

## IgE receptors on human basophils. Relationship to serum IgE concentration.

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*J Clin Invest.* 1978;62(1):176-181. <https://doi.org/10.1172/JCI109103>.

### Research Article

As reported previously, and confirmed here in 26 donors, the serum IgE level (2.6-5,500 ng/ml) correlates well ( $r_s = 0.95$ ,  $P$  less than 0.001) with the *in vivo* number of IgE molecules/basophil (6,000-600,000). The total number of IgE receptors/basophil was monitored by incubating them with an IgE-rich serum (15 microgram/ml), quantitatively stripping IgE from the cells at pH 3.7, and measuring eluted IgE by a direct radioimmunoassay test. Saturation of receptors for each donor was achieved with 15 nM IgE (3 microgram/ml). The proportion of receptors occupied *in vivo* correlated with the serum IgE ( $r_s = 0.84$ ,  $P$  less than 0.001) whereas the average association constant of the receptors was independent of serum IgE and ranged from  $7.1 \times 10(8)/M$  to  $2.8 \times 10(10)/M$ , averaging  $7.7 \times 10(9)/M$ . Unexpectedly, the total number of IgE receptors/basophil was closely related to the serum IgE level. ( $r_s = 0.92$ ,  $P$  less than 0.001). Thus, either there is genetic association between serum IgE and the number of basophil IgE receptors, or, more likely, the receptor number is modulated by the serum IgE concentration.

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# IgE Receptors on Human Basophils

## RELATIONSHIP TO SERUM IgE CONCENTRATION

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**ABSTRACT** As reported previously, and confirmed here in 26 donors, the serum IgE level (2.6–5,500 ng/ml) correlates well ( $r_s = 0.95, P < 0.001$ ) with the in vivo number of IgE molecules/basophil (6,000–600,000). The total number of IgE receptors/basophil was monitored by incubating them with an IgE-rich serum (15  $\mu$ g/ml), quantitatively stripping IgE from the cells at pH 3.7, and measuring eluted IgE by a direct radioimmunosorbent test. Saturation of receptors for each donor was achieved with 15 nM IgE (3  $\mu$ g/ml). The proportion of receptors occupied in vivo correlated with the serum IgE ( $r_s = 0.84, P < 0.001$ ) whereas the average association constant of the receptors was independent of serum IgE and ranged from  $7.1 \times 10^8$ /M to  $2.8 \times 10^{10}$ /M, averaging  $7.7 \times 10^9$ /M. Unexpectedly, the total number of IgE receptors/basophil was closely related to the serum IgE level. ( $r_s = 0.92, P < 0.001$ ). Thus, either there is genetic association between serum IgE and the number of basophil IgE receptors, or, more likely, the receptor number is modulated by the serum IgE concentration.

### INTRODUCTION

This laboratory has recently been concerned with understanding, in quantitative terms, the IgE components of the mediator-release process from human basophils (1). By measuring the IgE eluted from washed leukocytes at low pH, a technique first described by Ishizaka and Ishizaka (2), we previously reported a strong positive correlation between total serum and cell-bound IgE on human basophils (1). We have also observed, using anti-IgE as a stimulus, that

the release of histamine from human basophils is independent of the number of IgE molecules on their surface (1).

This manuscript describes additional studies aimed at direct assessment of the following additional parameters: (a) the average association constant ( $K_o$ )<sup>1</sup> of the interaction between the Fc of IgE and the basophil IgE receptor; (b) the degree of receptor saturation in vivo; and (c) the total number of IgE receptors/basophil. 26 unselected donors were evaluated with regard to serum IgE concentration and the number of IgE molecules/basophil in vivo. Their basophils were then passively saturated with a high IgE serum, and the increment in cell-bound IgE measured by elution at low pH. Among the individuals studied, there was a 10- to 100-fold range in the calculated  $K_o$  values for the basophil IgE receptors. The study further showed a correlation between basal serum IgE and IgE receptors/basophil. This finding suggests either that the two traits are genetically associated or that there is physiological modulation of basophil receptor display by circulating IgE.

