the design of protein adaptations to exploit this mechanism to improve survivals of other therapeutic proteins *in vivo*.**

Thirty-two years ago, Brambell published the hypothesis (1) bearing his name that remains as the cornerstone for thinking on IgG catabolism. To explain the long survival of IgG relative to other plasma proteins and its pattern of increased fractional catabolism with high concentrations of plasma IgG (2–4), Brambell and colleagues (1) proposed that specific IgG "protection receptors" (FcRp) bind IgG in pinocytic vacuoles and redirect its transport to the circulation; when the FcRp is saturated, the excess unbound IgG then passes to unrestricted lysosomal catabolism. Brambell similarly characterized the neonatal gut transport receptor (FcRn) and established its saturable nature (5) which was confirmed by others (6–8). The connection was made early and often between these two systems in which the same mechanism or receptor system was postulated (5, 6, 8), although it could not be demonstrated directly. Common features include IgG saturation and transendosomal transport (1–8), acid-enhanced binding (5–9), a shared site on the Fc for binding (10, 11), and widespread expression of both the heavy and light chain of the cloned FcRn in normal adult tissues (9, 12) that corresponds generally to diverse sites of IgG catabolism (13, 14). In 30 years, the FcRp has not been identified, and the problem has attracted little further attention in the absence of genetic markers to define its activity.

The intestinal receptor was cloned and characterized by Simister and colleagues (15, 16). It is a heterodimer of a membrane-integral class I-like heavy chain and a β_2-microglobulin (β_2m) light chain (15) in which both chains make essential contacts with Fc (11). When Fc is mutated in the domains contacting either FcR heavy or light chain (11), survival and transport are both adversely affected (10). In mice with a light chain deletion ($\beta_2m^{-/-}$), FcRn surface expression is lost and neonatal pups are devoid of maternal IgG transport (16). The same study noted that older $\beta_2m^{-/-}$ mice had autologous IgG levels 1/10th that of normal mice, which was proposed to reflect decreased IgG synthesis. We considered that this could instead be due to increased catabolism from parallel impairment of the IgG protection mechanism. Using a genetic knockout that disrupts the FcRn and intestinal IgG transport, we demonstrate that this lesion similarly disrupts the IgG protection receptor activity of these mice, providing genetic and functional links to support the identity of these two molecules.

MATERIALS AND METHODS

Animals. Wild-type and β_2m knockout ($\beta_2m^{-/-}$) mice were purchased from The Jackson Laboratory, with either a mixed C57BL/6 × 129/Ola background or an inbred C57BL/6J background. Animals were raised under low pathogen conditions (3, 4) to yield low endogenous IgG levels.

Proteins. Purified murine anti-Tac antibody was a gift of T. A. Waldmann (National Institutes of Health). Anti-Tac is an IgG2a,κ antibody against human interleukin 2 receptor α subunit and is not reactive with any mouse proteins or tissues. Isotype matched control antibody UPC was purified from ascites by protein A chromatography. Affinity-purified soluble Tac protein was a gift of J. Hakimi (Hoffmann–La Roche). Murine albumin was obtained from Inter-Cell (Hopewell, NJ). Proteins were radiolabeled with ^{125}I or ^{131}I with Iodobeads (Pierce) and separated from free iodide by size exclusion on a Sephadex PD10 G-25 column (Pharmacia). Final specific activities were 0.1–3 μCi/μg (1 Ci = 37 GBq), depending upon the experiment. Radioactivity was determined in a Beckman

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Abbreviation: β_2m, β_2-microglobulin.

†To whom reprint requests should be addressed.

model 5500 dual channel gamma counter, with corrections applied for radioactive crossover and decay.

