

Half-life of IgE in serum and skin: Consequences for anti-IgE therapy in patients with allergic disease

Monica G. Lawrence, MD,^{a,*} Judith A. Woodfolk, MBChB, PhD,^{a,*} Alexander J. Schuyler, BS, BA,^a
Leland C. Stillman, MD,^b Martin D. Chapman, PhD,^b and Thomas A. E. Platts-Mills, MD, PhD, FRS^a Charlottesville, Va

We present results from clinical studies on plasma infusion done in the late 1970s in patients with hypogammaglobulinemia in which we documented the short half-life of both total and allergen-specific IgE in serum. The development of specific allergic sensitization in the skin of those patients followed by the gradual decrease in sensitization over 50 days was also documented. The data are included here along with a discussion of the existing literature about the half-life of IgE in both the circulation and skin. This rostrum reinterprets the earlier clinical studies in light of new insights and mechanisms that could explain the rapid removal of IgE from the circulation. These mechanisms have clinical implications that relate to the increasing use of anti-IgE mAbs for the treatment of allergic disease. (J Allergy Clin Immunol 2016;■■■■:■■■-■■■.)

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It is generally recognized that the half-life of IgE in serum is short (ie, 2-3 days), whereas the half-life of IgG is much longer (23 days).¹ This long half-life of IgG is attributed to the protection of IgG from catabolism by binding to the neonatal Fc receptor (FcRn).^{2,3} However, the reason for the shorter half-life of IgE in comparison with other serum immunoglobulins, which also are not protected by FcRn (5-6 days for IgM and IgA), is not clear. Certainly none of our textbooks includes a coherent explanation of the reasons for the rapid removal of IgE from the circulation.^{1,4-7}

The original studies on the half-life of IgE were carried out in the early 1970s with radiolabeled IgE.¹ In those experiments it

Abbreviations used

FcRn: Neonatal Fc receptor

Gal: Galectin

PK: Prausnitz-Küstner

was difficult to exclude the possibility that the labeling procedure influenced IgE metabolism, although similar results were seen with unlabeled and C¹⁴-labeled IgE in individual experiments. In addition, the lack of known specificity of myeloma IgE used in the original studies meant that the presence of IgE could not be detected or monitored by using skin testing.

Here we present results from clinical studies on plasma infusion done in the late 1970s in patients with hypogammaglobulinemia, in whom we documented the short half-life of both total and allergen-specific IgE in serum. The development of specific allergic sensitization in the skin of those patients followed by the gradual decrease in sensitization over 50 days was also documented. These results were only reported in abstract form because we could not adequately explain where the serum IgE went or how it was catabolized. The data are included here along with a discussion of the existing literature about the half-life of IgE in both the circulation and the skin.⁸

This rostrum reinterprets the earlier clinical studies in light of newer developments in the field that could explain the rapid removal of IgE from the circulation. Understanding these mechanisms is of importance given the increasing use of anti-IgE mAbs for the treatment of allergic disease and might have clinical implications related to their use.

From ^athe University of Virginia Asthma and Allergic Diseases Center, and ^bIndoor Biotechnologies.

*These authors contributed equally to this work.

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Corresponding author: Thomas A. E. Platts-Mills, MD, PhD, FRS, Asthma and Allergic Diseases Center, PO Box 801355, University of Virginia, Charlottesville, VA 22908-1355. E-mail: tap2z@virginia.edu.

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PLASMA INFUSIONS FROM ALLERGIC DONORS IN PATIENTS WITH HYPOGAMMAGLOBULINEMIA AND A HEALTHY CONTROL SUBJECT

Studies were performed in 1976 and 1977 on 3 patients with hypogammaglobulinemia and 1 healthy nonallergic control subject.^{8,9} At that time, intravenous immunoglobulin had not been developed, and plasma infusions from healthy donors were occasionally used to treat patients with hypogammaglobulinemia.¹⁰ Because the plasma from allergic donors contained IgE and the patients with hypogammaglobulinemia did not have detectable levels of serum IgE at baseline, this allowed the half-life of transferred IgE in the patients' sera to be monitored. Each subject received 1 or 2 units of plasma containing high levels of both total and specific IgE to the grass pollen allergen Lol p 1 or the dust mite allergen Der p 1. This allowed specific IgE and IgG antibodies in the circulation to be monitored and the end point

sensitivity to these allergens in the skin to be assessed. For detailed methods, see the [Methods](#) section in this article's Online Repository at www.jacionline.org.