### METHODS

**Materials.** Tris-EDTA buffer contained 0.025 M Tris, 0.12 M NaCl, 0.005 M KCl, 0.03% human serum albumin, and 0.01 M EDTA. The acetate buffer used for elution consisted of 0.05 M acetate, 0.085% NaCl, 0.01 M EDTA, and 0.03% human serum albumin. This buffer was adjusted to pH 3.7 at 0°C. Goat antiserum against the Fc fragments of IgE myeloma protein (PS) was generously supplied by Dr. Kimishige Ishizaka (Johns Hopkins University at Good Samaritan Hospital). IgE-rich serum was obtained from a  $\mu$ g/ml. The AB serum used for diluting the reaginic serum was obtained from a normal donor whose serum IgE was 25 ng/ml.

**Preparation of cells.** Venous blood collected from each donor was mixed with 6% dextran 70 in saline (Cutter Laboratories, Inc., Berkeley, Calif.), 0.01 M EDTA, and 350 mg

Dr. Malveaux is the recipient of National Research Service Award AI 05385 from the National Institutes of Health. Dr. Conroy's present address is Ciba-Geigy Pharmaceutical Corporation, Ardsley, N. Y. 10502. Dr. Adkinson is the recipient of Allergic Disease Academic Award AI 71023 from the National Institutes of Health.

Received for publication 22 August 1977 and in revised form 9 March 1978.

<sup>1</sup> Abbreviations used in this paper:  $K_o$ , average association constant;  $r_s$ , proportion of receptors occupied.

dextrose and allowed to sediment for 60–90 min at room temperature (3). The leukocyte-rich plasma was centrifuged and the cell button was washed twice in an equal volume of Tris-EDTA buffer. The cells were suspended in 6.0 ml Tris-EDTA buffer and the basophils were counted in triplicate in a Spiers-Levy Chamber (Hausser Scientific Co., Blue Bell, Pa.) after staining with Alcian blue according to the method of Gilbert and Ornstein (4). To 50  $\mu$ l of the cell suspension mixed with 0.45 ml of 0.1% EDTA in saline was added 0.45 ml of a solution containing 0.076% acetyl pyridinium chloride, 0.7% lanthanum chloride, 0.9% sodium chloride, 0.21% Tween 20 (Atlas Chemical Industries, Inc. Wilmington, Del.), and 0.143% Alcian blue. After agitation of this mixture for 60 s, 50  $\mu$ l of 1 N HCl was added and an aliquot was transferred to the Spiers-Levy counting chamber. The actual number of basophils counted for each donor ranged from 200 to >1,200. The mean coefficient of variation for this method of basophil counting was 14%.

**Passive sensitization.** The method of sensitization was that described by Levy and Osler (5). Briefly, 10<sup>7</sup>–10<sup>8</sup> washed leukocytes (from 30 to 50 ml blood) suspended in 1.0 ml of Tris-EDTA buffer were combined with 0.5 ml heparin-EDTA and 1.0 ml of an appropriate dilution (in AB serum) of a single IgE-rich reaginic serum. An equal number of washed leukocytes was suspended in a comparable mixture containing autologous serum. Incubation proceeded for 90 min at 37°C on a reciprocal shaker.

**Elution of IgE.** After sensitization, leukocytes were washed twice with 5 ml Tris-EDTA buffer, suspended in 1 ml of the same buffer (chilled to 0°C), and allowed to sit 10 min at 0°C (fraction A). After centrifugation, the cells were resuspended in 1 ml of elution buffer, pH 3.7, for an additional 10 min at 0°C (fraction B). These cells were centrifuged and the supernatant fluid was immediately titrated to neutrality with 1 N NaOH. The acid elution technique was reported in detail by Ishizaka and Ishizaka (2). Two subsequent washings were carried out in 1.0 ml Tris-EDTA buffer (fractions C and D). All supernatant fluids were frozen for subsequent total IgE determination.

**Total IgE measurements.** The amount of IgE eluted and of serum IgE was measured by a direct (noncompetitive) radioimmunoassay test (6) which has been previously reported in detail (7). This assay is sensitive to <1 ng/ml IgE with a mean coefficient of variation for low range IgE samples of 10%.

