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Regulation of FcεRI Expression during Murine Basophil Maturation: The Interplay between IgE, Cell Division, and FcεRI Synthetic Rate

Asifa K. Zaidi and Donald W. MacGlashan

Expression of FcεRI on basophils and mast cells is modulated by IgE Ab. Previous studies have noted in vivo receptor expression dynamics that are discordant with expectations derived from in vitro studies. The current study presents a formal hypothesis to explain the discordant observations and tests two assumptions that underlie a proposed model of receptor dynamics. After first showing that a murine model of receptor expression on basophils recapitulates observations made using human basophils, the effect of changes in IgE on basophil egress rates was examined. In the proposed model, egress rates from bone marrow (BM) were assumed to be unaffected by changes in IgE concentration. Egress was tested by examining the labeling of BM and peripheral blood (BL) basophils at various times after injection of BrdU with and without injection with IgE. The IgE Ab did not alter the appearance of BrdU label in peripheral BL basophils. In addition, BM and BL basophils were responsive to the elevations in IgE, with receptor expression increasing on BM basophils before BL basophils. It was also noted that BL basophils expressed ~50% of the receptor density of BM basophils. There was a 3-fold greater synthetic rate of FcεRI on BM basophils that readily explained the difference. These results provide support for the proposed hypothesis of rapid changes in receptor expression being controlled by cell replacement. The studies also support a model whereby receptor expression is limited by cell division and that basophils, once mature, slow their rate of receptor synthesis. 


The high-affinity IgE receptor expression on the cell surface of basophils is critical for Ag-specific activation because of the receptor’s ability to bind Ag-specific IgE (1). IgE Ab upregulates cell surface FcεRI expression on basophils both in vitro and in vivo (2) by stabilizing the receptor on the cell surface and inhibiting its internalization. Dissociation of IgE from its receptor causes internalization of the receptor (3). The expression of FcεRI on the cell surface depends on several factors, such as synthesis of FcεRIα, FcεRIβ, FcεRIγ, and free IgE levels (4). Several in vitro studies of transfected cells demonstrated the specific role of FcεRIα and FcεRIβ in regulation of FcεRI cell surface expression. For example, association of FcεRIα with newly translated FcεRIγ is necessary for transport of FcεRIγ to cell surface and FcεRIβ acts as better chaperone for FcεRIα (5). However, very little is known about the dynamics of receptor expression on basophils and presence of IgE during maturation. There are several experiments that have provided insights into the regulation of FcεRI expression by IgE; however, these data are derived from in vitro studies. Furthermore, these studies of receptor expression regulation by IgE are derived from terminally differentiated peripheral blood (BL) basophils.

The in vitro studies suggested that the dissociation of IgE from its receptor on basophils is a slow process with a half life (T½) of 10 d and is not effected by IgE trap, such as the anti-IgE Ab omalizumab (6). Similarly, dissociation of IgE from its receptor on human skin mast cells is also slow process with a half life (T½) of 9.8 d in vitro (7). However, phase I clinical trial data of omalizumab treatment demonstrated receptor loss on circulating basophils with T½ of 3 d (2). In contrast, Beck et al. (8) have shown that omalizumab treatment reduced FcεRI expression on tissue mast cells (10 and 90% reduction of receptor at days 7 and 70, respectively) slowly compared with circulating basophils (90 and 99% reduction of receptor at days 7 and 70, respectively). These observations were discordant unless another aspect to the in vivo biology of receptor dynamics is considered. It was proposed (9) that the rate of cellular replacement could resolve the discrepant observations. Peripheral BL basophils are thought to be replaced approximately every 12 h (10, 11) whereas mast cells like those in the skin are considered long lived with replacement times on the order of months (12–15). Free IgE levels that were quickly reduced to near zero by treatment with omalizumab would stop the accumulation of receptor in maturing basophils. Rapid replacement of the circulating cell compartment with cells that had not significantly upregulated FcεRI during maturation would give the appearance of a rapid loss of FcεRI in the BL compartment. In contrast, slow replacement of skin mast cells would lead to changes in receptor expression that more properly mirrored the dissociation rate of IgE in vitro and therefore lead to changes in receptor expression that required weeks rather than days. Although a quantitative model of this concept has not been previously presented, even the qualitative model contained some underlying assumptions about the parameters of cell replacement that should remain constant for the model predictions to be valid.

The model predictions would be valid if the following assumptions are valid: 1) the IgE level in BL and bone marrow (BM) is equal; 2) the change in IgE level does not change the maturation and egress rate of basophil; 3) the basophil transit time in circulation is very
short and unaffected by IgE levels; and 4) the maturation time of basophil in BM is less than the dissociation rate of IgE from FcεRI. In the original formulation of the model, cell division was not considered. Because it is apparent that immature basophils that express FcεRI divide during maturation, this aspect of the biology needs to also be considered for its effects on the dynamics of receptor expression. To test some of these assumptions, it is necessary to use an animal model. Hence, we used a murine model to test these assumptions. We have determined the effect of IgE on the survival and egress of BM and BL basophils using BrdU staining technique. We also determined the pattern of FcεRI expression levels during maturation and after egress of BM basophils and BL basophils, respectively. In addition, the original qualitative formulation of the model was made into an explicit model that incorporated the effects of cell division on FcεRI expression dynamics. This model aided in interpretation of some of the unexpected observations found in the murine experiments.

Materials and Methods

**Experimental**

**Mice.** BALB/c mice were purchased from The Jackson Laboratory (Bar Harbor, ME) and IgE knockout mice were a gift from Dr. P. Leder (Harvard Medical School, Boston, MA). They were maintained in the research animal facility, and experimentation was conducted in accordance with current Johns Hopkins Animal Care and Use Committee guidelines.

**Detection of mouse BM and BL basophils by flow cytometry.** Freshly isolated mouse femoral BM cells were depleted of RBCs using red BL lysing buffer (Sigma-Aldrich, St. Louis, MO). BM RBCs and neutrophils were removed by centrifugation of the BM on a 1.08 density percoll (GE Healthcare, Piscataway, NJ). Hence, reporting of total BM leukocytes does not include neutrophils. For flow cytometry, cells were blocked with anti-mouse CD23 (clone B3B4) and anti-mouse CD16/32 (clone 2.4 G2) mAbs for 20 min and then incubated with mouse IgE anti-DNP mAb (5 μg/ml) for 1 h on ice (16). After washing with PIPES buffer, cells were stained with FITC–anti-mouse IgE Ab and PE–anti-mouse IgG4b (clone DX5) Ab for 30 min on ice. In some experiments, FITC-conjugated anti-mouse FcRRIα cells as basophils were analyzed using FACSCalibur (BD Biosciences, San Jose, CA). At least 100 double-positive (FcεRI+ CD49b+) cells as basophils were analyzed to calculate the mean and median fluorescence intensity of FITC.