***In Vivo* Studies.** Mice were injected by tail vein for pharmacokinetic studies. Blood was sampled at indicated times and processed for protein-bound counts by trichloroacetic acid precipitation as described (17). Rapidly catabolized proteins require confirmation of the protein-bound fraction of radioactivity to distinguish protein from radioactive catabolites in serum. Some animals were injected i.p. with ^{125}I-labeled human immunoglobulin (Miles) on schedules to maintain different steady-state blood levels of IgG for the duration of the experiment. Mice received 1–3 i.p. doses of human IgG before i.v. injection of ^{131}I-labeled anti-Tac, and 0–8 i.p doses after the i.v. injection of ^{131}I-labeled anti-Tac. Mice were injected i.v. with a single dose of ^{131}I-labeled anti-Tac, six hours subsequent to the last prior i.p. dose of human IgG. Blood levels of administered human IgG were determined from ^{125}I counts and IgG specific activity, and added to the estimated total murine IgG.

Monomeric Antigen-Antibody Complexes. ^{131}I-soluble Tac [0.3 μg (10 pmol)] was mixed with 100 μg (1200 pmol binding site) of nonspecific (UPC) or specific (anti-Tac) ^{125}I-antibody and injected i.v. as above. The concentration of specific antibody binding site ranged from 1200 to 300 nM in the plasma over the duration of the experiment, $\geq$1000$\times$ the antibody K_d, thus ensuring that antigen binding is essentially complete. Antibody survival is unaffected by antigen binding (17) and "antigen excess" is accordingly not represented in these tests. Samples were collected and processed for protein-bound counts as above.

Pharmacokinetic Modeling. Kinetic parameters were obtained by two-compartment modeling of the composite data of each group using PCNONLIN 4.2 (SCI, Durham, NC). The reported catabolic rate constant is k_{10} ($\pm$ fitting error) in standard pharmacokinetic nomenclature (catabolic $t_{1/2} = ln2/k_{10}$). The reported ratio of catabolic constants is between ^{131}I-labeled IgG and ^{125}I-labeled albumin as internal control. (It was previously noted that albumin catabolism is modestly faster in the mixed $\beta_2m^{-/-}$ than in wild-type mice; R.P.J., unpublished work.) For Fig. 2, the plasma loss kinetics of the administered ^{131}I-labeled anti-Tac antibody were analyzed for each group as above, except that the beta phase $t_{1/2}$ is shown that parallels previous representations (1–4) of the wild-type curve. A different experimental design including more early points (e.g., see Fig. 1) is required for accurate assessment of catabolic rates; however, it may be inferred from the extremes represented in the Fig. 1 data sets that the catabolic $t_{1/2}$ is 0.6 to 0.75 $\times$ the beta phase $t_{1/2}$ under the conditions of Fig. 2.

Immunoglobulin Levels. Plasma was prepared from blood obtained by cardiac puncture of anesthetized mice and measured by ELISA relative to purified mouse antibodies [UPC (an IgG monoclonal; Sigma) and bulk IgA (Sigma)]. Plates (Costar) were coated with polyvalent goat anti-(total mouse Ig) antibody, incubated with dilutions of plasma, and then developed with horseradish peroxidase-conjugated polyvalent antibodies specific to IgG or IgA and read against a standard curve.

RESULTS

Mice with the deletion for the FcRn β_2m light chain lose expression of the receptor on neonatal intestine and are devoid of maternal IgG transport (16). To test the hypothesis that the protection receptor (FcRp) and transport receptor (FcRn) are the same, we examined the impact of this same mutation on the survival of IgG in adult mice. Comparison of administered IgG in wild-type and $\beta_2m^{-/-}$ mice confirmed a marked acceleration of clearance in the latter (Fig. 1) (17). Compartmental modeling revealed catabolic rate constants of 0.14 $\pm$ 0.01 day^{-1} for wild-type (■) and 1.5 $\pm$ 0.12 day^{-1} for mutant (□)