In the patients with hypogammaglobulinemia who lacked detectable total IgE, antigen-specific IgE, and antigen-specific IgG at baseline, levels of total IgE and specific IgE antibody, as well as levels of specific IgG in serum, increased and then decreased rapidly after plasma infusion ([Fig 1, A](#),¹¹ and see [Table E1](#) in this article's Online Repository at www.jacionline.org). Total IgE levels decreased in parallel with specific IgE levels, with a half-life of 2 days. In the control subject, who also initially lacked detectable antigen-specific IgE or IgG and had a baseline total IgE level of 120 U/mL, total IgE levels initially increased and then decreased to preinfusion levels within 4 days ([Fig 1, B](#), and see [Table E1](#)), suggesting that endogenous IgE production was unperturbed and that the infused IgE had been rapidly cleared from the circulation. Serum specific IgG antibodies to these allergens were also followed and decreased with the expected half-life of IgG ([Fig 1](#) and see [Table E1](#)).

Skin sensitization was also monitored after infusion by using quantitative intradermal testing with purified Lol p 1 or Der p 1 serially diluted from 10^{-1} to 10^{-5} $\mu\text{g/mL}$. Each recipient showed a progressive decrease in skin sensitivity, such that it required approximately 10-fold more Lol p 1 or Der p 1 to produce an 8-mm wheal at each 16-day interval ([Table I](#) and see [Table E2](#) in this article's Online Repository at www.jacionline.org). Positive skin test results were demonstrated as late as 50 days after plasma infusion. Prausnitz-Küstner (PK) testing was then performed on the control subject using intradermal injections of serial 2-fold dilutions of donor serum from 1:25 to 1:1600. This method was used to load IgE directly onto skin mast cells and thus does not rely on the diffusion of IgE from the plasma into the skin, as in previous experiments. The subject was tested at 24 hours after injection with serial dilutions of Lol p 1 ranging between 10^{-1} and 10^{-4} $\mu\text{g/mL}$. Results suggested that a 2-fold decrease in the concentration of IgE antibody injected into the skin increased the quantity of allergen needed to produce an 8-mm wheal by 10-fold (see [Table E3](#) in this article's Online Repository at www.jacionline.org). Thus in combination with the results of the previous plasma infusion experiments, the half-life of IgE in the skin was calculated to be approximately 16 to 20 days.

Plasma infusion studies confirmed the dramatic difference in the half-life of IgE (2 days) and IgG (18-23 days) in the circulation ([Table I](#)). Persistence of IgE in the skin for at least 50 days occurred in both immunodeficient patients who previously lacked IgE and in a healthy nonallergic subject. This is consistent with other studies, which have reported persistence of passive sensitization up to 56 days in an IgE-deficient mouse model and up to 70 days after treatment with anti-IgE in patients with allergic rhinitis.¹²⁻¹⁴

DISTRIBUTION OF IgE IN THE BODY

Understanding the distribution of IgE in the body at steady state is of central importance to explain the observed difference in the kinetics of infused IgE in the circulation and skin. Although lymph nodes and bone marrow are thought to be the major sites of IgE antibody production, allergic sensitization is present in all parts of the skin, respiratory tract (nose and lungs), and conjunctivae, as well as in the gastrointestinal tract. This

phenomenon reflects normal passage of IgE into the circulation (the vascular compartment), extravasation into the primary and secondary lymphoid tissue and interstitial space (the extravascular compartment), and binding to tissue-resident mast cells distributed throughout the body (the cellular compartment). It is important to note that recent evidence suggests that perivascular mast cells can actively acquire IgE directly from the blood and that this process is not solely dependent on passive diffusion.¹⁵ IgE is also found on the surfaces of circulating basophils, dendritic cells, and Langerhans cells through binding to the high-affinity IgE receptor Fc ϵ RI. Although IgE is bound to the low-affinity IgE receptor Fc ϵ RII on B cells, monocytes, Langerhans cells, dendritic cells, and eosinophils,¹⁶ these cells likely account for only a fraction of cell-bound IgE because of their low receptor density and lower receptor affinity for IgE.

To address whether the cellular compartment is sufficient to serve as a "sink" for infused IgE, a principal question becomes calculating its capacity for binding molecules of IgE. Circulating basophils capture IgE through binding to Fc ϵ RI expressed at the cell surface. There are extensive published data about the numbers of IgE molecules and high-affinity IgE receptors on basophils in the circulation. The number of IgE receptors expressed on basophils varies widely between 6,000 and 900,000 molecules per basophil and correlates with the serum IgE level.¹⁶⁻¹⁸ However, the latest estimates are that the median number of Fc ϵ RI receptors is 150,000 per basophil for subjects with a normal serum IgE level.¹⁹ Based on the calculated number of circulating basophils in peripheral blood being 3.1×10^8 cells (see the [Methods](#) section in this article's Online Repository for detailed methods), with 150,000 Fc ϵ RI receptors per basophil, the number of Fc ϵ RI receptors on basophils as a whole is 4.5×10^{13} . Circulating basophils display increasing Fc ϵ RI occupancy (from 21% to 95%), which also correlates with the serum IgE level, with a reported 80% to 95% occupancy at a normal serum IgE level of 100 to 200 ng/mL; therefore an estimated 2 to 9×10^{12} unoccupied receptors are found on circulating basophils at steady state.¹⁸