**Sorting and culture of BM basophils.** BM basophils were sorted using the FACSARia cell sorter (BD Biosciences) and gating for the FcεRI+CD49b+ cell surface phenotype. After blocking, BM cells were stained with biotin-labeled anti-FcεRIα/Alexa Fluor 647-streptavidin and PE-conjugated anti-CD49b. The purity of the sorted basophils was >95%. For measurement of cell proliferation, sorted basophils were stained with CFSE (1 μl of 1 mg/ml CFSE solution, and mice were killed at different time intervals to determine the density of FcεRI DURING MURINE BASOPHIL MATURATION

**Kinetics of BrdU uptake by total cells and basophils in BL and BM in vivo.** Mice were injected i.p. with 1 mg BrdU and killed after different time intervals, as indicated in Results. BM and BL cells were processed as described above. Cells were stained for BrdU incorporation according to the allylophocyanin–BrdU staining kit instructions from BD Pharmingen (San Diego, CA). Briefly, after following the initial procedures outlined above for labeling with anti-IgE–FITC and CD49b–PE Ab, cells were fixed and permeabilized. Cells were then incubated with DNase for 1 h at 37°C water bath. After washing, cells were stained with anti–BrdU–allophycocyanin Ab for 20 min. Cells were then washed and resuspended in PBS containing 3% FCS for flow cytometric measurement. Double-positive cells for FcεRI and CD49b were gated to determine the percentage of BrdU-positive basophils. The percentage of BrdU-positive total BM and BL cells were also determined.

**Determination of effect of IgE in vivo on egress of basophils.** To determine the egress of basophils, mice were injected i.p. with 100 μl 10 mg/ml BrdU solution, and mice were killed at different time intervals to determine the percentage of BrdU-positive basophils. Staining of BrdU-positive basophils was performed as described above. To determine the effect of IgE on the egress of basophils, mice were injected i.p. with mouse IgE anti-lansydil (5-dimethylaminonaphthalene-1-sulphonyl) mAb (clone; 27–74) or IgG2α mAb (100 μg in 200 μl) or PBS (200 μl) daily for 3 d. On the third day, mice were injected i.p. with 100 μl of 1 mg/ml BrdU solution, and mice were killed 24 or 48 h after BrdU injection. BM and BL cells were collected for analysis of the percentage of BrdU+ basophils determined by flow cytometry.

**Bioavailability of BrdU in BL after i.p. injection.** To determine how long BrdU is available to the cells, mice injected with 1 mg BrdU in 100 μl PBS i.p. were killed after 0, 0.5, 1, 2, and 3 h. PL plasma and BM cells were obtained as described in the previous section. We used the dividing KU812, basophilic cell line, as an acceptor for BrdU. KU812 cells, stained with CFSE (1 μM) as described previously (17) and cultured for 1 d to confirm that the cells were dividing (by determining the mean fluorescence intensity of CFSE by flow cytometry on days 0 and 1), were incubated for 30 min in CO2 incubator with BL plasma obtained from mice injected with BrdU. Cells were washed with staining buffer, fixed, and then permeabilized according to the instructions in allophycocyanin-BrdU staining kit. The uptake of BrdU was measured by staining with anti-BrdU allophycocyanin Ab and flow cytometry measurement was performed. To confirm that there was uptake of BrdU, BM cells from same mice were also stained in a similar way for BrdU.

**Determination of FcεRI expression on basophils by IgE elution method.** To determine the density of FcεRI on BM and BL basophils, we first estimated the density of FcεRI expression on BM-derived mast cells (BMMC) by dissociation of IgE in acidic citrate stripping buffer (0.05 M sodium acetate, 0.085 M NaCl, 0.01 M EDTA, and 0.03% HSA [pH 2.9]) (18). The eluted IgE concentration was estimated by ELISA. BMMC were obtained by culture of murine BM cells in the IMDM containing 10% FCS, 10 ng/ml IL-3 and 10 ng/ml SCF for 6 wk with a change of medium every week. After 6 wk of culture, BMMCs were blocked with anti-mouse CD23 (clone B3B4) and anti-mouse CD16/32 (clone 2.4 G2) mAbs for 20 min and then sensitized with IgE for 1 h on ice. Cells were washed with buffer and then washed with isotonic saline once. Cells were first resuspended in 25 μl isotonic saline, followed by 200 μl ice-cold acetate buffer, and after 10 min of incubation on ice, supernatant recovered. The pH of the recovered supernatant was restored by adding 13.8 μl of 10× PIPES buffer and then 6.8 μl NaOH during gentle vortexing. The IgE eluted supernatants were supplemented with 50 μl of 4% BSA in PBS and were stored at −70°C until ELISA for IgE estimation.

**ELISA**

IgE was estimated after elution from BMMC by ELISA as per the BD OptEIA kit from BD Biosciences.

**Statistical analysis**

Statistical analysis was performed with Student t test (data in Figs. 4, 6, and 9). In general, the results of replicate experiments were expressed as mean or median plus or minus SEs of the mean or median.

**Simulation**

IgE regulates the expression of FcεRI by protecting the constitutively expressed receptor from endocytosis. This process has been modeled previously (19). It is described by the following coupled relationships:

\[
\frac{dR_{O}}{dt} = - V_{R} \left( \frac{R_{O}}{R_{O} + k_{RO}} \right) + L + k_{R} R_{F} - k_{J} R_{O} \text{IgE} \\
\frac{dR_{F}}{dt} = - k_{J} R_{E} + k_{J} R_{O} \text{IgE}
\]

where: \( R_{O} \) = unoccupied receptors; \( R_{F} \) = occupied receptors; \( \text{IgE} \) = solution IgE concentration; \( L \) = (loading), \( L \) is a constant synthesis and expression rate; \( V_{R} \) = maximum rate of removal; \( k_{RO} \) = concentration of unoccupied receptors for half maximal rate; \( k_{J} \) = forward rate binding constant for IgE binding to FcεRI; and \( k_{J} \) = reverse rate binding constant for IgE dissociation from FcεRI.

