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Research Article

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A Subpopulation of Normal Human Peripheral B Lymphocytes that Bind IgE

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ABSTRACT Isolated normal human peripheral lymphocytes were analyzed for their ability to bind IgE as shown by rosette formation with aldehyde-fixed ox red cells coated with an IgE myeloma protein (E_o'-IgE) as indicator cells. An average of 4.3% of the cells in the lymphocyte preparations of 12 donors formed E_o'-IgE rosettes. The lymphocyte preparations contained an average of 0.36% basophils which also formed E_o'-IgE rosettes, suggesting that about 4% of the lymphocytes bound IgE. The rosette formation was inhibited by IgE myeloma proteins or IgE Fc fragments but not by IgE Fab fragments or Ig of the other classes. On the average, the lymphocytes of the 12 donors contained 70.5% cells forming spontaneous rosettes with sheep erythrocytes (E), 10.6% cells having surface immunoglobulin (SIg), and 15.5% binding IgG as shown by rosette formation with IgG-coated ox red cells (E_oA). Fractionation of the lymphocytes into populations rich in spontaneously E-rosetting cells and cells with SIg indicated that the majority of the lymphocytes forming E_o'-IgE rosettes belonged to the SIg-positive lymphocytes. Analyses of lymphocyte populations lacking cells forming E_oA rosettes and of rosetting with mixed indicator cells both demonstrated that over 90% of the lymphocytes forming E_o'-IgE rosettes did not form E_oA rosettes and apparently had no Fc receptors for IgG. Pretreatment of the lymphocytes with trypsin in amounts that did not alter the number of E_oA-rosetting cells abolished the E_o'-IgE rosette formation. These data indicate that a subpopulation of normal human peripheral lymphocytes, probably belonging to the B-cell type, binds IgE presumably via trypsin-sensitive receptors specific for the Fc fragment of IgE. The surface markers of these lymphocytes resemble those of cultured human lymphoblastoid cells that have recently been shown to bind IgE.

INTRODUCTION

Lymphocytes can be grouped according to their cell surface markers. Human peripheral thymus-derived lymphocytes (T cells) have receptors for sheep erythrocytes (E)[1] with which they form rosettes (1, 2). Mature bone marrow-derived lymphocytes (B cells) have easily detectable surface immunoglobulin (SIg) (2, 3). A third population of lymphocytes forms neither E rosettes nor has SIg but is characterized by surface receptors for the Fc fragment of IgG (4, 5). Many B lymphocytes also have receptors for the Fc fragment of IgG (6) and, in addition, receptors for various complement components (7, 8), whereas few T cells have these two types of receptors (9, 10). Recently, several cultured human lymphoblastoid cell lines were found that bound IgE myeloma proteins, apparently with receptors specific for the IgE Fc fragment (11, 12). All cell lines that bound IgE had SIg but none formed rosettes with IgG antibody-coated ox red cells (E_oA), suggesting that these lymphoblastoid cells either may have derived from unusual B cells that lack receptors for IgG, or they may have changed their surface markers as a result of the culture conditions.

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[1] *Abbreviations used in this paper:* E, sheep erythrocyte; E_o, ox erythrocyte; E_oA, native ox red cell sensitized with rabbit IgG anti-ox red cell antibodies; E_o', fixed ox erythrocyte; E_o'-aIg, fixed ox red cells coated with a complex of rabbit Fab' anti-ox red cell antibody and rabbit F(ab')$_2$ anti-human Fab antibodies; E_o'-IgE, fixed ox red cell coated with IgE myeloma protein (Sha); E_o'-IgG, fixed ox red cell coated with normal human IgG; FH, Ficoll-Hypaque, density 1.077; Ig, immunoglobulin(s); MEM-FCS, minimum essential Eagle's medium containing 2.5% heat-inactivated fetal calf serum PBS, phosphate-buffered 0.15 M NaCl, ph 7.2; RFC, rosette-forming cells; Sha, IgE myeloma protein; SIg, cell surface immunoglobulin.

The purpose of this investigation was to analyze normal peripheral lymphocytes for the presence of a subpopulation that binds IgE. Since pure human IgE anti-red cell antibodies are not easily available, ox erythrocytes were aldehyde-fixed, coated with an IgE myeloma protein, and used as indicator cells in a rosette assay for detection of lymphocytes binding IgE.

METHODS

Immunoglobulins (Ig). Normal human IgG and IgG1, IgA1, IgD and IgE myeloma proteins, and an IgM macroglobulin were isolated from sera as previously described (13). A rat IgE myeloma protein that had been isolated from sera of rats bearing the plasma cell tumor IR-162 and provided by Dr. H. Bazin, Louvain, Belgium, was a gift of Dr. J. Feldman, Scripps Clinic, La Jolla, Calif.

