

IgE-regulated loss, not IgE-regulated synthesis, controls expression of Fc ϵ RI in human basophils

Donald MacGlashan, Jr.,* Han-Zhang Xia,[†] Lawrence B. Schwartz,[†] and Jianping Gong*

*Johns Hopkins Asthma and Allergy Center, Baltimore, Maryland, and [†]Virginia Commonwealth University, Richmond, Virginia

Abstract: Expression of the high-affinity receptor on basophils and mast cells is modulated by immunoglobulin E (IgE) antibody. Recent studies have shown that modulation occurs through interaction of IgE with the receptor itself, but the mechanisms underlying this control are not understood. Taking both a theoretical and experimental approach, we examined several competing models that focus on whether there is IgE-regulated loss, IgE-regulated synthesis, or both regulated loss and synthesis of the Fc receptor for IgE (Fc ϵ RI). We report that removing IgE from occupied Fc ϵ RI resulted in an accelerated loss only in the unoccupied receptor, with no loss of occupied receptors and no loss of total receptors when all receptors were occupied. Together with previous studies, these results establish that there was IgE-regulated loss of receptors. An examination of synthetic rates of Fc ϵ RI α using pulse-labeling with ³⁵S-methionine indicated no difference in synthetic rates in the presence or absence of IgE. Similarly, the presence or absence of IgE had no influence on the levels of mRNA for either α , β , or γ subunits of Fc ϵ RI. Using model simulations, we found that regulated-synthesis models could be distinguished from regulated-loss/constant-synthesis models on the basis of the relationship between starting Fc ϵ RI densities and changes in density after culture for 1 week in the absence of IgE. Experimental data from this type of study fit a regulated-loss model that did not include regulation of synthesis. Taken together, these results suggest that IgE regulates cell surface expression of Fc ϵ RI only by regulating the rate that receptor is lost from the cell surface. *J. Leukoc. Biol.* 70: 207–218; 2001.

Key Words: regulated loss · regulated synthesis · receptor

INTRODUCTION

Immunoglobulin (Ig) E antibody has been shown to up-regulate the expression of the Fc receptor for IgE (Fc ϵ RI) on basophils and mast cells [1–7], and this process is manifested in a relationship between total serum IgE titers and expression of Fc ϵ RI on peripheral blood basophils [8–10]. The induction of increased expression on human basophils appears to result

from binding of IgE to Fc ϵ RI itself [11]. The up-regulation requires only monomeric IgE because dimeric or higher oligomeric IgE actually inhibits up-regulation [3]. Conversely, elimination of IgE during culture of these cells results in down-regulation of cell surface expression. The mechanisms underlying the induction of increased expression or the down-regulation of expression in the absence of IgE are not known. The simplest model for this system that includes sensitivity to IgE concentrations is one in which either synthesis is inducible by the presence of IgE or removal is inhibitable by IgE. Precedence for the latter model can be found with Fc ϵ RI on rat basophilic leukemia (RBL) cells [12] or Fc ϵ RII on lymphocytes [13]. Cell surface expression of Fc ϵ RII is regulated by a metalloprotease whose enzymatic activity for proteolytic cleavage of the extracellular portion of Fc ϵ RII is blocked by bound IgE [14]. The mechanism of loss on RBL cells is unknown. Therefore, one working hypothesis for regulation of Fc ϵ RI has been that synthesis of Fc ϵ RI α is constitutive and that removal of cell surface Fc ϵ RI α (and presumably Fc ϵ RI $\beta\gamma$ 2) depends on whether IgE is bound. Without bound IgE, Fc ϵ RI α is susceptible to removal by means unknown.

Further reflection about a model in which IgE blocks the removal of Fc ϵ RI α from the cell surface led to some of the experiments carried out in this study. Previous in vitro studies indirectly suggested that removal was dependent on the presence of unoccupied receptors. The rate of Fc ϵ RI α down-regulation is slow; receptor expression in the absence of extracellular IgE is decreased by 50% in 13 days. But this appears to result from the slower than expected dissociation of IgE from Fc ϵ RI. Based on published dissociation rates, dissociation in our studies was slower by about 10-fold; published measurements yield a half-life for IgE bound to Fc ϵ RI α of 18–24 h [15–18] whereas in vitro culture data indicated that the half-life is closer to 7–10 days [3]. This dissociation rate is not altered by inclusion of an IgE trap to prevent its rebinding. These results imply that receptor is not lost until it becomes unoccupied. A second, subtler indication from the in vitro studies is that receptor loss is not strictly proportional to the number of unoccupied receptors. During the first 5 days of culture without IgE, Fc ϵ RI expression stays relatively constant,

Correspondence: Dr. Donald MacGlashan, Jr., Johns Hopkins Asthma and Allergy Center, 5501 Hopkins Bayview Circle, Baltimore, MD 21224. E-mail: dmacglas@jhmi.edu

Received November 25, 2000; revised February 11, 2001; accepted February 15, 2001.

but bound IgE levels begin to fall. It is not until there is a substantial increase in the number of unoccupied receptors that FcεRI expression begins to fall. Together these data suggest that receptor loss happens only to unoccupied receptors and the rate of loss is not a constant but in some manner is dependent on the density of unoccupied receptors. The study we report here was designed to test this hypothesis.

The other half of this process involves FcεRIα synthetic rates. Loading of FcεRI on the cell surface could be either constant or regulated/modulated by IgE. The *in vitro* studies suggest that synthetic rates are quite low, 300–500 molecules of FcεRI per h. This apparently low synthetic rate limits the kinds of studies that can be done with human basophils, but basic pulse-labeling experiments of FcεRIα protein are possible. As a first test of whether IgE induces FcεRIα synthesis, we examined basophils for changes in FcεRIα mRNA expression during culture with IgE antibody and performed some simple pulse-labeling experiments.

One additional aspect of the experimental data has not been readily explained by available information. The 50% effective concentration (EC₅₀) for up-regulation by IgE is 1–2 nM, 10- to 100-fold higher than would be expected based on the affinity of the IgE-FcεRIα interaction as either published [16–18] or recently estimated [3]. Using the experimental observations obtained from the experiments reported here, we present a mathematical model of receptor expression regulation with two purposes: (1) to develop some understanding of how the EC₅₀ for up-regulation is compatible with the known properties of the system and (2) to distinguish between IgE-regulated synthesis or constant-synthesis models.

MATERIALS AND METHODS

Human IgG, piperazine *N,N'*-bis-2 ethanesulfonic acid (PIPES), glucose, ethyleneglycol-bis-*N,N,N',N'*-tetraacetic acid, fetal calf serum, EDTA, bovine serum albumin, human serum albumin (HSA), sodium orthovanadate, benzamidine, aprotinin, phenylmethylsulfonyl fluoride (PMSF), sodium fluoride, 2-mercaptoethanol, Nonidet P-40, and Accuspin tubes were all purchased from Sigma Chemical Co. (St. Louis, MO). Sodium dodecyl sulfate (SDS), Tween, and Tris were from BioRad (Hercules, CA). Agarose was from Gibco BRL (Rockville, MD). Protein G sepharose and Percoll were from Pharmacia Biotech (Piscataway, NJ). Eight percent Tris-glycine gels and 2× sample buffer were from Novex (San Diego, CA), whereas biotinylated molecular-size markers were from New England Biolabs (Beverly, MA). The antibody cocktail and columns used in the negative selection of human basophils were from Miltenyi Biotec (Auburn, CA). Sheep anti-mouse Ig horseradish peroxidase, streptavidin horseradish peroxidase conjugate, enhanced chemiluminescence (ECL) Western blotting detection agents, and ECL hyperfilm were all from Amersham (Piscataway, NJ). Inhibitors that alter the class of metalloproteases involved in CD23 processing [14] were obtained from R. Mayer (SmithKline Beecham, King of Prussia, PA). All other reagents used were of the highest grade available. Purified IgE (PS) myeloma was a gift from T. Ishizaka [19].

Buffers

The buffers used in these experiments included PIPES (Sigma Chemical Co.) stock buffer—25 mM PIPES containing 110 mM NaCl, 5 mM KCl, and 40 mM NaOH adjusted to pH 7.3 and stored at 10-fold the above concentration; PIPES-albumin-glucose (PAG)—PIPES (1×) containing 0.003% HSA (Miles Laboratories, Inc., Elkhart, IN) and 0.1% glucose; PAG with 1.0 mM CaCl₂ and 1.0 mM MgCl₂; PAG-EDTA—PAG with 1.0 mM EDTA, lactic elution buffer containing 0.01 M sodium acetate, 0.14 M NaCl, 5 mM KCl, and 0.03% HSA at pH 3.9 [20]; lysis buffer containing 20 mM Tris (pH 7.8), 150 mM

sodium chloride, 1% Nonidet P-40, 5% glycerol, 1 mM PMSF, 1 mM sodium orthovanadate, 1 mM sodium fluoride, 1 mM benzamidine, and 1 μg/mL of aprotinin. In the electrophoresis studies, 2× sample buffer contained 0.5 M Tris-HCl (pH 6.8), 10% (w/v) SDS, 0.1% bromophenol blue, 20% glycerol, and 5% mercaptoethanol; TBST buffer contained 12 mM Tris base (pH 7.5), 150 mM NaCl, and 0.05% Tween-20; running buffer contained 25 mM Tris base, 192 mM glycine, and 0.1% SDS; transfer buffer contained 12 mM Tris base, 96 mM glycine, and 20% methanol; stripping buffer contained 7 M guanidine hydrochloride.