Surprisingly, there do not appear to have been many serious estimates of the number of mast cells in the body since Paul Ehrlich described these cells in 1878.²⁰ Ehrlich estimated that there were enough mast cells in the body to make up "an organ the size of the spleen" (approximately 150-200 g). More recent estimates have estimated the mast cells in the body to be "the size of a child's fist."²¹ Based on the surface area of the skin, mast cell numbers in the dermis, and average tissue weight and tissue density, it is possible to estimate the total number of mast cells in the human body as approximately 3×10^{11} cells; this is likely an overestimate because of the assumptions used in the calculation (see the [Methods](#) section in this article's Online Repository for detailed methods).²²⁻²⁴

In contrast to basophils (median diameter, 12 μm), mast cells display a high degree of size heterogeneity (diameter, 8-20 μm),²⁵ indicating the potential to express lower or higher numbers of Fc ϵ RI receptors on the surface (10,000 to 420,000 per mast cell) compared with basophils if the same receptor density is maintained.²⁶ Unlike basophils, tissue mast cells are not bathed in a milieu rich in IgE. Thus the Fc ϵ RI occupancy on mast cells is likely to be lower than in basophils. It is clear that mast cell Fc ϵ RI receptors are not fully saturated with IgE and thus have the ability to bind additional IgE molecules, even in allergic subjects. As evidence of this, it is possible to elicit a positive