Antisera. Four rabbits were repeatedly injected i.v. with washed ox erythrocytes (E_o) (14). Antisera having an agglutination titer of 1:32–1:64 were pooled and the IgG fraction was isolated by DEAE-cellulose chromatography in 0.015 M phosphate buffer at pH 8.0. Fab′ fragments of the anti-E_o IgG were prepared as described by Strausbauch et al. (15). $F(ab')_2$ fragments of a polyvalent rabbit anti-human Fab fragment antiserum were identical to those used in a previous study (13).

Lymphocyte isolation. Lymphocytes were isolated from defibrinated human peripheral blood by sedimenting red cells, removing phagocytic and adherent cells, and centrifuging through Ficoll-Hypaque (FH, Pharmacia Fine Chemicals Inc., Piscataway, N. J.) as described by Perlman et al. (16). The only changes in this method were the substitution of 3% dextran for gelatin and Eagle's minimum essential medium for the Tris-buffered Hank's balanced salt solution. The isolated lymphocytes were suspended at a concentration of 4×10^6 cells per ml Eagle's minimum essential medium containing 2.5% heat-inactivated fetal calf serum. More than 98% of the cells were viable as shown by trypan blue exclusion and had the appearance of lymphocytes when stained with Wright's stain, and 50–70% of the lymphocytes were recovered. The percent of basophils in the preparations was determined by counting 500–1,000 cells stained with toluidine blue as described by Dvorak et al. (17).

Red cells. Sheep and ox blood collected into Alsever's solution was purchased from the Colorado Serum Co., Denver, Colo. The blood was stored at 4°C for up to 4 wk and the cells were washed five times with phosphate-buffered 0.15 M NaCl (PBS), pH 7.2.

E rosette assay. For the demonstration of spontaneous E rosette formation, one volume of washed and packed E was pretreated with four volumes of the sulfidic compound, 2-aminoethylisothio-uronium bromide hydrobromide (0.28 M, pH 8.5), for 30 min at 37°C according to the method described by Pellegrino et al. (18).

For the rosette assay, 100 μl of 1% treated E and 100 μl of lymphocytes (4×10^6 cells/ml), both in minimum essential Eagle's medium containing 2.5% heat-inactivated fetal calf serum (MEM-FCS), were mixed and incubated 10 min at 37°C before centrifuging the cells at 200 *g* for 5 min at 4°C and incubating them for 60 min at 4°C. Two drops of 0.1% toluidine blue in PBS was then added, and the cells were gently resuspended. One drop of the cell mixture was placed on a microscope slide and covered with a coverslip. Usually, 300 cells were counted for enumerating the percentage or rosette-forming cells (RFC). A positive RFC was defined as a cell having at least three red cells adherent to the surface.

IgE rosette assay. E_o, which do not form spontaneous rosettes with human lymphocytes (14), were used as indicator red cells in the other rosette assays. To facilitate adsorption of proteins and allow their use for up to 5 wk when stored at 4°C, E_o were pretreated with trypsin, then fixed with pyruvic aldehyde and formaldehyde in a modification of the method described by Hirata and Brandriss (19). Equal volumes of 10% E_o and trypsin (Calbiochem, San Diego, Calif.; A grade, 0.2 mg/ml) in PBS were incubated for 60 min at 37°C with occasional mixing. After 0.2 mg/ml of soybean trypsin inhibitor (Calbiochem) was added, the cells were washed three times in PBS and suspended as a 10% solution. An equal volume of 3% pyruvic aldehyde (ICN Pharmaceuticals Inc., Cleveland, Ohio) in PBS (pH adjusted to 7.2 with 10 N NaOH) was added to the cells and incubated for 20 h at room temperature with continuous mixing on a rotator at 60 rpm. The cells were then washed five times with PBS and fixed with 3% formaldehyde in PBS in the same manner for 20 h at room temperature. The fixed cells (E_o') were washed and stored in PBS at 4°C.

For adsorption of the IgE myeloma (Sha) onto the E_o', the following conditions were found to be optimal. First, an equal volume of 4% E_o' and 0.4 mg/ml IgE (Sha) in 0.15 M acetate buffer, pH 5.0, was incubated for 2 h at room temperature and continuously mixed on a rotator. The sensitized cells (E_o'—IgE) were then washed three times in PBS and stored in a 1% suspension in PBS for up to 3 wk. In preliminary experiments, it was found that sensitization of the E_o' at concentrations of up to 2 mg/ml IgE protein Sha did not yield higher numbers of IgE-rosetting cells employing either normal or Wil-2WT lymphocytes. Wil-2WT cells were used for routine standardization of the indicator cells because about 80% of these cells form rosettes (12) and they proved to be very sensitive to inadequately sensitized indicator cells.