Cell preparation

Two types of basophil preparations were employed. Most of the studies used cells obtained from leukapheresis and were prepared as previously described [21]. Basophil purities in these preparations ranged from 15–95% with a median of 33%. In some experiments, cells were isolated by the double Percoll method used for fresh blood [22]. The blood was diluted with EDTA-saline, centrifuged at 500 *g* for 15 min to obtain a buffy coat. The buffy coat cells were diluted in saline and layered onto a two-step Percoll gradient, 1.065 g/mL–1.079 g/mL, as described previously [23]. After centrifugation at 450 *g* for 15 min, the interface between the 1.065 Percoll/plasma upper layer and the 1.079 lower Percoll layer was harvested and washed as above (basophil purities of 8–45%). Which cell preparations were used are noted below.

Cell counting

Basophils were stained with Alcian blue and counted in a Spiers Levy hemocytometer [24]. Viability was also assessed by trypan blue exclusion, and, for these studies, basophil viability was >95%.

Cell culture

Enriched basophil preparations were cultured in Iscove's modified Dulbecco's medium (Life Technologies, Rockville, MD) containing 2% fetal calf serum, 40 μg/mL of gentamycin, and 10 ng/mL of interleukin (IL)-3. The total cell density was 2×10^6 /mL, and culturing was done in 96-, 24-, or 6-well tissue culture-treated plates (Costar, Cornell, NY). Cell viability in short cultures, <2 days, averages 95% whereas for longer cultures (>2 days), it averages 89%.

Flow cytometry

A flow-cytometric technique incorporating light scatter characteristics was used to quantify cell surface IgE and FcεRIα chain expression on basophils as described [25]. Cell surface IgE was detected using a monoclonal anti-human IgE (TES-19 or E10-95-3; Tanox Biosystems, Houston, TX). Cell surface expression of FcεRIα chain was detected using a mouse IgG1 anti-human FcεRIα chain mAb 22E7; generously provided by J. Kochan, Roche Biochemicals, Indianapolis, IN [26]) and was compared with labeling with the same concentration of irrelevant mouse IgG1 (Coulter, Hialeah, FL). The 22E7 antibody has been shown to recognize an epitope that is unaffected by FcεRIα occupancy [26]. The mAb 15A5 (also a gift from J. Kochan) was used to detect unoccupied FcεRIα [26]. Aliquots of cells were labeled in phosphate-buffered saline containing 0.2% HSA with 1 mg/mL of human IgG to minimize non-specific binding to FcγR [25]. Each of the monoclonal antibodies was used at concentrations predetermined to be optimal for labeling. Binding of monoclonal antibodies was detected using saturating concentrations of R-phycoerythrin-conjugated polyclonal goat anti-mouse IgG (Tago, Burlingame, CA). An EPICS Profile flow cytometer (Coulter, Inc., Hialeah, FL) was used to analyze fluorescent signals after excitation at 488 nm. "Bitmap" gates, intermediate between the forward and side scatter characteristics of lymphocytes and monocytes, were used to select for a population of cells which were predominantly basophils. Since the cells were already enriched in basophils, these bitmaps could select a population of cells that is generally greater than 80% basophils, with the primary contaminants being lymphocytes. Data were expressed as the mean fluorescence in labeled cells minus the mean fluorescence of IgG1 controls. Day-to-day variability in the sensitivity of the flow cytometer was corrected by noting or adjusting the photomultiplier tube voltage to generate the same signal for a set of standard calibration beads (Immunotech; Coulter).

In previous studies, the flow-cytometric measurements were calibrated by examining the fluorescence staining of six donors' basophils, which spanned a moderate range of staining intensities (7–120 fluorescence units or 8,000–

140,000 Fc ϵ RI per basophil) and simultaneously assessing receptor or IgE density by the acetate elution method [3]. A linear plot ($R=0.963$) of 22E7 staining (ordinate) compared with total Fc ϵ RI density by acetate elution yielded a conversion factor in which a fluorescence measurement of 100 represented approximately 120,000 receptors.

Statistics

Some statistical comparisons were made with a Student's *t*-test, and others where made with a nonparametric Wilcoxon signed-rank statistic. Results were expressed as means plus or minus standard errors of the means unless otherwise indicated.

Metabolic labeling

Cells were preincubated for 30 min at 37°C in methionine-free RPMI-1640 medium (Gibco-BRL Life Technologies), and then labeled with 0.1 mCi/mL of ³⁵S-methionine (NEN Life Science, Boston, MA) in methionine-free RPMI-1640 containing 40 μ g/mL of gentamycin and 10 ng/mL of IL-3 for 6 h at 37°C in the presence or absence of IgE (5 μ g/mL). Cells were collected and lysed in ice-cold lysis buffer containing 1% Triton X-100, phosphate-buffered saline (pH 7.4), 5 mM EDTA, 5 mM dithiothreitol, 1 mM PMSF, 20 μ g/mL of leupeptin, and 100 μ g/mL of aprotinin. For autoradiography of beta particles, the membranes were exposed to high-performance autoradiography film (Amersham) with intensifying screens (Kodak) at -80°C for 24–100 h.

Lysate preparation

Purified human basophils (>97%) were counted (see above) and then directly spun down (14,000 *g* for 10 s) and lysed in 1 $\times$ sample buffer at a final concentration of 25×10^6 per mL, then boiled at 100°C for 5 min. Samples were then stored at -130°C until analyzed by SDS-polyacrylamide gel electrophoresis and Western blotting.

Immunoprecipitation

For some preparations, immunoprecipitation was also performed. Briefly, centrifuged lysates, as obtained above, were precleared with protein G-Sepharose beads for 30 min at 4°C. The precleared lysates were then washed and incubated with 5 μ g/mL of 22E7 prebound to protein G-Sepharose beads. After gentle rotation for 1 h at 4°C, the beads were washed and the immunoadsorbed proteins were eluted from the beads by boiling in 2 $\times$ SDS sample buffer. Control experiments revealed that an irrelevant IgG antibody or 22E7 in the absence of lysate did not pull down Fc ϵ RI α in the immunoprecipitates.

SDS-polyacrylamide gel electrophoresis and Western blotting

Proteins were separated in a 4–20% Tris-glycine gel under reducing conditions and electrotransferred on to a nitrocellulose membrane. The free binding sites were blocked by incubating the membrane overnight at 4°C with 4% bovine serum albumin in TBST. After exposure of hyperfilm to the nitrocellulose membranes, they were then Western blotted with 22E7 or UBI anti-Fc ϵ RI α (Upstate Biotechnology, Inc., Lake Placid, NY). The membrane was then washed with TBST prior to the addition of an anti-mouse or anti-rabbit horseradish peroxidase conjugate (1:3,000 dilution) for 1 h at room temperature. After further washing of the membranes with TBST, the proteins were visualized using ECL. The nitrocellulose membrane was exposed to ECL hyperfilm for various times ranging from 15 s to 5 min, a time course previously determined to provide exposures that yielded reasonably linear quantification of Fc ϵ RI α protein. After exposure to chemiluminescence detection agents, the intensity of each band was determined using densitometric analysis with a Kodak DC120 digital camera and acquisition software.

mRNA expression

Total RNA was isolated from enriched human basophils by RNazol according to the manufacturer's instructions. Briefly, 0.5×10^6 basophils were resuspended in 0.6 mL of RNazol. The total mRNA was then extracted by a chloroform/isopropanol technique. Any contamination was then removed by an ethanol wash. Dried ethanol precipitates were resuspended in diethyl pyrocarbonate-treated water. All samples were stored at -70°C until mRNA was

quantified using dilutional PCR, competitive reverse-transcriptase (RT)-PCR, or real-time PCR.

Competitive RT-PCR

Cells lysates processed by RNazol B were analyzed by competitive RT-PCR using primers, competitor cRNA molecules, and procedures as previously described [27]. For competitive RT-PCR, the amounts of competitor cRNAs included in the reverse-transcriptase mixture were determined empirically. For PCR, 5 μ L of the cDNA products were mixed with 1 $\times$ reaction buffer [10 mM Tris-CL (pH 8.3), 50 mM KCl, 1.5 mM MgCl₂, and 0.02% gelatin], 200 μ M deoxynucleotide triphosphate mix (Boehringer Mannheim, Indianapolis, IN), 400 nM primers, and 0.625 U of AmpliTaq (Perkin-Elmer Corp., Foster City, CA) in a total volume of 25 μ L. The hot start technique was used to reduce nonspecific priming, and reactions were run for 35 cycles, each consisting of denaturation at 94°C for 1 min., annealing at 55°C for 1 min and extension at 72°C for 1 min. After each PCR, 10 μ L of the products were analyzed by electrophoresis in a 10% polyacrylamide gel containing 89 mM Tris, 89 mM boric acid, and 2 mM EDTA at pH 8.3 (Novex) and photographed. Films were scanned and band intensities were measured by densitometry. Data were analyzed as described previously [27]. Briefly, the ratio of target to competitor was determined, and concentrations of the target calculated from this ratio and the known concentration of competitor.

