

Expression and modulation of FcεRIα and FcεRIβ in human blood basophils

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Background: The IgE receptor (FcεRI) may exist as a tetramer (αβγ₂) or a trimer (αγ₂) because FcεRIβ is dispensable for membrane expression of FcεRIα. FcεRIβ amplifies signaling of FcεRI so that regulation of FcεRIα:β stoichiometry would affect cellular responsiveness.

Objective: We examined basophils from a variety of donors for differences in their expression of FcεRIα and FcεRIβ protein.

Methods: Enriched blood basophils were assessed at baseline and after IL-3 culture for FcεRIα and FcεRIβ protein by Western blotting, surface FcεRIα by flow cytometry, and FcεRIβ mRNA by real-time PCR. Basophil functional response was measured by allergen-triggered histamine release.

Results: For the FcεRIα subunit, 2 protein bands with molecular weights of 50 kd and 60 kd were identified by Western blots. The 60-kd band correlated to surface-expressed FcεRIα detected by flow cytometry (Spearman $R = 0.78$, $P < .01$). Surface FcεRIα also correlated with FcεRIβ protein (Spearman $R = 0.92$, $P < .01$). FcεRIβ protein levels increased disproportionately with higher surface FcεRIα expression. The ratio of FcεRIβ to FcεRIα varied 10-fold among donors and correlated with surface FcεRIα. Basophil 50-kd α protein levels were similar despite a 10-fold range in surface FcεRIα expression, implying stores of this protein such as those found in eosinophils. Unlike eosinophils, the basophil 50-kd protein was lost with culture and was absent from supernatants. Levels of β protein and mRNA were enhanced by IL-3 culture, whereas FcεRIα expression (by flow cytometry and 60 kd) was not.

Conclusion: These findings demonstrate variable stoichiometry of FcεRIα:β in whole cells and that this stoichiometry can be altered by IL-3 culture. With the assumption that all detected β protein is surface expressed, these findings suggest a variable stoichiometry for FcεRIα:β that is also related to FcεRIα surface expression. (*J Allergy Clin Immunol* 2001;107:832-41.)

Key words: IgE receptors, basophils, IL-3

Abbreviations used

BPO:	Benzylpenicilloyl
HSA:	Human serum albumin
MFI:	Mean fluorescence intensity
PAG:	PIPES buffer containing 0.003% human serum albumin and 0.1% glucose
PIPES:	Piperazine-N,N'-bis-2-ethanesulfonic acid
RBL:	Rat basophil leukemia
Rs:	Spearman rank correlation coefficient
TBST:	Buffer containing Tris buffer, NaCl, and Tween-20

The high-affinity IgE receptor (FcεRI) expressed on the surface of human mast cells and basophils is involved in the mediation of allergic responses.¹ On these leukocytes, the FcεRI complex has been considered to be a tetramer with a single α and β chain and 2 γ chains. Free IgE binds with high affinity to the α subunit of the receptor, whereas the β and 2 γ chains are involved with signal transduction and bear immunoreceptor tyrosine-based kinase activation motifs in their cytoplasmic tails. Transfection studies using human, mouse, and rat FcεRI receptor complex subunits first demonstrated that coexpression of the human α and γ subunits was sufficient to display functional surface FcεRI complexes.² Human studies have confirmed the existence of trimeric FcεRI (αγ₂) receptor complexes on cells such as Langerhans' cells,³ dendritic cells,⁴ and monocytes.⁵ The ability of the receptor to be expressed as a trimer or tetramer has led to the hypothesis that both αγ₂ and αβγ₂ complexes may coexist on the surface of mast cells and basophils. In transfected cells and transgenic mice, the presence of the β subunit in the FcεRI complex has been shown to amplify IgE-triggered signaling events by 5- to 7-fold.^{6,7} Recently, the β subunit has also been shown to amplify surface IgE receptor expression.⁸ In addition, the locus of the β gene has been linked in genomic studies to the condition of atopy.⁹ Given its amplifier functions, the relative presence of β in FcεRI complexes of mast cells and basophils may be an important regulator of cellular responsiveness to allergen-triggered events.

In this study we examined the total expression of FcεRIα and β protein levels in human blood basophils as an indicator of their surface stoichiometry. We further examined the effects of IL-3 exposure on basophil receptor subunit levels and its effects on basophil mediator release.

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METHODS

Buffers

PIPES (piperazine-N,N'-bis-2-ethanesulfonic acid) obtained from Sigma Chemical Co (St Louis, Mo) was used in a stock buffer composed of 25 mmol/L PIPES, 110 mmol/L NaCl, 5 mmol/L KCl, and 40 mmol/L NaOH, adjusted to pH 7.47. PAG buffer consisted of PIPES buffer containing 0.003% human serum albumin (HSA; Miles Laboratories Inc, Elkhart, Ind) and 0.1% glucose; PAG-EDTA buffer consisted of PAG buffer with 1 mmol/L EDTA. TBST buffer consisted of 50 mmol/L Tris buffer (pH 7.5) and 0.15 mol/L NaCl, with 0.1% Tween-20, whereas boric-buffered saline buffer contained 100 mmol/L boric acid and 80 mmol/L NaCl, with 0.5% Triton X-100.

Human subjects

After giving informed consent, adult volunteers (aged 26-54 years; 5 men/7 women) either with (n = 6) or without (n = 6) a history of atopic disease (positive skin test results and a clinical history of allergic rhinitis) underwent venipuncture under a protocol approved by the Johns Hopkins Bayview institutional review board.

Basophil isolation

Venous blood samples (60-120 mL) were drawn into syringes containing 5 mmol/L EDTA as an anticoagulant. Basophils were enriched from blood with a PIPES-buffered, Percoll-based density-gradient centrifugation technique and Accuspin separation tubes (Sigma), as described.^{10,11} Basophil purity ranged from 2% to 6% (mean 4%, n = 21), as enumerated by alcian blue staining and light microscopic counts.¹² Aliquots of basophils were removed at this point for flow cytometric analysis of FcεRIα surface expression (see below). For Western analysis and culture experiments, basophils were further purified by negative selection with the basophil-separation kit (Miltenyi Biotech, Auburn, Calif) according to the manufacturer's instructions. The resulting basophils were of higher purity (range 50%-100%, mean 80%, n = 21). For some experiments, positively selected leukocytes (mainly monocytes and lymphocytes) were also examined (n = 10). Total cell counts and viability (98% ± 1%, mean ± SEM; n = 9) after negative selection were determined by light microscopy and erythrosin B dye exclusion.