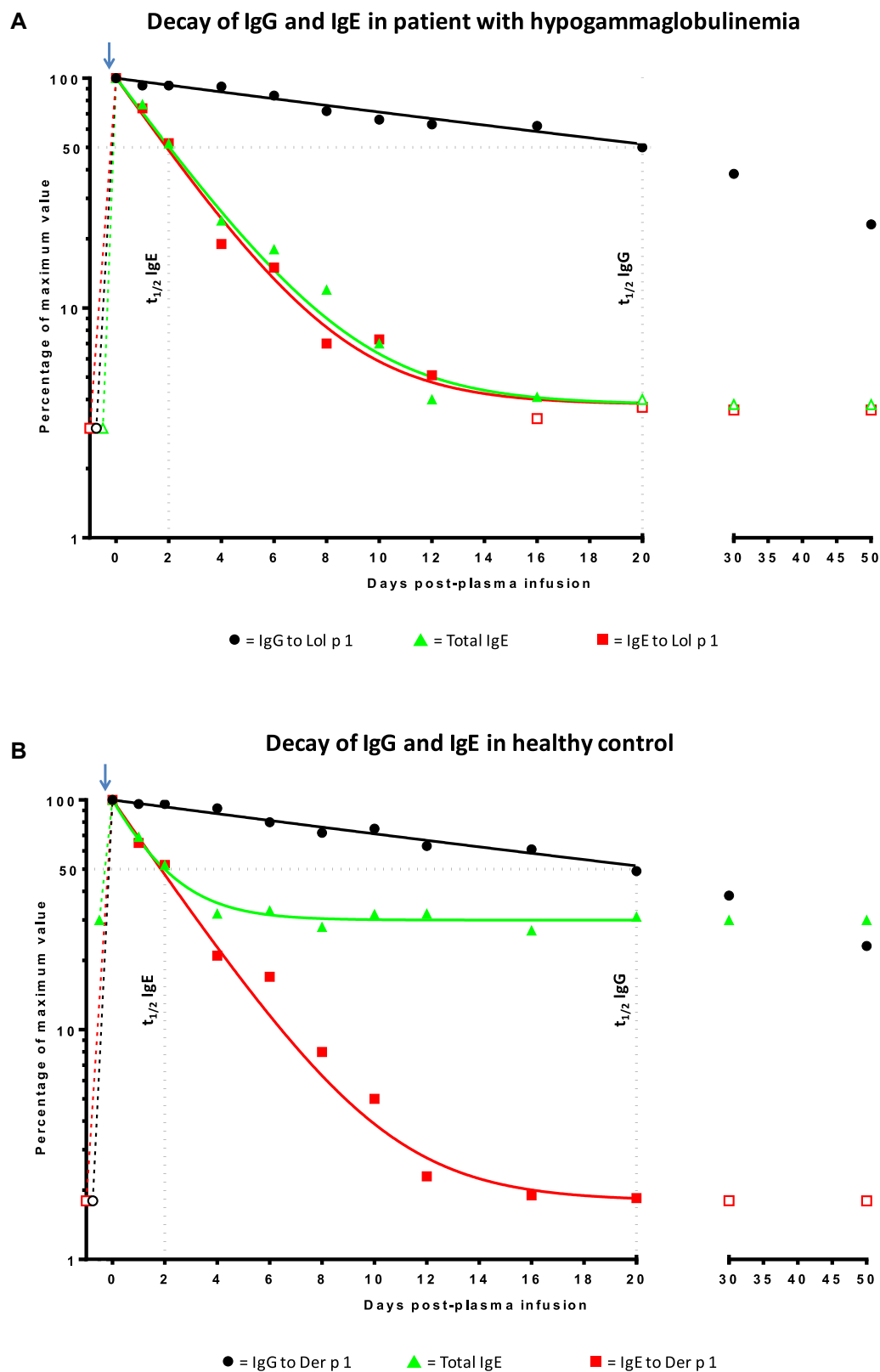


FIG 1. Decay of serum IgG and IgE after plasma infusion. Serum antibody decay is shown for a patient with hypogammaglobulinemia (**A**) and a healthy nonallergic control subject (**B**). *Closed markers* indicate positive IgG or IgE values (ie, ≥ 10 and ≥ 3 U/mL, respectively), whereas *open markers* denote negative values (ie, < 10 and < 3 U/mL, respectively). For further details on the antigen-binding assays performed, see Platts-Mills et al.¹¹

TABLE I. Estimates of the half-life of IgE and IgG antibodies in serum, as well as the half-life of allergen skin tests after plasma infusion

Subject	Serum antibodies			Skin tests	
	Total IgE antibody	sIgE antibody	sIgG antibody	Allergen	Half-life
Hypogammaglobulinemia #1	~2 d	~2 d	~23 d	Lol p 1	27 d
Hypogammaglobulinemia #2	~2 d	~2 d	~20 d	Lol p 1	16.6 d
Hypogammaglobulinemia #3	~2 d	~2 d	~18 d	Lol p 1	23 d
Healthy control subject	Not done	~2 d	~20 d	Der p 1	20 d

PK test result in allergic subjects who lack baseline sensitization to the specific IgE present in the transferred serum.²⁷ Therefore we have made a conservative receptor occupancy estimate of 60%.^{12,15} Based on the estimated total number of mast cells in the human body (3×10^{11}) and using a published FcεRI density value of 130,000 to 200,000 molecules/mast cell with an assumed 50% to 60% receptor occupancy,^{25,28} we estimate there are 1.4 to 1.9×10^{16} unoccupied FcεRI receptors that are available for IgE binding.

EXPLANATIONS FOR THE SHORT SERUM HALF-LIFE OF IgE

To investigate whether the infused IgE could be rapidly redistributed from the vascular to the cellular compartment, we have estimated the capacity for IgE binding on mast cells and basophils (cellular compartment) compared with the total number of IgE molecules present in the circulation (vascular compartment). The cellular compartment has the capacity to bind 1.4 to 1.9×10^{16} additional IgE molecules through unbound FcεRI at steady state in a person with a normal serum IgE level. We can estimate the number of IgE molecules infused into the healthy control subject in the previously described experiments to be 4.13×10^{16} molecules (see the [Methods](#) section in this article's Online Repository for detailed methods). Thus the number of injected molecules is at least 2- to 3-fold larger than can be accommodated by the cellular compartment. This suggests that there are additional mechanisms contributing to the rapid removal of IgE from the circulation.

Additional evidence that the half-life of serum IgE is not solely determined by the size or characteristics of the cellular space is derived from the previously described plasma infusion experiments. In these the calculated half-life of serum IgE was identical in patients and control subjects, despite the fact that the 3 patients with hypogammaglobulinemia had no detectable serum IgE at baseline and thus would be predicted to have lower basal expression of FcεRI with low/no receptor occupancy, whereas the healthy control subject had a baseline serum IgE level of 120 IU/mL and would be predicted to have upregulation of FcεRI, as well as accompanying receptor occupancy.

Because the calculations above demonstrate that binding of IgE to the cellular compartment is not sufficiently large to explain the rapid disappearance of infused IgE from the circulation, what are the other possibilities? The fractional catabolic rate of IgE is much higher than that of other immunoglobulins,^{1,28} suggesting that there is either enhanced destruction/degradation of IgE relative to IgG and other immunoglobulin isotypes or lack of protection from degradation.

Endogenous proteases, such as mast cell-derived β-tryptase, as well as proteases produced by helminths during infection, can

directly bind to and digest IgE molecules *ex vivo* and *in vitro*.²⁹⁻³¹

There is also some evidence that polymorphisms or copy number variations in other mast cell–derived proteases, including α-tryptase and chymase, are correlated with the serum IgE level.³²⁻³⁴ However, *in vivo* human IgE has been postulated to associate with other plasma proteins, including α₁-antitrypsin, to protect it from degradation.³⁰