For the IgE rosette assay, the coated indicator red cells were mixed and incubated with isolated lymphocytes as described above for the spontaneous E rosette assay except that they were not incubated first at 37°C. The cells were usually incubated for 1 h at 4°C and the rosettes were counted after 60 min. Unsensitized E_o or E_o' were always used as a control to exclude nonspecific rosette formation, which was found to be less than 0.5%.

To elute IgE bound to lymphocytes in vivo that might not have washed off during the normal isolation procedure, lymphocytes of two donors were first treated with acetate buffer, pH 4.0, for 10 min as described by Ishizaka and Ishizaka (20).

IgG rosette assays. For enumeration of cells binding IgG, two types of indicator red cells were used. First, fixed ox red cells coated with normal human IgG (E_o'-IgG) were prepared exactly like the E_o'-IgE cells except that normal IgG was substituted for the IgE myeloma protein. Lymphoblastoid cells of the Daudi line were initially used to determine the best conditions for sensitization of the indicator red cells. Second, E_o were sensitized with rabbit IgG anti-E_o antibodies (14) to prepare the E_oA indicator cells. Pooled hyperimmune rabbit IgG anti-E_o was used at a 1:40 dilution (0.5 mg/ml), which was the highest possible concentration that did not cause agglutination of the ox red cells. The rosette assay for IgG-binding cells was performed identically to the E_o'-IgE assay.

Inhibition of rosette formation. For inhibition of rosette formation, the lymphocytes were incubated for 30 min at 4°C with different concentrations of myeloma proteins before addition of the indicator red cells.

SIg-positive lymphocytes. For detection of cells with SIg, E_o' were coated with rabbit $F(ab')_2$ anti-human Fab fragments coupled to Fab' fragments of a rabbit anti-E_o antiserum (E_o'-aIg) and used as a protein-E_o bridge (15). The Fab' bridge was used because $F(ab')_2$ fragments did not adhere well to E_o' cells shown by the low numbers of rosettes formed with cultured Wil-2WT cells which have SIg. With the Fab' bridge, over 90% of the Wil-2WT cells formed rosettes (12). 6 mg/ml of $F(ab')_2$ anti-Fab and 3 mg/ml of Fab' anti-E_o were conjugated for 60 min at room temperature with 0.1% glutaraldehyde. The reaction was terminated with 10 times excess sodium bisulfite, and the complexes were dialyzed overnight at 4°C against PBS. Plateau sensitization of E_o' was achieved by incubating a 2% cell suspension with a final concentration of 0.5 mg protein complexes in 0.1 M acetate buffer, pH 5.0, for 2 h at room temperature as described above for the E_o'-IgE cells.

Mixed rosettes. In each mixed rosette assay, two types of indicator red cells were used (final concentration of 1%) that could be distinguished microscopically on the basis of size and toluidine blue staining. E, E_o, and E_oA cells appeared smaller than E_o' cells and did not stain with toluidine blue, whereas the E_o' of the E_o'-IgE, E_o'-IgG, and E_o'-aIg indicator cells appeared violet and larger than the native cells. A mixed rosette was scored when the lymphocytes were surrounded by at least two indicator cells of each type.

Lymphocyte fractionation. Lymphocytes were incubated with appropriate indicator red cells and RFC were separated from the nonrosetting cells by FH density centrifugation. 25×10^6 cells in 2.5 ml MEM-FCS were mixed with 2.5 ml of a 1% indicator red cell suspension, centrifuged, and then incubated for 2 h at 4°C to allow rosette formation. The cells were then gently resuspended and 5 ml FH underlayered in a 15-ml conical plastic tube. E RFC were centrifuged at 4°C for 8 min at 1,050 *g* (21). E_oA and E_o'-aIg RFC were centrifuged at room temperature for 30 min at 1,200 *g*. The cells at the interface (depleted population) and at the bottom of the tube (enriched population) were collected separately and washed three times with MEM-FCS. Red cells of the enriched E and E_oA RFC were lysed with 0.83% NH_4Cl. E_o' did not lyse under these conditions. Therefore, these rosettes were mechanically disrupted by using a Vortex mixer (Scientific Industries, Inc., Bohemia, N. Y.) and the red cells were separated from the lymphocytes by a second FH centrifugation at room temperature for 30 min at 1,400 *g*. The fractionated lymphocytes were washed, suspended at 4×10^6 cells/ml MEM-FCS, and tested for rosette formation as described above. The yields of E-rosetting lymphocytes were up to 84% of the expected total but only 13–30% of the E_o'-aIg- and E_oA-rosetting lymphocytes.

Trypsin treatment of lymphocytes. The effect of trypsin treatment on rosette formation was studied as described previously (12) by employing 100 μg trypsin/ml lymphocytes incubated for 15 min at 37°C.