Immunoprecipitation and Western blotting analysis

For determination of FcεRIα and β protein levels in fresh or cultured basophils, 500,000 enriched basophils (or an equal number of positively selected cells) were lysed on ice for 5 minutes in SDS sample buffer (8% SDS, 25 mmol/L Tris [pH 6.8], 2% mercaptoethanol, 0.1% bromophenol blue, 4% glycerol) and boiled for 5 minutes. Lysates of a single passage of rat basophilic leukemia (RBL)-SX38 cells transfected with the human αβγ chains (kind gift of Dr Jean Pierre Kinet, Harvard University, Boston, Mass) were included with each Western blot experiment as a standard to allow comparisons between immunoblots. Cell lysates were analyzed by SDS-PAGE electrophoresis, and proteins were transferred to polyvinylidene difluoride membranes (Bio-Rad, Hercules, Calif). Membranes were blocked overnight in TBST -4% BSA at 4°C to reduce nonspecific binding. Membranes were divided to separate paired basophil lysates and immunoblotted with either a rabbit polyclonal anti-FcεRIβ antibody or anti-FcεRIα antibody (Upstate Biotechnology, Saranac, NY) or a mouse monoclonal anti-FcεRIα antibody (22E7, IgG1; J. Kochan, Hoffman-La Roche Pharmaceuticals, Nutley, NJ). Membranes were washed 4 times for 10 minutes each in TBST before incubation for 45 minutes with the appropriate horseradish peroxidase-conjugated secondary antibodies (Phar-

macia Biotech, Uppsala, Sweden). Membranes were subjected to 4 additional washes before proteins were visualized by enhanced chemiluminescence reagent (Amersham, Burlington Heights, Ill). Intensities of detected protein bands were measured with a Kodak DC120 digital camera (Eastman Kodak, Rochester, NY) and NIH Image software (Bethesda, Md) and expressed relative to the band intensity of the standard lysate of RBL αβγ transfectants. In pilot studies with a standard lysate of RBL αβγ transfectants, the ability to detect up to 10-fold differences in FcεRIβ protein levels was maintained at a variety of exposure times (30 seconds to 5 minutes).

For immunoprecipitation experiments, basophils were lysed in boric-buffered saline solution lysis buffer with 200 mmol/L phenylmethylsulfonyl fluoride and 10 μg/mL each leupeptin, aprotinin, and pepstatin A (Sigma). In the case of RBL αβγ transfectants, cells were first sensitized with human myeloma IgE (5 μg/mL myeloma IgE) for 20 minutes at 37°C in PAG containing 1 mmol/L EDTA and 10 μg/mL heparin before lysis. Cell lysates were incubated overnight at 4°C with a monoclonal mouse antihuman IgE mAb (gift of Dr Frances Davis, Tanox Corp, Houston, Tex) or 22E7 bound to protein G Sepharose (Pharmacia Biotech). The beads were washed several times in lysis buffer, 20 μL of SDS sample buffer was added, and the samples were boiled for 5 minutes. Endoglycosidase H digestion of 22E7 immunoprecipitates was performed according to the manufacturer's instructions (New England Biolabs, Beverly, Mass).

Basophil and eosinophil culture

Enriched basophils (mean purity 69%, n = 8) were cultured in Iscove's modified Dulbecco's media with 2% heat-inactivated FCS, 10 μg/mL gentamicin (GIBCO BRL, Gaithersburg, Md), and 10 ng/mL recombinant IL-3 (R&D Systems, Minneapolis, Minn) at a final concentration of 1×10^6 to 2×10^6 total cells per milliliter. Cells were incubated at 37°C in 5% CO₂ in 6-well tissue culture plates (Costar, Cambridge, Mass) and harvested after an average of 22 hours. After culture, viability was 89% ± 2% (mean ± SEM; n = 7), and total cell recovery was 75% ± 6% (mean ± SEM; n = 7). For eosinophil cultures, eosinophils were purified from peripheral blood with Percoll density-gradient sedimentation (specific gravity 1.090) followed by negative selection with anti-CD16 antibodies to remove neutrophils (purity > 98%).¹³ Eosinophils were cultured in RPMI 1640 media, 5% FCS, 1% penicillin/streptomycin (GIBCO BRL), and 10 ng/mL IL-5 (R&D Systems), as described previously.¹⁴ Cultured basophils and eosinophils were processed as described for Western analysis and flow cytometry.

Surface and intracytoplasmic flow cytometry

Enriched basophil preparations were labeled for direct and indirect immunofluorescence and flow cytometry in the presence of 4 mg/mL human IgG to minimize nonspecific binding to FcγR as previously described.¹⁵ Antibodies for these studies included an irrelevant mouse IgG₁ control (Coulter Corp, Hialeah, Fla), FITC-conjugated polyclonal goat antihuman IgE and its control FITC-conjugated normal goat IgG (Kirkegaard and Perry, Gaithersburg, Md), and mouse antihuman FcεRIα subunit (22E7, unaffected by FcεRI occupancy).¹⁶ Samples were analyzed on a Coulter EPICS Profile flow cytometer. At least 5000 events per experimental condition were counted. Data were expressed as net mean fluorescence intensity (MFI) (Actual MFI - MFI of irrelevant IgG control, the latter averaging 0.4 ± 0.4 [mean ± SEM; n = 12]).

For intracytoplasmic detection of FcεRIα, fresh low-purity basophils (3.6% purity, n = 3) were compared with basophils after IL-3 culture, as described.^{17,18} In brief, leukocyte samples were fixed for 5 minutes in 4% paraformaldehyde, permeabilized by incubation in PBS containing 0.1% saponin for 1 hour on ice, and then labeled for flow cytometry as indicated above.