Real-time PCR

Forward and reverse oligo primers for Fc ϵ RI α and β were constructed using Primer Express software and previously published cDNA sequences. For Fc ϵ RI α , the forward primer was 5'-GTGAACCTGTGTACCTGGAAGTCTT-3' (nucleotide position 408; Genbank accession number X06948), and the reverse primer was CATCCAGTTCCTCCAACCA (position 524). For Fc ϵ RI β the forward primer and reverse primer were 5'-CCAGGAAGTATCTTCAGGCA-GACT-3' (nucleotide position 140; Genbank accession number M89796) and 5'-TCAAAACTGTCAGCCATGTATGC-3' respectively. The probe sequences were 5'-TGACTGGCTGCTCCTTCAGGCCTC-3' and 5'-TTGAAGTCGGCCT-CATCCCCACC-3' for Fc ϵ RI α and β respectively. The primers for IL-4 were 5'-CGACTGCACAGCAGTTCCA-3' and 5'-CAGGCCCCAGAGGTTCC-3', and the probe was 5'-TCCGATTCTGAAACGGCTCGACA-3'. For all probes the 5'-reporter dye was 6-carboxyfluorescein and the 3'-quencher dye was 6-carboxytetramethylrhodamine. The real-time PCR method is based on the cleavage of fluorescent-dye-labeled probes by the 5'-3' exonuclease activity of the *Taq* DNA polymerase during PCR and measurement of fluorescence intensity by a Sequence Detection System (Perkin-Elmer, 7700) [28]. With this instrumentation, seven measurements of fluorescence per well per PCR cycle are made during a 40-cycle amplification, and the point at which fluorescence exceeds a predetermined threshold (occurring within the linear region of the amplification curve) determines a cycle number (the so-called "C_T") for the sample. Because multiple measurements are made during each cycle, the analytical software produces fractional cycle numbers for each sample. This system provided small coefficients of variation for mRNA levels for Fc ϵ RI α and β . In pilot experiments, serial dilutions of our Fc ϵ RI α mRNA standard were evaluated, and it was determined that each unit increase in cycle number (average $\Delta C_T = 1.02 \pm 0.03$ for multiple twofold dilutions) was equivalent to a twofold decrease in mRNA for both Fc ϵ RI α and β . The final PCR product was run on a 2.5% agarose gel to confirm that a single product at the correct base pair length was produced.

Model simulations

Mathematical modeling of this system was simplified to address broader questions about the need for IgE-regulated loss or synthesis. We focused on models with some heuristic properties (i.e., general properties rather than explicit representations of what is actually occurring in the cell) that required as few parameters as possible. There were two basic goals. The first goal was to determine what conditions led to the higher than expected EC₅₀ for receptor up-regulation. Our second interest was to determine whether there were parameters or conditions that would easily discriminate between a model in which synthesis of the receptor was constitutive versus one in which occupied receptor signaled the cell to make more receptor. One additional variation was considered; two compartments for the receptor with or without bound IgE were postulated, one being the plasma membrane surface where there is free

exchange of IgE with the receptor and one internal compartment from which receptors are removed if they are unoccupied. The primary motivation for exploring this later model is derived from the assumption that our experimental measurements of IgE dissociation from the receptor reflect its actual dissociation rate. A two-compartment model allows for faster intrinsic dissociation rates to be masked by a pool of IgE and receptor that is cycling to (and from) the cell membrane. **Figure 1** summarizes the general schemes examined by simulation. It should be stressed that this modeling effort was intentionally kept very simple because the wealth of possible reactions can not be known. In essence, our model was akin to a simple steady-state problem, e.g., the filling of a basin with water flowing in and out. The difference that makes this model more useful is that the interaction between IgE and FcεRIα is well known and also a potential regulator of the process. In other words, the flow in (loading of the plasma membrane with FcεRI) and the flow out (loss of receptor from the plasma membrane) might be controlled by the presence of IgE in the receptor.

The model in which synthesis was assumed to be constitutive was patterned from the current model for CD23 expression. One heuristic representation of down-regulation—the rate of loss is an inverse exponential function—has the advantage of requiring only two parameters as well as providing a means to limit the rate of loss if necessary. However, with the correct choice of parameters, an inverse exponential is quantitatively very similar to the hyperbolic function that describes an enzymatic process (they differ by at most 10–12% within two regions of the two curves). Therefore, because there is no meaningful advantage of choosing one heuristic function over the other except for consistency with the prevailing model of CD23, we extensively evaluated only down-regulation, using an enzymatic rate equation. However, no specific enzymatic reaction is implied. Because the experiments on FcεRIα down-regulation indicate that the rate of loss is markedly faster when a 5- to 10-fold-greater number of unoccupied receptors is present, the parameters for this heuristic expression can be chosen also to fit this constraining information. The model sets the down-regulation of receptor to match a process in which the K_m for the reaction is high enough that the rate of down-regulation increases when the number of unoccupied receptors increases from a few thousand to

tens of thousands. As noted, synthesis is set to be constitutive, and this rate is readily determined from the rate the receptors are acquired by basophils incubated with high IgE concentrations—conditions which should stop all loss.

Models in which synthesis is induced are more involved because of the wide variety of ways one can view induction. There are several aspects to consider. First, we briefly examined models in which loss was effectively constant. These failed to replicate the characteristics of down-regulation across a large range of starting receptor densities and were rejected (more on this below). Second, if induction of synthesis is strictly a function of the number of occupied receptors, the up-regulation curves “accelerate” as receptor expression increases. This occurs because accumulating receptor densities generate a greater signal, which increases the signal for synthesis (in a positive-feedback loop). Therefore, we dictated that synthetic rates saturated at some value that could be estimated from the rate of up-regulation in the presence of high concentrations of IgE. At high levels of “stimulation,” this model is similar to that of constitutive synthesis; i.e., at high densities of occupied receptors, the rate of up-regulation reaches its saturation point and appears constant as in the constitutive model. However, at the low end of the curve, the rate of synthesis is variable, and it should be possible to detect changes in synthetic rate experimentally under the correct conditions. These models of IgE-regulated synthesis of FcεRIα can be viewed two ways, one in which a signal for increased receptor synthesis is always generated by an occupied receptor and one in which the signal from an occupied receptor exists for only a short time after the initial binding of IgE to an unoccupied receptor. As it turns out, these two approaches result in qualitatively and quantitatively similar simulations with only modest differences in some of the parameters.

Figure 1C shows that we considered regulated loss and constant synthesis, regulated synthesis and constant loss (with two variations in regulated synthesis), regulated loss and regulated synthesis, and finally a two-compartment model with regulated loss and constant synthesis. The rate relationships for the regulated loss/constant synthesis model are as follows:

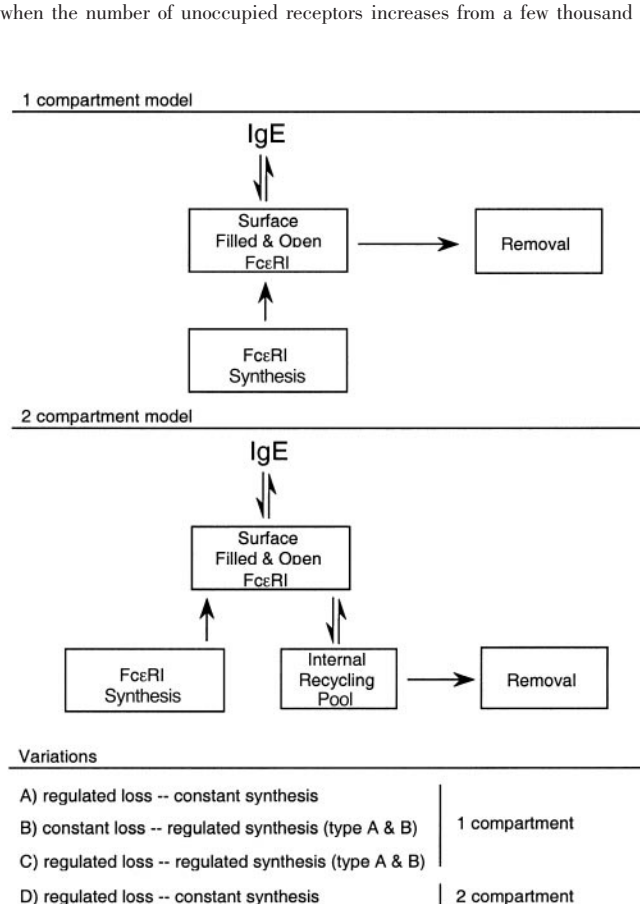


Fig. 1. Schematic representation of the four models of receptor expression examined.

$$\frac{dR_0}{dt} = -V_R \left(\frac{R_0}{R_0 + K_{R0}} \right)^\alpha + L + (k_i R_F - k_r R_0 \text{IgE}) \quad (1)$$

$$\frac{dR_F}{dt} = k_i R_0 \text{IgE} - k_r R_F \quad (2)$$

$$\text{IgE} = (\text{IgE}_T - R_F \rho) / (6.02 \times 10^{20}) \quad (3)$$

where R_0 is unoccupied receptors; R_F is occupied receptors; IgE is the solution IgE concentration; L is loading in this version of the model, and L is constant synthesis and expression rate; V_R is the maximum rate of removal; K_{R0} is the concentration of unoccupied receptors for half-maximal rate; k_i is the forward rate-binding constant for IgE binding to FcεRI; and k_r is the reverse rate-binding constant for IgE dissociation from FcεRI; α is the adjustable parameter controlling the steepness of the rate expression for loss, and ρ is cell density in culture (cells per mL).

Equation 1 shows the simplicity of the model; receptor is lost from the plasma membrane or loaded into the plasma membrane, and once in the membrane, the amount of unoccupied versus occupied receptor is a result of interaction with solution phase IgE (the expressions of which are simple reaction kinetics from two-component binding). The middle term in Equation 1, L , is used to represent the loading rate, a term which is chosen to encompass all the factors that contribute to expressing new receptor on the cell surface. This would include both synthesis and transport from the endoplasmic reticulum (ER) to the Golgi and to the plasma membrane, processes that might be independently regulated. As discussed above, the loss is a standard expression for enzyme kinetics. The initial conditions specify values for R_F , R_0 , and IgE_T , i.e., the initial density of occupied and unoccupied receptors (known from many previous studies) and the solution phase IgE (a variable parameter both experimentally and in this model). The model includes some fixed conditions, volume of the reaction and cell densities (ρ), that are consistent with the conditions used experimentally. Equation 2 is the reaction rate expressions for association of IgE and FcεRIα, keeping in mind that in this model, only unoccupied receptor is loaded into the membrane or lost from the membrane

(as experiments in this study indicate). Equation 3 is just the mass balance for IgE in solution.