## RESULTS

**Elution of IgE after passive sensitization.** A representative experiment showing the quantity of IgE eluted from basophils at different steps in the experimental protocol is shown in Fig. 1. At least 90% of the eluted material was present in the acid-treated fraction and the first postelution wash. Previous studies done in our laboratory have shown that a second exposure to acid is of no benefit in eluting more IgE (1). For calculation of the total number of molecules eluted, the IgE eluted at pH 3.7 and the first postelution wash [fractions B and C] were combined. The number of molecules/basophil was calculated as follows: molecules/basophil = (ng IgE eluted  $\times$  6.023  $\times$  10<sup>23</sup> molecules/mol) / (2  $\times$  10<sup>6</sup> g/mol IgE  $\times$  10<sup>9</sup>  $\times$  number basophils per elution tube).

**Saturation of receptors.** Experiments were per-

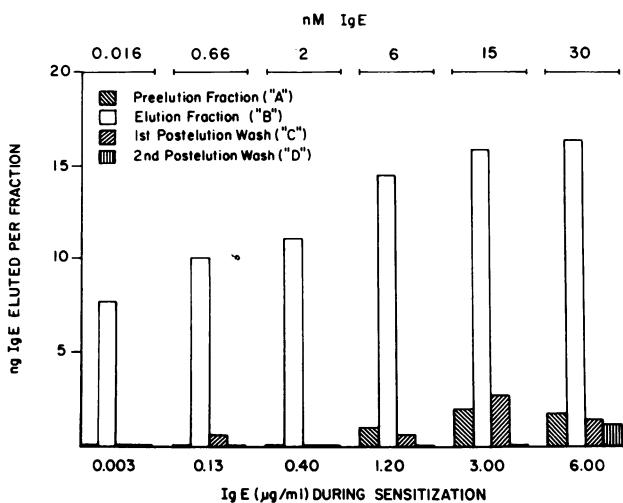


FIGURE 1. Quantity of IgE measured in the supernatant fluids at different steps of the experimental protocol after passive sensitization. Sensitization at 3 ng/ml was carried out in autologous serum. (A) Preelution with Tris-EDTA (pH 7.4) at 0°C for 10 min; (B) Acetate-EDTA (pH 3.7) elution at 0°C for 10 min; (C and D) Postelution washings with Tris-EDTA.

formed to establish whether human basophils could be saturated with IgE. Fig. 2 shows the results when the cells of three individuals were incubated with increasing concentrations of the same IgE-rich serum. The elutable IgE increases with increasing IgE concentrations until a plateau is reached. Apparent saturation of receptors occurs at about 15 nM [3  $\mu$ g/ml] IgE for each donor. This was later shown to be independent of total receptor number. Concentrations of IgE > 3  $\mu$ g/ml did not increase elutable basophil-bound IgE. That functionally significant passive sensitization

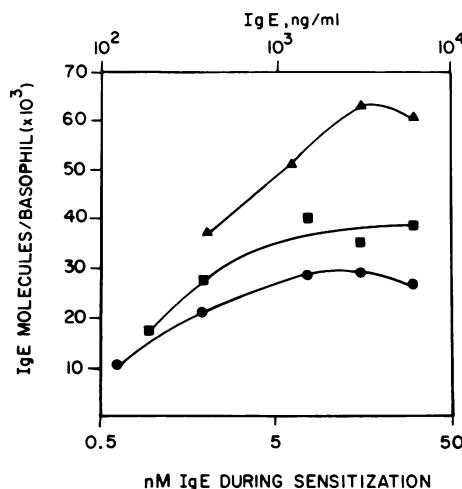


FIGURE 2. Number of IgE molecules eluted from basophils of three different donors after passive sensitization. Autologous serum IgE levels for the donors were 2.6 (●), 6.7 (■), and 8.3 ng/ml (▲).

occurs under these conditions has been demonstrated previously (5) and was confirmed by challenge of the sensitized cells of nonallergic donors with ragweed antigen E [data not shown].