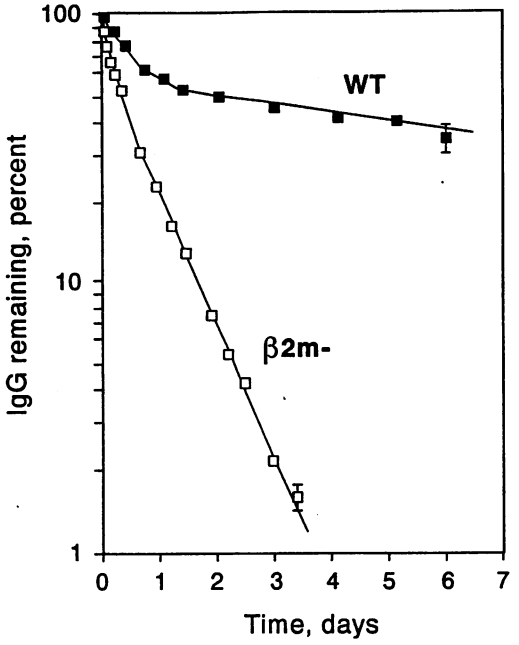

FIG. 1. Abbreviated IgG survival in $\beta_2m^{-/-}$ mice. Animals were injected with mixtures of ^{131}I-labeled murine anti-Tac antibody (■, wild-type mice; □, mutant mice) and ^{125}I-labeled murine albumin (Inter-Cell) (data not shown), and blood samples were processed for protein bound counts. Five mice were used per group. Error bars = $\pm$1 SE, shown only on last points; fractional errors of other points are similar or less.

mice.§ When normalized to albumin coadministered in these tests, the wild-type mice catabolized IgG at a rate of 0.15 relative to albumin, reflecting an $\approx$7-fold relative protection of IgG, whereas the mutant mice catabolized IgG and albumin at identical rates (ratio = 0.97 $\pm$ 0.05), hence displaying no protection of IgG.

These results thus confirm disruption of the protection receptor (FcRp) that parallels FcRn disruption. Further, these data allow quantitation of the protection by the FcRp: the IgG in these normal mice was recycled through cellular endosomes an average of seven times (relative to albumin) before it was finally catabolized.

These studies were repeated in wild-type and mutant mice of inbred C57BL/6 background in which plasma immunoglobulin levels were also assayed. Similar to the data of the mice of mixed background in Fig. 1, the catabolic rate constants for IgG were 8-fold faster in the knockout (1.34 day^{-1}) than in the wild-type mice (0.18 day^{-1}). Correspondingly, plasma IgG levels were measured as 7-fold lower in mutant than in wild-type mice, which is comparable to the difference reported previously (16), whereas IgA was similar between groups (Table 1). This direct relation of decreased steady state blood levels and increased catabolism of IgG in FcRp-deleted mice is compatible with pharmacokinetic predictions with an unaltered IgG synthetic rate (17).

As a corollary of its role in protecting IgG from catabolism, the disruption of the FcRp is predicted to disrupt the classical pattern of decreased IgG survival with higher IgG concentration (1–6). An experiment was undertaken to examine this hypothesis. We recapitulated procedures developed by Fahey and Sell (3, 4) using human IgG, which competes equally for

§The catabolic $t_{1/2}$ values for IgG were 4.9 $\pm$ 0.4 days in wild-type versus 0.47 $\pm$ 0.02 day in the mutant mice. The $t_{1/2}$ values of the beta phase of the curves were longer for both (8.2 and 0.64 days, respectively) but beta phase constants are a complex composite of distribution and catabolism and are not appropriate for judging catabolic rates or steady states.

Table 1. Selective depression of plasma IgG concentration in $\beta_2m^{-/-}$ mice

	IgG	IgA
Wild-type	2200 ± 100	110 ± 20
Mutant	260 ± 30	110 ± 20
Ratio	8.4:1 ± 0.9	1.0:1 ± 0.2