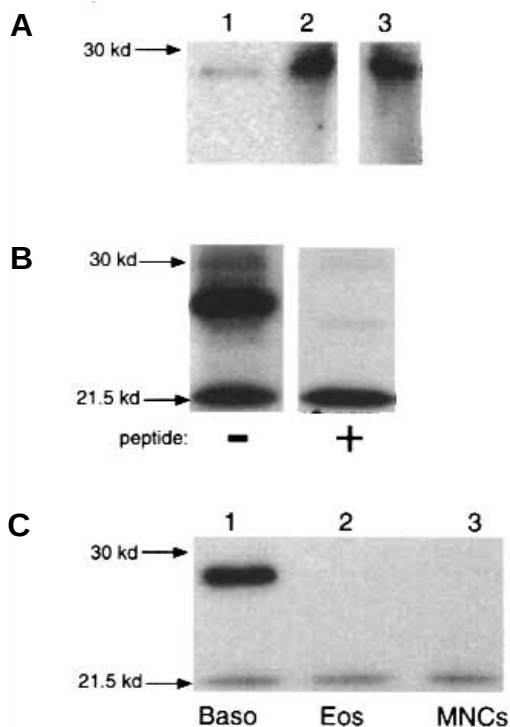


FIG 1. Detection of Fc ϵ RI β protein in human basophils. **A**, Shown is an anti-Fc ϵ RI β immunoblot of basophil whole cell lysates (lane 1, 5×10^5 cells) and antihuman IgE immunoprecipitates of basophils (lane 2, 4×10^6 cells) and RBL $\alpha\beta\gamma$ transfectants (lane 3, 4×10^6 cells sensitized with human myeloma IgE). A 28-kd protein band is identified in all 3 lanes. **B**, Specific inhibition of Fc ϵ RI β protein detected in RBL $\alpha\beta\gamma$ transfectants by immunogenic peptide. Shown are identical RBL $\alpha\beta\gamma$ transfectant whole cell lysates immunoblotted with anti-Fc ϵ RI β in the absence (left) or presence (right) of the immunogenic peptide (MDTESNRRRLALPQE, 10 μ g/mL) used to generate the anti-Fc ϵ RI β rabbit polyclonal antibody. Specific inhibition of the prominent 28-kd band is observed in the presence of the peptide. **C**, Absence of Fc ϵ RI β protein in eosinophils and mononuclear whole cell lysates. Shown is an anti-Fc ϵ RI β immunoblot of basophil whole cell lysates (Baso; lane 1, 5×10^5 cells), eosinophils (Eos; lane 2, 5×10^5 cells), and mononuclear cells (lymphocytes and monocytes, MNCs; lane 3, 5×10^5 cells). No immunoreactive protein is observed in either eosinophil or mononuclear cell lysates. Data shown are representative of $n = 2$ eosinophil preparations and $n = 10$ mononuclear preparations.

Antigen-specific basophil histamine measurements

In separate experiments, low-purity basophils before or after IL-3 culture were sensitized with various concentrations (5 μ g/mL as the highest concentration and a series of 1:3 dilutions) of benzylpenicilloyl (BPO)-specific IgE antibody to generate a series of cell surface IgE densities. Previous studies demonstrated that sensitization with the highest concentration of BPO-specific IgE within the time used to sensitize the cells was just sufficient to load all unoccupied receptors.¹⁹ Purified BPO-specific IgE was obtained by procedures described previously.²⁰ Cells were incubated with IgE antibody for 20 minutes in PAG containing 1 mmol/L EDTA and 10 μ g/mL heparin at 37°C. After being washed once, the cells were

layered over 1 mL of chelated newborn calf serum and centrifuged for 5 minutes to rapidly separate the cells from their diluted sensitization buffer. The cells were washed 2 more times in PAG. The cells being tested for histamine release were challenged with BPO₂₁-HSA at 0.5 μ g/mL (previously determined to be the optimum for histamine release at all levels of basophil sensitization).

Real-time PCR

Low-purity basophils (average 4%, $n = 3$) and high-purity basophils (average 74%, $n = 2$) were cultured for 4 hours with or without IL-3 (10 ng/mL) and subjected to total RNA extraction with the RNeasy method according to the manufacturer's instructions (Tel-Test Inc, Friendswood, Tex). Samples were subjected to reverse transcription and real-time PCR amplification with primers specific to the human Fc ϵ RI β gene²¹ (5'-CCA-GGA-AGT-ATC-TTC-AGG-CAG-ACT-3', 5'-TCA-AAA-CTG-TCA-GCC-ATG-TAT-GC-3') along with an internal reporter construct containing FAM and TAMRA fluorochromes (5'-FAM-TTG-AAG-TCG-GCC-TCA-TCC-CCA-CC-TAMRA-3'; Biosource International, Camarillo, Calif) by use of the Taq Man kit (Perkin-Elmer, Foster City, Calif). Reaction conditions were as follows: reverse transcription at 48°C for 30 minutes followed by PCR amplification (95°C for 10 minutes; 40 cycles at 95°C for 15 seconds/60°C for 1 minute). The number of cycles to reach an equivalent number of specific mRNA copies was determined. Pilot studies demonstrated that 2-fold dilutions of a preparation of Fc ϵ RI β mRNA resulted in 1.02 ± 0.15 difference in cycle number for each 2-fold dilution.

Statistical methods

All values are expressed as mean $\pm$ SD or mean $\pm$ SEM unless otherwise noted. Data analysis was performed with JMP software (SAS Institute, Cary, NC). Correlations were calculated with Spearman rank or as indicated in the Results section.

RESULTS

Detection of Fc ϵ RI β in human basophils

The Fc ϵ RI β -specific antibody from Upstate Biotechnology (Saranac, NY) has not been previously characterized; therefore Fig 1 shows some of the preliminary characterization. We found that the antibody is not useful for direct immunoprecipitation (not shown), so Fc ϵ RI β could be detected only in whole cell lysates or from cell lysates that had been immunoadsorbed with anti-IgE antibody under conditions favorable for retaining the association of Fc ϵ RI subunits.²² Fig 1, A, shows that a whole cell lysate from peripheral blood basophils demonstrated a band migrating somewhat faster than the 30-kd molecular weight marker. Anti-IgE antibody immunoprecipitates from lysates of basophils and RBL-SX38 cells showed a similar band. This band was not present in immunoblots in which the Fc ϵ RI β subunit peptide immunogen was included during the incubation with anti-Fc ϵ RI β antibody (Fig 1, B). Fig 1, C, shows that the band was not detected in whole cell lysates of mononuclear cells (lymphocytes and monocytes) or eosinophils (as expected from previous studies using the anti-Fc ϵ RI β antibody obtained from J. P. Kinet,¹⁴ in which Fc ϵ RI β was also not detected). These results indicated that the UBI antibody could reasonably detect Fc ϵ RI β protein in Western blots.