For the purposes of the current simulation, receptor binding does not decrease the free IgE concentration, although this was allowed in the previous modeling (19). The original model was developed for a non-dividing mature peripheral BL basophil. However, in the BM, immature basophils accumulate receptor but are dividing. Therefore, overlaid on the nondividing kinetics, the simulation divides the accumulated receptor by one-half each division time (T0). An additional component of the simulation is to define a time when the dividing basophil stops dividing (Tm = mature). We can speculate that at the point of the last division, the cell can be declared mature and that it resides in the BM for a defined amount...
of time before leaving the BM; this time period is labeled as $T_1$. After this time, the cell leaves the BM for the circulation and spends some time ($T_2$) in the circulation before dying or possibly, entering some tissue (e.g., the spleen). Fig. 1A summarizes the relationships between the various times used and varied in the simulation. There is one additional time to consider, the time when free IgE is altered. The dotted line in Fig. 1A shows where free IgE became relevant once the cell experiences an environment where free IgE is 0 (not shown is the small loss that follows the slow dissociation of IgE from existing receptor). The absissa for the plot in Fig. 1A represents number of cell divisions. Because $T_D$ is a parameter that is varied, for convenience, the data are expressed in units of division time. The general characteristics of the simulation remain similar as $T_D$ is varied, although absolute values of receptor expression change as $T_D$ is varied.

From the perspective of the experimentalist, where only the circulating basophils are monitored in humans, any abrupt change in free IgE at $T_3$ can only be observed at $T_P$, and thus, if only one cell were being monitored, one would have to wait for $T_F-T_S$ to see that particular cell. Of course, the BM is composed for a continually renewing source of basophils at all stages of maturation, so $T_S$ is occurring at points throughout the temporal range shown in Fig. 1. Therefore, a plot of $T_F-T_S$ shows what is expected for basophils appearing in the BL having experienced the change in IgE at the time before egress of $T_F-T_S$. This is the cell replacement process that explains the receptor loss curve. As will be discussed in Results, we also allowed the simulation to alter the constitutive loading rate of receptor in the period between $T_S$ and $T_E$. For these studies, free IgE could not be manipulated with an Ab like omalizumab, so mice were injected with IgE. The half-life of this IgE is considered quite short, but it is not a step function. Therefore, to better understand how this pulse of IgE affected the kinetics of receptor expression in the two compartments, the simulation superimposed a narrow rise and fall in IgE concentrations, and changes in receptor expression were followed. The IgE pulse followed the following kinetics:

$$IgE(t) = A(e^{-kt_1}) \left(1 - e^{-kt_2}\right) + B.$$  (3)

In this case, $A$, $k_1$, and $k_2$ were adjusted to generate a peak of IgE and a rise and decay rate that approximated the data found in mice. $B$ is a denogenous baseline IgE level.

To determine $T_F-T_S$ curves, multiple simulations were run, varying $T_S$, $T_E$, and $T_D$, changes in $L$ (constitutive synthetic rate of receptor) in BM basophils waiting for egress or changes in $V_R$ (maximum receptor removal rate). In addition, BM is not a homogeneous synchronized population of cells. In the experimental study, only average data were analyzed from flow cytometry. Therefore, an average of many simulations representing a BM population was needed. Rather than run multiple simulations sequentially, the software was designed to simulate a population of "cells," each running the same receptor accumulation model. As shown in Fig. 1B, the simulation starts with no "cells," and with a probability, $p_1$, at each increment in the simulation clock, a "cell" is created. Each cell created begins running the simulation clock, a "cell" is created. Each cell created begins running the simulation clock. In the software, the cell is a data structure with a set of Boolean data fields that indicate whether the cell has transitioned to the next step in its "development." Once the "mature" field is set for the basophil, the software monitors receptor accumulation for a period of time defined by $T_F$, before setting the field that the cell is now declared to be "circulating." In either period (between $T_M$ and $T_C$ or after $T_C$), these fields are used by the software to possibly alter the characteristics of the receptor accumulation model, as will be discussed in Results. The fields also allow for the software to extract information for the two types of basophils, BM versus circulation. For most of the plots shown in Results, the average receptor expression in these populations of parallel simulations is shown. To round out the simulation, another data field that is associated with each cell is the amount of BrdU incorporated at each division. The BrdU concentration that determines the amount of incorporation at each division is in arbitrary units but follows a pulse shape like that shown in Equation 3, where $k_1$ and $k_2$ were adjusted to approximate the BrdU pulse shape determined experimentally; a pulse that decayed with a half-life of 30 min (in this case, Equation 3, $B = 0$). Before simulating the effects of an IgE change or BrdU incorporation, the simulation was allowed to run until a relatively stable number of cells was obtained, usually 12–20 divisions. Table 1 lists the values of the various parameters. $T_M$ and $T_F$ vary according to the values $p_2$ and $T_T$ ($T_F = T_M + T_T$). Typically, $T_M$, on average, is 5.5 divisions.

### Results

#### Model

**Embedded Receptor Expression Model.** The underlying receptor accumulation model is dynamic but it has been shown that it can be reduced to two possible steady states. If no IgE is present, the steady-state value of receptor expression ($SS_{NE}$) is given by:

$$SS_{NE} = R_T \left( \frac{L/R}{1-L/R} \right)$$  (4)

where $L$ is the synthetic rate (loading rate), $R$ is the removal rate, and $R_T$ is the threshold constant for removal.

The second possible steady state is in the presence of IgE ($SS_E$). It is given by:

$$SS_E = SS_{NE} \left(1 + (K(IgE)/(1+c)) \right)$$  (5)

where $K$ is the affinity constant between receptor and IgE, $IgE$ is the free IgE concentration, and $c$ is a variable that is related to cell concentration, $SS_{NE}$, and a constant (19). However, it is difficult for the value of "c" to exceed very small numbers, so "c" is essentially 0 and the equation reduces to:

$$SS_E = SS_{NE} \left(1 + (K(IgE)) \right)$$  (6)

An example is useful. Previous in vitro studies with human basophils found that $L = 500$ (molecules/cell/hour), $R = 2000$ (molecules/cell/hour), $R_T = 25,000$ molecules/cell, which leads to $SS_{NE} = 8333$ receptors/cell (a value that is found experimentally in vivo as well (19)). In humans, the affinity constant $K$ is approximately $3 \times 10^{10}$ (forward rate $= 3 \times 10^4$ $M^{-1} s^{-1}$, reverse rate $= 1 \times 10^{-6}$ $M^{-1}$) (19) (see also Table I). At 500 ng/ml IgE $(2.5 \times 10^{-8} M)$, then $SS_E$ is 76 times greater than $SS_{NE}$. This would be a value of $\sim 630,000$ receptors (also an expression level that is plausible in humans (20)). An important caveat is that at the loading rate of $L = 500$ (2), it would require 2–3 mo to achieve this steady-state value. Because the available evidence doesn’t support BM residence of several months, in vivo receptor accumulation must be much faster. In addition, the plateau may never be reached; based on studies of omalizumab (2), the ratio of $SS_E$ to $SS_{NE}$ is closer to 15–25.

