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Research Article

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ABSTRACT Lymphocytes from normal nonallergic donors and patients with atopic disorders were analyzed for subpopulations bearing Fc receptors for immunoglobulin (Ig)E (Fc_ϵ) and IgG (Fc_γ), surface IgM (sIgM) and IgD (sIgD), and for T cells forming spontaneous rosettes with sheep erythrocytes (E). The patients were divided into three groups according to serum IgE concentrations and systemic corticosteroid treatment. Group I consisted of 12 atopic patients with either normal or moderately increased IgE levels up to 4,000 U/ml. Four patients of group II and three of group III had 10,500–31,000 U/ml and severe atopic dermatitis. Patients of group III, but not I and II, were receiving corticosteroids systemically. The percentage (mean ±SD) and total number of Fc_ϵ^+ lymphocytes were 1.2±0.5%, 41±24/mm³ in 12 normals; 1.6±0.9%, 59±43/mm³ in patients of group I: 7.0±2.0%, 187 ±67/mm³ in group II; and 0.3±0.1%, 13±5/mm³ in patients of group III. The increase in group II and decrease in group III of Fc_ϵ^+ cells were statistically significantly different from the normal persons and patients of group I. In contrast, the patients did not differ significantly from the donors in $sIgM^+$, $sIgD^+$, Fc_γ^+, and E^+ cell populations. As shown by depletion of sIg^+ cells in four patients with atopic disorders, the great majority of the Fc_ϵ^+ lymphocytes were B cells. However, two patients with elevated Fc_ϵ^+ cell numbers had small numbers of mixed E- and Fc_ϵ-rosetting cells, presumably T cells. Two patients of group II were examined during an acute herpes simplex infection. Both showed an ≅80% decrease of Fc_ϵ^+ cells at that time. No apparent correlation between numbers of Fc_ϵ^+ cells and IgE level existed in patients of group I. Injection of an IgE myeloma protein into two monkeys did not significantly change their percentages of Fc_ϵ^+ lymphocytes.

The data indicate that Fc_ϵ^+ lymphocytes are increased in patients with markedly elevated serum IgE and severe atopic disease, suggesting that these cells may be involved in the regulation and(or) synthesis of IgE antibody formation.

INTRODUCTION

It is well known that immunoglobulin (Ig)E antibodies bind to high affinity Fc receptors (Fc_ϵ)[1] on basophils and mast cells and, after reacting with antigen, cause these cells to release vasoactive substances (1). Recently, we described a subpopulation of lymphocytes that also has Fc_ϵ receptors (2–6). The majority of normal (3, 6), cultured (4), and chronic lymphatic leukemic (5) lymphocytes with Fc_ϵ receptors had cell surface bound immunoglobulin (sIg) indicating that the cells were B lymphocytes. Fc_ϵ receptors on lymphocytes differ most likely in structures from those on basophils and mast cells, since their affinity for monomeric IgE is low and because an antilymphocyte Fc_ϵ receptor antiserum failed to release histamine from basophils (7). The number of Fc_ϵ^+ lymphocytes in the peripheral blood of normals is small. In our first report we found an average of 4% (3); however, in

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[1] *Abbreviations used in this paper*: E, sheep erythrocyte; E_0 and E_0', native and fixed ox erythrocyte; Fc_ϵ and Fc_γ, Fc receptors for IgE and IgG; sIg, cell surface bound immunoglobulin; TH, Tris-buffered Hanks' balanced salt solution containing 2.5% heat-inactivated fetal calf serum.