If synthesis (included under the generic term of loading) is regulated by IgE, a simple heuristic expression using an inverse exponential is used to provide a maximal rate of synthesis and a midpoint ($1/e$) for reaching this maximal rate:

$$L = V_s(1 - e^{-k_s R_F}) \quad (4)$$

where L is the rate of synthesis (loading), V_s is the maximal rate of synthesis, and k_s is the $1/e$ constant. In this particular expression, occupied receptors generate the signal for synthesis and continue to generate a signal as long as they are occupied. For a transient signal, the simulation keeps track of receptors that have just become occupied and uses this value for R_F in Equation 4. Parameters were optimized from a relatively crude five-dimensional grid of possible choices, with attention first paid to reasonable choices of the IgE-binding parameters followed by refinement of the remaining parameters.

RESULTS

Loss regulated by IgE occupancy

Previous studies strongly indicated that receptors were lost only when not occupied by IgE. To extend these studies we took advantage of the technique developed to rapidly dissociate IgE from its receptor and retain cell viability, the lactic acid technique of Pruzansky et al. [29]. We have previously demonstrated that very short treatments with this mildly acidic buffer, i.e., <30 s, result in modest dissociation of IgE but functionally intact basophils as assessed by a technique that determines cellular sensitivity [20]. Longer treatments modify cell functions considerably. Moderately enriched basophil preparations were treated with lactic acid buffer for 15 s, and a portion was fully resensitized with $10 \mu\text{g/mL}$ of PS-myeloma for 20 min. All cells were analyzed for either total Fc ϵ RI α (22E7 mAb), total IgE (mAb TES-19 or 10-95-3), or unoccupied Fc ϵ RI α (mAb 15A5) by flow cytometry at the start of culture or after culture for 24 h. **Figure 2** summarizes the

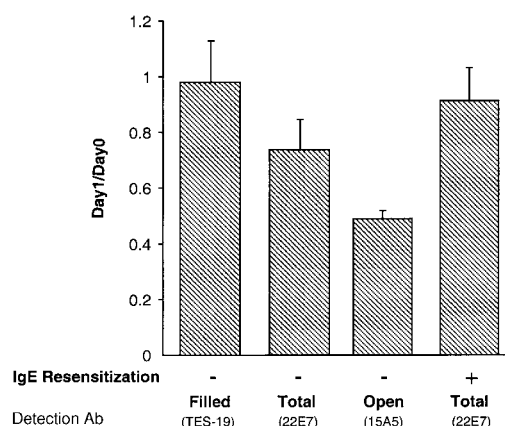


Fig. 2. Changes in receptor densities after lactic acid elution of IgE. Enriched basophils were first treated with lactic acid to remove some cell-bound IgE. After this dissociation, a portion of the cells was rapidly resensitized to saturate unoccupied receptors and then placed into culture with the remaining cells. Flow-cytometric measurements using 22E7, 15A5, and TES-19 were made on cells before and after a 24-h culture. The ordinate plots the ratios of these measurements for each antibody probe. The data shown represent the means of three experiments ($\pm$ SE), and the mean starting intensity of 22E7 staining was 88 ± 11 ($\approx 104,000$ receptors).

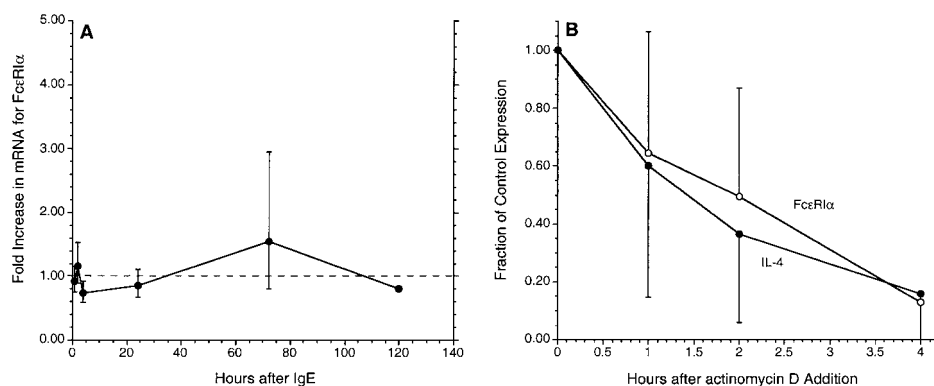
results of three experiments. In an important control for the effects of lactic acid treatment as well as to determine whether occupied receptors are lost, cells that had been resensitized with IgE showed no loss of Fc ϵ RI α (with 22E7 mAb as the detection reagent), indicating both that occupied receptors are not lost and that lactic acid treatment was not deleterious to cell survival and function. Stripped cells lost 50% of their 15A5 binding in 24 h and 25% of total receptors (22E7), but there was no loss of bound IgE (mAbs TES-19 or 10-95-3).

Although previously published studies noted $\sim 5\%$ loss of receptors in 1 day, the studies reported here showed a 25% loss of total receptors and a 50% loss of unoccupied receptors in 1 day. This magnitude of change provided a means to test for the effects of metalloprotease inhibitors on loss of Fc ϵ RI α expression. We examined compounds under development at Smith-Kline Beecham; all except one of these compounds were known to be active in the 0.1 – $1.0 \mu\text{M}$ range as inhibitors of the metalloprotease that removes CD23 [14]. These compounds included SKB202968 (batimastat), SKB235854 (marimastat), and SKB257702, 245210, 249737, 242113, 238746, 236502, and 224002, the last of which is inactive. We were unable to alter the loss of receptors in a 24-h culture of lactic-acid-treated basophils with any of the inhibitors at concentrations up to $2 \mu\text{M}$ [three- to sixfold their reported 50% inhibitory concentration for CD23 loss (data not shown)].

Synthesis regulated by IgE

A more difficult question was whether IgE changed the rate of Fc ϵ RI α synthesis. We initially approached this problem by examining the cells for changes in expression of Fc ϵ RI α mRNA, using dilutional RT-PCR (data not shown), real-time RT-PCR and competitive RT-PCR. The more extensive data set involves real-time PCR for Fc ϵ RI α , and data for these experiments are shown in **Figure 3**. Basophils were cultured $\pm 3 \mu\text{g/mL}$ of PS myeloma for the times shown. This plot represents a composite of a variety of experiments in which the times examined varied. Our previous up-regulation studies indicated that up-regulation begins immediately after starting a culture with IgE antibody although this can be hard to detect because the differences between expression values for most donors' basophils on day 0 and day 1 are small. Nevertheless, this trend in the previously published data suggested that if IgE were exerting an influence on synthetic rates of Fc ϵ RI α , it should occur within hours. Thus, the largest number of experiments focused on times that were ≤ 24 h of culture, but we also examined later time periods. For many of these experiments, portions of the cells were also evaluated for Fc ϵ RI α expression by flow cytometry, but for experiments in which the analysis of mRNA was done on cells cultured for less than several days, a subset of cells was cultured for 7 days to obtain data that could be compared with previous 7-day results. In Figure 3A are shown the relative levels of Fc ϵ RI α mRNA for cells cultured with and without IgE. There was no change in mRNA at any of the time points examined. In those experiments in which flow cytometry was done in parallel, there was an average increase in Fc ϵ RI α expression of 2.8-fold, a value consistent with previous studies (average starting receptor 22E7 mean fluorescence intensity = 24 or $\approx 29,000$ receptors).

Fig. 3. Changes in mRNA levels for FcεRIα during culture in the presence and absence of IgE. (A) Ratio of mRNA levels $\pm$ 3 μ g/mL of IgE for the times shown, as determined by real-time PCR. The plot is a composite of eight experiments, but not all experiments included the time points shown ($n=5$ for 1-, 2-, and 24-h time points; $n=2$ for the 4-h time point; $n=3$ for the 72-h time point; and $n=1$ for the 120-h time point). (B) Changes in mRNA for FcεRIα (○) culture $\pm$ actinomycin D at 5 μ M. The ordinate is the ratio of mRNA levels determined by real-time PCR for +actinomycin/-actinomycin ($n=3$). As a reference, similar measurements were made for IL-4 mRNA (●) whose time course has been previously described.



A similar experimental design was used to examine changes in mRNA for FcεRIα, β, and γ using a competitive RT-PCR technique. Times up to 24 h were examined. The results for FcεRIα were similar to real-time PCR data in Figure 3; for times of 1–4 h, +IgE/–IgE ratios were 0.96 ± 0.22 ($n=6$), and for 24 h, the ratio was 0.96 ± 0.10 ($n=3$). Results for FcεRIβ and γ are shown in **Figure 4**. As observed for FcεRIα, there were no consistent changes in the mRNAs for these receptor subunits when comparing cultures with and without IgE (3 μ g/mL). The competitive RT-PCR results also allowed an estimation of the absolute mRNA levels in these samples, 12–120, 1–10, and 10–100 molecules per basophil for FcεRIα, β, and γ, respectively.

FcεRIα mRNA appeared to be a very stable species in that no significant changes were observed throughout these exper-

iments. To determine whether this mRNA species is particularly stable (as often found for so-called housekeeping genes), we treated cells with actinomycin D and monitored mRNA levels for both FcεRIα and IL-4 over the course of 4 h. Previous studies have shown that the low constitutive level of IL-4 in basophils decays with a half-life of approximately 1 h [in the absence of stimulation (J. T. Schroeder, personal communication)]. Figure 3B shows that FcεRIα mRNA also decayed with a half-life similar to that of IL-4 mRNA.