The quantitative results obtained with this experimental protocol were corroborated independently by Dr. Teruko Ishizaka during a joint opportunity we had to study a patient with chronic myelocytic leukemia and 75% basophilia whose serum IgE was 20 ng/ml. Dr. Ishizaka studied the incremental binding of  $^{125}\text{I}$  IgE to the purified [90–93%] basophils from the patient using the technique which Kulczycki et al. (8) applied to similar studies of rat basophil leukemia cells. The data of Dr. Ishizaka were obtained from two separate determinations using  $10^6$  and  $2 \times 10^6$  basophils per condition. Our data on the same cells by the elution technique were obtained using  $1.2 \times 10^6$  basophils/elution tube. Fig. 3 shows good agreement between the acid-elution technique and that obtained by  $^{125}\text{I}$  IgE binding. With both methods, the number of receptors was about  $3 \times 10^4$ /basophil. Basophils used in this study were normal in microscopic appearance and demonstrated normal kinetics of histamine release by anti-IgE (9).

*Determination of IgE receptor occupancy in autologous serum and after receptor saturation.* 26 donors were studied by passive sensitization using two conditions: autologous serum and 3  $\mu\text{g}/\text{ml}$  IgE from an IgE-rich serum. Table I shows the results of this study. Both atopic and nonatopic individuals were

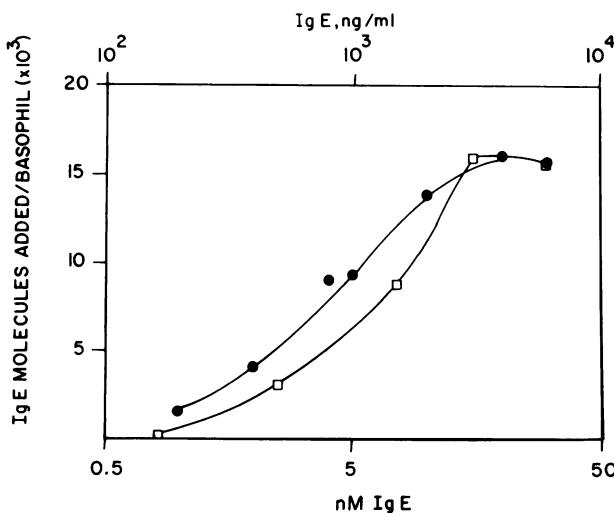


FIGURE 3 Saturation of IgE receptors in a patient with chronic myelogenous leukemia by acid elution and  $^{125}\text{I}$ -IgE binding. In the latter procedure carried out in Dr. Teruko Ishizaka's laboratory, cells ( $1 \times 10^6$  and  $2 \times 10^6$  basophils per condition in two separate determinations) were incubated with  $^{125}\text{I}$ -myeloma E (0.4–20 nM) for 2 h at 37°C with constant shaking and the radioactivity of the cell pellets was counted directly.  $\square$ , acid elution;  $\bullet$ ,  $^{125}\text{I}$ -IgE binding.

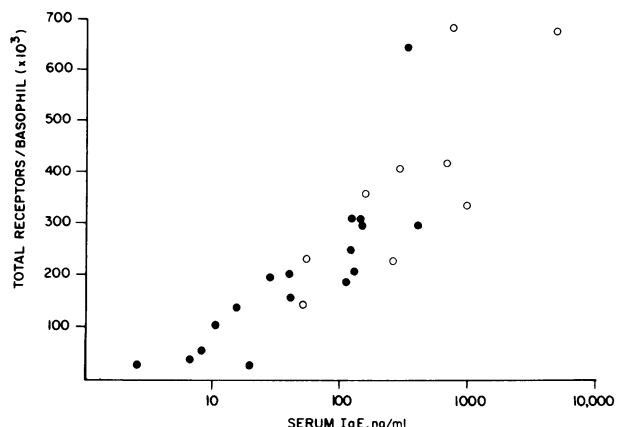


FIGURE 4 Relationship between total number of IgE receptors per basophil and serum IgE concentration.  $\bullet$ , Normal donors;  $\circ$ , donors with a history of allergic rhinitis, asthma, and/or atopic dermatitis.