TABLE I
Clinical Data of Patients with Atopic Diseases

Patient	Age/ Sex	Diagnosis	IgE	Fc_ϵ^+	Fc_ϵ^+	Treatment			
						Systemic steroid	Local steroid	Anti-histamine	Theophylline
			μg/ml	%	mm³				
Mild to moderate atopics									
B. All	47 F	AR, A, FA	3.87	1.0	51.8	−	+	+	−
D. All	27 F	AR	4.60	1.8	37.5	−	−	+	−
Bar	23 F	AR, A	1.09	2.0	63.4	−	−	+	−
DeH	33 M	AD, AR, A	9.68	1.4	25.2	−	−	−	+
Fit	32 F	AR	0.02	3.0	106.0	−	+	+	−
Mat	39 M	AR	0.09	1.1	62.0	−	−	−	−
Neh	36 F	AR, A	1.40	3.3	171.6	−	−	+	+
Rey	26 F	AR	0.03	0.4	14.6	−	−	−	−
Sch	40 F	AD, AR, A	4.72	1.2	44.5	−	−	+	+
Sim	27 F	AR, A	0.44	1.0	41.9	−	+	−	−
Sno	30 F	AR	0.12	1.0	31.5	−	−	−	−
Wil	28 F	AR, A	1.40	1.4	51.9	−	−	−	−
Severe atopics without systemic corticosteroid treatment									
Fer	28 F	AD, AR, A	75.02			−	+	+	−
Gre	21 F	AD, AR, A	47.19			−	+	+	−
Kan	27 M	AD, AR, A	41.14			−	+	+	+
Pra	32 F	AD, AR, A	29.04			−	+	+	+
Severe atopics with systemic corticosteroid treatment									
Bur	40 M	AD	35.09			+	+	+	−
Nic	63 F	AD, AR, A	25.41			+	+	+	+
Rus	19 F	AD, AR, A	35.09			+	+	+	+

Abbreviations used in this table: AD, atopic dermatitis; AR, allergic rhinitis; A, asthma; FA, food anaphylaxis.

subsequent studies only 1–2% Fc_ϵ^+ lymphocytes could be demonstrated (5, 6).

Because patients with atopic disorders usually have elevated serum IgE concentrations, we investigated if they differ from normals in Fc_ϵ^+ lymphocytes. We also examined their lymphocytes for subpopulations having Fc receptors for IgG as well as B and T cells. Lymphocytes of four atopic patients were analyzed for the presence of Fc_ϵ^+ T cells by sIg depletion and mixed rosette experiments. Because Fc_ϵ^+ cells were increased in patients with very high IgE concentrations, two monkeys were injected with an IgE myeloma protein to determine if high IgE plasma levels influence the numbers of Fc_ϵ^+ lymphocytes.

METHODS

Subjects. 12 healthy laboratory workers who had no history of atopic[2] disorder and who had taken no medication for 72 h before testing, volunteered to serve as the normal controls.

The clinical data of the atopic patients are summarized in Table I. 12 patients with mild to moderate disease (group I) were randomly selected from the practice of one of the authors at the Cecil and Ida M. Green Hospital of Scripps Clinic. Most of these patients were studied at a time when their atopic disease was in spontaneous remission or controlled with medications taken with 72 h of blood sampling. The clinical diagnoses of allergic rhinitis and(or) asthma in these patients was confirmed by significant titers of skin sensitizing antibodies (IgE) to historically relevant allergens. The four patients of group II and three of group III with severe atopic dermatitis were selected from patients at the University Hospital, University of California at San Diego, San Diego, Calif., by Dr. O'Connor. The patients in these two groups had serum IgE levels >10,000 U/ml and atopic dermatitis involving at least 75% of total body surface. Patients in groups II and III were using local corticosteroid at the time of study. In addition, group III patients were receiving systemic corticosteroids, and had been for at least 3 yr. Patients Nic and Rus (group III) were on alternative day prednisone, 15 and 10 mg respectively, and were studied 48 h after their last dose. Bur received 4 mg i.m. of betamethasone weekly. The patients' lymphocytes were studied when they were clinically stable and not infected. Two patients in group II were subsequently studied during an acute herpes simplex infection. Patient Pra had bilateral ophthalmic herpes, and patient Gre had cutaneous herpes of both arms, chest, abdomen, and face.