**Receptor model behavior with cell division.** An examination of the accumulation of receptors in the presence of IgE shows that for periods of time long before reaching the steady state ($SS_{NE}$), that the accumulation is essentially linear. This is observed in in vitro cultures of human basophils (19). But with cell division included in the model, it is apparent that linear accumulation does not occur (certainly not for individual cells (see Fig. 1) and not for the population average). Indeed, cell division imposes a new kind of steady state on the accumulation of any cell molecule that is not otherwise constrained by other mechanisms. This certainly applies to FcγRI.

An example is useful. Previous in vitro studies with human basophils found that $L = 500$ (molecules/cell/hour), $R = 2000$ (molecules/cell/hour), $R_T = 25,000$ molecules/cell, which leads to $SS_{NE} = 8333$ receptors/cell (a value that is found experimentally in vivo as well (19)). In humans, the affinity constant $K$ is approximately $3 \times 10^{10}$ (forward rate $= 3 \times 10^4$ $M^{-1} s^{-1}$, reverse rate $= 1 \times 10^{-6}$ $M^{-1}$) (19) (see also Table I). At 500 ng/ml IgE $(2.5 \times 10^{-8} M)$, then $SS_E$ is 76 times greater than $SS_{NE}$. This would be a value of $\sim 630,000$ receptors (also an expression level that is plausible in humans (20)). An important caveat is that at the loading rate of $L = 500$ (2), it would require 2–3 mo to achieve this steady-state value. Because the available evidence doesn’t support BM residence of several months, in vivo receptor accumulation must be much faster. In addition, the plateau may never be reached; based on studies of omalizumab (2), the ratio of $SS_E$ to $SS_{NE}$ is closer to 15–25.

**Cell Cycle**

<table>
<thead>
<tr>
<th>Cell Cycle</th>
<th>Receptor Accumulated</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>L</td>
</tr>
<tr>
<td>1</td>
<td>L/2 + L</td>
</tr>
<tr>
<td>2</td>
<td>L/4 + L/2 + L</td>
</tr>
<tr>
<td>...</td>
<td>...</td>
</tr>
</tbody>
</table>
| N          | $\Sigma L/2^n$       | (7)
This infinite sum would converge on $2L$, where $L$ is the amount of receptor loaded in a single cell cycle. Fig. IA shows the patterns for single cells, and the dotted line follows the peaks in the saw tooth pattern so the plateau can be visualized.

Additional issues addressed using the model

Several questions were asked of the simulations:

1) How does the inclusion of a cell division component alter our understanding of receptor expression dynamics and what may be happening during basophil maturation?

2) How does the addition of cell division into the simple model previously proposed (19) alter the analysis and interpretation of the receptor loss rate during the period of time following a precipitous drop in free IgE level (as found when patients are placed on omalizumab treatment)?

3) Are there useful characteristics of BrdU labeling and receptor expression kinetics that help dissect what happens during maturation?

4) Based on current understanding of receptor expression dynamics, what are the expected characteristics of expression following an artificial rapidly transient increase in serum IgE?

1) Receptor loading ($L$), without division, is essentially linear and accumulates at $L \times N$ (N is the number of division cycles). Receptor accumulation with division accumulates to a maximum of $2L$, so for $n > n_0$, the effect of cell division is to blunt the final amount of receptor accumulation by $2/N$ ($2L/LN$). For example, for eight divisions, cell division suppresses the amount of receptor loaded to $\sim 25\%$ of loading without division. This simple result has interesting implications on how the process must work, because in humans, the ultimate loading of receptor is 10–25 times the SSNE. It raises questions about how many divisions must occur, the required loading rates to achieve 10–25 times SSNE, and whether there are periods during maturation, before circulation, that the basophil doesn’t divide. In other words, cell division alters the relationship between SSNE and SSNE. By imposing an additional form of steady state on the process of FcεRI accumulation, the ratio of SSRI and SSNE expected for nondividing cells is suppressed. For any given final expression level, the loading rate per cell division must be 50% of the final density. For example, to achieve a final density of 500,000 receptors, the cell needs to load 250,000 receptors in one cycle, a rate 25 times faster than in vitro studies have found for human basophils in culture. IgE still controls the rate, but the synthetic rate is rapid.

2) Despite the saturating (inverse logarithm) nature of the whole population accumulation curve (Fig. 1A), a rapid reduction of IgE will continue to generate a receptor loss kinetics for cells in circulation that is initially a linear decline. Without cell division, the model shows that the decrease in circulation, when IgE is reduced to 0, is indeed a mirror reflection of the linear accumulation during maturation (i.e., the slope of loss = $-1 \times$ the slope of accumulation [data not shown]). With cell division, the slope is more reflective of the average slope across multiple cell divisions. The precise slope is more ambiguous without further information about the number of cell divisions, but it is still faster than would be predicted from the rate of IgE dissociation. The central characteristic of the model, irrespective of precise nature of up-regulation, is that apparent receptor loss in circulation reflects an inversion of the receptor accumulation curve. Fig. 2A shows the expected decrease in receptor expression if there was no cell replacement (i.e.,
just natural loss due to IgE dissociation and the intrinsic receptor loss mechanisms) and the loss rate in the cell division model (with replacement of circulating cells with new basophils). The gray “envelope” shows the range of possible decay curves that depend on the average number of cell divisions experienced in the BM.

The primary question that initiated this study was whether a change in the rate of egress or maturation time of basophils in the BM would be able to account for, or significantly modify, the rapid apparent loss of receptor expression in circulating basophils when IgE levels were decreased to 0. Experimentally, the rate of receptor loss in vivo in circulating cells is 4-fold faster than would be expected from in vitro measurements. The model shows that cell cycle division rates and/or egress rates would have to increase by ∼6 fold to alone explain this drop in receptor expression. For example, if normal division times were 24 h, receptor expression would fall if cell division time were induced to decrease to 4 h, even without a change in serum-free IgE concentration (line a in Fig. 2B). This period seems too brief to allow differentiation, but T cells have been observed to decrease their division time from 24 to 5 h (21). In addition, a change in cell division time would alter the rate of decrease in receptor expression that occurred when free IgE levels decreased to 0 (and, therefore, also affect receptor-cycling dynamics). For example, using the kinetics shown in Fig. 2A as a starting point, imposing a 4 fold change in cell division rate, increases the rate of apparent receptor loss ∼3 fold (compare lines b and d in Fig. 2B). A decrease in TD also shifts the apparent rate of receptor loss, although the effect is more modest, ∼1.5-fold change in rate (compare lines b and c in Fig. 2B) for TL decreasing from 24 to 0 h. Therefore, with this model, alterations in cell division time and egress rates could influence the apparent loss of receptor expression, emphasizing the need to explore changes in BrdU labeling kinetics.