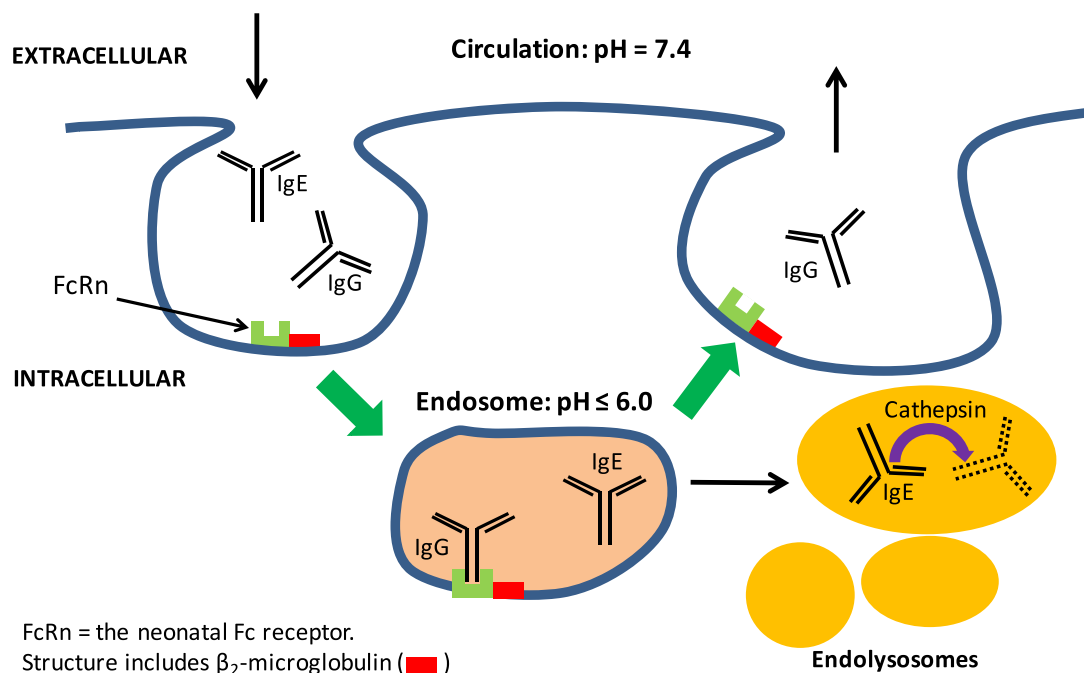
Another possibility is that glycans found on IgE bind to membrane-bound lectins (carbohydrate binding proteins) and target IgE for destruction. Mac-2, also known as galectin (Gal) 3, carbohydrate binding protein 35, and IgE-binding protein, is a galactose-specific lectin that can specifically bind IgE but not IgG or IgM.^{35,36} This molecule exists in both soluble and membrane-bound forms found on macrophages, dendritic cells, mast cells, eosinophils, neutrophils, and some epithelial cells. Another lectin, Gal-9, binds to IgE and blocks access of antigen to the IgE, thus preventing IgE-antigen complex formation and inhibiting mast cell degranulation triggered by FcεRI crosslinking. In a mouse model, however, there was no change in serum IgE levels associated with administration of exogenous Gal-9.³⁷ It is important to note that several lectins important in immunity are capable of binding other immunoglobulins but do not bind IgE. These include mannose-binding lectin/protein, which does not bind IgE because of the presence of an additional CH2 hinge domain compared with IgG and jacalin.^{38,39}

Lastly, there is some evidence that both the high- and low-affinity IgE receptors can contribute to IgE homeostasis. CD23, the low-affinity IgE receptor, has been shown to contribute to the negative regulation of serum IgE levels in murine models.^{40,41} Recently, it was found that the high-affinity IgE receptor on human dendritic cells, monocytes, and macrophages is internalized constitutively and that the bound IgE is endocytosed and degraded in lysosomes, a process known as receptor-mediated endocytosis.⁴² This same process of cyclic receptor recycling occurs in mast cells and basophils but is abolished on IgE binding.⁴³ However, it is possible that for a brief period of time immediately after IgE binding, the receptor is not yet stabilized in the membrane, and thus a small amount of receptor-bound IgE could be delivered to the catabolic pathway.

Because there is not a clear mechanism for enhanced destruction/degradation specifically targeting IgE, the remaining possibility that needs to be considered for IgE is lack of protection from catabolism occurring in pinocytic endosomes in vascular endothelial cells, which can digest immunoglobulin molecules that are not protected by FcRn.^{2,3} FcRn is a β₂-microglobulin-associated MHC class I-like molecule that binds all IgG subclasses (although IgG₃ binds poorly) and plays a critical role in prolonging the half-life of IgG in serum.⁴⁴⁻⁵⁰ The best-supported mechanism by which FcRn prolongs the half-life of IgG is by binding it in endosomes in vascular endothelial