While receptor mRNA expression was unaltered by incubation with IgE, it remained possible that synthesis was under translational control. However, receptor synthesis is extremely slow (see below); <500 molecules per h is a reasonable estimate. Nevertheless, we have found that weak bands can be quantitated. Basophils were cultured with and without IgE in medium containing only ^{35} S-methionine as its source of methionine. Cultures were limited to 6 h due to a low absolute concentration of methionine, which appears to disallow a good response of the basophil to the IL-3 in the cultures and therefore significant apoptosis with longer cultures. Basophils were cultured for 6 h with and without IgE (5 μ g/mL), and cell lysates were analyzed by immunoprecipitation with 22E7, electrophoresis, and Western blotting. Hyperfilm MP was exposed to the nitrocellulose for 24–100 h to develop for ^{35} S-methionine, and then the nitrocellulose was analyzed for FcεRIα by Western blotting with UBI anti-FcεRIα and detection by ECL. **Figure 5** shows one of the ^{35} S-methionine exposures and the ECL blot for of this experiment. After correcting for lane loading (based on the Western blot) and averaging two separate experiments, the ratio of +IgE/–IgE was 0.84 ± 0.16 .

Model simulations

Figure 6 shows previous experimental data superimposed on curves generated from a model for regulated loss/constant synthesis. However, it should be noted that the goal was not to obtain these parameters for the purposes of making an explicit statement regarding the basophil system but to ask general questions about the behavior of the system. Nevertheless, the four types of experiments provide strong constraints on a model which has five basic parameters (six with regulated synthesis) and one additional constant if α is varied. For example, experimental dissociation rates of IgE from the receptor constrain k_r . As discussed below, multiple combinations of k_r and k_f

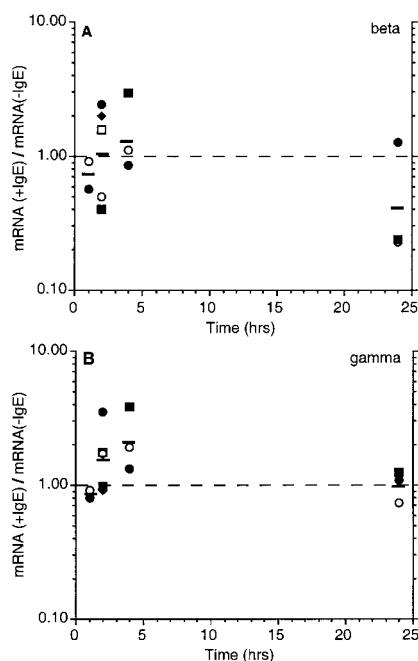


Fig. 4. Changes in mRNA levels for FcεRIβ and γ during culture in the presence and absence of IgE. (A) Ratio of mRNA levels $\pm$ 3 μ g/mL of IgE for the times shown as determined by competitive RT-PCR for FcεRIβ; (B) Data for FcεRIγ. Individual experimental points were plotted (each symbol representing a separate experiment), and not all time points were part of each experiment.

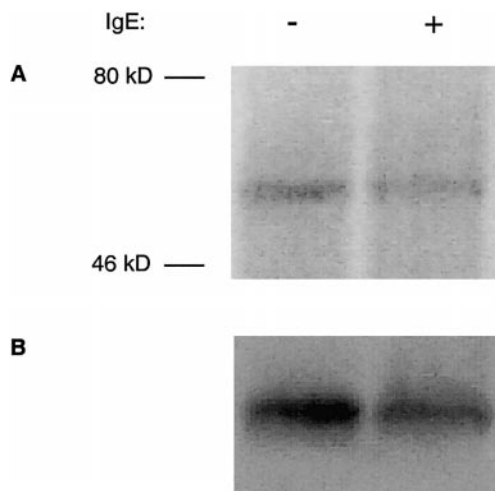


Fig. 5. Synthesis of FcεRIα as determined by ^{35}S -methionine labeling. Basophils (average purity, 29%) were cultured in RPMI-1640 supplemented with all amino acids except methionine, which was replaced with ^{35}S -methionine for 6 h prior to harvesting and lysis for immunoprecipitation with 22E7. Electrophoresis of the IP material was followed by film exposure for 24 h (A). The nitrocellulose membrane was then Western blotted with UBI anti-FcεRIα, and film was developed for ECL (B).

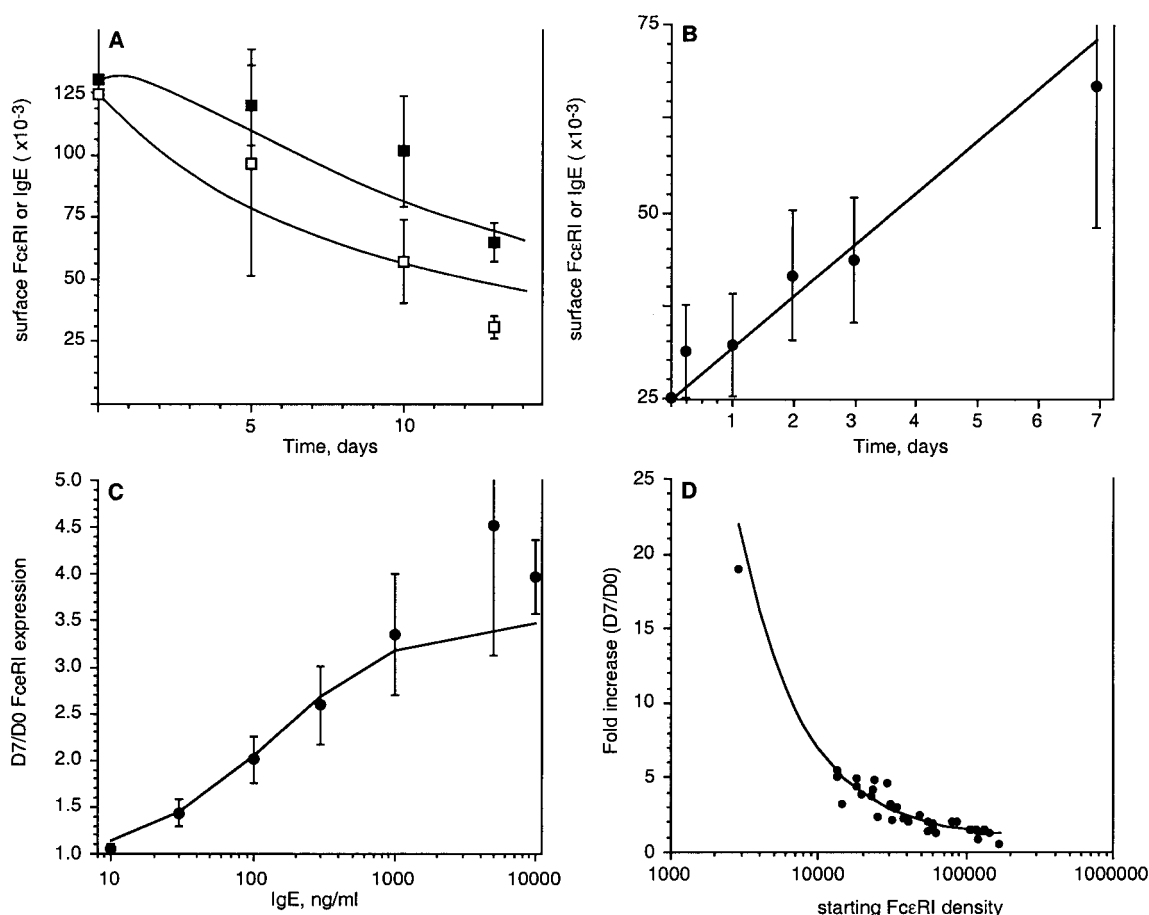
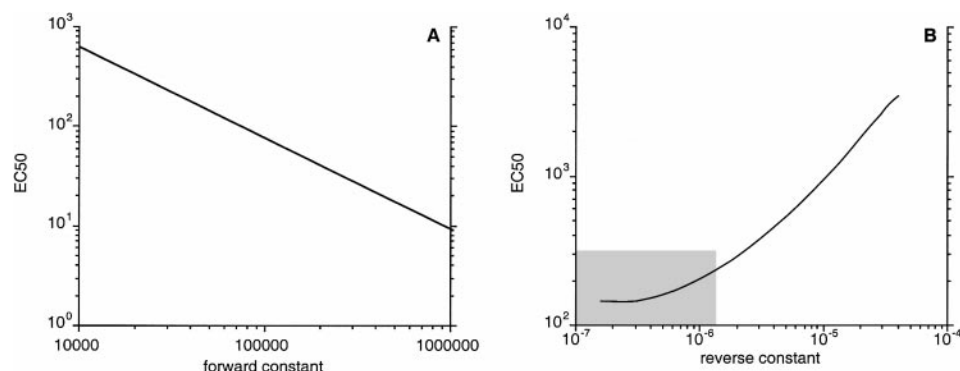


Fig. 6. Relationship of experimental to model simulation data. These four plots of FcεRI expression changes in basophils were derived from previous studies. The lines in the plots show the data derived from the model simulation in which $k_i = 3 \times 10^4 \text{ M}^{-1} \text{ s}^{-1}$; $k_r = 1.25 \times 10^{-6} \text{ M}^{-1}$; $V_R = 4,600$ receptors/h/basophil; $K_{RO} = 125,000$ receptors/basophil; $L = 450$ receptors/h/basophil; and $\alpha = 1$. The data points were taken from the previously published data [3]. (A) Changes in total receptor (■) and IgE (□) on basophils cultured in the absence of IgE; (B) Changes in total receptor on basophils cultured in the presence of 500 ng/mL of IgE; (C) Relationship between up-regulation of receptors (D7/D0 values) in 7-day culture at different concentrations of IgE; (D) Relationship between starting density of receptor and the fold up-regulation during 7 days of culture with 500 ng/mL of IgE.