studied and the serum IgE levels ranged from 2.6 to 5,500 ng/ml. Basophils from individuals with serum IgE levels  $> 400$  ng/ml failed to show an increase in elutable IgE when incubated with the IgE-rich serum. Within the limitation of our method, these cells appear to be virtually saturated in vivo. Elutable cell-bound IgE, after incubation in autologous serum, ranged from  $6 \times 10^3$  to  $6.7 \times 10^5$  molecules/basophil and, confirming our previous findings, was significantly correlated with serum IgE ( $r_s = 0.95$ ;  $P < 0.001$ ). Moreover, the total number of IgE receptors on the basophils studied correlated equally well with the basal serum IgE level of the donor, as shown in Fig. 4 ( $r_s = 0.92$ ;  $P < 0.001$ ).

Table I also shows the proportion of receptors occupied ( $r$ ) under in vivo conditions for each patient. In general,  $r$  increases as serum IgE increases ( $r_s = 0.84$ ;  $P < 0.001$ ). This was true for both atopic and nonatopic subjects.

An average association constant ( $K_o$ ) for IgE binding to basophil Fc receptors under in vivo conditions was calculated for each donor as follows:  $K_o = r/\text{IgE}[1 - r]$  where  $r$  = the proportion of occupied receptors at basal serum IgE level [expressed in molar concentration]. Where no increment of cell-bound IgE could be detected after incubation at 3  $\mu\text{g}/\text{ml}$  IgE,  $K_o$  was not calculated.  $K_o$  values range from  $7.1 \times 10^8/\text{M}$  to  $2.8 \times 10^{10}/\text{M}$  and were unrelated to basal IgE level.

## DISCUSSION

The data indicating that the number of IgE molecules on human basophils correlates highly with serum IgE level confirms our previous observations on a somewhat different population (1). The same conclusion was also reached by Stallman et al. (10) using a micro-

fluorescence technique. The principal novel observation reported here is the association between total receptors for IgE on human basophils and circulating levels of IgE in the same individuals. Stallman et al. (10) was unable to detect an increase of IgE after passive leukocyte sensitization using either IgE myeloma protein PS or high IgE sera. Preliminary experiments performed by one of us (Dr. Conroy) using IgE myeloma protein PS were unsuccessful because of the low avidity adherence of myeloma protein to cell preparations which could not be prevented even when the myeloma protein was mixed with normal human serum or ultracentrifuged.

The only previously reported attempt to quantitate the total number of IgE receptors on human basophils

was that of Ishizaka et al. (11), using the C1q fixation technique. Among the 13 patients they studied, there was no clear relationship between the IgE level and either the degree of receptor saturation or the total number of receptors. They observed a 1.1- to 6.9-fold increase in receptor number after incubation with IgE myeloma protein PS, and reported a maximal receptor number of about 85,000. Although this estimation of total IgE receptors is lower than ours (up to 600,000), the C1 fixation transfer technique would provide a minimal estimate, whereas the method of acid elution probably represents a maximal estimate of the number of receptors. When, however, the cells of the patient with 75% basophils were studied with the acid elution technique and with  $^{125}\text{I}$ -IgE binding