RESULTS

Percentage of lymphocytes forming rosettes with different indicator red cells. Isolated lymphocytes from 12 normal adults ranging in age from 24 to 43 yr were tested for rosette formation with E_o'-IgE, E_o'-aIg, E, E_o'-IgG, and E_oA indicator cells (Table I). An average of 4.3% (range, 1.5–7.5%) of the cells formed E_o'-IgE rosettes. Since basophils have receptors for IgE and isolate in part with the lymphocytes, the number of basophils was counted in all lymphocyte preparations and averaged 0.36% with a range of 0.15–0.8%. Although we attempted to differentiate between lymphocytes and basophils when counting the rosettes (Figs. 1*a* and 1*b*), about half of the center white cells were covered with so many red cells that often no clear distinction was possible. Whenever a basophil was recognized, it was always surrounded by E_o'-IgE indicator cells but no other types. Therefore, the number of "mononuclear"

TABLE I
Percent Lymphocytes Forming Rosettes with Different Indicator Red Cells

Donor	Sex	Age	% Rosettes					Basophils
			E'_o-IgE*	E'_o-aIg	E	E'_o-IgG	E_oA	
								%
E. P.	M	31	5.2	12.5	81.3	17.2	15.6	0.30
B. D.	F	27	4.9	8.2	71.6	17.5	19.0	0.18
J. R.	F	27	5.7	8.0	72.0	10.3	15.4	0.52
M. D.	M	30	4.5	9.7	70.3	12.8	14.8	0.27
H. S.	M	43	3.8	11.0	73.0	14.2	16.0	0.61
R. Z.	M	33	3.2	8.6	83.7	19.4	16.3	0.31
M. H.	F	24	2.7	6.5	73.3	6.6	7.1	0.20
H. K.	M	29	5.0	12.0	67.0	22.0	21.4	0.31
R. L.	M	24	1.5	13.2	69.0	17.0	NT	0.25
V. H.	F	33	3.0	10.0	63.0	15.6	15.6	0.80
M. G.	M	30	4.5	10.6	81.0	12.0	17.3	0.15
D. H.	F	28	7.5	16.8	71.7	21.0	NT	0.40
Average, ±SD			4.3±1.6	10.6±2.8	73.1±6.1	15.5±4.3	15.9±3.7	0.36±0.19

* Includes all cells that formed E'_o-IgE rosettes. NT, not tested.