Lymphocyte isolation. Lymphocytes were isolated from

[2] "Atopic" is herein used meaning "associated with but not necessarily caused by reaginic (IgE) antibodies" as defined in Allergy Principles and Practice. 1978. E. Middleton, C. E. Reed, E. F. Ellis, editors. The C. V. Mosby Co., St. Louis.

anticoagulated venous blood by a slight modification of the method described by Perlmann et al. (8). Briefly, erythrocytes were sedimented in 3% dextran, the mononuclear cell-rich supernate was incubated with colloidal iron, and the lymphocytes isolated by Ficoll-Hypaque centrifugation. Because <2% of these cells ingested latex particles, no attempts were made to remove any remaining phagocytic cells adherent to plastic. For rosette assays, 5×10^6/ml lymphocytes were suspended in Tris-buffered Hanks' balanced salt solution, pH 7.4, containing 2.5% heat-inactivated fetal calf serum (TH). As shown (3), the contamination of such isolated lymphocytes with basophils is very low.

Rosette assays. The reagents and methods for detecting Fc_ϵ and Fc_γ receptors, cells with sIgM and sIgD and T cells by rosette assays were exactly as described (5). Lymphocytes with Fc_ϵ receptors were usually detected with ox erythrocytes (E_0) coated with glutaraldehyde coupled rabbit IgG Fab' anti-E_0 and Fc IgE fragments. Fixed E_0 (E_0') (3) coated with IgE myeloma protein (Sha) were used only for mixed rosettes. To ensure specificity of the IgE rosettes, the rosette assays were also performed by including 2 mg/ml IgE myeloma protein Sha as an inhibitor. The percentage of uninhibitable IgE rosettes, which varied from 0 to 0.7%, was subtracted from the percentage obtained in the absence of inhibitor. Between 300 and 1,000 cells were counted to determine the percentage of rosetting cells. Data were analyzed statistically with a Student's *t* test. *P* values above 0.05 were not considered statistically significant.

B- and T-cell depletion. For B-cell depletion $10-30 \times 10^6$ lymphocytes were incubated with a mixture of E_0 coated with $F(ab')_2$ anti-IgM, IgD, κ-, or λ-chain (5). Neuraminidase-treated E were used for T-cell depletion (6). The cell mixtures were centrifuged at 800 *g* for 5 min and incubated for 1.5 h at 4°C. The cells were then gently resuspended, underlayered with Ficoll-Hypaque and centrifuged for 20 min at 1,600 rpm (3). The interphase cells were collected, washed three times with TH, and used in rosette assays. The erythrocytes of the rosetting cells in the cell button were lysed with 0.9% NH_4Cl, and the remaining lymphocytes were washed three times with TH. Mixed rosette assays were performed by employing E_0'-IgE (3) and neuraminidase-treated E (6).

IgE determination. Serum IgE concentrations were determined by double antibody radioimmunoassay as described (9). 1 U of IgE was equivalent to 2.42 ng of IgE (10).

Monkeys. 10 Macaca monkeys were tested for lymphocyte markers by employing the same reagents used for human cells. Two pigtail monkeys (*Macaca nemestrinus*) were selected for the experiments because both were 7-yr-old females weighing 6 kg. One had a high number (3.3%) and the other a low number (0.3%) of Fc_ϵ^+ lympocytes. IgE myeloma protein Sha (20 mg/ml) was ultracentrifuged to remove aggregates (11) and 40 mg was injected i.m. into each of the monkeys on the 1st d, then 20 mg/animal per d on the 2nd, 3rd and, through the 4th d. 10 ml blood was drawn daily from the saphenous vein for lymphocyte isolation.

RESULTS

Lymphocyte subpopulations in normals. The age, sex, IgE levels, and the Fc_ϵ^+ lymphocytes of 12 normal donors are shown in Table II. The age and sex distributions of the normal donors were similar to those of the patients. The IgE levels were within normal range. Many donors were examined repeatedly. The differences between individual tests were small and the data, therefore, averaged. The 12 normals had

TABLE II
IgE Concentrations and Fc_ϵ^+ Lymphocytes of 12 Normal Nonallergic Donors