Plasma was prepared from blood obtained by cardiac puncture of anesthetized mice and measured by ELISA. In this series, inbred C57BL/6J mice were used. Values are averages of five mice ± SE. Two significant figures are reported but all calculations were done with complete figures. The ratio standard errors were obtained by standard formulas. The catabolic $t_{1/2}$ values were measured in these animals as 3.78 ± 0.21 and 0.52 ± 0.02 days (k_{10} of 0.18 and 1.34 day^{-1}) for wild-type and mutant mice, predicting steady state IgG ratios of 7.3 ± 0.5, which is not significantly different from the observed ratio of 8.4 ± 0.9 ($P > 0.3$ by t test).

the protection mechanism as mouse IgG. ^{125}I-labeled human IgG was injected i.p., which transports to blood over several hours; by the dose quantity and frequency, different mean levels of plasma IgG were maintained. The catabolism of tracer ^{131}I-labeled mouse IgG injected i.v. was then evaluated. The expected survival pattern was confirmed (Fig. 2). Wild-type mice exhibited suppression of IgG survival with increased total IgG as shown previously (1–4, 6). The mutant mice showed no similar effect, with IgG behaving essentially as expected for subunit albumin, which does not share the IgG protection receptor and whose clearance is unaffected by IgG concentration (1–5).

Finally, studies were performed with monomeric antigen-antibody complexes. Our previous metabolic studies (17) with soluble Tac antigen (soluble interleukin 2 receptor α) and anti-Tac antibody showed that antibody binding greatly prolonged antigen survival by blocking renal glomerular filtration—the principal mode of catabolism of free soluble Tac—whereas antigen binding had no influence on antibody survival. However, these studies also noted that antigen-in-complex clears faster than antibody-in-complex in normal mice, termed "differential catabolism." This was interpreted (17) as antigen dissociation in the acidic endosome, where Fc-FcRp binding is stabilized, with return of antibody to circulation through the protection receptor but passage of antigen to lysosomal catabolism. The results of Fig. 3*A* confirm the observation of differential catabolism in the wild-type mice of this experiment, with longer survival of antibody than antigen associated with antibody. When performed with mutant mice (Fig. 3*B*), bound antigen was now cleared at the same accelerated rate as (unprotected) antibody. These results confirm that the protection receptor is central to the differential catabolic mechanism for antigen-in-complex and antibody-in-complex.

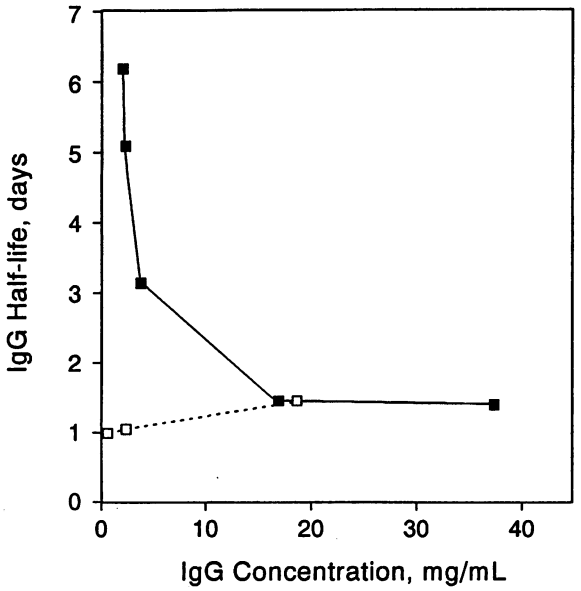

FIG. 2. Suppression of antibody survival by increased IgG concentration in wild-type but not in $\beta_2m^{-/-}$ mice. The survival $t_{1/2}$ for wild-type (■) or mutant (□) mice is plotted against plasma concentration of IgG, represented as the sum of human and endogenous murine IgG. Each point represents the average of two to five mice. Error bars not shown: fitting error of the $t_{1/2}$ was 10% or less, and the midquartile range for the IgG concentrations over the duration of the experiments was approximately ±25%.