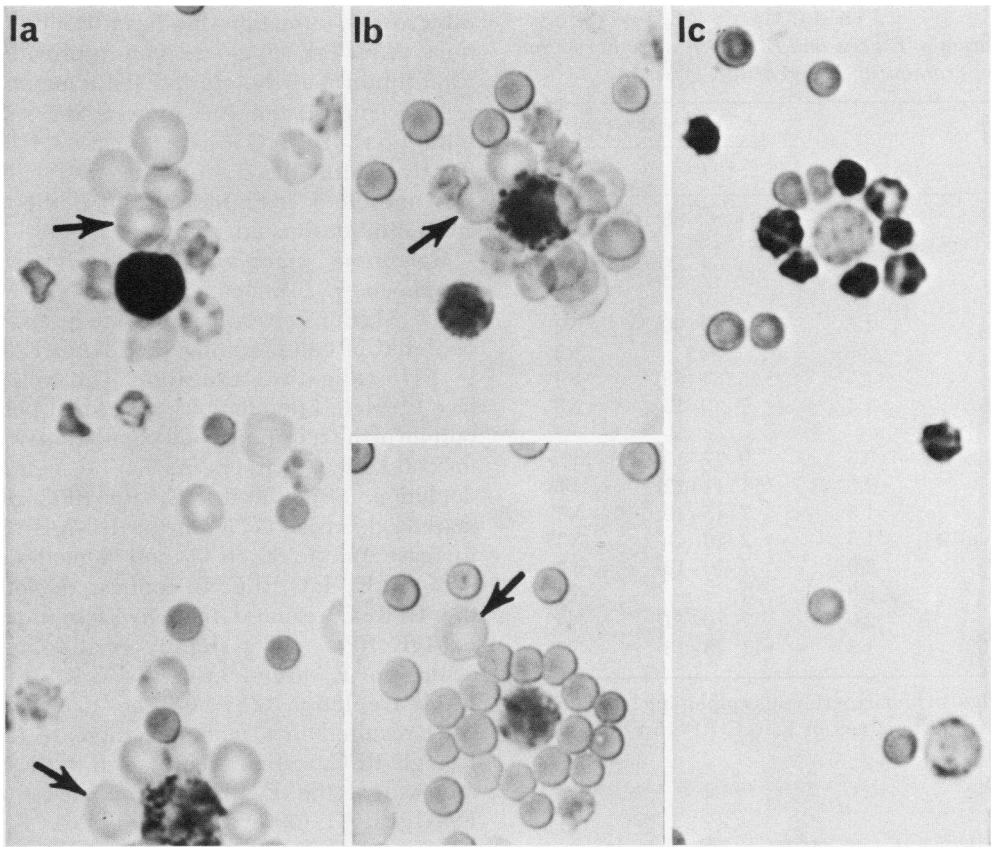

FIGURE 1 Rosettes formed with E_o'-IgE and E_oA indicator cells (1*a* and *b*) and E_oA and E_o'-IgG indicator cells (1*c*). (*a*) A lymphocyte (top) and a basophil (bottom), each forming an E_o'-IgE rosette but not a mixed rosette. (*b*) A basophil forming an E_o'-IgE rosette (top) and a lymphocyte forming an E_oA rosette (bottom) but again, not a mixed rosette. The fixed E_o' cells (arrows) appear larger and less dense than the E_oA cells. (*c*) A mixed rosette; 0.5% instead of the usual 0.1% toluidine blue dye was added to these cells to increase the contrast between fixed E_o'-IgG (violet shown as black) and native E_oA cells (red shown as grey).

lymphocyte-like cells forming E_o'-IgE rosettes was corrected by subtracting the number of basophils in the preparation from the total number of E_o'-IgE RFC. This lowered the number by only about 10%. The average corrected number of lymphocytes forming E_o'-IgE rosettes was 3.94%. Seven donors were tested two or three times within a 2–5-mo time span. The number of E_o'-IgE RFC varied by not more than 1.7%, except for donor B.D., who showed 3.2, 5.5, and 6.1% E_o'-IgE RFC. Since normal IgE, bound to lymphocytes in vivo and not removed during isolation, might have prevented E_o'-IgE rosette formation, lymphocytes of two donors were washed with pH 4.0 buffer which was previously shown to elute IgE from basophils (20). In three different experiments, no significant change in the number of E_o'-IgE RFC was found between acid-treated and untreated cells.

The lymphocytes of the 12 donors were also tested for RFC with other indicator cells. The average number of lymphocytes forming E_o'-aIg rosettes was 10.6%. In contrast to the rosettes formed by all other indicator cells tested, these rosettes were relatively stable and resisted even vigorous shaking of the tubes. The average number of E RFC was 73.1%.

When lymphocytes were tested for binding of IgG by employing two types of indicator cells, E_oA and E_o'-IgG, the resulting averages were almost identical, 15.5 and 15.9%. However, lymphocytes from some individual donors differed up to 5% for unknown reasons.

The lymphocyte preparations that were treated with pH 4.0 buffer were also analyzed for binding of E_oA RFC and compared to untreated cells. As with the E_o'-IgE RFC, no significant difference in rosetting between acid-treated and untreated cells was found.

Inhibition of E_o'-IgE and E_o'-IgG rosette formation.

TABLE II

*Percent Inhibition of E'_0-IgE and E'_0-IgG Rosette Formation by Proteins of Different Ig Classes**

Inhibitor	mg/ml	% Inhibition (range) E'_0-IgE	% Inhibition (range) E'_0-IgG
IgE (Sha)	0.5	84 (81–86)	6
IgE (Sha)	2.5	>95	16
IgE (Be)	0.5	82 (75–89)	NT
IgE (Be)	1.5	>95	NT
IgE (He)	1.5	85 (79–90)	NT
IgE (He)	2.5	>95	NT
Fc IgE (Sha)	1.5	65 (61–68)	NT
Fab IgE (Sha)	1.5	7 (0–13)	NT
IgG (normal)	0.5	9 (7–10)	83 (81–84)
IgG (normal)	2.5	11 (11–12)	>95
IgG1–4	1.5	7 (5–10)	NT
Fc IgG (normal)	1.5	2 (0–4)	NT
IgA1 (Pu)	2.0	7 (0–13)	NT
IgM (Vi)	2.0	0	NT
IgD (Ac)	2.0	7 (0–14)	NT
Rat IgE (IR-162)	1.5	11 (8–13)	NT

* Average (range) of four experiments employing lymphocytes of four donors; the numbers of E'_0-IgE RFC were 3, 4, and 7%.
NT, not tested.

The specificity of the E_0'-IgE rosette formation was tested by preincubating the lymphocytes with three different IgE myeloma proteins, IgE Fc and Fab fragments, normal IgG, myeloma proteins of different classes and subclasses, and a macroglobulin. As shown in Table II, the IgE myeloma proteins and IgE Fc fragments significantly inhibited the rosette formation at concentrations of 0.5–2.5 mg/ml whereas IgE Fab fragments or Ig of the other classes did not inhibit rosette formation significantly at these concentrations. A rat IgE myeloma protein provided no significant inhibition when tested at 1.5 mg/ml.

As a control, the IgE protein Sha was tested for inhibition of E_0'-IgG rosette formation. It did not significantly inhibit the E_0'-IgG rosettes whereas normal IgG inhibited over 95% RFC at the two protein concentrations tested.