result in similar fits of the model to the experimental data that make up Figure 6C (the EC50 for up-regulation). However, only one of the k_r/k_i pairs that results in a good fit for Figure 6C also generates a good fit to Figure 6A because of the constraint on the dissociation rate. With this constraint, the best k_r/k_i pair becomes $1 \times 10^{-6} \text{ s}^{-1}$ and $3 \times 10^4 \text{ M}^{-1} \text{ s}^{-1}$, respectively. This is a particularly interesting result because previous studies of the forward binding constant have also given the estimate of $3 \times 10^4 \text{ M}^{-1} \text{ sec}^{-1}$ [3, 30]. The rate of receptor loss is also constrained by the data in Figure 6A, in which rates of loss average about 200 per h for the density of unoccupied receptors in these experiments. Factoring in the experimental data presented above, V_R and K_{RO} (Equation 1) can be estimated. The synthetic rate is well defined by data in Figure 6B and the high end of the curve in Figure 6C. In the presence of a high concentration of IgE, there is essentially no loss of receptor, so the loading rate can be readily estimated (450 receptors per h per cell). This fit of the rate based on the kinetics of up-regulation (combined with knowledge of the removal rates) predicts the curves in Figures 6C and D, which can be seen to readily fit the experimental data for these plots. Thus, despite the use of five or six parameters, the three ways of measuring

Fig. 7. Relationship between the EC50 for receptor up-regulation in the presence of IgE and the forward or reverse rate constants for IgE binding to the receptor. (A) Reverse-rate constant held constant at $1 \times 10^{-6} \text{ M}^{-1}$ while the forward rate constant was varied in the model simulation. Calculated results obtained from the concentration-dependence relationship between IgE and fold up-regulation in a simulated 7-day culture were used to obtain an EC50 of up-regulation for each value of k_f used in the simulation. (B) Calculated results for the reverse-rate constant while holding the forward-rate constant at $3 \times 10^4 \text{ M}^{-1} \text{ s}^{-1}$. The gray zone indicates the range of k_r that seems to best fit our experimental data using a one-compartment model.



receptor changes experimentally constrain the choice of parameters, and the choices then further fit the fourth experimental data set shown in Figure 6D. The Figure 6 legend summarizes the values of the parameters that optimally fit these four plots. It should be noted that, with this choice of parameters and starting with the number of unoccupied receptors that were generated in the lactic acid strip experiments shown in Figure 2, results in the model predict a 17% loss of total receptors in 1 day, similar to that found experimentally.

We included the α parameter as a refinement to the regulated-loss aspect of the model because the data in Figure 6A suggested that a value of α greater than one would better replicate the experimental relationship between the rate of loss of IgE and receptor. However, exploration of other values of α did not significantly improve the ability of the model to simulate the experimental data (this is not to say that changing α had little on the outcome of the various simulations—it did—only that as all parameters were adjusted to the new value of α and for optimal fit of the various plots, the fitting was not markedly improved), so α was fixed to a value of one for the rest of the models in which it was applicable.

The initial goal for these modeling efforts was to determine whether a simple model of expression would also result in an EC50 for up-regulation on the order of 200–250 ng/mL. This was found to be the case for all the models examined. The simulated curves in the plots in Figure 6 can be generated for all four types of model. **Figure 7** demonstrates the relationship between the value of either the forward or reverse binding constants and the EC50 for up-regulation (as determined by the model). In Figure 7A, the forward-rate constant is varied while the reverse-rate constant is held constant at $1 \times 10^{-6} \text{ s}^{-1}$, whereas in Figure 7B, the forward-rate constant was held constant at $3 \times 10^4 \text{ M}^{-1} \text{ s}^{-1}$, while the reverse-rate constant was varied. The curves were generated using the regulated-loss/constant-synthesis model, but similar curves could be generated with any of the models (data not shown). These results indicate that the forward-binding constant has a linear relationship with EC50 over a broad range whereas the reverse-binding constant has a marked effect on the EC50 only at values that are not likely to represent the dissociation of IgE from Fc ϵ RI α .

The second goal was to determine how the various models differ in their ability to replicate the experimental data. As

noted above, it was possible to chose parameters for each of the models that would simultaneously fit the four relationships shown in Figure 6. In other words, based only on these previously published experimental results, we could not distinguish between a model in which synthesis was constitutive and one in which it was regulated by IgE. However, there was a relationship that markedly differentiated these two models. **Figure 8** shows a plot of the relationship between the starting Fc ϵ RI α density and its change during a 7-day incubation in the *absence* of IgE. The lower generated curve for the regulated-synthesis/regulated-loss model (based on parameters that allow all four relationships in Figure 6 to be fit) has the attribute that the lower the starting density, the lower the ratio of D7/D0 expression; i.e., at low starting densities, there continues to be loss of receptor. In contrast, for the constant-synthesis/regulated-loss

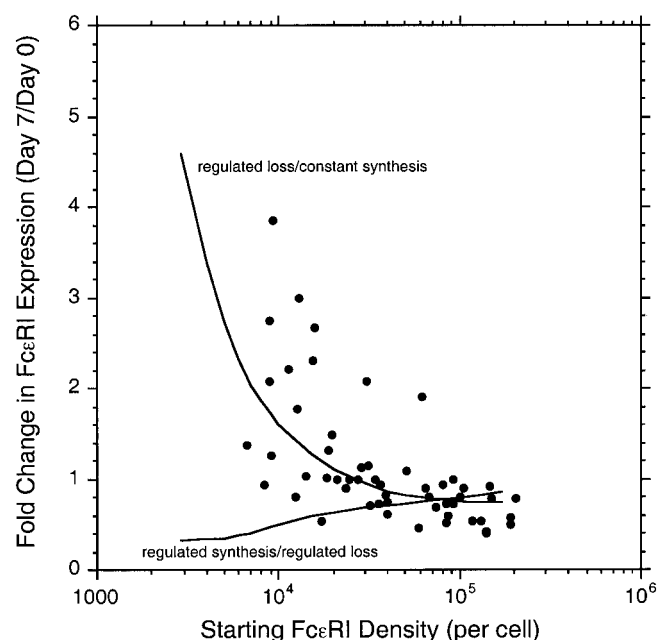


Fig. 8. Experimental and simulated relationship between the starting receptor density and its final density after a 7-day culture in the absence of IgE. Two of the models were used for the calculated results; both included regulated loss although one used constant synthesis and the other used IgE-regulated synthesis. The data points were derived from experiments performed under these conditions.

model, starting with low densities results in an increase over the course of 7 days (this counterintuitive result is discussed below). The model generates a steady-state point that occurs at approximately 25,000 receptors. If the cell starts with less than this number, it increases to this number. If it starts with expression greater than 25,000 receptors, it decreases to this number (but not below). For many of the experiments used to generate the parameters used for the simulations (but only those that utilized freshly isolated basophils), the experimental conditions included 7-day culture without IgE. These data are shown in Figure 8 and indicate that the experimental data set most reasonably follows the constant-synthesis/regulated-loss model. An alternative way of analyzing this experimental data is to focus on those experiments in which the starting receptor expression was below 25,000; this value would be $17,000 \pm 1,700$ ($n=24$) on day 0 and $25,000 \pm 2,600$ after 7 days in culture without IgE.

As noted in Materials and Methods, we also examined a two-compartment model because such a model appeared to have the potential to change the relationship between the actual dissociation constant (k_d) and the observed dissociation constant. We found that, if the internal compartment pool of receptor and IgE was large enough, the slow appearance of IgE and receptor from this internal pool could mask a faster dissociation rate. For example, if the size of the plasma membrane compartment and the internal pool were set to be equal, the dissociation rate could be reduced by approximately two- to threefold. However, there was an additional effect. After setting the compartments equal and refitting the parameters so that the four relationships in Figure 6 were satisfied, the relationship shown in Figure 8 was altered. The internal pool increased the steady-state point to higher values that were dependent on the size of the internal pool. For the equal-pool simulation, steady-state values (the crossover-point marked in Figure 8) shifted from 25,000 to 35,000, a value we did not observe experimentally but one not grossly outside the bounds of error in these studies.

DISCUSSION

These studies explored both the loss and gain of FcεRI during culture in the presence of IgE, with an eye towards demonstrating whether IgE regulated either loss or gain or both. Our previous *in vitro* studies suggested that the loss of receptors was very slow (5% per day once sufficient IgE had dissociated) and also raised the possibility that only unoccupied receptors were lost. Dissociating IgE from basophils with lactic acid supported this viewpoint and also indicated that the maximal rate of loss was not restricted to the low rates estimated in previous studies. Indeed, 50% of unoccupied receptors could be lost in 1 day. The data in Figure 2 suggest that we had opened up $\approx 50,000$ receptors (the starting receptor density was $\approx 100,000$, 25% of which were lost in 1 day, and only unoccupied receptors were lost) and that 25,000 would be lost in 24 h. Although these receptors were lost (as reflected by decreases in 15A5 binding), there was no loss of IgE (mAb TES-19 or 10-95-3), and if the cells were fully sensitized, there was no loss of receptor (22E7). These studies solidified our

previous interpretation of the data, i.e., that occupied receptor is protected from loss. How loss is accomplished remains unknown. In this context, we also found that a variety of metalloprotease inhibitors (all with hydroxamic acid moieties and able to bind zinc), some reasonably selective for the CD23 metalloprotease [14], do not appear to influence the rate of FcεRIα loss. The concentrations used for these experiments have been shown to be effective in a variety of other systems, but these drugs are also toxic in cell culture, so significantly higher concentrations could not be examined.

The other side of this process relates to whether IgE induces synthesis of FcεRIα. Because receptor up-regulation, even in the presence of high IgE concentrations, is very slow, we initially examined basophils for any IgE-mediated changes in mRNA expression. This was done by three techniques, all of which returned the same result. IgE had no meaningful influence on expression of mRNA for FcεRIα, β, and γ. We also could find no evidence for a change in synthetic rates as determined by ^{35}S -methionine labeling of FcεRIα. It is important that an ideal radiolabeling study for this purpose would be designed to inhibit loss so that any changes in label accumulation could not be ascribed to changes in the loss rate rather than the synthesis rate. However, in these studies we can put the model simulation to work and demonstrate that for a short incubation, changes in the amount of labeling can be primarily attributed to changes in synthetic rates. This occurred because there was already an existing large pool of receptor, relative to the labeled receptor being generated, that diluted out the labeled receptor so that any loss mechanism sampled labeled receptor from this surface pool rather than from the synthetic pool. Simulating a model in which the receptor can be radio-labeled (or not) shows that within the first 12 h, loss could contribute only a few percent to the accumulation of receptor under the conditions of our experiments. Having said this, the data indicate that there was no difference in the incorporation of ^{35}S -methionine into FcεRIα.