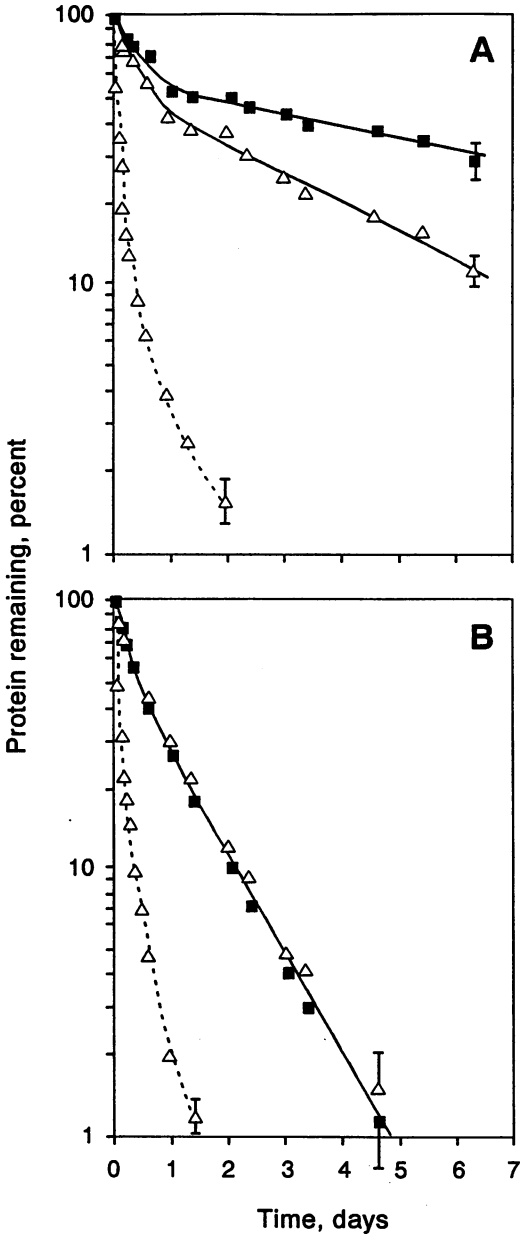

FIG. 3. Abrogation of differential catabolism mechanism for antigen-in-complex and antibody-in-complex in $\beta_2m^{-/-}$ mice. Monomeric antigen-antibody complexes were prepared and injected. Both panels show the survival in wild-type (*A*) or $\beta_2m^{-/-}$ (*B*) mice of soluble Tac antigen in the presence of nonspecific (– –△– –) or specific (—△—) antibody. Also shown is the survival of specific antibody (anti-Tac, ■) The nonspecific isotype control antibody (UPC) is not shown. Five mice were used in each group. Samples were collected and processed for protein-bound counts. Error bars = ±1 SE, shown only on last points; other points are similar or less.

DISCUSSION

IgG has a much prolonged survival relative to other serum proteins, but this survival decreases at higher concentrations of IgG (2–4). To explain this, Brambell *et al.* (1) proposed in 1964 that there was a saturable IgG protection receptor (FcRp) in cellular endosomes that selectively recycles endocytosed IgG back to the circulation. This concept, called the "Brambell hypothesis," remains as the cornerstone of thinking on IgG catabolism. Brambell subsequently demonstrated a neonatal intestinal receptor (FcRn) that transported maternal IgG with similar saturation behavior (5). Waldmann later showed preferential binding of IgG to an FcR at low pH using neonatal intestine (FcRn) or eviscerated adult carcasses (FcRp) (6, 7). Following cloning of the FcRn (15), both chains of the dimer were shown to be expressed much more broadly than neonatal gut (9), corresponding to the similarly wide distribution of sites of IgG catabolism previously shown (13). Other studies showed that mutations in IgG Fc at sites of contact with the FcRn (11) that suppressed intestinal transport also increased IgG catabolism (10).