Membrane markers of E_0'-IgE rosette-forming lymphocytes. To characterize the lymphocytes forming E_0'-IgE rosettes, lymphocyte preparations were depleted of cells forming E, E_0A, or E_0'-aIg rosettes by FH gradient centrifugation. The cells at the interface (depleted population) and, when possible, at the bottom of the tube (enriched population) were analyzed for cell surface markers. As can be seen in Table III, depletion (88%) of the E_0'-aIg RFC resulted in a marked decrease (77%) of the E_0'-IgE RFC, a slight increase in the E RFC, and a moderate decrease (45%) in E_0'-IgG RFC. In contrast, depletion (80%) of the E RFC resulted in a five-fold increase of the E_0'-IgE RFC and a slightly smaller increased percentage of E_0'-aIg (4.5 times) and E_0'-IgG (3.7 times) RFC. Depletion (78%) of the E_0A rosette-forming cells resulted in a moderate increase (2.1 times) of E_0'-IgE RFC, a slight decrease in the E_0'-aIg, and an increase of the E RFC. Lymphocytes enriched in E_0'-aIg RFC (69%) contained 18% E_0'-IgE RFC, whereas lymphocytes enriched in E or E_0A RFC contained only 0.5% E_0'-IgE RFC.

In two experiments, lymphocyte preparations were depleted of E and E_0'-aIg or E and E_0A RFC in two steps. Again, a significant decrease of the E_0'-IgE RFC occurred only after depletion of E_0'-aIg rosetting cells. The highest percentage of E_0'-IgE RFC (31%) occurred in cells depleted first of E-rosetting cells and then of E_0'-IgG-rosetting cells.

TABLE III

Rosettes Formed with Fractioned Populations of Lymphocytes

Lymphocyte fraction	% Rosettes* E'_0-IgE	E'_0-aIg	E	E'_0-IgG	Basophils
					%
Unfractionated	4.7±1.9	12.1±2.4	71.6±5.5	16.2±3.8	0.41±0.2
E'_0-aIg depleted	1.1±0.9	1.4±0.6	79.0±9.4	9.0±4.6	NT
E depleted	23.4±8.7	51.9±17.5	5.7±2.8	58.2±13.4	2.3±0.8
E_0A depleted	9.8±4.1	10.5±5.1	77.0±9.4	1.3±0.7	NT
E and E'_0-aIg depleted	2.8±2.3	2.1±0.1	6.5±5.5	63.8±19.2	NT
E and E_0A depleted	31.1±4.3	42.4±14.6	9.0±3.1	2.6±1.2	NT
E'_0-aIg enriched	18.0±6.3	69.0±9.3	11.6±5.1	48.1±5.7	NT
E enriched	0.5±0.4	3.8±2.5	85.4±4.3	6.7±4.2	0.02
E_0A enriched	0.5±0.1	55.1±8.6	16.4±4.3	69.0±12.1	NT

* Mean±SD of four to six experiments employing lymphocytes of six different donors.
NT, not tested.

Basophils were counted in the lymphocyte preparations depleted of and enriched with E RFC. They increased to 2.3% in the E-depleted cells; however, they still represented only about 10% of the E_0'-IgE RFC.

Mixed rosettes. The data presented above suggested that most of the E_0'-IgE RFC did not belong to the E or E_0A RFC. Therefore, lymphocytes were incubated with two types of indicator cells that could be distinguished by size and stainability with toluidine (Fig. 1*a–c*). No mixed rosettes formed when the lymphocytes were incubated with E + E_0'-IgE indicator cells (Table IV). However, mixed rosettes were found when the cells were incubated with E_0'-IgE + E_0A indicator cells, particularly in preparations depleted of E RFC (5.4%) with which 66.6% of the cells had formed rosettes, whereas only few mixed rosettes (0.2%) were found in unfractionated cell preparations. Mixed rosette experiments were also performed with other pairs of indicator cells. The average number of mixed rosettes was 1.8% for E and E_0'-aIg RFC, 4.9% for E and E_0'-IgG RFC, and 33.6% for E_0'-aIg and E_0A indicator cells. When lymphocytes were incubated with E_0'-IgG + E_0A indicator cells that presumably react with the same receptors for IgG, 82.5% of the cells formed mixed rosettes.

Rosette formation with trypsin-treated lymphocytes. Lymphocytes of two donors were first treated with trypsin before the rosette assays. In both experiments, E_0'-IgE and E rosette formation was reduced to less than 0.1%, whereas the number of E_0'-IgG rosettes was essentially unchanged.