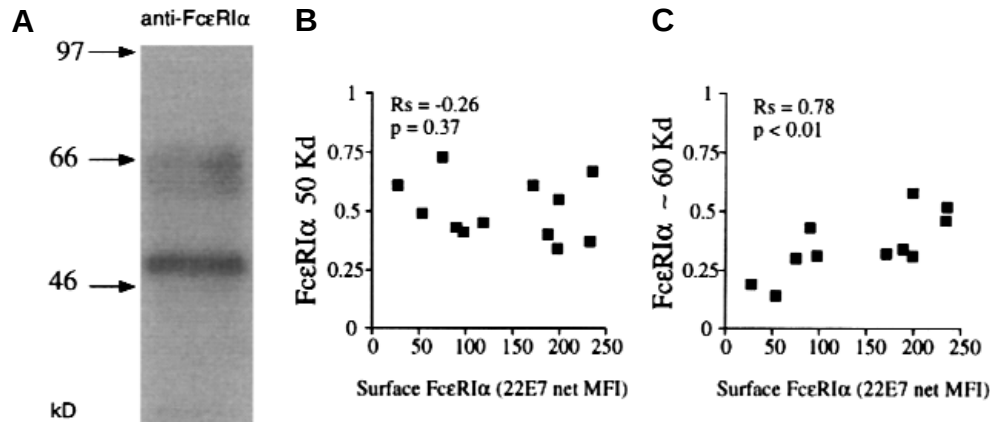


FIG 2. Detection of FcεRIα protein relative to levels of surface FcεRIα among basophil donors. **A**, Shown is an anti-FcεRIα (mAb 22E7) immunoblot of a basophil lysate from an individual. Surface expression of FcεRIα by flow cytometry for this donor was performed with mAb 22E7 (net MFI = 170). Two immunoreactive bands were detected for FcεRIα: a broad band of approximately 60 kd and a dense 50-kd band (**B** and **C**). In **B** and **C**, the *x-axis* represents the surface FcεRIα level for each individual donor in net MFI units (as detected by the 22E7 mAb). The *y-axis* represents the level of the indicated FcεRIα protein expressed relative to the FcεRIα protein in the standard RBL transfectant lysate. Levels of approximately 60-kd FcεRIα (**C**) are positively correlated to surface FcεRIα expression, whereas levels of 50-kd FcεRIα protein (**B**) are not. Note that *n* = 11 for the approximately 60-kd data because of technical issues in digital image analysis of this band in a single subject.

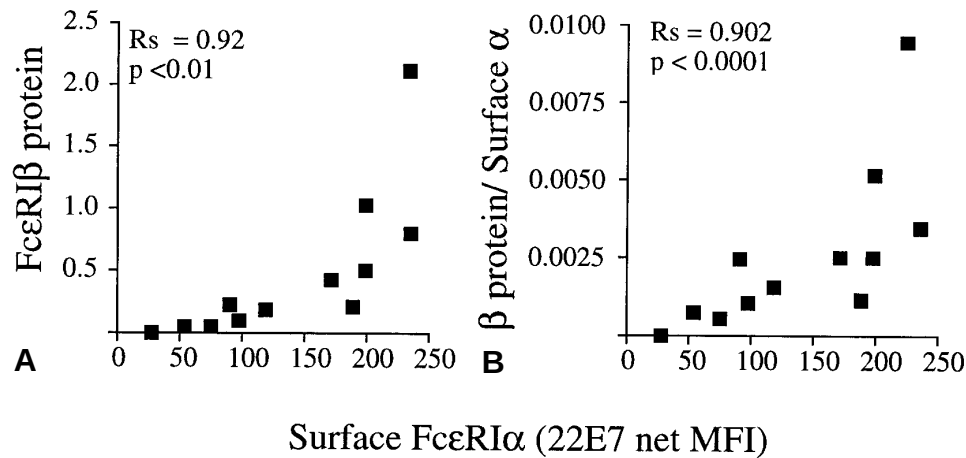


FIG 3. Expression of FcεRIβ protein relative to levels of surface FcεRIα among basophil donors. In both **A** and **B** the *x-axes* represent the surface FcεRIα level for each individual donor in net MFI units as detected by the 22E7 mAb. In **A** the *y-axis* represents the level of FcεRIβ protein expressed relative to the corresponding β protein in the standard RBL transfectant lysate. Levels of the FcεRIβ protein are positively correlated to surface FcεRIα. In **B** the *y-axis* represents a ratio of total FcεRIβ to surface FcεRIα. Levels of FcεRIβ protein are not constant across the range of surface FcεRIα but increase disproportionately in individuals with higher surface FcεRIα.

Relative expression of FcεRIα and FcεRIβ proteins in freshly isolated human basophils

To study the relative expression of FcεRIα and β, we recruited 12 donors (6 atopic and 6 nonatopic) with a 10-fold range in cell surface FcεRIα expression as determined from flow cytometry using 22E7 antibody for detection. We have previously calibrated this antibody

under flow cytometric conditions, and using this information, we found that the receptor density for these subjects ranged from 32,000 to 280,000 per basophil (27 to 235 MFI).²³ In addition to the measurements of surface expression by flow cytometry, we examined the same preparations for FcεRIα and β using whole cell lysates and Western blotting. Blotting for FcεRIα with 22E7 resulted in an unexpected band pattern, as shown in Fig

