

The relationship between serum IgE and surface levels of FcεR on human leukocytes in various diseases: Correlation of expression with FcεRI on basophils but not on monocytes or eosinophils

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Background: Expression of receptors for IgE (FcεR) have been mainly studied on mast cells and blood basophils in the context of allergic disease. Some reports have noted limited expression of FcεR on other leukocytes, including blood monocytes and eosinophils in certain patients. An association between human blood basophil expression of FcεRIα and serum IgE has been noted among allergic subjects.

Objective: Recent evidence supports regulation of FcεRIα by free IgE on both mast cells and basophils. We hypothesized that this relationship would exist across an extremely wide range of IgE levels for human basophils, irrespective of underlying disease. We further examined whether a similar relationship existed between serum IgE and FcεRIα or FcεRII (CD23) expression on monocytes and eosinophils in these same subjects.

Methods: Blood was obtained from nonallergic subjects (n = 3) and subjects with allergic asthma (n = 5), atopic dermatitis (n = 3), hypereosinophilic syndromes (n = 7), hyper-IgE syndrome (n = 6), helminth infestation (n = 6), or IgE myeloma (n = 1). Levels of serum IgE were determined by using RIA and ranged from 3 to 4.7 mg/mL. Levels of cell surface FcεRIα, FcεRII, and IgE were measured by using immunofluorescence and flow cytometry.

Results: Basophil surface IgE density and FcεRIα expression correlated with serum IgE levels ($r = 0.67$ and $r = 0.46$, respectively; $P < .01$; n = 31) regardless of the disease state. In contrast, monocyte FcεRIα expression did not correlate with serum IgE ($r = 0.09$, $P > .5$, n = 29), and low-level eosinophil FcεRIα expression was only detected in a single asthmatic subject. CD23 expression was not detected on basophils or eosinophils, except for the eosinophils from the donor with IgE myeloma. CD23 was present on monocytes from some donors but did not correlate with serum IgE levels.

Conclusions: In a variety of disease states, FcεRIα expression by basophils, but not monocytes or eosinophils, correlated with serum IgE levels across a 6-log range of IgE. These data support the concept of *in vivo* regulation of FcεRIα on basophils by serum IgE and further demonstrate that this is independent of allergic disease per se. (*J Allergy Clin Immunol* 2000;106:514-20.)

Key words: IgE receptors, IgE antibody, basophils, monocytes, eosinophils

Cell surface receptors specific for IgE couple antibody-allergen interactions to cellular responses. The high-affinity receptor for IgE on mast cells and basophils, FcεRI, is a tetrameric complex composed of one α subunit, one β subunit, and two γ subunits. The α chain binds monomeric IgE at its third constant domain,^{1,2} whereas the β and γ chains are involved in signal transduction by an aggregated complex.³

An association between serum IgE levels and expression of FcεRI on human basophils from allergic subjects has been known for over 20 years⁴ and was recently confirmed.⁵ Several lines of evidence suggest a regulatory role for IgE in the expression of its high-affinity receptor on mouse and human mast cells and basophils. Reduction of circulating free IgE in allergic subjects by infusions of an mAb (E25) directed against the receptor-binding domain of IgE led to parallel declines in surface IgE and FcεRIα densities on circulating blood basophils.⁶ Furthermore, exposure of basophils to IgE, either *in vivo* by stopping E25 or *in vitro* by using myeloma IgE, led to an enhancement of FcεRIα expression.⁷ *In vitro*, IgE-dependent enhancement of basophil FcεRIα requires IgE binding to the high-affinity receptor⁸ and results in increased protein mass of the α subunit of the complex.⁹ Thus IgE appears to regulate the levels of high-affinity receptor expression on human basophils *in vitro* and *in vivo*. Studies with murine mast cells, cord blood-derived human mast cells, and fetal human liver mast cells have all shown similar regulation.¹⁰⁻¹²

Other cells reported to express FcεRI or FcεRII (CD23) on their surface include monocytes,^{13,14} Langerhans cells,¹⁵ dendritic cells,¹⁶ and possibly eosinophils.¹⁷⁻²⁰ However, FcεRI expression appears to be restricted to certain disease states, such as atopy for blood monocytes

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Abbreviations used

HES:	Hypereosinophilic syndrome
HIE:	Hyper-IgE syndrome
MFI:	Mean fluorescence intensity
PAG:	PIPES buffer containing 0.003% human serum albumin and 0.1% glucose
PE:	Phycoerythrin
PIPES:	Piperazine-N,N'-bis-2-ethanesulfonic acid

and Langerhans cells,^{13,15} and hypereosinophilic syndrome (HES) or helminth infection for eosinophils.¹⁸⁻²⁰ These cells also differ from mast cells and basophils in that their FcεRI exists as a trimeric complex (αγ2) lacking the β subunit.¹³⁻¹⁶ Factors responsible for the regulation of FcεRI on these cell types are not known.