It is becoming increasingly evident that several factors lead to expression of FcεRIα [31]. One aspect that we have not examined which might still result in altered “loading” rates relates to the regulatory mechanisms for transport of completed FcεRIα to the plasma membrane. These cells might synthesize receptor at a higher rate than it is exported to the plasma membrane, which would lead to an accumulation internally such that the export rate could be modulated by IgE up to the point of the synthetic rate. For example, 500 receptors per h might be synthesized, but under conditions of low IgE, only 400 per h might be transported to the plasma membrane. There would then be an accumulation of 100 receptors per h internally (e.g., in the ER or Golgi apparatus) under this scheme. Recently published results by Donnadieu et al. [31] raise the possibility of an accumulating internal pool in the absence of FcεRIβ expression. As will be shown in a forthcoming study, there does appear to be an internal pool of receptors in freshly isolated basophils [32]. Based on the sensitivity of this pool to endoglycosidase H, this internal pool appears to be a pre-Golgi pool in human basophils, but a clear understanding of what this pool is doing in basophils is not yet in hand. Its presence does open up the possibility for another level of control for receptor loading on the plasma membrane.

To address this possibility, we searched for a relationship in our experimental data set that could be used to distinguish between a model that used regulated versus constant synthesis. We found that although the two models were not easily distinguished on the basis of the four relationships shown in Figure 6, they were distinguished by the relationship shown in Figure 8. This relationship indicated that the better model would be one of constant “loading,” which would therefore indicate that there is no level at which the “loading” rate is modified by the presence of IgE, including the scheme discussed above in which transport to the plasma membrane was a potential area of regulation.

This notion of constant synthesis/regulated loss leading to an increase in receptor expression in the absence of IgE when the starting density is low is counter-intuitive. For any model in which there is other than zero synthesis in the absence of IgE, there will necessarily be a steady state at which loading is balanced by loss. The system is always migrating to the appropriate steady state, and, because we force the model to start with certain experimental conditions that are not necessarily steady state for the model, the system naturally migrates to this steady state. Therefore, the question becomes why this happens in the *in vitro* experimental system? If the model parameters represent what happens *in vivo*, then it should not be possible to observe an increase in culture in the absence of IgE. The answer to this question is not currently known, but we can speculate that culturing basophils in media containing 10 ng/mL of IL-3 results in a change in the synthetic or export rate of FcεRIα. Thus, it is not IgE which regulates the loading rate but IL-3. This may be testable under the right conditions, although excluding IL-3 from these cultures is not possible for extended periods due to the induction of apoptosis. However, it is unlikely that many individuals have circulating IL-3 levels as high as 10 ng/mL, and IL-3 at these concentrations is known to induce a variety of phenotypic changes in basophils. It is possible that the loading rates of FcεRIα are one of the processes controlled by IL-3. If so, transferring cells from *in vivo* conditions to *in vitro* conditions would shift the steady-state point higher, thus causing basophils with low levels of expression to increase expression even in the absence of IgE. A combination of recent results by our group and other data recently published suggest one possibility. As noted above, Donnadieu et al. [31] have shown that FcεRIβ regulates the rate at which FcεRIα₂ is transferred from the ER to post-ER processing steps (Golgi and transport). We have recently shown that IL-3 markedly up-regulates the expression of FcεRIβ (increases in protein and mRNA) [32]. When these results are taken together, we could suggest that once placed in culture, donor basophils that begin with very low receptor levels (and therefore do not express much FcεRIβ) would increase the rate of ER-to-Golgi processing, effectively increasing the loading rate and therefore altering the steady-state set point upwards.

The other goal of the modeling was to determine whether a simple model could explain the unexpectedly high IgE EC50 for FcεRIα up-regulation. Parameters that allowed a good fit with the down-regulatory and up-regulatory kinetics resulted in an excellent fit with the EC50 relationship. For all the models examined, the forward rate constant played a dominant role in positioning the EC50 point. This is an intuitively correct result;

if loss is controlled by receptor occupancy, then the rate that IgE can occupy the receptor would influence the ability of the cell to accumulate receptor. The parameter k_r that fits the data set best is entirely consistent with previously determined values [3, 30].

We also examined the effect of including a second circulation compartment in the model. One interesting aspect to the two-compartment model is that it allows for a faster dissociation constant to be used in the model and still retains the quality of data fitting shown in Figure 6. Rates of dissociation faster than the one we have used in the one-compartment model would be more consistent with reported dissociation rates. However, it is not yet known what happens to receptor removed from the cell surface. Studies in RBL cells in the 1980s indicated that monomeric receptors undergo coendocytosis with aggregated FcεRI cycles through an internal compartment [33]. On the other hand, under resting conditions, unoccupied receptors on RBL cells disappear by mechanisms unknown [12]. One could conclude that when receptors are lost under these conditions, they become unable to bind IgE because the technique used to isolate receptor depends on IgE binding. This result would suggest that there is no receptor recycling under resting-cell conditions in RBL cells. The simulated two-compartment model also appears to shift the steady-state receptor density (the value the system settles into in the absence of IgE) upward significantly, to values that are not as compatible with the experimental results we have found. Whether, this is an indication of the absence of a cycling second compartment requires experimental verification.

In summary, these studies strongly suggest that regulation of FcεRIα expression on basophils is mediated by a process of IgE-regulated loss in the presence of constant synthesis. We have strengthened our data which indicate that loss occurs only with unoccupied receptors. The nature of the down-regulatory process remains unknown. At both the mRNA level and protein level, there was no indication that IgE regulates synthetic rates.

APPENDIX

If the model of constitutive synthesis and regulated loss is correct, there are two specific steady solutions that have biological implications. The steady-state solution with no IgE present for Equations 1–3 is calculated as follows:

$$R_0 = \frac{(L/V_R)^{1/\alpha} K_{RO}}{1 - (L/V_R)^{1/\alpha}}$$

The variables are the same as described in Materials and Methods. Using the parameters listed in the legend to Figure 6, the steady-state expression of FcεRI should be 13,554 receptors. This result appears different from the one shown in Figure 8. However, the data in Figure 8 indicate a crossover point for the *in vitro* system under study; i.e., the point where basophils cultured in the absence of IgE do not lose and do not gain receptors appears to be $\approx 25,000$. The cause for this discrepancy lies in the method by which the experiment was per-

formed in Figure 8. To simulate what is happening in the experimental cultures, it was necessary to include the fact that the basophils come preloaded with some IgE. In the low range of receptors where the cells gain receptors even in the absence of IgE, experimental measurements indicate that receptor occupancy ranges from 30 to 70% depending on the starting density. For the purposes of the simulation, a variety of experimental studies led to a simple relationship between starting density and occupancy which was used to do the simulation. The effect of including some resident IgE on the basophil is that the starting receptor density is approximated by the unoccupied receptor density in this type of experiment. Some of the resident IgE dissociates (and reaches an approximate equilibrium with the receptors) so that the final result is a steady state loosely based on the unoccupied-receptor density. At 25,000 receptors, occupancy is such that there is no net gain or loss of total receptors in a 7-day culture. This result is therefore different from a situation in which IgE concentrations are very low in vivo. Here, the cell surface density of receptors more closely approaches the prediction of the steady-state solution. For example, for values of IgE <1 ng/mL, 1 week of accumulation from 0 (see below) would be within 10% of the steady-state value noted above.

A second issue relates to the maximum numbers of receptors that could be expressed on basophils. In this model, during the first weeks of receptor accumulation, the process appeared linear, but a maximum does exist in this model. Although the accumulation to a maximum occurs asymptotically, the time to reach this asymptote is extremely long for concentrations of IgE considered typical for atopic patients (at 500 ng/mL of IgE, reaching this maximum requires over a year). Furthermore, the asymptote is a linear function of the IgE concentration (as is the time required to closely approximate the asymptote at concentrations above ≈ 3 ng/mL); at 500 ng/mL of IgE, the maximum number of receptors would be approximately 720,000 (using the parameters supplied in the Figure 6 legend). It is apparent that the in vitro loading rate for basophils is fairly slow such that if no receptors are lost because the IgE concentration is high, 450 receptors per hour translates to only 75,000 receptors per week in the first weeks of the process. Atopic donors have receptor densities that average 250,000 per cell. The implication is that the basophils must circulate for weeks to obtain this value. The only published reports for the human basophil life span conclude that it is on the order of 2 days [34]. Other anecdotal reports have placed the life span closer to 1–2 weeks. In either case, further information is necessary. Prior to being moved into the circulation, developing basophils express receptors [35]. However, the following information is missing: (1) the residence time of the basophil in the bone marrow, (2) when, during its development, the basophil begins behaving like a circulating basophil with respect to the control of Fc ϵ RI expression, and (3) the titers of IgE in the marrow. As noted in the discussion, the regulation of Fc ϵ RI α synthetic rates may not be a function of IgE but of other factors, such as the cytokine environment. Therefore, the actual rates of loading may be different in the marrow than in the circulation. Therefore, although the analysis is incomplete due to a lack of information, there are clearly some issues raised which, if addressed, may shed new insight into the process of Fc ϵ RI expression.

ACKNOWLEDGMENT

We thank Dr. Byron Goldstein, Theoretical Biology and Biophysics Group, Los Alamos National Laboratories, for his review and suggestions.