3) If BrdU is added in a pulse that has a decay rate of 30 min (see Fig. 5), the time of appearance of BrdU in circulating basophils depends on the length of time between the last division and egress to the circulation. If the egress occurs immediately after the last division that defines a mature basophil, then the appearance of BrdU labeling in circulation is rapid. Immediate egress after the last division influences average receptor expression in the circulation; the average receptor in circulation might be lower than observed in the BM. The similarity of receptor expression in peripheral BL relative to BM would depend on the amount of time the basophil circulates and whether the cell continues to accumulate receptors while circulating. If there is a lag in time between the last division and egress to the circulation, then BrdU labeling will appear at a rate that depends on the relative lag time and the division time but the two parameters are not independently determined by this methodology. A change in either division time or lag time moves the time point for the peak in the circulation BrdU percent positivity. The peak fraction of cells labeled with BrdU will occur within one-fourth division time for immediate egress and one-fourth division time plus lag time if there is a lag time. The most
obvious characteristic for the presence of a lag time is an early period where no labeling appears (relative to the peak). The breadth of the kinetic curve of BrdU labeling is dependent on the pulse width of BrdU, the rate of division in the BM and the number of divisions a basophil experiences in the BM. The change in BrdU labeling in the circulating compartment would appear as shown in Fig. 2B (no time lag versus a one-division period time lag). The percent positivity, the peak of the curves, depends on the amount of nonspecific binding and where one arbitrarily sets a threshold; the useful measure from these curves is the timing of the peak.

4) Described in the section Simulation of a transient increase in IgE.

**Basophil detection**

In pilot studies, we found that anti-mouse FcεRIα Ab (MAR-1 Ab) does not detect FcεRI that is occupied by IgE. As shown in Fig. 3A, we noted that detection of FcεRI by MAR-1 Ab does not show a difference in expression of FcεRI even after 100 μg of IgE-i.v. injection, which (see below) upregulates FcεRI expression both in vivo and in vitro. Using BMMCs, with or without sensitization with IgE, we verified that MAR-1 Ab binding was blocked with full sensitization (Fig. 3B). Therefore, in subsequent studies, we used anti-IgE to detect FcεRI expression, and cells were sensitized with IgE to fully load receptors before detection with anti-IgE Ab. As shown in Fig. 3C, basophils were gated as double positive FcεRI+/CD49b+ cells by flow cytometry. Receptor levels in these mice were calibrated to absolute numbers using the technique we have used to study human basophils. The anti-IgE Ab used to measure receptor expression was calibrated on murine BMMCs. The mast cells were sensitized with IgE, washed well, and counted, and the IgE was dissociated by weak lactic acid. The eluted IgE concentration was measured in an ELISA and together with the cell count and parallel flow cytometric measurements led to a rough calculation of 830 mean fluorescence intensity units (by flow cytometry) to be equivalent to 110, 000 molecules of IgE/basophil.

**Mouse model recapitulates human IgE/receptor downregulation on basophils**

In human studies, two observations characterize receptor loss during treatment with omalizumab: 1) slow dissociation of IgE and loss of FcεRI expression on basophils in vitro, and 2) rapid loss of FcεRI expression following rapid decrease in free IgE in vivo. These observations motivated the proposed explanation of receptor loss by cell replacement. We asked whether these two characteristics could be recapitulated in the mouse model. First, the loss of receptor in vitro should be slow (reflecting a slow dissociation of IgE). Fig. 4A shows that for both BL and BM-derived basophils, there was little or no loss of FcεRI expression after an overnight culture in the absence of IgE. It is difficult to detect basophils in culture after 24 h; hence, data are presented for only 24 h of culture. In contrast, there is a rapid loss of receptor expression in vivo (Fig. 4B). There is no available mouse equivalent of omalizumab, but it is known that IgE is rapidly lost in circulation after injection. Instead of using anti-IgE Ab to rapidly decrease free IgE, we used IgE knockout (IgE−/−) mice, injected 100 μg of IgE-i.v. and followed how rapidly IgE falls and monitored the effect on FcεRI expression. Fig. 4B shows that IgE levels were very transient. After an injection that resulted in at least 1 μg/ml serum IgE, 10 min postinjection, there were very low levels after 24 h. To be consistent with this result, the half-life was estimated to be <3 h. The mathematical model presented, which includes the concept of cell replacement, predicted several features for this experiment. Some of these will be discussed further below, but one feature was that after the peak in FcεRI expression, receptor expression should rapidly settle back to preinjection levels. The experiment showed (Fig. 4B, also see Fig. 2D) that following the peak of expression, FcεRI expression fell with a “half-life” of ~24 h. These data suggest that the mouse model recapitulates the kinetics observed in human studies (2, 8, 9).

**Basic BrdU characteristics**

To interpret the results of a BrdU study, the temporal characteristics of BrdU bioavailability after i.p. injection were explored. As a rough assessment, serum was obtained from mice after injection and included in a culture of KU812 cells. The relative labeling of KU812 cells that divided during a 0.5-h period was assessed. Fig. 5A shows that the bioavailability was short-lived, as found in other studies (22, 23), with a T1/2 of decay of ~30 min. Fig. 5B demonstrates that BrdU was rapidly incorporated into BM cells, both total cells and FcεRI+/CD49b+ basophils, although basophils labeled somewhat more slowly. When expressed as median fluorescence of BrdU (Fig. 5C), it is apparent that there is an impulse that decays with a T1/2 of ~2 d for total BM cells and 1 d for FcεRI+/CD49b+ basophils. Coupled with the very short impulse of BrdU bioavailability, these results suggest that FcεRI+/CD49b+ basophils divide approximately once per 24 h. To support this interpretation, FcεRI−/CD49b− basophils from BM were sorted, labeled with CFSE, and cultured in the presence of 10 ng/ml IL-3 for 96 h, with sampling every 24 h. Fig. 5D shows the appearance of CFSE labeling at 96 h. Analysis of the distribution supports a division time of 24 h.
To test the effect of IgE on egress time, BrdU labeling for 24 h cultures of BM basophils with and without IL-3 was injected into mice using an IL-3/anti–IL-3 Ab mixture to sustain the presence of IL-3 (data not shown). To further examine the influence of IL-3, IL-3 was injected into mice using an IL-3/anti–IL-3 Ab mixture to sustain the presence of IL-3 (24–26). Receptor expression in both BL and BM basophils did not change (Fig. 9B). Similarly, receptor expression in BM basophils (FceRI+ / CD49b+ cells) also correlated to serum IgE levels and were rapidly responsive to the injection of IgE (Fig. 7B). Within 24 h, receptor expression on BM or BL basophils increased ∼4-fold in mice loaded with IgE, relative to the IgG2a-injected controls. As shown in Table II, using the calibration discussed above, we calculated the FceRI expression on BL and BM basophils in wild-type and IgE−/− mice with and without IgE-i.v.