In the present study we examined whether IgE or other disease-specific factors were involved with basophil FcεRIα expression. We hypothesized that if IgE regulates expression of its own receptor, then serum IgE levels should determine levels of FcεRI on basophils, regardless of disease state. Whether there is a similar relationship between serum IgE and FcεRII is not clear. In the present study we have examined the relationship between serum IgE and surface IgE and FcεRIα and FcεRII expression for human blood basophils across a 6-log range of IgE levels, a much larger range of IgE levels than previously studied. Furthermore, we have examined the relationship in the context of 7 diseases or conditions where other modulating factors might participate in regulation of FcεRI expression. We further examined whether a similar relationship existed between IgE and FcεRIα or FcεRII for circulating monocytes and eosinophils and have expanded the analyses to include a wide range of diseases associated with high levels of eosinophilia and IgE, including nonallergic diseases.

METHODS

Study subjects

A total of 31 subjects participated in the study (12 male and 13 female subjects, 6 unidentified to preserve anonymity; age range, 17-70 years; mean age, 41 years). Subjects included nonallergic subjects (negative skin test responses and no clinical symptoms of rhinitis, asthma, or eczema) and those with allergic asthma (AA; positive skin test responses and a history of asthma symptoms and demonstrated reversible obstruction, a positive methacholine challenge result, or both), those with atopic dermatitis (AD; diagnosed by the criteria of Hanifin and Rajka²¹), those with HES (either as idiopathic HES,²² eosinophilic gastroenteritis,²³ or a member of a familial hypereosinophilic cohort²⁴), those with hyper-IgE syndrome (HIE; characterized by abscesses, pneumonia, and elevated IgE²⁵), parasitized hosts (active infection by *Loa loa* or *Filaria* species), or those with IgE myeloma. After informed consent, subjects agreed to participate in the study by a protocol of blood donation approved by the Johns Hopkins Bayview Institutional Review Board (nonallergic subjects and subjects with AA, AD, HES, and IgE myeloma). Subjects with familial HES, HIE, and actively parasitized hosts were referred from ongoing study protocols at the National Institutes of Health. Samples obtained at the National Institutes of Health were

kept on ice and immediately transported by a messenger. Despite the transport time (always <3 hours), no difference was noted in leukocyte markers in samples subjected to immediate or delayed isolation and analysis at Johns Hopkins (data not shown).

Buffers

Piperazine-N,N'-bis-2-ethanesulfonic acid (PIPES) obtained from Sigma Chemical Co (St Louis, Mo) was used in a stock buffer composed of 25 mmol/L PIPES containing 110 mmol/L NaCl, 5 mmol/L KCl, and 40 mmol/L NaOH, adjusted to pH 7.4. PAG buffer consisted of PIPES buffer containing 0.003% human serum albumin (Miles Laboratories Inc, Elkhart, Ind) and 0.1% glucose.

Leukocyte enrichment

Mononuclear cells (basophils, lymphocytes, and monocytes) were enriched from 30 mL of venous blood treated with EDTA by using a Percoll-based density gradient centrifugation technique, as previously described.²⁶ Basophil purity ranged from 1% to 10% (4.2% ± 0.4%, n = 31, mean ± SEM), as enumerated by Alcian blue staining and light microscopic counts.²⁷ Total cell counts and viability (98.8% ± 0.14%, n = 21, mean ± SEM) were determined by using light microscopy and erythrosin B dye exclusion. Eosinophils (purity range, 6%-88%; 34.0% ± 5.5%, n = 23, mean ± SEM) were obtained from granulocyte pellets of the same Percoll gradients after brief hypotonic lysis of red blood cells, as previously performed.²⁸

Flow cytometry

Mononuclear or granulocyte specimens were labeled for direct and indirect dual-color immunofluorescence and flow cytometry in the presence of 4 mg/mL human IgG to minimize nonspecific binding to FcγR, as previously described.⁶ For each leukocyte subset, both light-scatter characteristics and specific surface markers were used for analysis (anti-IgE for basophils, CD14 for monocytes, CD9 for eosinophils, and CD19 for B cells). Antibodies used for these studies included an irrelevant mouse IgG₁ control (Coulter-Immunotech, Hialeah, Fla), FITC-conjugated polyclonal goat anti-human IgE and its control FITC-conjugated normal goat IgG (Kirkegaard and Perry, Gaithersburg, Md), FITC-conjugated mouse anti-human CD14 (MY4, IgG2b; Coulter-Immunotech), FITC-conjugated mouse anti-human CD9 (MM2/57, IgG2b; RDI, Pleasant Hill, NJ), FITC-conjugated irrelevant mouse IgG₁ (Biosource, Camarillo, Calif), mouse anti-human FcεRIα subunit (22E7, IgG₁, binding unaffected by FcεRI occupancy, kindly provided by J. Kochan, Roche Pharmaceuticals, NJ²⁹), mouse anti-FcεRII (CD23, 9P25, IgG1; Coulter-Immunotech), and phycoerythrin (PE)-conjugated polyclonal goat anti-mouse Ig (Biosource) as a secondary reagent. For monocyte and eosinophil experiments, leukocytes were first incubated with a single primary antibody (FcεRI, CD23, or irrelevant IgG) followed by the secondary PE-conjugated goat anti-mouse Ig. After washing, the cells were next incubated for 15 minutes with a 10-fold excess of mouse IgG (Sigma) to saturate open sites on the goat anti-mouse PE-conjugated secondary antibody before the addition of leukocyte-specific conjugated antibodies (either FITC CD14 or FITC CD9).³⁰⁻³³ Samples were analyzed by using a Coulter EPICS Profile II flow cytometer with appropriate compensation. Identical photomultiplier tube settings were used for each sample, and the flow cytometer was fully aligned and calibrated with fluorospheres daily. At least 5000 events per experimental condition were counted. All leukocyte samples were examined for surface marker expression the same day as venipuncture. Data were expressed as net mean fluorescence intensity (MFI; sample MFI minus MFI of the irrelevant IgG control).