TABLE IV
*Lymphocytes Forming Mixed Rosettes with Two Types of Indicator Cells**

Lymphocyte fraction	Total rosettes	Total rosettes containing one type of indicator cells		Mixed rosettes‡
		%		
		E	E'_0-IgE	E + E'_0-IgE
Unfractionated	75.5±2.4	95.4±7.4	4.6±1.2	0.0
		E'_0-IgE	E_0A	E'_0-IgE + E_0A
Unfractionated	17.9±4.0	21.0±5.0	79.1±5.1	0.2±0.02
E depleted	66.6±6.0	27.0±6.2	69.5±9.4	5.4±3.3
		E	E'_0-aIg	E + E'_0-aIg
Unfractionated	86.6±2.1	84.3±5.4	13.8±3.8	1.8±0.4
		E	E'_0-IgG	E + E'_0-IgG
Unfractionated	83.5±1.7	73.7±6.3	21.4±3.6	4.9±2.8
		E'_0-aIg	E_0A	E'_0-aIg + E_0A
Unfractionated	18.2±6.7	15.2±3.6	51.3±4.8	33.6±3.6
		E'_0-IgG	E_0A	E'_0-IgG + E_0A
Unfractionated	18.9±2.2	9.5±2.9	7.9±4.7	82.5±5.3

* Mean±SD of six different experiments involving lymphocytes of six donors.
‡ A mixed rosette was scored if it contained at least two indicator cells of each type.

DISCUSSION

On the average, 4% of the normal adult human peripheral lymphocytes bound IgE as shown by rosette formation with E_0'-IgE. This rosette formation was specifically inhibited by intact IgE myeloma proteins and IgE Fc fragments, but not by IgE Fab fragments, Ig of other classes, or rat IgE myeloma protein, suggesting that these lymphocytes had receptors specific for the Fc fragment of human IgE. Examination of lymphocyte preparations depleted of or enriched with cells having SIg or forming E rosettes demonstrated that most, if not all, of the lymphocytes that bound IgE had SIg but did not form spontaneous E rosettes. Lymphocytes having SIg are generally assumed to be bone marrow-derived (2, 3), thus the lymphocytes binding IgE most likely belonged to the B-cell type. The lymphocytes that bound IgE differed, however, from previously characterized B cells (3) in that the majority did not have Fc receptors for IgG as shown by lack of mixed rosetting with IgG-coated ox red cells and by an incease of lymphocytes binding IgE in cell preparations depleted of lymphocytes binding IgG. Incubation of the cells with concentrations of trypsin that did not affect the E_0A rosetting almost completely abolished E_0'-IgE rosette formation, suggesting that the postulated receptor was a protein and that the IgE did not bind to the Fc receptor for IgG. The normal lymphocytes that bound IgE were remarkably similar to cultured human lymphoblastoid cell lines, e.g., Wil-2WT and RPMI-8866, that bound IgE (11, 12). These cultured cells also had SIg, did not form E_0A rosettes, and no longer bound radiolabeled IgE after pretreatment with trypsin. Therefore, the cultured lymphoblastoid cells that bound IgE were probably derived from normal, IgE-binding lymphocytes much like those characterized in this study.

In evaluating the results of the rosette formation assays, the purity of the lymphocyte preparations is critical. In this case, the basophilic granulocytes were the most important possible contaminants because they have Fc receptors for IgE (20). However, they averaged only 10% of the E_0'-IgE-rosetting cells. Conceivably, some basophils could have degranulated during the isolation procedure and were not recog-

nized, but if so, it is unlikely that such cells were present in a large number. Basophils were often found in the center of the E_0'-IgE rosettes but never in the E_0'-aIg rosette assays used for demonstrating SIg. Therefore, the fact that depletion of the E_0'-aIg RFC removed about 80% of the E_0'-IgE RFC indicates again that native and degranulated basophils, which did not react with these indicator cells, could not account for more than 20% of the total E_0'-IgE RFC. Because the cell centered in a rosette was often obscured by the red cells, probably the most accurate number of IgE-binding lymphocytes is obtained by simply subtracting the percentage of basophils in the lymphocyte preparation from the total E_0'-IgE RFC. Since monocytes do not seem to bind IgE myeloma proteins (13), the few possibly present were unlikely contributors to the number of E_0'-IgE RFC in our lymphocyte preparations.

Bound IgE in vivo that did not wash off during isolation of the lymphocytes was probably not a factor in causing a false low number of IgE-binding lymphocytes since washing with a buffer of low pH did not increase rosette formation. Even if IgE remained bound to the lymphocytes, it probably did not occupy all receptors, therefore, the lymphocytes could still form rosettes.

Unquestionably, the preparation of the indicator red cells is an equally important factor in obtaining the highest possible number of RFC. Of several methods initially attempted, coating aldehyde-fixed ox erythrocytes with IgE proved to be the most reliable and reproducible as shown by examination of cultured lymphoid cells of the Wil-2WT line of which over 80% formed E_0'-IgE rosettes with optimally sensitized indicator red cells (12). Conceivably, if high titer IgE anti-E_0 antisera would be available for sensitization, a higher number of IgE-binding cells could be detected. Definitive conclusions on the most accurate number of lymphocytes binding IgE has to await future investigations employing different methods for determining binding of IgE to lymphocytes. When lymphocytes of donors were tested repeatedly with the same as well as with different batches of indicator cells, the percentage of E_0'-IgE RFC varied somewhat. This resulted probably either from variations of the IgE-binding lymphocytes of the donor or from counting errors rather than variation of different batches of indicator cells.