A

Endocytosis and the processing of IgG and IgE within endothelial cells



B

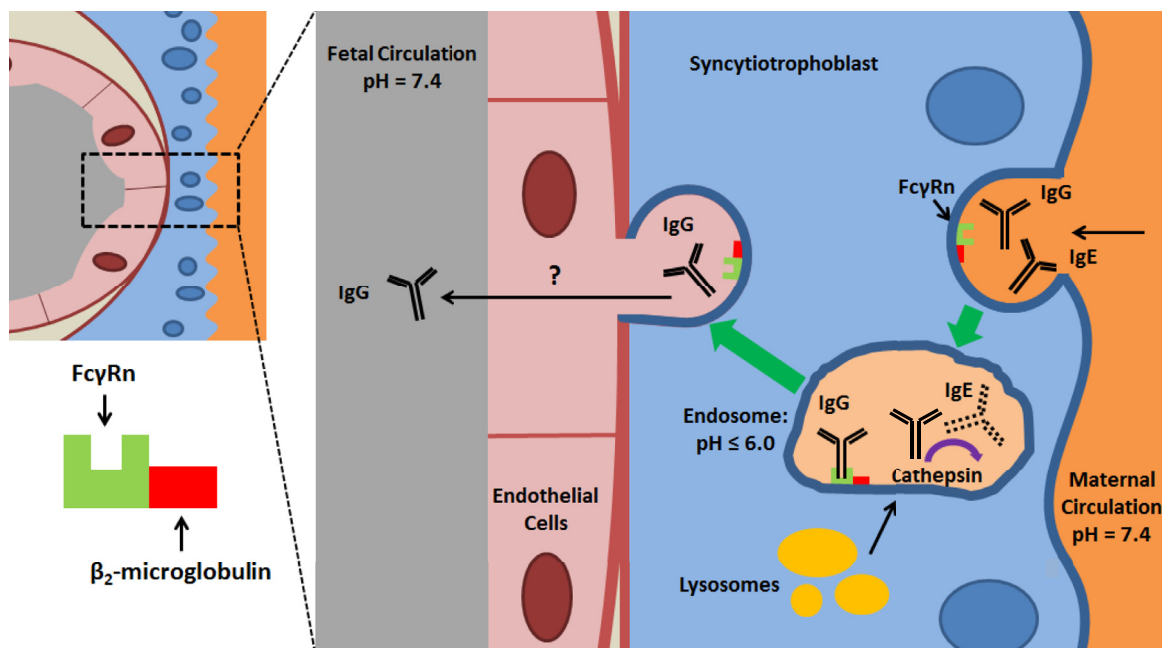


FIG 2. Protection of IgG, but not IgE, by the neonatal receptor FcRn. **A**, Endocytosis and processing of IgG and IgE in vascular endothelial cells. In endosomes, the pH falls below 6.0. At this pH, the affinity of FcRn for IgG increases and cathepsins are activated. Under these conditions, immunoglobulins such as IgE that do not bind to FcRn will be digested in the endolysosome, while IgG is either recycled back into the circulation or transcytosed into tissues. **B**, Role of FcRn in protection of maternal IgG in transplacental transfer.

cells and protecting it from degradation. IgG is either recycled back into the circulation or transcytosed into tissues for eventual return to the circulation through the lymphatics (Fig 2, A). The transplacental transfer of IgG from the mother to the fetus is also well known to occur through FcRn (Fig 2, B).^{47,51-53}

Thus the importance of FcRn in IgG homeostasis is clear. However, what is not well recognized is that monomeric IgE is not capable of binding FcRn, as evidenced by the fact that the concentration of IgE in cord blood is less than 1% of that in the mother's blood.⁵² Thus IgE is not protected from digestion in the endolysosomes of the endothelial cells. This catabolism of unprotected IgE would be expected to occur as rapidly as IgG is catabolized in β_2 -microglobulin and FcRn knockout mice (ie, approximately 10-times more rapidly than in wild-type mice).^{45,46} This 10-fold difference is similar to the difference in the observed serum half-life of IgG in human subjects (23 days) compared with that of IgE (2-3 days). Other non-FcRn-salvaged isotypes, including IgA, IgD, IgM, and most notably IgG₃ (the only IgG subclass that does not bind well to FcRn) also have shortened half-lives compared with IgG₁, IgG₂, and IgG₄, ranging from 3 to 7 days, emphasizing the importance of this mechanism in determining immunoglobulin homeostasis.

DISCUSSION

There are several possible explanations for the short half-life of IgE in serum: rapid removal of free IgE from the circulation by binding to tissue-resident mast cells and circulating basophils; degradation of IgE by extravascular and membrane-bound proteases; binding of IgE glycans to membrane-bound lectins, leading to delivery for degradation; receptor-mediated endocytosis; and digestion of IgE in endolysosomes caused by lack of protection by FcRn. All of these mechanisms are likely to contribute to a varying extent. We would argue that the latter is a major and underappreciated contributor to the short half-life of IgE in serum.