We have previously determined the limits of receptor detection with flow cytometry for basophils (1%-2% purity) to be 5000 receptors per cell³⁴ and, by extrapolation, that for eosinophil preparations (purity >5% purity) to be greater than 3000 receptors per cell.

TABLE I. Subject characteristics

Subject	Age (y)	Sex	Diagnosis	Serum IgE (ng/mL)	Total eosinophil count (cells/dL)
1	30	F	Nonallergic	3	ND
2	53	F	Nonallergic	4	ND
3	32	M	Nonallergic	29	ND
4	24	M	AA	1115	ND
5	44	F	AA	145	ND
6	26	F	AA	451	ND
7	39	F	AA	2469	ND
8	69	M	AA	130	520
9	NA	F	AD	2628	ND
10	NA	M	AD	4715	ND
11	40	F	AD	13,884	900
12	60	M	Lymphatic filariasis	792	1800
13	21	F	Loasis	48,696	10,400
14	28	M	Loasis	5359	1410
15	26	F	Loasis	108	120
16	27	M	Loasis	3218	4396
17	27	F	Loasis	638	5274
18	40	M	Eosinophilic gastroenteritis	534	1600*
19	66	F	IHES	30,840	3100
20	17	F	IHES	31	6390
21	74	M	FHES	10	4270
22	25	F	FHES	<12	2360
23	56	M	FHES	29	5292
24	46	M	IHES	394	2566
25	NA	NA	HIE	985	ND
26	NA	NA	HIE	33,470	ND
27	NA	NA	HIE	38,861	ND
28	NA	NA	HIE	15,490	ND
29	NA	NA	HIE	184†	ND
30	NA	NA	HIE	28,834	ND
31	70	M	IgE myeloma	4.7 × 10 ⁶	ND

IHES, Idiopathic HES; FHES, familial HES²⁴; NA, not available; ND, not determined.

*Taking prednisone at the time of blood draw.

†Previously elevated serum IgE with recent decline, as described in certain subjects with HIE.²⁵

In previously published studies the flow cytometric measurements were calibrated by examining the fluorescence staining of 6 donors' basophils that spanned a moderate range of staining intensities (7-120 fluorescent units or 8000 to 140,000 FcεRI per basophil) and simultaneously assessing receptor or IgE density by the acetate elution method, as previously described.^{9,35} 22E7 staining compared with total FcεRI density by acetate elution was linear with a slope of 0.00084 (ie, a fluorescent measurement of 100 represents approximately 120,000 receptors) with an *R* value of 0.963.

Measurement of serum IgE and lactic acid elution of IgE

Serum samples were collected separately but simultaneously with whole blood for leukocyte phenotyping and analyzed by RIA for total serum IgE levels.³⁶

In some samples to determine whether high serum IgE levels potentially interfered with labeling and detection of FcεRIα, we examined mononuclear cells before and after IgE removal. For removal of IgE, mononuclear pellets were resuspended in ice-cold lactic acid buffer (pH 3.7) and incubated in an ice bath for 3 to 5 minutes, as previously described.⁶ After incubation, cold PAG was added, and cells were allowed to equilibrate for 10 minutes before centrifugation and labeling for flow cytometry. Effective basophil IgE stripping was examined by surface labeling with FITC anti-human IgE of separate cell aliquots at each step of the protocol.

Statistical methods

All values are means ± SD unless otherwise noted. Correlations were calculated by using simple regression analysis.

RESULTS

Clinical characteristics of subjects

A total of 31 subjects participated in the study (Table I). Serum IgE levels ranged from 3 to 4.7 mg/mL. Group means for serum IgE by disease category were as follows: nonallergic subjects, 12 ng/mL (*n* = 3); subjects with AA, 862 ng/mL (*n* = 5); subjects with AD, 7076 ng/mL (*n* = 3); subjects with HES, 4550 ng/mL (*n* = 7); subjects with HIE, 19,637 ng/mL (*n* = 6); and helminth-infested hosts, 9802 ng/mL (*n* = 6). Eosinophil counts were measured in all subjects with HES and helminth infestation, with group means of 3654 cells/dL and 3900 cells/dL, respectively.