Only rarely has the binding of IgE to normal lymphocytes been reported. Lawrence et al. (13) observed radioiodinated aggregated IgE myeloma proteins bound to normal peripheral lymphocytes that probably corresponded to the lymphocytes characterized in this study. Using autoradiography, which can be less sensitive than rosette formation, Ishizaka et al. (22) found only about 1% of normal human medium-to-large lymphocytes bind IgE. Lymphocytes, like cultured basophilic rat leukemia cells, could vary in the number of Fc receptors depending on the cell cycle (23), and autoradiography may detect only those cells with the largest number of receptors. The presence of Fc receptors for IgG on human B lymphocytes is still controversial. B cells bind heat-aggregated IgG as originally described by Dickler and Kunkel (24), but only very few or none at all form rosettes with human erythrocytes coated with human (Ripley) anti-CD antibodies (4, 25). In two recent reports (26, 27), it was suggested that the binding of heat-aggregated IgG may be different from that of Fc receptor binding to lymphocytes of the third population called K, L, or null cells. In our study, 33.6% (Table IV) of the SIg-positive B cells formed mixed rosettes with ox red cells coated with rabbit IgG antibodies. Whether the rabbit IgG bound to Fc receptors or via the aggregation mechanism has not been investigated. Although we might not have detected binding of IgG to a portion of B cells because of the sensitivity of our system, the fact that the cells that formed IgG rosettes did not bind IgE suggests that the two subsets of B lymphocytes binding either IgG or IgE differ at least quantitatively in their numbers of receptors. Jondal (28) reported that about 3% of the SIg-positive peripheral lymphocytes lacked both Fc IgG receptors and C3 receptors. We have not yet analyzed lymphocytes that bind IgE for receptors to the different complement components. Lymphocytes obtained from lymph nodes, spleens, and thoracic ducts of rats (29) and mice (30) have a particularly high proportion of SIg-positive, Fc IgG receptor-negative cells which Parish and Hayward (29) suggested may originate from a particular phase of the cell cycle (S-phase). However, they may, at least in part, represent the subpopulation of B lymphocytes binding IgE described in this study.

No mixed rosettes with E and E_0'-IgE indicator cells were formed demonstrating that none or only a few T lymphocytes bound IgE. In contrast, mixed rosettes were found with E_0'-IgE and E_0A indicator cells, suggesting that some lymphocytes have receptors for both IgG and IgE. However, it was difficult to find such cells in unfractionated lymphocytes, and the 5% of mixed rosettes in lymphocyte preparations depleted of E RFC could be partially artifactual. The numbers of lymphocytes forming mixed rosettes with either E and E_0'-aIg (2%) or E and E_0'-IgG (5%) indicator cells in this study were similar to those previously reported by others (9, 10, 31, 32). The distribution of cells having Fc receptors for IgG among the different types of lymphocytes indicated that about one-third of each of the IgG-binding cells belonged to the T, B, and third population of lymphocytes. In the case of the B cells, the absolute num-

ber of 5% amounts to about half of the B cells, a large portion of the remainder being the E_0'-IgE RFC. Although further studies will be necessary on E_0'-IgE RFC from lymph nodes, spleens, Peyer's patches, etc., to determine the overall percentage of B lymphocytes that bind IgE, this percentage appears to be relatively large and comprised of those which do not have Fc receptors for IgG.

The finding that, like basophils and mast cells, lymphocytes binding IgE do not bind IgG and the fact that basophils isolated with lymphocytes suggest that the two may be related. Several investigators working with thymus cell cultures have observed large numbers of mast cells that seemed to originate from thymus precursor cells (33, 34). Conceivably, thymus cells may also play a role in the differentiation of the IgE-binding lymphocytes. Demonstration of the IgE receptor's chemical identity on basophils, mast cells, and lymphocytes will further help in the evaluation of the relationship between these three different cell types.

Of the secondary biological functions of IgE, only release of vasoactive substances from basophils and mast cells, and activation of the alternative pathway of complement are known (35). IgE bound to lymphocytes could play a role in immunoregulation, as has been speculated for the Fc IgG receptors (36), or it could "arm" lymphocytes for secondary functions such as antibody-mediated cellular cytotoxicity (37) and release of lymphokines (38, 39). The finding that a large proportion of B lymphocytes binds IgE suggests that cytophilically bound IgE on lymphocytes may play an important role in immunity.

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