Questions about IgE metabolism have become more relevant with increasing use of IgG anti-IgE mAbs, such as omalizumab, in the treatment of allergic disease. Although treatment with this mAb decreases free IgE levels in the circulation, at the same time, there is an increase in the overall quantity of IgE in the circulation.^{13,54,55} The majority of these IgE molecules are in the form of complexes with anti-IgE mAb. It has been suggested that the increase in total IgE levels reflects decreased binding of these anti-IgE mAb-IgE complexes to Fc ϵ RI on mast cells and basophils, either through steric hindrance or through downregulation of Fc ϵ RI caused by the decrease in free IgE levels.^{54,55} However, an alternative explanation is that binding of anti-IgE mAb to the Fc portion of the IgE molecule prevents catabolism of IgE. Protection of the molecule could occur through a variety of mechanisms: (1) the presence of anti-IgE mAb bound to the Fc portion of IgE prevents its degradation because of steric interference, perhaps involving the interruption of macrophage-anchored lectins binding to glycans found on IgE; (2) the Fc portion of the IgG anti-IgE mAb binds the complex to FcRn and protects it from endolysosomal degradation; or (3) IgE-anti-IgE mAb complexes do not undergo pinocytosis and endolysosomal degradation because of their size. Thus the observed increase in serum total IgE levels after treatment with anti-IgE mAb might not reflect simply a decreased ability to

transfer bound IgE to the cellular fraction but also enhanced protection from catabolism.

Understanding the mechanisms whereby monoclonal anti-IgE leads to an increase in serum IgE levels might also have implications for understanding how this treatment works *in vivo*. Recent evidence indicates that FcRn can mediate the transfer of IgE complexed to IgG anti-IgE molecules both across the placenta and in breastmilk.⁵⁰ If anti-IgE mAb-IgE complexes do in fact bind to FcRn, as we suggest, then it might be possible for them to cross the placenta and be transferred in breastmilk. Thus, in theory, fetuses of mothers treated with anti-IgE mAb could become passively sensitized through this route if the IgE were to somehow become dissociated from the anti-IgE mAb complex.⁵⁰

With the increasing use of targeted mAbs as therapeutics for asthma and chronic urticaria, as well as other atopic diseases, better understanding of IgE homeostatic control mechanisms and the direct immunologic effect or effects of these biologic agents on IgE regulation is critical. This is an area that merits further investigation, and it is our hope that this discussion will serve as a catalyst for future research.

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METHODS

Human studies research was carried out before the institution of federal regulations requiring institutional review board approval. However, research was performed in accordance with the standards outlined in the 1964 Declaration of Helsinki.

Plasma infusions

Two patients with atopic dermatitis but who were otherwise healthy were used as plasma donors: the first donor (age 35 years) had well-controlled atopic dermatitis, a total IgE level of 6400 IU/mL, and 230 arbitrary units of IgE antibodies to grass pollen, and the second donor (age 21 years) had moderate atopic dermatitis, a total IgE level of 8900 IU/mL, and 140 arbitrary units of IgE to dust mite allergens. Plasma, which was group and cross-match compatible with the plasma recipients, was obtained by using a sterile plasmapheresis technique under supervision of the Blood Transfusion Service at Northwick Park Hospital. The plasma was further tested for hepatitis B antigen and stored at -35°C . After obtaining informed consent, 1 to 2 units of plasma was infused into 3 patients with hypogammaglobulinemia and 1 healthy nonallergic control subject. IgG and IgE antibodies to the antigens (ie, Lol p 1 and Der p 1) were quantified by using an isotype-specific double-antibody binding assay.^{E1,E2} Total IgE antibody levels were quantified by using a double-antibody inhibition radioimmunoassay.

Intradermal skin testing and PK testing

Intradermal skin tests were carried out with 0.02 mL of antigen. Reactions were measured at 15 minutes, and an 8-mm wheal was regarded as positive.

Twenty-four hours after local intradermal injection of donor serum at the listed dilutions, PK testing was carried out by testing the sites with 0.02 mL of antigen at the listed dilutions.^{E3} An 8×8 -mm wheal was regarded as a 2+ end point, and a 4×4 -mm wheal was regarded as a 1+ end point.

Calculation of cellular fraction and vascular pool

The number of circulating basophils was estimated by using the following equation:

Circulating basophil frequency (cells/L) $\times$ Average human blood volume (L).

The average circulating basophil frequency used was 52×10^6 cells/L. The average human blood volume used was 6 L. Based on this, the number of circulating basophils is equal to 3.12×10^8 cells.

The number of dermal mast cells was estimated by using the following equation:

Dermal mast cell density (mast cells/mm³) $\times$ Dermal depth (mm) $\times$ Average human body surface area (m²) $\times$ (10⁶ mm²/m²).