IgE, FcεRIα, and FcεRII surface expression

Basophil surface IgE and FcεRIα densities were measured and correlated with serum levels of IgE. As previously observed in atopic subjects, a significant correlation

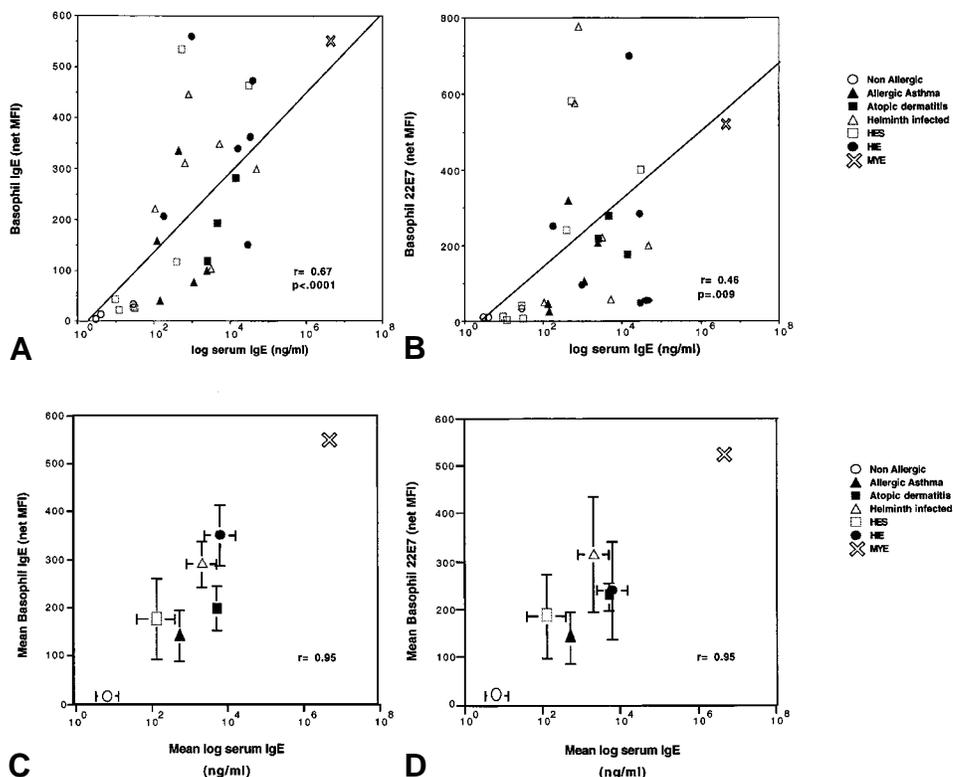


FIG 1. A, Levels of basophil cell surface expression of IgE as measured by labeling with FITC-conjugated goat anti-human IgE antibodies and flow cytometry ($r = 0.67$, $P < .0001$, $n = 31$). B, Levels of basophil surface expression of FcεRIα as measured by labeling with mAb 22E7 and flow cytometry expressed relative to a subject's log serum IgE value. Note the low expression by certain subjects with HIE ($r = 0.46$, $P = .009$, $n = 31$). C and D, Disease-specific means of basophil surface IgE and FcεRIα intensity expressed relative to group mean serum IgE levels.

between surface IgE and serum IgE was observed ($r = 0.67$, $P < .0001$; Fig 1, A). Regarding basophil surface FcεRIα, expression was detectable among all subject groups, with a range of 10 to 777 MFI units (which translates to a range of 8000-930,000 basophil surface receptors) and an overall mean of 211 ± 219 MFI units ($n = 31$). A significant correlation with serum IgE levels was seen across the 6-log range of IgE ($r = 0.46$, $P = .006$, $n = 31$; Fig 1, B). Analysis by disease group revealed that mean basophil surface IgE and FcεRIα density were closely related to mean serum IgE levels in each category ($r = 0.95$ for both; Fig 1, C and D). Unexpectedly, certain subjects with HIE demonstrated low FcεRIα intensity despite high levels of surface IgE binding (compare HIE; Fig 1, A to B). To explore this, basophils of subsequent subjects with HIE (subjects 28-30) were examined by using flow cytometry before and after removal of surface-bound IgE (Fig 2). In the case of subject 28 with HIE, no change in mAb 22E7 labeling was noted after removal of IgE, whereas increased levels of mAb 22E7 labeling were seen with subjects 29 and 30 with HIE after IgE stripping, suggesting that in some subjects there was interference with mAb 22E7 binding.