An unexpected observation was that BL basophils expressed ∼50% of the receptor observed on BM basophils, and this difference exists in both control and IgE-i.v.-treated mice. As discussed in the modeling background (methods–analytical framework), cell division results in a steady state level of expression that in combination with the logarithmic accumulation of older cells, generates a narrow distribution of expression that is at a steady-state value. Therefore, the 2-fold difference in expression between BM and BL basophils was not expected unless certain conditions were met. For example, if prior to egress, the basophil divides one last time but either leaves the BM immediately after division or resides in the BM for a period of time but does not accumulate additional receptors, then a 2-fold difference might be expected. The BrdU studies indicate that there is a 24- to 36-h lag between the injection of BrdU and its appearance in BL basophils; therefore, the hypothesis that after their last division, the basophils immediately leave the BM appears false. To test whether mature circulating basophils can accumulate receptor, mixed peripheral BL leukocytes and BM cells were cultured for 24 h in the presence of IgE (Fig. 8A). A difference in receptor accumulation rate was found. Fig. 8B shows that in BL basophils, receptors accumulated at an absolute rate of 1100/h in the presence of 1 μg/ml IgE. In contrast, BM basophils accumulated receptor at a rate of 3000/h under the same conditions. As noted in the modeling section, the rate of receptor accumulation in a mature cell only needs to be ∼2- to 3-fold slower to explain the 2-fold difference in expression between BL and BM basophils as a result of the steady-state nature of the process.

Effect of IL-3 on receptor expression

It could be argued that the rate of receptor accumulation for the BL basophil is compromised in the in vitro culture because of the absence of cytokines like IL-3 to reverse apoptosis. Therefore, the experiment shown in Fig. 8A was repeated with the inclusion of 10 ng/ml IL-3 (Fig. 9). Relative to the absence of IL-3, the rate of accumulation of FceRI (1100/h) did not change for BL basophils but was altered in the BM basophils (Fig. 9A). The inclusion of IL-3 altered the level of receptor in the 1-d culture without IgE. The receptor accumulation rate, comparing day 1 with or without IgE was ∼2300/h. The cause of the difference between day 0 and day 1 +IL-3 (no IgE) might have been due to IL-3 altering cell division in the BM cells. However, no difference was found in BrdU labeling for 24 h cultures of BM basophils with and without IL-3 (data not shown). To further examine the influence of IL-3, IL-3 was injected into mice using an IL-3-anti–IL-3 Ab mixture to sustain the presence of IL-3 (24–26). Receptor expression in both the BL and BM basophils did not change (Fig. 9B).

Parenthetically, the rate of receptor accumulation on BM basophils, in the presence of 1 μg/ml IgE would predict an accumulation of 75,000 receptors/d. If the cell division rate is 24 h, as found experimentally, then the model would predict ∼2 times this value as the maximum level of receptor expression. Table II shows that in mice injected three times with IgE, receptor expression showed a median level of expression of 218,000/cell.
Simulation of a transient increase in IgE
Without a tool like omalizumab to rapidly suppress free IgE in the mice, the experiment shown in Fig. 4 relied on the rapidly transient increase and decrease in injected IgE to explore the dynamics of receptor expression under conditions of cell replacement. However, it was useful to also explore how the model behaved under these conditions. Fig. 2 shows three features predicted by the model in this type of experiment. Note that the time points chosen to be highlighted by symbols at points along the gray curves are roughly similar to the time points used in the experimental study but also highlight features of this data set. The model used our experimental observations about the time lag in the BrdU labeling and the 66% decrease in synthetic rate of receptor in the “mature” basophil relative to the maturing BM basophil. The first feature is the difference in the absolute level of receptor expression between BM and BL basophils. This difference is the result of a difference in the absolute synthetic rate postulated for an immature and mature basophil. In other words, if the model incorporates a reduction in receptor synthesis when the basophil reaches its final mature state prior to egress, there will be a difference in expression of receptor in circulating basophils and BM basophils. The magnitude of the difference in loading rate does not need to be marked to observe a 1.5- to 2-fold difference in the two populations, because receptor expression remains a steady-state problem of both loading and loss by endocytosis. Without the steady-state aspect, when the immature basophil divides for the last time (halving the receptor number for each daughter cell), a 2-fold difference between BM and BL basophil would require no further synthesis of the receptor. But considering that the steady state does continue to define the expression levels, only a 2- to 3-fold reductions in loading rate are necessary to observe a 1.5- to 2-fold reductions in BL basophil expression level for simulated single cells. Alternatively, an increase in the removal rate of unoccupied receptor could change the accumulation rate but the change in needs to be large (10-fold). Even with 10-fold increases, the influence of increasing receptor loss is often blunted because under most circumstances, receptor is already occupied with IgE. This leaves only newly synthesized receptor subject to the effects of increasing the rate of endocytosis.
The second feature is that the time of peak up-regulation of receptor is different for BM and BL basophils, with the peak for BM occurring approximately one cell division time prior to the BL peak. The BL basophil peak lagging behind the BM basophil peak is also a result of the difference in receptor loading rate between “mature” and “immature” basophils. Without a difference in loading rate, the difference between BM and BL would be difficult to discern because the model predicts that one would see a closely spaced biphasic peak for the BL basophils that experimentally would look like a peak at the point observed for BM cells (data not shown). In Fig. 2D, the gray line that represents the model output at many time points (rather the selected points that are chosen to highlight points of interest) shows this initial but modest first peak for the condition where the loading rate in the “mature” basophil is one-third the rate for BM basophils (see below). With the coarse intervals of measurement used experimentally, this subtly in the kinetics for BL basophils is lost, appearing only as a slow rise in receptor expression.