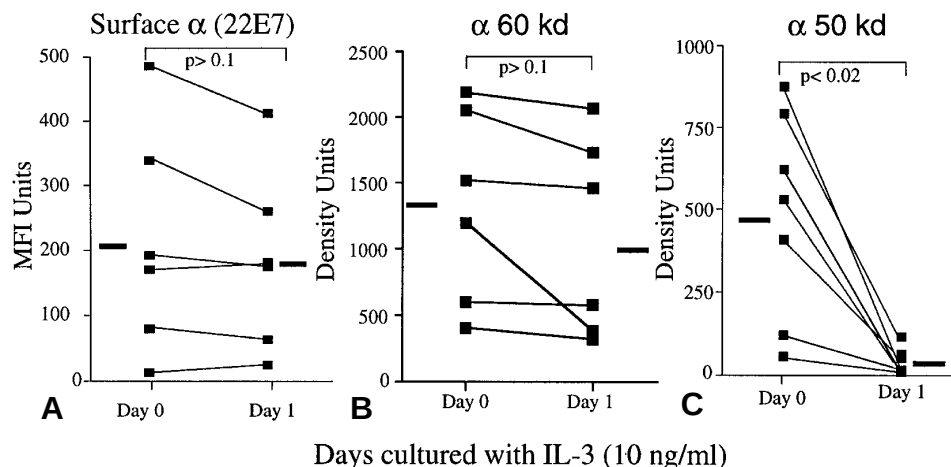


FIG 4. Expression of surface FcεRIα and FcεRIα proteins (50 kd and ~60 kd) before and after 22 hours of IL-3 culture (10 ng/mL). **A**, Levels of surface FcεRIα on basophils as detected by 22E7 net MFI were not altered by IL-3 culture. **B** and **C**, The indicated FcεRIα protein is expressed in density units measured from the same immunoblot for both time points. FcεRIα surface levels and approximately 60-kd FcεRIα protein are relatively stable ($P > .1$) compared with the dramatic decrease in 50-kd FcεRIα protein in cultured basophils ($P < .02$). Horizontal bars indicate means (statistics: paired *t* test day 0 vs day 1).

2, A, 22E7 detected a broad protein band between 55 and 65 kd (60 kd average) as well as a more homogeneous band migrating at around 50 kd. The appearance of the homogeneous 50-kd band was consistent among subjects' basophils, irrespective of atopic status, and was not correlated with the purity of the basophil preparations, which ranged between 50% and 100%. Development of blots with a UBI antibody specific for FcεRIα also resulted in both 50- and 60-kd bands, suggesting that both bands were a form of FcεRIα. To further characterize the 50-kd species, we treated FcεRIα immunoprecipitates with endoglycosidase H, which cleaves mannose-rich sugars such as those found on newly synthesized FcεRIα.²⁴ As expected, only the 50-kd band was sensitive to endoglycosidase H digestion, suggesting that it is an immature form of FcεRIα (data not shown). For control of differences in Western blots, each blot included a standard lane of material from a single preparation of RBL-SX38 αβγ transfectant cells that had been stored as frozen aliquots to avoid freeze/thaw cycles. Comparisons of scanned blots were made by expressing the data as a fraction of the standard RBL-SX38 transfectant band run within the same blot. On this basis, we found the 2 relationships shown in Fig 2, B and C. There was no correlation between cell surface FcεRIα, as detected by flow cytometry, and the 50-kd band (detected by Western blot). Indeed, the intensity of this band was remarkably consistent among preparations, 0.51 ± 0.12 (fraction of standard $\pm$ SD) for these 12 subjects. In contrast, there was a reasonable correlation between cell surface FcεRIα (by flow cytometry) and the 55- to 65-kd band (Fig 2, C, Spearman *R* [Rs] = 0.78, $P < .02$). We conclude that the 55- to 65-kd band largely represented cell surface FcεRIα, which is consistent with the known heterogeneity of its glycosylation.²⁴

We next examined the relationship between FcεRIα as detected by flow cytometry and the expression of FcεRIβ. For comparisons among Western blots (as done for FcεRIα bands), digitized band intensities were expressed as a fraction of an RBL-SX38 transfectant standard included as a separate lane within each blot. Fig 3, A, shows that there was a good rank correlation between surface FcεRIα and FcεRIβ ($R_s = 0.92$, $P < .0001$).²⁵ However, there was a distinct curvilinear relationship to this plot, which suggested that the FcεRIβ/FcεRIα ratio of was not constant across the range of cell surface FcεRIα expression. The data were re-expressed as the ratio of FcεRIβ/FcεRIα versus FcεRIα and shown in Fig 3, B. Statistical analysis of these data verified that the ratio was not constant (slope $\neq 0$, $P < .0001$) across the range of cell surface FcεRIα expression (see Appendix). For 9 of the subjects, we repeated the measurements of FcεRIβ and surface FcεRIα, and the results were similar to those found in the first measurements. Not shown are Western blots for FcεRIα or β of whole cell lysates obtained from the contaminating leukocytes (positively selected by the Miltenyi column) that failed to express detectable levels of either subunit.

Effects of IL-3 culture on FcεRIα expression

While performing pilot experiments of FcεRI expression under various culture conditions, we made 2 unexpected observations. First, a 24-hour culture in the medium containing 10 ng/mL of IL-3 resulted in a marked loss of the 50-kd band of FcεRIα with no change in the 55- to 65-kd band. Fig 4 summarizes these results. Fig 4, A, shows that, as expected, there was no change in cell surface FcεRIα, as detected by flow cytometry with 22E7, during the 24-hour culture. Likewise, there was no change in the band intensity of the 55- to 65-kd band of