In contrast to basophils, monocyte FcεRIα expression was not a universal finding among donors. Eight of 29

subjects had detectable surface FcεRIα expression (range, 1.5-13.7 net MFI units; mean, 5.1 MFI units). In every case monocyte expression was at least 5- to 194-fold lower (average, 93-fold lower) than for basophils in the same subject. FcεRIα expression did not correlate with levels of serum IgE (Fig 3, A). Although the numbers of allergic subjects were small, 2 of 3 subjects with AD and 2 of 5 subjects with AA expressed significant levels of FcεRIα. Among other disease categories, one of 6 subjects with HIE (subject 28) had a positive test result and in fact had the highest level of monocyte FcεRIα expression that was seen. The remaining subjects with positive test results included one of 6 parasitized host donors and 2 of 7 HES donors. Questionable detection (net MFI, >0.1 but <1.0) was seen in a total of 11 subjects (1 nonallergic subject, 1 subject with AA, 1 subject with AD, 4 parasitized hosts, 2 subjects with HES, 1 subject with HIE, and 1 subject with IgE myeloma), but no correlation was seen with levels of serum IgE. Clear eosinophil FcεRIα was detected on cells from one asthmatic subject (net MFI, 1.2; data not shown), whereas questionable expression (net MFI, >0.1 but <1.0) was seen in 6 of 28 subjects tested (1 subject with AA, 2 parasitized hosts, 2 subjects with HES, and 1 subject with IgE myeloma).

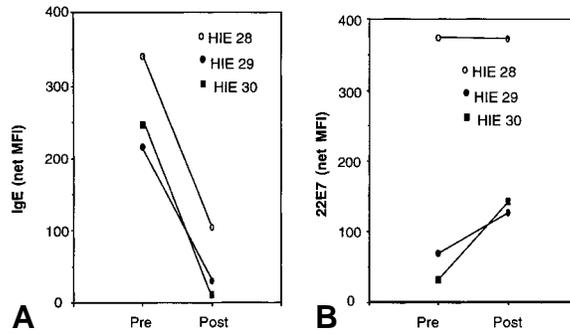


FIG 2. The indicated subjects with HIE were examined for surface IgE labeling (A) and surface FcεR1α (B) before (*pre*) and after (*post*) lactic acid stripping of IgE. Note the increase in 22E7 net MFI for subjects 29 and 30 with HIE after stripping of IgE.

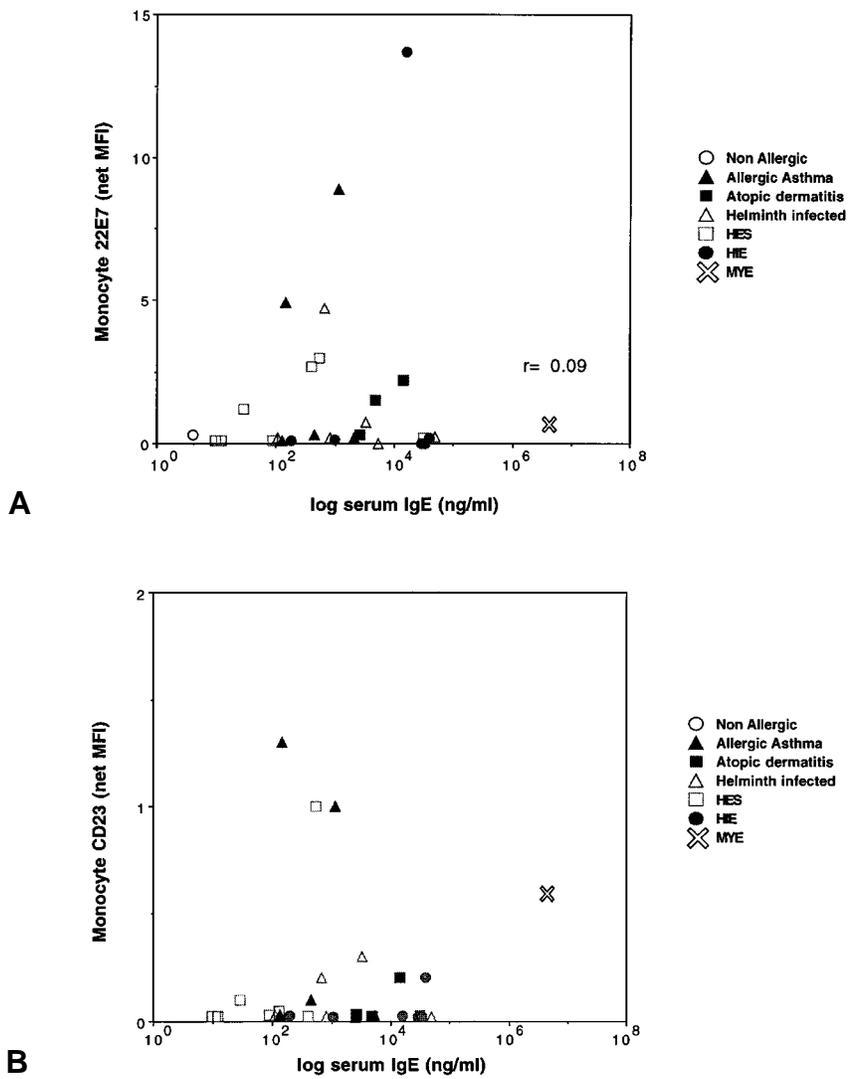


FIG 3. A, Expression of FcεR1α by monocytes from various subjects as measured by surface labeling with mAb 22E7. Expression was most commonly seen among subjects with atopic diseases and yet was absent in the subject with the highest IgE level (IgE myeloma). Note the lower scale of the y-axis compared with Fig 1. **B,** Expression of CD23 by monocyte donors.