TABLE I  
*IgE Receptors on Human Basophils*

Donor	Serum IgE <sup>†</sup>	Basophils/ml ( $\times 10^6$ ) <sup>§</sup>	Ig eluted* after sensitization with		Occupied receptors ( $\times 10^3$ ) after sensitization with		$r^{\parallel}$	$K_o$ $\times 10^6/M\ddagger$
			Autologous serum	High IgE serum	Autologous serum	High IgE serum		
1	2.6	6.1	1.3	5.9	6	29	0.21	16
2	6.7	8.5	4.6	10.8	17	38	0.45	24
3	8.3	8.8	7.7	18.5	26	63	0.41	17
4	10.9	5.0	3.4	16.6	21	101	0.21	4.9
5	15.9	3.5	5.3	16.6	44	138	0.32	5.9
6	20	9.6	5.0	9.3	15	29	0.52	11
7	29	1.4	2.3	9.4	47	193	0.24	2.2
8	41	8.8	23	59	78	200	0.39	3.1
9	42	4.8	14	25	83	154	0.54	5.6
10**	52	10.5	45	52	123	140	0.88	28
11**	56	11	46	84	125	229	0.55	4.4
12	118	9.3	31	57	100	183	0.55	2.1
13	122	5.4	33	45	183	249	0.73	4.4
14	127	2.8	24	29	253	308	0.82	7.2
15	131	9.7	49	66	151	203	0.74	4.3
16	150	5.5	44	56	239	308	0.78	4.7
17	151	7.2	28	71	114	291	0.39	0.85
18**	166	3.5	34	42	294	356	0.83	5.9
19**	270	8.1	53	61	198	225	0.88	5.4
20**	307	4.8	33	64	209	402	0.52	0.71
21	358	2.0	38	42	576	643	0.90	5.0
22	422	9.3	90	90	291	291	0.95 $\ddagger\ddagger$	—
23**	725	8.1	113	109	417	404	0.95 $\ddagger\ddagger$	—
24**	825	3.1	70	70	675	681	0.95 $\ddagger\ddagger$	—
25**	1040	18.1	200	194	333	323	0.95 $\ddagger\ddagger$	—
26**	5500	2.4	55	55	678	678	0.95 $\ddagger\ddagger$	—

\* Total IgE (nanograms) eluted after incubation for 90 min at 37°C with autologous or high IgE serum (3  $\mu\text{g}/\text{ml}$ ).

† Expressed in nanograms per milliliter.

§ Number of basophils per elution tube (1.0 ml).

¶ Proportion of receptors occupied in vivo.

¶ $K_o$  under in vivo conditions. See text for details of calculation.

\*\* Personal history of allergic rhinitis, asthma, and (or) atopic dermatitis.

†† Endogenous number of molecules eluted equal to that of cells sensitized with 3  $\mu\text{g}$  IgE/ml.  $r$  is arbitrarily assigned a value of 0.95.

(Fig. 3) there was good agreement on the total number of molecules/basophil. The range of  $r$  in vivo and the calculated association constants reported by Ishizaka et al. in 1973 (11) are similar to our current data.

It is possible that our data overestimate the number of receptors at high serum IgE levels due to binding of IgE to other cells. The contribution to the apparent number of receptors by IgE binding to nonbasophilic elements is unknown at this time. Teruko Ishizaka<sup>2</sup> has noted IgE adsorption to monocytes when exposed to high concentrations of IgE myeloma at 37°C. She had also detected IgE on the monocytes from some atopic patients with high serum IgE levels by autoradiography. Stallman and Aalberse (12) noted relatively weak binding of IgE to monocytes when compared to that of basophils using their microfluorometry technique. Conroy et al. (1), however, did not demonstrate significant binding of IgE to basophil-poor lymphocyte preparations. Gonzalez-Molina and Spiegelberg (13) have recently reported that a subpopulation of bone marrow-derived (B) lymphocytes have trypsin-sensitive Fc receptors specific for IgE, but not IgG. Among human peripheral blood lymphocytes, those capable of binding IgE constitute a small percentage (<4%). Although the avidity of these lymphocyte receptors for IgE has not been quantitated, the data of Conroy et al. (1), Stallman and Aalberse (12) and Ishizaka et al. (14) suggest that the IgE binding to these receptors is limited in quantity and/or avidity as compared to the IgE receptors on basophils. Because the influence of these lymphocytes is likely to be more significant at high IgE concentrations, the maximal number of IgE receptors/basophil may represent an over-estimation. However, assuming that contaminating levels of monocytes and IgE-bearing lymphocytes are randomly distributed over the donor population, such nonbasophil IgE can not invalidate the observation that the total number of IgE receptors is related to the serum IgE level.