Historically, the identity of the intestinal and protection receptors was suggested by these several features, prompting the hypothesis underlying the present study; however, the FcRp was never previously identified with any specific protein species. The common functional disruption of the FcRp and FcRn from genetic deletion of a subunit of the molecule is the most concrete evidence that the FcRp and FcRn are one and the same, which, by now, represents the most straightforward interpretation of these accumulated data. As the heavy chain and light chain (β_2m) subunits are each encoded by single copy genes, the expression of this FcR in these two contexts should be regulated from the same loci by temporal and tissue-specific factors. As FcRn, the receptor is expressed in intestinal tissue only in the first 2 weeks of neonatal life and then is down-regulated (5, 7, 8, 15, 16), in contrast to its systemic expression that persists through life (8, 9). Of these two settings, therefore, it is as the FcRp that this FcR has its broadest and most durable expression. Further studies will be needed to define aspects of cellular expression that differentiate its transient superexpression in neonatal gut from the constitutive expression observed in the majority of other tissues.

The unifying feature of the FcRn and the protection receptor is high affinity binding at low pH, present both in bowel and in the endosome, and low affinity at normal plasma pH. In the protection setting, we expect that IgG is not bound to FcRp on the cell surface at all, but only after IgG is passively internalized by ongoing pinocytosis into endosomes where low pH levels (5–6.5) foster tight binding to the FcRp, which then redirects the IgG to the cell surface where it is returned to circulation with reversal of binding at neutral physiologic pH. It is noted finally that the other known FcγRs, which mediate diverse effector and clearance functions (18) and also recycle (19), by inference do not participate importantly in the bulk catabolism of monomeric IgG, confirming previous data (20). The present studies show that normal IgG catabolism is regulated principally through the Brambell receptor because deletion of a subunit of the receptor renders its catabolism indistinguishable from that of albumin in the same mice.

These studies also provide further information on the differential catabolism mechanism for antigen-in-complex and antibody-in-complex (17), and establish the central role of the FcRp in the expression of this function, which now merits explicit representation (Fig. 4). The acidic endosome environment promotes dissociation of antigen from antibody and stimulates binding of IgG to FcRp, with return of IgG to circulation and passage of dissociated antigen to the lysosome, thus yielding the different catabolic rates. This mechanism thus "cleanses" the antibody of antigen and harvests antigen for presentation without antibody destruction, as occurs with mIg on B cells (21). That vesicles may be thus topologically "divided" was previously demonstrated with transferrin and horseradish peroxidase: both colocalize in early endosomes, but transferrin returns to the surface bound to its receptor, whereas horseradish peroxidase proceeds to the late endosomes and lysosomes (22).

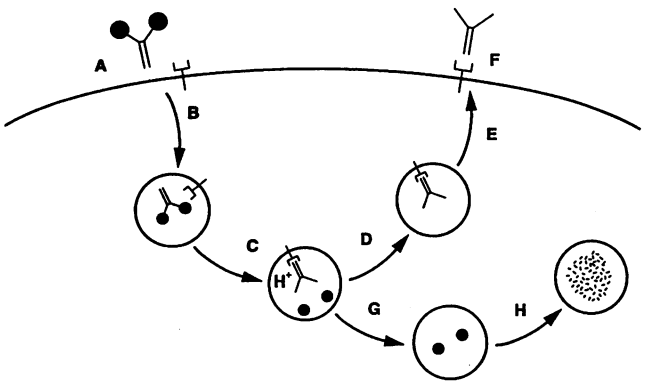

FIG. 4. Differential catabolism model incorporating Brambell receptor function. Circulating monomeric IgG plus antigen (*A*) is internalized into endosomes passively (*B*), without prior FcRp binding. In the low pH of the endosome (*C*), antigen dissociates from antibody, whereas binding of IgG to FcRp is promoted. The endosome then divides into two pathways. (*D–F*) Antibody retained by the FcRp is recycled to the cell surface and dissociates in the neutral pH of the extracellular fluid, returning IgG to circulation, free of antigen. (*G* and *H*) Unbound antigen is shunted with the endosomal contents to the lysosomes for degradation. When the Brambell receptor is deleted, the antigen and antibody pass together to lysosomal catabolism.