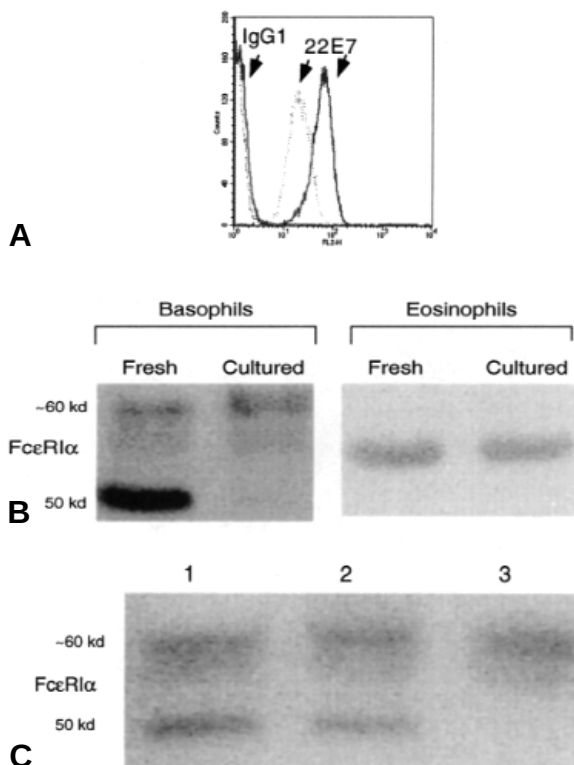


FIG 5. Detection of FcεRIα in basophils before and after IL-3 culture (A and B) or degranulation (C). Fresh (solid line) or cultured (dotted line, 10 ng/mL IL-3, 22 hours) basophils were fixed, permeabilized, and labeled for indirect immunofluorescence by flow cytometry with either control (mouse IgG1) or FcεRIα (22E7) antibodies. Cultured basophils had reduced levels of surface and intracellular FcεRIα expression (MFI = 21) as compared with fresh basophils (MFI = 107). Control IgG1 MFI: fresh, 1.2; and cultured, 1.1. Shown is 1 of 3 separate experiments each with similar results. **B**, Shown are FcεRIα immunoblots of whole cell lysates before or after 22 hours of culture with either IL-3 (basophils) or eosinophils (IL-5). Cultured basophils show a loss of the 50-kD FcεRIα protein (UBI anti-FcεRIα), whereas eosinophils maintain FcεRIα protein in culture (mAb 22E7). **C**, FcεRIα immunoblots of basophil whole cell lysates at baseline (lane 1), after stimulation with anti-IgE (lane 2) (100 ng/mL, 30 minutes, 37°C), or after 22-hour IL-3 culture (lane 3). Despite significant histamine release (28% of total histamine content), the 50-kD protein is preserved as compared with IL-3-cultured basophils.

FcεRIα ($P = .143$). In contrast, after 24 hours of culture, the median 50-kD band intensity decreased 35-fold (Fig 4, C). A decrease in FcεRIα immunoreactivity was also noted in permeabilized basophils after culture. Fig 5, A, shows a representative flow cytograph of basophils permeabilized with saponin and stained with 22E7 before and after 24-hour culture. For experiments performed with this technique, 22E7-detected FcεRIα in permeabilized cells decreased 3.7-fold compared with before and after culture preparations ($n = 3$). Our previous studies with cultured eosinophils demonstrated the presence of FcεRIα protein in culture supernatants with maintenance of intracellular stores of FcεRIα. Therefore a comparison of cultured basophils and eosinophils was performed. As

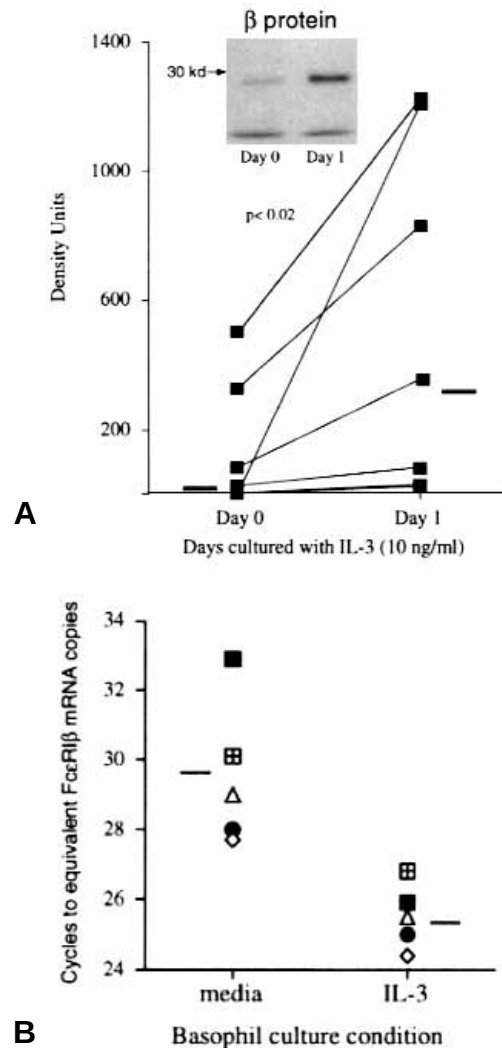


FIG 6. Expression of FcεRIβ protein and mRNA before and after IL-3 culture. **A**, Levels of FcεRIβ protein are significantly increased 13-fold after 22 hours of IL-3 culture (horizontal bars indicate median density units, $n = 7$). **B**, Real-time PCR for FcεRIβ mRNA in basophil cultures. Basophils isolated from 5 subjects (open symbols, mean purity 4%; filled symbols, mean purity 74%) were cultured for 4 hours in media or media supplemented with IL-3 (10 ng/mL) followed by RNA extraction. Shown is the number of amplification cycles required to reach an equivalent number of FcεRIβ mRNA copies. IL-3-cultured basophils required 2 to 7 fewer cycles than their media counterparts, indicating a 4- to 128-fold difference in baseline copies of FcεRIβ mRNA. (horizontal bars indicate mean cycle number; $P < .003$, paired t test).

shown in Fig 5, B, whole cell lysates of basophils demonstrated a loss of 50-kD FcεRIα after culture, whereas eosinophils did not (slightly heavier ~53-kD FcεRIα band, as described previously¹⁴). As expected, eosinophils lacked a detectable 55- to 65-kD FcεRIα band, which is consistent with an absence of surface-expressed FcεRIα on human eosinophils.^{14,26} In addition, attempts to demonstrate FcεRIα protein in basophil culture supernatants have been unsuccessful (data not shown). To examine the possibility that this internal pool

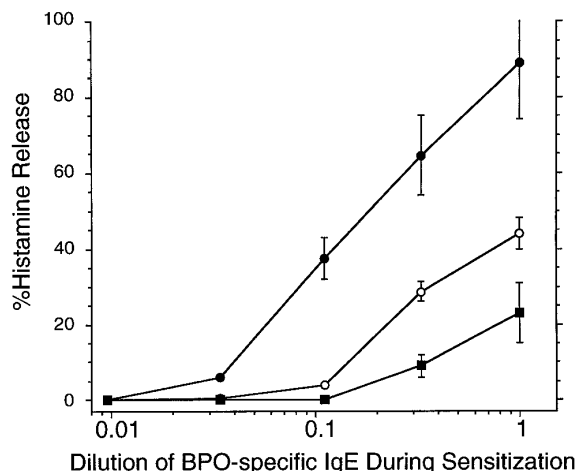


FIG 7. Effects of IL-3 culture on antigen-specific histamine release from basophils. Fresh human basophils (open circles) or those cultured for 1 day in the absence (filled squares) or presence (filled circles) of IL-3 were sensitized with the indicated dilution of BPO-specific IgE (5 μ g/mL) for 45 minutes and challenged with an optimal dose of BPO₂₁-HSA. Basophils treated with IL-3 demonstrated greater maximal histamine release and greater sensitivity to allergen (lower level of sensitization required to achieve 25% of maximal histamine release; $n = 3$, $P = .0021$).

of 50-kd Fc ϵ RI α was associated with the histamine-containing granules and that IL-3 induced some secretion, we examined basophil lysates before and after stimulation of degranulation by polyclonal anti-IgE antibody. As shown in Fig 5, C, there was no loss of the 50-kd subunit despite significant degranulation, as measured by histamine release. Furthermore, the same basophils cultured with IL-3 demonstrated loss of the 50-kd subunit without evidence of spontaneous histamine release in culture supernatants.