The affinity of receptors on human basophils for IgE is high and ranges from  $7.1 \times 10^8$  to  $2.8 \times 10^{10}/M$  (Table I). The average  $K_0$  ( $7.7 \times 10^9/M$ ) is comparable to that described by Kulczycki and Metzger (15) for the intact rat basophil leukemia cell line ( $6 \times 10^9/M$ ). We recognize that such estimates of average equilibrium constants as we have derived reflect not only the intrinsic binding constants of the molecular interaction but also the contribution of membrane placement and distribution of the receptors (16, 17). The differences in calculated association constants among our donors may be due to one or more of the following: (a) heterogeneity of IgE receptors on human basophils; (b) structural heterogeneity of the Fc portion of IgE;

(c) contribution to binding by IgE-bearing lymphocytes or monocytes at high serum IgE levels. Of these possibilities, we feel the heterogeneity of IgE receptors is the most likely explanation for the observed differences. Whereas the range of calculated  $K_0$  was 40-fold among our donors, Ishizaka et al. (11), using the C1q fixation technique, reported >100-fold variation among their 13 donors. There is further precedence for the heterogeneity of ligand-receptor binding among peptide hormone (18, 19) and catecholamine (20) systems. The heterogeneity observed in our system may represent positional changes of the receptor in the basophil membrane rather than structural heterogeneity of the receptor itself. With regard to the second possibility, subclasses reflecting heterogeneity of the Fc portion of IgE have not been described. However, such subclasses, if they do exist, cannot explain our findings because the same serum was used throughout our study to saturate IgE receptors. The third possibility encompassing the role of IgE-bearing B lymphocytes and monocytes was discussed above.

The need for purified human basophils to characterize quantitatively and more accurately the IgE receptor-ligand interaction is obvious. Likewise, solutions to questions of cell viability and binding activity of the ligand must await methodology for purifying basophils. Whereas the viability of the basophils ( $\geq 1\%$  of the leukocyte preparation) could not be studied microscopically, these cells were capable of releasing histamine in a normal manner after passive sensitization for 2 h at 37°C in the presence of 40% human serum (data not shown). Functionally, the cells appear normal under these experimental conditions. Binding activity of IgE and precise calculation of equilibrium constants may be influenced not only by a heterogeneous cell population, but by the presence of aggregated IgE in the sensitizing mixture. Although it is possible in this study that our IgE-rich serum contained aggregated ligand, such aggregates of IgE would be expected to have an equal effect on all individuals studied because a single sensitizing serum was employed throughout the experiments reported here. The presence of aggregated IgE or of biologically inactive IgE may well influence the absolute value of the calculated  $K_0$ , but would not be expected to alter the basic observation of a strong positive correlation between total IgE receptor number and basal serum IgE concentration.

The strong correlation between total IgE receptors and serum IgE was an unexpected finding which suggests that either the two are associated by primary genetic programming, or that the presence of one induces or modulates the other. It is most reasonable to assume that circulating IgE has some influence on the number of available IgE receptors on circulating basophils. Fluctuation of the number of receptors per

<sup>2</sup> Personal communication.

cell is known to occur during the cell cycle of a cultured rat basophil leukemia (21). In the rat system, new receptors are acquired on cells arrested in the  $G_1$  phase of the cell cycle. Resting human basophils, however, are nondividing, end-stage cells. Whereas it is currently not known whether IgE receptors on basophils, or Fc receptors on lymphocytes and macrophages are subject to modulation by circulating levels of ligand, it would not be surprising that immunoglobulins may control receptor density, because hormones have been shown in some cases to modulate the number of hormone receptor molecules on a cell surface (22). It is known, for example, that circulating insulin levels in part determine receptor concentration on a variety of cells, but in this system the relationship between insulin levels and receptor number is an inverse one (23). The possibility of modulation of receptors by IgE is suggested by our findings, and experiments are currently in progress to test this hypothesis.

#### ACKNOWLEDGMENTS

We wish to thank Dr. Teruko Ishizaka for providing data on the binding of  $^{125}\text{I}$ -IgE to basophils and for her helpful suggestions and constructive criticism. We also thank Mrs. LaVerne Grove for excellent technical assistance.

This work was supported by grant AI 07290 from the National Institute of Allergy and Infectious Diseases, National Institutes of Health.

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