REFERENCES

1. Hsu, C., MacGlashan, D. W., Jr. (1996) IgE Antibody up-regulates high affinity IgE binding on murine bone marrow derived mast cells. *Immunol. Lett.* 52, 129–134.
2. MacGlashan, D. W., Jr., Bochner, B. S., Adelman, D. C., Jardieu, P. M., Togias, A., Mckenzie-White, J., Sterbinsky, S. A., Hamilton, R. G., Lichtenstein, L. M. (1997) Down-regulation of Fc ϵ RI expression on human basophils during in vivo treatment of atopic patients with anti-IgE antibody. *J. Immunol.* 158, 1438–1445.
3. MacGlashan, D. W., Jr., White-Mckenzie, J., Chichester, K., Bochner, B. S., Davis, F. M., Schroeder, J. T., Lichtenstein, L. M. (1998) In vitro regulation of Fc ϵ RI α expression on human basophils by IgE antibody. *Blood* 91, 1633–1643.
4. Yamaguchi, M., Lantz, C. S., Oettgen, H. C., Katona, I. M., Fleming, T., Miyajima, I., Kinet, J. P., Galli, S. J. (1997) IgE enhances mouse mast cell Fc ϵ RI expression in vitro and in vivo: evidence for a novel amplification mechanism in IgE-dependent reactions. *J. Exp. Med.* 185, 663–672.
5. Lantz, C. S., Yamaguchi, M., Oettgen, H. C., Katona, I. M., Miyajima, I., Kinet, J. P., Galli, S. J. (1997) IgE regulates mouse basophil Fc ϵ RI expression in vivo. *J. Immunol.* 158, 2517–2521.
6. Yamaguchi, M., Sayama, K., Yano, K., Lantz, C. S., Noben-Trauth, N., Ra, C., Costa, J. J., Galli, S. J. (1999) IgE enhances Fc epsilon receptor I expression and IgE-dependent release of histamine and lipid mediators from human umbilical cord blood-derived mast cells: synergistic effect of IL-4 and IgE on human mast cell Fc epsilon receptor I expression and mediator release. *J. Immunol.* 162, 5455–5465.
7. Xia, H. Z., Du, Z., Craig, S., Klisch, G., Noben-Trauth, N., Kochan, J. P., Huff, T. H., Irani, A. M., Schwartz, L. B. (1997) Effect of recombinant human IL-4 on tryptase, chymase, and Fc epsilon receptor type I expression in recombinant human stem cell factor-dependent fetal liver-derived human mast cells. *J. Immunol.* 159, 2911–2921.
8. Malveaux, F. J., Conroy, M. C., Adkinson, N. F. J., Lichtenstein, L. M. (1978) IgE receptors on human basophils: relationship to serum IgE concentration. *J. Clin. Invest.* 62, 176–181.
9. Sihra, B. S., Kon, O. M., Grant, J. A., Kay, A. B. (1997) Expression of high-affinity IgE receptors (Fc epsilon RI) on peripheral blood basophils, monocytes, and eosinophils in atopic and nonatopic subjects: relationship to total serum IgE concentrations. *J. Allergy Clin. Immunol.* 99, 699–706.
10. Saini, S. S., Klion, A. D., Holland, S. M., Hamilton, R. G., Bochner, B. S., MacGlashan, D. W., Jr. (2000) The relationship between serum IgE and surface levels of FcepsilonR on human leukocytes in various diseases: correlation of expression with FcepsilonRI on basophils but not on monocytes or eosinophils. *J. Allergy Clin. Immunol.* 106, 514–520.
11. MacGlashan, D. W., Jr., Lichtenstein, L. M., McKenzie-White, J., Chichester, K., Henry, A. J., Sutton, B. J., Gould, H. J. (1999) Upregulation of Fc ϵ RI on human basophils by IgE antibody is mediated by interaction of IgE with Fc ϵ RI. *J. Allergy Clin. Immunol.* 104, 492–498.
12. Furuichi, K., Rivera, J., Isersky, C. (1985) The receptor for immunoglobulin E on rat basophilic leukemia cells: effect of ligand binding on receptor expression. *Proc. Natl. Acad. Sci. USA* 82, 1522–1552.
13. Lee, W. T., Rao, M., Conrad, D. H. (1987) The murine lymphocyte receptor for IgE IV. The mechanism of ligand-specific receptor upregulation on B cells. *J. Immunol.* 139, 1191–1198.
14. Marolewski, A. E., Buckle, D. R., Christie, G., Earnshaw, D. L., Flamborg, P. L., Marshall, L. A., Smith, D. G., Mayer, R. J. (1998) CD23 (Fc ϵ silonRII) release from cell membranes is mediated by a membrane-bound metalloprotease. *Biochem. J.* 333, 573–579.
15. Ishizaka, T., Helm, B., Hakimi, J., Niebhl, J., Ishizaka, K., Gould, H. (1986) Biological properties of a recombinant human immunoglobulin epsilon-chain fragment. *Proc. Natl. Acad. Sci. USA* 83, 8323–8327.
16. Ishizaka, T., Dvorak, A. M., Conrad, D. H., Niebhl, J. R., Marquette, J. P., Ishizaka, K. (1985) Morphologic and immunologic characterization of human basophils developed in cultures of cord blood mononuclear cells. *J. Immunol.* 134, 532–540.

17. Miller, L., Blank, U., Metzger, H., Kinet, J. P. (1989) Expression of high-affinity binding of human immunoglobulin E by transfected cells. *Science* 244, 334–337.
18. Hakimi, J., Seals, C., Kondas, J. A., Pettine, L., Danho, W., Kochan, J. (1990) The alpha subunit of the human IgE receptor (FcεRI) is sufficient for high affinity IgE binding. *J. Biol. Chem.* 265, 22079–22081.
19. Ishizaka, K., Ishizaka, T., Lee, E. H. (1970) Biologic function of the Fc fragments of E myeloma protein. *Immunochemistry* 7, 687–702.
20. MacGlashan, D. W., Jr. (1993) Releasability of human basophils: cellular sensitivity and maximal histamine release are independent variables. *J. Allergy Clin. Immunol.* 91, 605–615.
21. MacGlashan, D. W., Jr., White, J. M., Huang, S. K., Ono, S. J., Schroeder, J., Lichtenstein, L. M. (1994) Secretion of interleukin-4 from human basophils: the relationship between IL-4 mRNA and protein in resting and stimulated basophils. *J. Immunol.* 152, 3006–3016.
22. Schroeder, J. T., MacGlashan, D. W., Jr., Kagey-Sobotka, A., White, J. M., Lichtenstein, L. M. (1994) The IgE-dependent IL-4 secretion by human basophils: the relationship between cytokine production and histamine release in mixed leukocyte cultures. *J. Immunol.* 153, 1808–1817.
23. Warner, J. A., Reshef, A., MacGlashan, D. W., Jr. (1987) A rapid Percoll technique for the purification of human basophils. *J. Immunol. Methods* 105, 107–110.
24. Gilbert, H. S., Ornstein, L. (1975) Basophil counting with a new staining method using alcian blue. *Blood* 46, 279–286.
25. Bochner, B. S., McKelvey, A. A., Schleimer, R. P., Hildreth, J. E., MacGlashan, D. W., Jr. (1989) Flow cytometric methods for the analysis of human basophil surface antigens and viability. *J. Immunol. Methods* 125, 265–271.
26. Riske, F., Hakimi, J., Mallamaci, M., Griffin, M., Pilson, B., Tobkes, N., Lin, P., Danho, W., Kochan, J., Chizzonite, R. (1991) High affinity human IgE receptor (FcεRI): analysis of functional domains of the α-subunit with monoclonal antibodies. *J. Biol. Chem.* 266, 11245–11251.
27. Xia, H.-Z., Kepley, C. L., Sakai, K., Chelliah, J., Irani, A.-M. A., Schwartz, L. B. (1995) Quantitation of tryptase, chymase, FcεRIα and FcεRIγ mRNAs in human mast cells and basophils by competitive reverse transcription-polymerase chain reaction. *J. Immunol.* 154, 5472–5480.
28. Kalayci, O., Saraclar, Y., Adalioglu, G., Sekerel, B., Tuncer, A. (1995) The effect of cetirizine on sulfidoleukotriene production by blood leukocytes in children with allergic rhinitis. *Allergy* 50, 964–969.
29. Pruzansky, J. J., Grammer, L. C., Patterson, R., Roberts, M. (1983) Dissociation of IgE from receptors on human basophils. I. Enhanced passive sensitization for histamine release. *J. Immunol.* 131, 1949.
30. Goldstein, B., Dembo, M., Malveaux, F. J. (1979) Some quantitative aspects of the passive sensitization of human basophils. *J. Immunol.* 122, 830–833.
31. Donnadieu, E., Jouvin, M. H., Kinet, J. P. (2000) A second amplifier function for the allergy associated FcεRIβ subunit. *Immunity* 12, 515–523.
32. Saini, S., Richardson, J. J., Wofsy, C., Lavens-Phillips, S. E., Bochner, B., MacGlashan, D. W., Jr. (2001) Expression and modulation of FcεRIα and FcεRIβ in human blood basophils. *J. Allergy Clin. Immunol.*, 107, 832–841.
33. Furuichi, K., Rivera, J., Buonocore, L. M., Isersky, C. (1986) Recycling of receptor-bound IgE by rat basophilic leukemia cells. *J. Immunol.* 136, 1015–1022.
34. Parwaresch, M. R. (1976) *The Human Blood Basophil*. Springer-Verlag, New York.
35. Thompson, H. L., Metcalfe, D. D., Kinet, J. P. (1990) Early expression of high-affinity receptor for immunoglobulin E (Fc epsilon RI) during differentiation of mouse mast cells and human basophils. *J. Clin. Invest.* 85, 1227–1233.