Some monocyte donors had detectable CD23 staining, but again, expression did not correlate to serum IgE levels. In terms of disease states, 2 of 4 subjects with AA and one of 7 HES donors had low-level CD23 staining (mean, 1.1; Fig 3, *B*). Expression was negative in 21 donors, with questionable expression (net MFI, >0.1 but <1.0) observed in 5 donors (1 subject with AD, 2 parasitized hosts, 1 subject with HIE, and 1 subject with IgE myeloma). As expected, CD23 was not detected on basophils of any of the 31 subjects tested, whereas only the subject with IgE myeloma had detectable eosinophil CD23 expression (net MF, 3.1; data not shown). As a positive control for CD23, CD19⁺ B cells analyzed in the same mononuclear cell preparations were found to have readily detectable CD23 (data not shown).

DISCUSSION

We have found that basophil IgE and FcεRIα expression correlates to serum IgE in a variety of disease states, extending the previous observation of such a relationship beyond atopic individuals. The two prior studies by Malveaux et al⁴ and Sihra et al⁵ focused on normal or atopic donors without the diversity in disease states in the present study. Furthermore, our inclusion of serum IgE levels from quantities in nanograms per milliliter to milligrams per milliliter encompasses a much wider range of this Ig (Fig 1, *A*). The scatter of data points around the log-linear regression line was somewhat greater than expected. For example, looking at the band of data around 1 μg/mL serum IgE, there was a 10-fold range of receptor densities. In previous studies the range was more restricted (~4-fold). This degree of scatter suggests that there may be other factors than IgE that contribute to the expression of FcεRIα. However, whatever these factors may be, they were not manifested in specific disease states because the data points for each disease examined fell on a similar regression line (Fig 1, *C* and *D*). The log-linear relationship from the current studies was similar to those in the Malveaux studies. For example, by using our current data set, an IgE level of 100 ng/mL would predict 150,000 to 170,000 receptors per basophil, whereas the Malveaux data set would predict 200,000 receptors. The relationship between IgE and FcεRI expression across the different diseases that were part of this study provides further support for the IgE concentration being the strongest determinant of FcεRI expression.

In contrast to the basophil, FcεRIα expression on monocytes was more restricted among disease states than for basophils. Surface expression was most common in allergic donors (4 of 8 donors), as previously reported,¹³ followed by hypereosinophilic subjects. We found no correlation between levels of monocyte FcεRIα and serum IgE across diseases or among our atopic donors, although such a relationship has been described for monocytes of atopic subjects.⁵ Levels of monocyte FcεRIα expression were nearly 2 logs lower than corresponding basophils for a given subject. An explanation for this difference may relate to the altered composition of the FcεRI complex on

monocytes, which lack the β subunit. Given the limited range of FcεRI expression within a particular disease state, other regulatory factors and a functional role for FcεRI expression remain unknown.

In our hands eosinophils lacked surface FcεRIα in all but one of 31 subjects tested. This is consistent with the levels of expression observed by most groups.^{5,19,20,37} Furthermore, unlike previous publications,¹⁸ we failed to observe detectable eosinophil FcεRIα in disease states associated with significant eosinophilia and elevations of IgE, such as helminth infestation and HES. Although we were limited to loasis among parasitic diseases, it is not unreasonable to expect a similar relationship among other parasitic diseases given the range in IgE levels and disease states in the present study. Functionally, FcεRI has been proposed to promote eosinophil host defense from parasites, but recent findings failed to demonstrate eosinophil activation or degranulation by binding of FcεRI.²⁰

An unexpected observation was the possible interference of mAb 22E7 labeling of basophils from some subjects with HIE. An explanation for this finding is not clear; however, subjects with HIE have been described to have altered IgE metabolism,³⁸ IgE production,³⁹ and varied IgE glycoforms,⁴⁰ which in theory may alter epitopes or accessibility for mAb 22E7 binding to FcεRIα. To our knowledge, this is the first such report of interference by IgE when using 22E7 to detect FcεRIα. Previous studies have otherwise demonstrated time and again that bound IgE does not normally interfere with the ability of 22E7 to detect occupied receptors.

Finally, expression of FcεRII (CD23) was restricted to monocytes, but it was not present on all monocytes. Despite the fact that IL-4 has been shown to induce CD23 expression on monocytes *in vitro*,^{41,42} no clear association between the atopic state and monocyte CD23 expression was observed. Although two FcεRII isoforms (FcεRIIa and FcεRIIb) have been reported, they share structural and antigenic identity in their C-terminal extracellular regions.⁴³ The absence of CD23 surface expression on eosinophils among our subjects is consistent with other groups by using the same antibody clone, as well as other anti-CD23 isoform antibodies.^{20,44} However, permeabilized eosinophils have been shown to express a significant intracellular pool of FcεRII that is similar to B-cell FcεRII and rapidly mobilized on eosinophil stimulation.⁴⁴ Thus the lack of a correlation between CD23 expression and circulating IgE levels is not readily explained by technical issues or FcεRII isoforms.