The survival of Tac-in-complex in wild-type (catabolic $t_{1/2}$ 1.7 days) versus $\beta_2m^{-/-}$ mice ($t_{1/2}$ 0.55 day) (Fig. 3) suggests that Tac bound to antibody in normal animals recycles through the endosome an average of three times before it is dissociated and passed to the lysosome for catabolism versus eight times for the antibody itself in this experiment. By this model, the survival of other antigens traversing the endosome will depend on antigen-antibody affinity (off-time) at acidic endosomal pH levels and on the endosomal transit time, expected to be of the order of a few minutes from data on other recycling receptors (22). The normal off-time for Tac from anti-Tac complexes is $t_{1/2}$ 100 min under physiologic conditions (23); the endosome environment evidently accelerates this dissociation rate to account for the 50% catabolism of Tac-in-complex on a few brief passages through the cell.

It is notable that the catabolic $t_{1/2}$ of 0.43 ± 0.06 day previously estimated for the 10% nonrenal fraction of Tac catabolism (90% is renally filtered) (17) approximates the value of 0.47 ± 0.02 day for IgG and albumin in the $\beta_2m^{-/-}$ mice (Fig. 1), and is also comparable with the nonrenal component of L chain and Fab catabolism (24) and to total catabolism for IgM (6), which is not filtered. This correspondence suggests that the dispersed pinocytotic activities of virtually all cells capture and process all soluble plasma proteins at a rate of ≈2× per day with equivalent degradative rates unless they are protected by specific mechanisms, as studied here with the FcRp and as available to transferrin and other recycled proteins through their cognate receptors (22). Although all nucleated cells perform pinocytosis, our data show that cells of the compartment in rapid equilibrium with the blood are relatively more active in this catabolism on a volume basis than is the extravascular compartment. This is apparent by the difference in the beta and catabolic rate constants for IgG: they would be identical if intravascular and extravascular catabolism were the same.

As a final point, there is apparently no feedback mechanism to regulate synthesis of IgG to maintain specific blood levels.

Although catabolism of IgG is 7- to 10-fold faster in mice deleted for the FcRp, resulting in markedly diminished blood levels, the direct correspondence of IgG blood level changes to catabolic changes implies a constant rate of synthesis (17) of IgG despite wide differences in plasma concentration.

Pharmacokinetic models of bulk metabolic processes in live animals with controlled genetic defects have enabled our correlation of long-established observations with newer information on these processes. In all respects, these data support the wisdom of early insights by Brambell, Waldmann, Fahey, and others who pioneered these concepts more than 30 years ago. The recent decade has been marked by major advances in understanding of the molecular features of this receptor and its intestinal expression. The present studies rejoin the link between these systems governing the transport and catabolism, respectively, of IgG and thereby provide the basis for a renewed examination of this receptor in the dominant metabolic role of its systemic expression. With the increased use of therapeutic antibodies in humans, the understanding of mechanisms of catabolism of the administered IgG will enable improvements in the design and application of these new clinical modalities. Other therapeutic non-Ig proteins may even be modified by "surface reshaping" to adapt to this recycling protection receptor system and thereby adopt a correspondingly long survival.

In this field, it was said, we are "standing on the shoulders of a giant" (8). In honor of the late Professor Brambell, who defined both the FcRp and FcRn activities, we propose that the generic and genetic names for this molecule be assigned as FcRB, and FcRBα or FcRB heavy chain for the class I-related subunit, with the specific designations of FcRn and FcRp preserved to distinguish its separate expressions as transporter or protection receptor for this most important of all immunoglobulins.

Note Added in Proof: We wish to call attention to concurrent efforts we learned of after completing our own studies, which also show faster IgG clearance in $\beta 2m^{-/-}$ mice (ref. 25; E. J. Israel, D. F. Wilsker, K. C. Hayes, D. Schoenfield & N. E. Simister, unpublished work). We note, however, that a conclusion of reduced IgG biosynthesis in these mice (25) contrasts with our analysis that it is essentially unaltered.

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