The feature is that the time of peak up-regulation of receptor is different for BM and BL basophils, with the peak for BM occurring approximately one cell division time prior to the BL peak. The BL basophil peak lagging behind the BM basophil peak is also a result of the difference in receptor loading rate between “mature” and “immature” basophils. Without a difference in loading rate, the difference between BM and BL would be difficult to discern because the model predicts that one would see a closely spaced biphasic peak for the BL basophils that experimentally would look like a peak at the point observed for BM cells (data not shown). In Fig. 2D, the gray line that represents the model output at many time points (rather the selected points that are chosen to highlight points of interest) shows this initial but modest first peak for the condition where the loading rate in the “mature” basophil is one-third the rate for BM basophils (see below). With the coarse intervals of measurement used experimentally, this subtly in the kinetics for BL basophils is lost, appearing only as a slow rise in receptor expression.

**Table II. FcεRI expression on wild-type and IgE<sup>−/−</sup> BL and BM basophils**

<table>
<thead>
<tr>
<th>Time After IgE i.v. (h)</th>
<th>IgE i.v.</th>
<th>Tissue</th>
<th>Wild-Type FcεRI (&lt;i&gt;×&lt;/i&gt; 10&lt;sup&gt;3&lt;/sup&gt;)</th>
<th>N</th>
<th>IgE&lt;sup&gt;−/−&lt;/sup&gt; FcεRI (&lt;i&gt;×&lt;/i&gt; 10&lt;sup&gt;3&lt;/sup&gt;)</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>— —</td>
<td>— —</td>
<td>BL</td>
<td>29.99 (8.5–90)</td>
<td>38</td>
<td>5.60 (3.6–20)</td>
<td>9</td>
</tr>
<tr>
<td>— —</td>
<td>— —</td>
<td>BM</td>
<td>53.28 (14–130)</td>
<td>39</td>
<td>24.12 (12–112)</td>
<td>13</td>
</tr>
<tr>
<td>24 +</td>
<td>+</td>
<td>BL</td>
<td>132.9 (105.8–152.8)</td>
<td>4</td>
<td>83.25 (53.6–112.9)</td>
<td>2</td>
</tr>
<tr>
<td>24 +</td>
<td>+</td>
<td>BM</td>
<td>218.40 (185.0–239.1)</td>
<td>4</td>
<td>232.10 (207.3–256.8)</td>
<td>2</td>
</tr>
<tr>
<td>48 +</td>
<td>+</td>
<td>BL</td>
<td>105.8 (97.1–156.4)</td>
<td>3</td>
<td>107.00</td>
<td>1</td>
</tr>
<tr>
<td>48 +</td>
<td>+</td>
<td>BM</td>
<td>134.4 (130.8–182.1)</td>
<td>3</td>
<td>107.00</td>
<td>1</td>
</tr>
</tbody>
</table>

Wild-type and IgE<sup>−/−</sup> mice i.v. injected with PBS (−) or 100 μg of IgE in 200 μl of PBS (IgE i.v.) daily for 3 d (+) and BL and BM basophils were isolated after 24 h of IgE i.v., and estimation for FcεRI expression was performed by flow cytometry as described in Materials and Methods. FcεRI expression was shown here as median with range in parenthesis.
FIGURE 7. Differential FcεRI expression in BL and BM basophil in vivo. A, BALB/c mice were killed, and BL was collected from heart. After separation of plasma for IgE estimation, RBCs were removed by percoll gradient. Cells were then blocked with anti-CD-16/32 (2.4G2) and anti-CD23 (B3B4) Ab and incubated with 5 μg/ml IgE for 1 h on ice. Cells were washed and then were stained with PE-conjugated anti-mouse CD49b (DX5) and FITC-conjugated anti-mouse IgE Ab for 30 min on ice. Basophils (FcεRI⁺CD49b⁺) were analyzed by flow cytometry. Mean fluorescence intensity of FITC was converted to number of receptors per basophil versus IgE (estimated by ELISA in plasma) was plotted (n = 21). B, Mice were i.v. injected with either 200 μl of PBS (−IgE-i.v.; n = 29) or 100 μg of IgE in 200 μl of PBS (+IgE-i.v.; n = 7) daily for 3 d. Mice were killed after 24 h, and FcεRI expression was measured as median fluorescence of FITC of BL and BM basophils (FcεRI⁺CD49b⁺), and afterward, conversion to number of receptors per basophil was plotted. Mean number of receptors per basophil ± SEM was written above each group.

The third feature is that following the peak, only 1.5 to 2 division-equivalent time cycles are needed to observe a 50% decrease in receptor expression. For example, if a division cycle requires 24 h, then the peak for BM occurs at 1 d, the peak for BL occurs at 2.5 d and by the day 4–5, receptor expression in the BL basophil is approximately one-half of the peak.

Discussion

The rapid loss of receptor expression observed for peripheral BL basophils in patients treated with omalizumab is not understood except in theoretical terms. And yet, provided certain assumptions are met, it may provide a useful tool toward exploring receptor behavior on basophils in vivo. The issue to understand is the reason for the difference in the rate of receptor loss in vitro (T1/2 of 13 d) versus the rate of apparent loss in vivo (T1/2 of 2–3 d) following a marked reduction in free IgE. In a 2004 letter to the editor (9), it was first proposed that the discrepancy could be explained by the in vivo decrease resulting from basophil replacement rather than a difference in receptor handling. This explanation was kept fairly abstract but did not explicitly discuss the influence that cell division may have on the analysis. This is considered in the more explicit model presented in this article. In addition, the current study examines two assumptions that were part of the original and current formulation. The first is the assumption that IgE is present in the BM environment during leukogenesis. The second is that IgE does not alter the egress time of maturing basophils from the BM. With respect to the first assumption, it is apparent that in mice, both BM and circulating basophil receptor expression correlates with serum IgE. In addition, the injection of IgE-i.v. leads to a rapid change in receptor expression on BM basophils (Fig. 7B). If IgE were not present in the interstitial fluids of BM, then the only way the cells could sense the injected IgE is to have come from a non-BM site where IgE was present and then reside in the BM for only a short period of time. There has been speculation that some BM basophils are derived from a splenic basophil progenitor that is already expressing receptor (27). However, even if this did occur, there are two counterarguments. The first is that splenectomy does not eliminate all basophils from the BM. Second, because the cell is dividing with a 24-h cycle, any residence time >24 h in an environment without IgE would blunt the increase in receptor expression because of poor reaccumulation after division (see Simulation in Materials and Methods). Because it appears that basophils reside for >24 h in the BM, this argues against the BM being an IgE-free site. The experimental maneuver of injecting IgE also is predicted to generate an increase in FcεRI expression in the BM prior to the BL if IgE is present in the BM (Fig. 4B). Because the experiment in mice recapitulated the model prediction, this is further support for the presence of IgE concentrations in the BM that are equivalent to those in BL. Previous studies of murine basophils have also noted the responsiveness of BM basophils to IgE (16).