Modulation of Fc ϵ RI β protein and mRNA by culture with IL-3

The second observation made in these culture experiments relates to changes in expression of Fc ϵ RI β . Despite stable cell surface expression of Fc ϵ RI α , there was a 13-fold increase in Fc ϵ RI β after IL-3 culture (Fig 6, A). Both high and lower purity basophils were cultured with or without IL-3 at 10 ng/mL for 4 hours; they were then harvested, and extracted Fc ϵ RI β mRNA was measured by real-time PCR. The data in Fig 6, B, are expressed as the number of cycles required for an equivalent fluorescent signal, which translates to a 16-fold increase in mRNA in the IL-3-treated cells.

Effects of IL-3 on cellular function and sensitivity

It has been well documented that overnight culture of basophils with IL-3 dramatically alters a wide variety of functions. However, there has not yet been a demonstration of a change in basophil sensitivity, with sensitivity defined as the amount of cell surface antigen-specific IgE required to reach either 50% of maximal release or a fixed

amount of release. Fig 7 shows the results of experiments in which basophils were sensitized with several different levels of BPO-specific IgE and then challenged with an optimal concentration of antigen BPO₂₁-HSA and analyzed for histamine release. Some cells were analyzed for histamine release immediately after sensitization. The remaining cells were cultured with or without IL-3 for 24 hours, harvested, and challenged for histamine release. Not surprisingly, basophils treated with IL-3 showed both greater maximal histamine release and greater sensitivity (lower level of sensitization required for 25% histamine release) than cells examined on day 0 or day 1 without IL-3. There remained a difference in sensitivity even when the data were normalized for the differences in maximal release (comparing day 0 with day 1 + IL-3).

DISCUSSION

In the human system, the Fc ϵ RI receptor complex is unique in that expression of the Fc ϵ RI β subunit is not obligatory for surface receptor display, as has been observed in the rodent system. In this study we have examined the relative expression of Fc ϵ RI α and Fc ϵ RI β subunits in freshly isolated and short-term IL-3-cultured basophils from allergic and nonallergic donors. We have demonstrated that variable ratios of Fc ϵ RI β /Fc ϵ RI α can be observed under different conditions. First, examination of freshly isolated circulating basophils indicated that across a broad range of cell surface Fc ϵ RI α densities, Fc ϵ RI β expression was not strictly proportional to Fc ϵ RI α expression. Although these studies cannot specifically conclude that cell surface Fc ϵ RI β /Fc ϵ RI α stoichiometry is variable, the results nevertheless indicate that expression of these 2 subunits does not follow a constant stoichiometry at the whole cell level. A statistical analysis of these results demonstrated that the ratio was not constant (Fig 3, B). Another way to view the data is to divide the data set into 2 equal groups containing the 6 lowest and 6 highest surface Fc ϵ RI α expressors. The average-fold difference in surface Fc ϵ RI α levels for these 2 groups was 2.6, whereas the difference in Fc ϵ RI β expression was 8.7 ($P < .05$ for statistical difference in the 2 ratios). Perhaps it was not surprising that the Fc ϵ RI β /Fc ϵ RI α ratio was variable among both allergic and nonallergic donors, but it was unexpected that the stoichiometry showed a relationship to surface Fc ϵ RI α expression.

The second observation indicated that Fc ϵ RI β expression was rapidly and markedly responsive to the presence of IL-3 without any changes in Fc ϵ RI α protein (surface or whole cell). At one extreme, Fc ϵ RI β protein expression was altered by a factor of 80. These changes were accompanied by 16-fold changes in mRNA expression in basophils cultured in IL-3 for only 4 hours. Recent studies using Fc ϵ RI subunits transfected into KU812 cells indicate that Fc ϵ RI β associates with Fc ϵ RI α shortly after synthesis of both components, certainly before Golgi processing.⁸ This would imply that the presence of Fc ϵ RI β in whole cell lysates is indicative of the stoichiometry of Fc ϵ RI β /Fc ϵ RI α because the 2 subunits plus Fc ϵ RI γ

should not be found separate from each other. Whether they are all on the cell surface remains to be determined, but if they track together, whole cell lysate stoichiometry should ultimately reflect cell surface FcεRI stoichiometry. Together these observations strongly suggest that cell surface FcεRI has a variable stoichiometry that might be very sensitive to local cytokine conditions.

These observations should have functional implications. First, the disproportionately high FcεRIβ expression in cells with high surface density of FcεRIα (and the higher FcεRIβ/FcεRIα stoichiometry that should result) would predict that cells with greater FcεRI density would show greater cellular sensitivity to IgE-mediated stimulation. Previous studies contain such data, although not analyzed in this manner. These studies reveal that cellular sensitivity, whether defined as maximal IgE-mediated histamine release or as the density of antigen-specific IgE required for half-maximal histamine release, has no relationship to cell surface density of FcεRI.²⁷ The correlation coefficient for these 2 measures of basophil function (vs cell surface FcεRIα expression) were -0.17 and 0.07, respectively. Because the donor set used for the current studies also showed the expected positive correlation between serum IgE and receptor density^{28,29} ($r = 0.83$, $n = 8$), we tentatively conclude that the expected amplifier functions of an increased FcεRIβ presence do not occur. However, this requires a more direct study because the conclusion would otherwise be based on linking 2 imperfect correlations.

On the other hand, overnight incubation of basophils with IL-3 resulted in increases in both FcεRIβ and IgE-mediated functions of the cell. However, the change in function is difficult to interpret given that many changes occur in basophils cultured with 10 ng/mL of IL-3 for 24 hours. For example, the overall cytosolic calcium response increases for any stimulus, not just for IgE-mediated stimulation, suggesting that more global changes in the processing of calcium signals are altered by IL-3 exposure.^{30,31} It is now clear that downstream signaling element expression is also a significant variable in IgE-mediated responses. For example, changes in the level of syk kinase expression operate independently of FcεRI expression³² and in our hands have been variably altered by the IL-3 conditions used for FcεRIβ enhancement. Understanding the functional significance of the change in expression of FcεRIβ under the influence of IL-3 will require a much better understanding of how IL-3 alters IgE-mediated functions and whether these changes in FcεRIβ occur in the surface-expressed receptor.