Taken together, our study confirms and extends to a broader patient population previous analyses demonstrating that serum IgE positively correlates with surface levels of IgE and FcεRI in human basophils. This does not hold for other cell types or for FcεRII, suggesting that other factors are involved in the regulation of these receptors on these other cell types.

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REFERENCES

- Vangelista L, Laffer S, Turek R, et al. The immunoglobulin-like modules Ce3 and $\alpha 2$ are the minimal units necessary for human IgE-Fc ϵ RI interaction. *J Clin Invest* 1999;103:1571-8.
- Garman SC, Kinet JP, Jardetzky TS. Crystal structure of the human high-affinity IgE receptor. *Cell* 1998;95:951-61.
- Lin S, Cicala C, Scharenberg AM, Kinet JP. The Fc ϵ RI β subunit functions as an amplifier of Fc ϵ RI γ -mediated cell activation signals. *Cell* 1996;85:985-95.
- Malveaux FJ, Conroy MC, Adkinson NF Jr, Lichtenstein LM. IgE receptors on human basophils: relationship to serum IgE concentration. *J Clin Invest* 1978;62:176-81.
- Sihra BS, Kon OM, Grant JA, Kay AB. Expression of high-affinity IgE receptors (Fc ϵ RI) on peripheral blood basophils, monocytes, and eosinophils in atopic subjects: Relationship to total serum IgE concentrations. *J Allergy Clin Immunol* 1997;99:699-706.
- MacGlashan DW, Bochner BS, Adelman DC, et al. Down-regulation of Fc ϵ RI expression on human basophils during in vivo treatment of atopic patients with anti-IgE antibody. *J Immunol* 1997;158:1438-45.
- Saini SS, MacGlashan DW, Sterbinsky SA, et al. Down-regulation of human basophil IgE and Fc ϵ RI α surface densities and mediator release by anti-IgE-infusions is reversible in vitro and in vivo. *J Immunol* 1999;162:5624-30.
- MacGlashan D, Lichtenstein LM, McKenzie-White J, et al. 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* 1999;104:492-8.
- MacGlashan D, White J, Chichester K, et al. In vitro regulation of Fc ϵ RI α expression on human basophils by IgE antibody. *Blood* 1998;91:1633-43.
- Yamaguchi M, Lantz CS, Oettgen HC, et al. 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* 1997;185:663-72.
- Hsu C, MacGlashan D Jr. IgE antibody up-regulates high affinity IgE binding on murine bone marrow derived mast cells. *Immunol Lett* 1996;52:129-34.
- Yamaguchi M, Sayama K, Yano K, et al. IgE enhances Fc ϵ 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 ϵ receptor I expression and mediator release. *J Immunol* 1999;162:5455-65.
- Maurer D, Fiebiger E, Reininger B, et al. Expression of functional high affinity immunoglobulin E receptors (Fc ϵ RI) on monocytes of atopic individuals. *J Exp Med* 1994;179:745-50.
- Polla BS, Ezekowitz RA, Leung DY. Monocytes from patients with atopic dermatitis are primed for superoxide production. *J Allergy Clin Immunol* 1992;89:545-51.
- Bieber T, Salle HDI, Wollenberg A, et al. Human epidermal langerhans cells express the high affinity receptor for immunoglobulin E (Fc ϵ RI). *J Exp Med* 1992;175:1285-90.
- Maurer D, Fiebiger E, Ebner C, et al. Peripheral blood dendritic cells express Fc ϵ RI as a complex composed of Fc ϵ RI α - and Fc ϵ RI γ -chains and can use this receptor for IgE-mediated allergen presentation. *J Immunol* 1996;157:607-16.
- Mawhorter SD, Stephany DA, Ottesen EA, Nutman TB. Identification of surface molecules associated with physiologic activation of eosinophils—application of whole-blood flow cytometry to eosinophils. *J Immunol* 1996;156:4851-8.
- Gounni AS, Lamkhioou B, Ochial K, et al. High-affinity IgE receptor on eosinophils is involved in defence against parasites. *Nature* 1994;367:183-6.
- Seminario MC, Saini SS, MacGlashan DW, Bochner BS. Intracellular expression and release of Fc ϵ RI α by human eosinophils. *J Immunol* 1999;162:6893-900.
- Kita H, Kaneko M, Bartemes KR, et al. Does IgE bind to and activate eosinophils from patients with allergy? *J Immunol* 1999;162:6901-11.
- Hanifin JM, Rajka G. Diagnostic features of atopic dermatitis. *Acta Derm Venereol* 1980;92:44-7.
- Spry CJF, Davies J, Tai PC, Olsen EGG, Oakley CM, Goodwin JF. Clinical features of fifteen patients with the hypereosinophilic syndrome. *Q J Med* 1983;205:1-22.