The second issue that was directly addressed is whether there are changes in the egress time for basophils that are dependent on the presence or absence of IgE. As shown in Fig. 6, BrdU labeling of the circulation pool of basophils wasunaltered by the injection of IgE, and this was true in both wild-type and IgE knockout mice. There is no indication in these experiments that IgE changes the division,egress rate of basophils in/from the BM.

There was one unexpected observation regarding basophil FcεRI expression. In every situation where FcεRI expression on basophils in the BM and peripheral BL was examined, there was an ~2-fold lower expression of receptor on the peripheral BL.
basophils (Fig. 8B). This observation could result from several possible behaviors. For example, if the circulation time of basophils is short, then a BM basophil that divided and immediately left the BM would appear to express $\approx$-fold less receptor (there not being enough time in circulation for the normal accumulation to occur). However, the BrdU studies indicate a significant lag in not being enough time in circulation for the normal accumulation to occur). Mean fluorescence FITC and mice were killed after 3 d of injection. FcεRI expression on basophils (FcεRI $^{CD49b^+}$) were estimated as mean fluorescence of FITC on day 0 (without incubation) or on day 1 (after 24 h of incubation) and plotted as number of receptors per basophil $\pm$ SEM ($n = 7$). B. Mice were i.v. injected with 200 μl of PBS (−IL-3-i.v.) or 10 μg of IL-3 plus 5 μg of anti–IL-3 Ab in 200 μl of PBS (+IL-3-i.v.), and mice were killed after 3 d of injection. FcεRI expression on BL and BM basophils was estimated by flow cytometry. Mean fluorescence FITC of basophils were converted to number of receptors per basophil $\pm$ SEM and plotted from three experiments, and differences between −IL-3 versus +IL-3 group are statistically nonsignificant determined by Student $t$ test.

The effect of IL-3 on receptor expression in vitro and in vivo was examined. By and large, IL-3 had little effect on receptor expression in vivo and in vitro with one exception. We noted that culturing BM basophils in IL-3–reduced receptor expression. We speculated that this might occur if cell division accelerated but did not find an effect on basophil division rates in 24 h. There was a modest decrease in the receptor accumulation rate in the presence of IgE (3000/h to 2300/h) that may indicate a change in receptor synthesis. At this point in time, we can’t fully explain the change in receptor expression and there may be a multiplicity of behaviors in these mixed cultures that we do not yet understand. In addition, we did not make these observations in vivo.

Recent studies have noted that high concentrations of IL-3 alters the number of basophils in circulation (24, 28, 29). Some results lead to the interpretation that IL-3 alters the generation of immature basophils from progenitors without necessarily altering the maturation process itself. The absence of change in FcεRI expression levels in our studies and the dependence of these levels on division times suggest that IL-3 is not altering division times of basophils (unless there were a change in division time that was perfectly counterbalanced by a change in FcεRI loading rates).

These studies established that IgE is present in the BM interstitial environment and that IgE does not alter the maturation rate or egress of BM basophils. These studies also demonstrated that 1) absolute levels of receptor expression in mice are similar to those found in humans, 2) that basophils divide with a cell cycle of $\approx$24 h, 3) that following their last division, they reside in the BM for $\approx$24–36 h before entering the BL, and 4) that there is a likely during one division cycle. This means that to achieve 300,000–500,000 receptors/basophil in humans, basophils must load 150,000–250,000 receptors in one cell division period. In vitro studies of human basophils suggested only 12,000 receptors/d so that if one division cycle required 24 h, then this rate is too slow by a factor of 15–25 to explain receptor expression levels for most atopic patients. The in vitro culture of mouse basophils suggested an accumulation of 75,000–100,000 receptors/d (the amount of IgE used to generate the loading rate experimentally was 1 μg/ml, an amount that leads to $\approx$80% of the maximal rate based on studies in human basophils (19)). This rate is compatible with the levels of receptor observed, 185,000–240,000.

However, the model also suggests that for every fold increase in the rate of receptor accumulation there must be a commensurate increase in the rate that receptor is lost when IgE is removed from the receptor or the steady state level of receptors in the absence of IgE also shifts (see Simulation in Materials and Methods). In other words, to achieve the correct numerical value of 5,000–10,000 receptors at the “no IgE” steady-state (a value that appears to apply to both human and mouse basophils, see methods for the model and Table II), then the loss rate must be 400,000 receptors/d for a loading rate of 100,000 receptors/d. This loss rate appears to occur in humans (3). It is also worth noting that the two rates do not have to change together. If the synthetic/loading rate increases without a change in the loss rate, then the steady-state value of receptor expression in the absence of IgE increases. This may occur in cultures of BM-derived mast cells where receptor expression without IgE ranges between 50,000 and 100,000 receptors (unpublished data).

The second model prediction that was captured in the experimental observations was the characteristics of the IgE injection experiments. Using the measured values for cell division times and receptor dynamics in maturing and mature basophils, the model predicted the difference in time for maximal receptor expression for BM and BL basophils, the relative levels of receptor and the overall kinetics of receptor expression (Fig. 2D).

The effect of IL-3 on receptor expression in vitro and in vivo was examined. By and large, IL-3 had little effect on receptor expression in vivo and in vitro with one exception. We noted that culturing BM basophils in IL-3–reduced receptor expression. We speculated that this might occur if cell division accelerated but did not find an effect on basophil division rates in 24 h. There was a modest decrease in the receptor accumulation rate in the presence of IgE (3000/h to 2300/h) that may indicate a change in receptor synthesis. At this point in time, we can’t fully explain the change in receptor expression and there may be a multiplicity of behaviors in these mixed cultures that we do not yet understand. In addition, we did not make these observations in vivo.

Two other sets of experimental observations support the underlying model. Perhaps the most significant insight provided by the modeling is that repetitive cell division has a marked effect on how receptors can accumulate. Rather than having many days to load receptor onto the cell surface, the steady state established by cell division enforces a limitation to accumulation such that the final level of expression is only two times the level of loading that occurs...
reduction in receptor synthetic or loading rate once a fully mature basophil is generated. It should also be noted that the underlying model applies to any IgE-bearing cell whose turnover and replacement is rapid. Furthermore, the limitations on protein accumulation during cell division apply to any protein whose accumulation is not limited by other factors.

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