The dermal mast cell density^{E4} used was 6,377 to 10,584 cells/mm³ (depending on depth), and dermal depth used was 1.2 mm.^{E5} The average human body surface area^{E6} used was 1.6 m². Based on this, the number of dermal mast cells is equal to 1.22×10^{10} to 2.03×10^{10} cells.

The number of tissue mast cells was estimated by using the following equation:

Dermal mast cell density (mast cells/mm³) $\times$ (10³ mm³/mL) $\times$ Tissue weight (kg) $\times$ (10³ g/kg) $\div$ Average human density (g/mL).

Mast cells are assumed to be present at the same density throughout all tissues as in the skin; this is likely an overestimate of total body mast cells. The dermal mast cell density^{E4} used was 6,377 to 10,584 cells/mm³ (depending on depth). The average human weight^{E7} used was 62 kg, with 25% subtracted for the weight of the human skeleton, hair, nails, skin, teeth, and gastrointestinal contents^{E8} to produce a tissue weight of 46.5 kg. The average human density^{E9} used was 1.061 g/mL. Based on this, the number of tissue mast cells is equal to 2.79×10^{11} to 4.64×10^{11} cells.

The number of total body mast cells is the sum of dermal and tissue mast cells equal to 2.92×10^{11} to 4.84×10^{11} cells.

The number of IgE molecules infused into the healthy control subject was calculated by using the following equation:

Measured donor serum total IgE (IU/mL) $\times$ 2.44 ng/IU $\times$ Volume infused (mL) $\times$ (1 mg/10⁶ ng) $\div$ Molecular weight IgE (mg/ μ mol) $\times$ (6.023 $\times 10^{17}$ molecules/ μ mol). The measured donor serum IgE level was 8900 IU/mL. Volume infused was 2 units of plasma or 600 mL. The molecular weight of IgE used was 190 mg/ μ mol. Based on this, the number of IgE molecules infused into the healthy control subject was 4.13×10^{16} molecules.

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TABLE E1. Measured serum values at baseline and after plasma infusion in a patient with hypogammaglobulinemia and a healthy control subject

Day	Patient with hypogammaglobulinemia		
	IgG to Lol p 1 (U/mL)	IgE to Lol p 1 (U/mL)	Total IgE (U/mL)
Baseline	<10.0	<3.0	<3.0
Infusion	1300	73.2	77.8
1	1211	54.2	59.9
2	1209	38.8	40.5
4	1196	13.9	18.7
6	1092	11.0	14.0
8	936	5.1	9.3
10	858	5.3	5.5
12	819	3.7	3.6
16	806	<3.0	3.1
20	650	<3.0	<3.0
30	499	<3.0	<3.0
50	301	<3.0	<3.0

Day	Healthy control subject		
	IgG to Der p 1 (U/mL)	IgE to Der p 1 (U/mL)	Total IgE (U/mL)
Baseline	<10.0	<3.0	120
Infusion	742	240	400
1	712	156	276
2	712	127	208
4	683	50.4	128
6	594	40.8	132
8	534	19.2	112
10	557	12.0	127
12	467	5.5	128
16	453	4.2	108
20	364	4.4	124
30	285	<3.0	120
50	172	<3.0	120

TABLE E2. Wheal size of intradermal skin tests performed on a healthy control subject at specified intervals after plasma infusion

	Days after plasma infusion								
	0	1	3	5	12	28	40	56	97
Concentration of Lol p 1 used ($\mu\text{g/mL}$)									
10^{-1}	Neg	20 × 23	ND	ND	ND	12 × 13	15 × 12	9 × 10	4 × 5
10^{-2}	ND	12 × 13	ND	11 × 11	12 × 12	10 × 9	12 × 11	8 × 7	4 × 4
10^{-3}	ND	11 × 11	11 × 12	10 × 10	11 × 10	6 × 5	9 × 7	4 × 5	3 × 3
10^{-4}	ND	8 × 9	8 × 8	9 × 7	8 × 6	4 × 4	4 × 4	5 × 5	Neg
10^{-5}	ND	Neg	Neg	5 × 6	Neg	Neg	Neg	Neg	Neg

Measurement of skin test wheal in millimeters is shown.

Neg, Wheal not detectable; *ND*, test not done.

TABLE E3. Wheal size after PK testing performed on the healthy control subject

	Concentration of Lol p 1 used ($\mu\text{g/mL}$)			
	10^{-1}	10^{-2}	10^{-3}	10^{-4}
Dilution of serum used locally				
1:25	++	++	++	++
1:50	++	++	++	++
1:100	++	++	++	+
1:200	++	++	++	Neg
1:400	++	+	Neg	Neg
1:800	+	Neg	Neg	Neg
1:1600	Neg	Neg	Neg	Neg

+, 3-mm wheal or greater; ++, 5-mm wheal or greater; *Neg*, wheal not detectable or less than 3 mm.