- Wershil BK, Walker WA. The mucosal barrier, IgE-mediated gastrointestinal events, and eosinophilic gastroenteritis. *Gastroenterol Clin North Am* 1992;21:387-404.
- Lin AY, Nutman TB, Kaslow D, et al. Familial eosinophilia: clinical and laboratory results on a US kindred. *Am J Med Genet* 1998;76:229-37.
- Grimbacher B, Holland S, Gallin JI, et al. Hyper-IgE syndrome with recurrent infections—an autosomal dominant multisystem disorder. *N Engl J Med* 1999;340:692-702.
- Warner JA, Reshef A, MacGlashan DW Jr. A rapid percoll technique for the purification of basophils. *J Immunol Methods* 1987;105:107-10.
- Gilbert HS, Ornstein L. Basophil counting with a new staining method using alcian blue. *Blood* 1975;46:279-86.
- Georas SN, McIntyre BW, Ebisawa M, Bednarczyk J, Schleimer RP, Bochner BS. Expression of a functional laminin receptor ($\alpha 6\beta 1$, VLA-6) on human eosinophils. *Blood* 1993;82:2872-9.
- Riske F, Hakimi J, Mallamaci M, et al. High affinity human IgE receptor (Fc ϵ RI): analysis of functional domains of the α -subunit with monoclonal antibodies. *J Biol Chem* 1991;266:11245-51.
- Bochner BS, Sterbinsky SA, Saini SA, Columbo M, MacGlashan DW. Studies of cell adhesion and flow cytometric analyses of degranulation, surface phenotype, and viability using human eosinophils, basophils, and mast cells. *Methods* 1997;13:61-8.
- Grayson MH, Van der Vieren M, Sterbinsky SA, et al. α 4 β 2 integrin is expressed on human eosinophils and functions as an alternative ligand for vascular cell adhesion molecule 1 (VCAM-1). *J Exp Med* 1998;188:2187-91.
- Taylor ML, Noble PW, White B, Wise R, Liu MC, Bochner BS. Extensive surface phenotyping of alveolar macrophages in interstitial lung disease. *Clin Immunol* 2000;94:33-41.
- Georas SN, Liu MC, Newman W, Beall WD, Stealey BA, Bochner BS. Altered adhesion molecule expression and endothelial activation accompany the recruitment of human granulocytes to the lung following segmental antigen challenge. *Am J Respir Cell Mol Biol* 1992;7:261-9.
- Bochner BS, McKelvey AA, Schleimer RP, Hildreth JEK, MacGlashan DW Jr. Flow cytometric methods for analysis of human basophil surface antigens and viability. *J Immunol Methods* 1989;125:265-71.
- MacGlashan DW Jr. Releasability of human basophils: cellular sensitivity and maximal histamine release are independent variables. *J Allergy Clin Immunol* 1993;91:605-15.
- Adkinson NF Jr. Measurement of total serum immunoglobulin E and allergen-specific immunoglobulin E antibody. In: Rose NR, Friedman H, eds. *Manual of clinical immunology*. 2nd ed. Washington, DC: The American Society of Microbiology; 1980. p. 800-5.
- Terada N, Konno Y, Terada S, Fukuda T, Yamashita T. IL-4 upregulates Fc ϵ RI alpha-chain messenger RNA in eosinophils. *J Allergy Clin Immunol* 1996;96:1161-9.
- Dreskin SC, Goldsmith PK, Strober W, Zech LA, Gallin JI. Metabolism of immunoglobulin E in patients with markedly elevated serum immunoglobulin E levels. *J Clin Invest* 1987;79:1764-72.
- Garraud O, Mollis SN, Holland SM, et al. Regulation of immunoglobulin production in hyper-IgE (Job's) syndrome. *J Allergy Clin Immunol* 1999;103:333-40.
- Robertson MW, Liu FT. Heterogeneous IgE glycoforms characterized by differential recognition of an endogenous lectin (IgE-binding protein). *J Immunol* 1991;147:3024-30.
- Alderson MR, Armitage RJ, Tough TW, Ziegler SF. Synergistic effects of IL-4 and either GM-CSF or IL-3 on the induction of CD23 expression by human monocytes: Regulatory effects of IFN α and IFN γ . *Cytokine* 1994;6:407-13.
- Vercelli D, Jabara HH, Lee BW, Woodland N, Geha RS, Leung DY. Human recombinant interleukin 4 induces Fc epsilon R2/CD23 on normal human monocytes. *J Exp Med* 1988;167:1406-16.
- Yokota A, Kikutani H, Tanaka T, et al. Two species of human Fc epsilon receptor II (Fc epsilon RII/CD23): tissue-specific and IL-4-specific regulation of gene expression. *Cell* 1988;55:611-8.
- Sano H, Munoz NM, Sano A, et al. Upregulated surface expression of intracellularly sequestered Igepsilon receptors (FcepsilonRII/CD23) following activation in human peripheral blood eosinophils. *Proc Assoc Am Phys* 1999;111:82-91.