Our findings also indicate the presence of another pool of FcεRIα, one found in freshly isolated basophils and that has an electrophoretic mobility suggesting a molecular weight of approximately 50 kd. Because a different broad band between 55 and 65 kd correlates with cell surface FcεRIα, as determined by flow cytometry, we suggest that the 50-kd band is an internal pool. This conclusion is further supported by the observation that 24 hours of IL-3 culture results in a rapid loss of this pool and such changes are observed only by flow cytometry if

the cells are first permeabilized. We do not yet know whether this internal pool represents a precell or postcell surface receptor. The molecular weight is consistent with a pre-Golgi pool of FcεRIα that accumulates in transfected KU812 in the absence of FcεRIβ. The homogeneity of the 50-kd band, relative to that of the 55- to 65-kd band we associate with the cell surface receptor, is also consistent with its being a post-endoplasmic reticulum, pre-Golgi protein. Its sensitivity to endoglycosidase H supports this view as well. At this stage of processing, only the initial simple glycosylation steps, including initial terminal sugar trimming, have occurred, leading to a homogeneous species of receptor. In recent studies by Donnadieu et al,⁸ the relative presence of the FcεRIβ could expand or contract this pool because it appears that the presence of the associated FcεRIβ accelerates movement of the FcεRIα from this stage into the Golgi processing stage. The relationship we demonstrate between disproportionately greater FcεRIβ expression and cell surface FcεRIα would be consistent with the Donnadieu study. Furthermore, a plot of the density of 50 kd as a ratio of the total α pool density (50 kd and 60 kd) compared with total β protein levels reveals a significant negative correlation ($R_s = -0.83$, $P = .002$), as might be expected from the observations of the roles of FcεRIβ in expression. However, the nearly constant level of expression for this 50-kd pool across a 10-fold range of cell surface FcεRIα expression is not consistent with this interpretation. One would expect there would be an inverse relationship between the presence of 50-kd FcεRIα and FcεRIβ expression. However, we cannot exclude that the net effect of IL-3 culture is a change in number of αβγ2 surface receptors without an overall increase in total receptor number. Although not the focus of the present work, further studies will be necessary to examine the identity of the 50-kd species and its relationship to expression of surface FcεRI.

It is not clear where this pool of 50-kd α protein goes during the 24-hour culture. Recent data showing that FcεRIβ accelerates the processing of FcεRIα, as well as our observation that FcεRIβ expression increases markedly during culture, might suggest a mechanism that involves FcεRIβ-induced processing of FcεRIα during the 24-hour period. However, if this were the operative mechanism, one would expect to see increases in the 55- to 65-kd pool (or surface FcεRIα as detected by flow cytometry), and this did not occur. We also could not detect FcεRIα in the extracellular medium or its loss after basophil degranulation. Given the similarity in the internal basophil pool of FcεRIα and an internal pool in eosinophils, this was surprising. However, the internal pool in eosinophils does not decrease but instead appears to give rise to a steadily increasing presence of FcεRIα in the extracellular medium.¹⁴ Perhaps this pool in basophils represents a receptor that is removed from the cell surface; culture may simply induce the cell to complete its dissolution. An initial impulse would be to consider that the homogeneous molecular weight of this pool would mitigate against this explanation, but in our previous stud-

ies of FcεRI downregulation in the absence of IgE, a similarly homogeneous band at around 50 kd, although with a much weaker presence, would appear and disappear when IgE was reintroduced. Thus it remains unclear where it originates. Studies in progress are exploring the nature of the 50-kd pool of FcεRIα, but in retrospect, its disappearance explains why we have not detected its presence in the many studies we have made using basophils obtained from leukocyte packs.^{33,34} These basophils were studied after 1 day of processing and culturing.

There is a strong relationship between serum IgE concentrations and cell surface expression of FcεRIα.^{15,29,35,36} Taken together with results from the study of Donnadieu et al,⁸ the results in Fig 3 are consistent with the view that it is the expression of FcεRIβ that is a strong determinant of cell surface FcεRIα expression. It is not yet clear how to reconcile these 2 views. However, although the relationship between IgE levels and FcεRIα expression is a good one, it is not without significant scatter. We recently published a study indicating that various disease states also resulted in a concordant relationship between serum IgE and basophil FcεRIα expression.²⁸ However, this study did make note of this wide scatter of data points around a single regression line. It seems possible that this scatter about the basic relationship results from the differential expression of cytokines among donors and therefore differential expression of FcεRIβ. One could predict that a multiple regression that included FcεRIβ expression levels and IgE levels might account for all of the variance observed in cell surface densities of FcεRIα.

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APPENDIX

Parametric and nonparametric statistical methods were used to investigate the stoichiometry of the interaction of the α and β chains on the cell surface (ie, to determine whether the ratio of β to α chain expression was constant among donors and remained constant under conditions that altered the expression of each chain). Then the β chain level on the cell surface should be a constant multiple of the α chain level. However, linear regression indicated a poor fit of a line ($\beta = m[\alpha] + c$) to the data ($R^2 = 0.52$). The relation is closer to exponential ($\beta = Ae^{-k[\alpha]}$) fit with the equivalent linear relation $\ln(\beta) = \ln A + k(\alpha)$ ($R^2 = 0.81$). A plot of the ratio β/α , as a function of α , should be constant, but as illustrated in Fig 3, there is an increasing

trend that appears even stronger than linear. Linear regression indicates the poor fit of a line to the data ($R^2 = 0.48$). A test based on the linear relation would result in rejection of the model $\beta/\alpha = \text{constant}$ ($P = .013$).

A nonparametric test of the null hypothesis of a constant ratio of β to α chain expression on the cell surface, based on the Spearman rank correlation, is more appropriate than the parametric methods described. The alternative is that the ratio changes monotonically with the level of α (or β). The advantage of the test is that it does not require an assumption about the parametric form of the relation between β and α levels. On the basis of the Spearman statistic ($R_s = 0.902$), the hypothesis that the stoichiometry is constant is rejected ($P < .0001$).

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