Donor	Age/ Sex	IgE	Fc_ϵ^+	Fc_ϵ^+
		μg/ml	%	mm³
Bal	30 M	0.09	0.5	15.7
Bla	38 M	0.09	1.1	29.3
Bre	30 F	0.05	2.0	81.2
Dai	31 M	0.20	1.0	21.7
Fri	33 M	0.14	1.5	50.7
Hei	21 F	0.02	1.4	34.9
Pat	33 F	0.02	0.7	20.2
Spi	45 M	0.05	1.3	34.6
Sto	49 F	0.05	0.7	17.8
Tru	59 F	0.09	1.5	66.0
Wod	28 M	0.05	0.7	32.2
Yor	34 F	0.09	1.9	84.4
Average		0.08	1.2	40.7
±SD		0.05	0.5	24.3

7,470±1,430 leukocytes/mm³ and 3,008±889 lymphocytes/mm³ (mean±SD). They had 18.7±7.1% (572 ±212/mm³) Fc_γ^+ cells, 9.7±2.2% (318±129/mm³) $sIgM^+$ cells, 10.4±5.0% (341±202/mm³) $sIgD^+$ cells, and 69.6±10.7% (2,260±887/mm³) E-rosetting T cells.

Lymphocyte subpopulations in atopic patients. As described in Methods, patients were divided into three groups based upon severity of disease, serum IgE concentration, and systemic corticosteroid treatment. The 12 patients of group I had more lymphocytes (3,837±1,167) than the normal individuals. The had only slightly more Fc_ϵ^+ cells (1.6±0.9% or 54±43 Fc_ϵ^+ cells/mm³) than the normal subjects (Table I). No obvious correlation between the numbers of Fc_ϵ^+ cells and the IgE concentration was apparent. The patients of group I had 20.1±5.9% (821±373/mm³) Fc_γ^+ cells, 14.2±6.5% (552±336/mm³) $sIgM^+$ cells, 15.5±6.0% (604±320/mm³) $sIgD^+$ cells, and 68.3±8.2% (2,632 ±904/mm³) E-rosetting cells. All these values were statistically not significantly different from the cells in the normal group. The Fc_ϵ^+ and the B cells were proportionally slightly more increased than the T cells.

The lymphocyte subpopulations of four patients of group II with severe atopic dermatitis and IgE levels of 29–75 μg/ml are shown in Table III. These patients had statistically significantly ($P < 0.001$) more Fc_ϵ^+ cells (7.0±2.0; 187±67/mm³) than the normals and patients of the other groups. They had the lowest number of Fc_γ^+ cells; this difference was, however, not statistically significant. The percentage and absolute numbers of B and T cells were similar to the normals.

Two patients (Gre and Pra) were retested during an acute severe herpes simplex infection. As shown at the bottom of Table III, both patients had lower

TABLE III

Lymphocyte Subpopulations of Patients with Severe Atopic Disease

Patient	Fc receptors				B cells				T cells	
	Fc_ϵ	Fc_ϵ	Fc_γ	Fc_γ	sIgM	sIgM	sIgD	sIgD	E	E
	%	mm^3	%	mm^3	%	mm^3	%	mm^3	%	mm^3
Fer										
6/29/78	4.7	117	14.3	356	7.0	174	5.0	125	77.0	1,917
8/14/78	8.3	257	11.0	341	8.3	257	11.7	363	72.7	2,254
Average Fer	6.5	187	12.7	349	7.7	216	8.4	244	74.9	2,086
Gre										
6/8/78	4.6	193	18.0	756	15.7	659	14.7	617	75.2	3,158
11/29/78	6.5	156	10.1	242	10.7	257	16.7	401	76.3	1,831
Average Gre	5.6	175	14.1	499	13.2	458	15.7	509	75.8	2,495
Kan										
6/8/78	7.3	238	24.3	792	17.4	567	17.3	564	69.3	2,259
7/5/78	14.0	313	21.0	470	16.3	365	13.7	307	66.0	1,478
11/29/78	8.6	NT	14.3	NT	5.3	NT	22.0	NT	44.0	NT
Average Kan	10.0	275	19.9	631	13.0	466	17.7	436	59.8	1,869
Pra										
6/26/78	6.4	133	13.5	279	10.0	207	10.0	207	75.3	1,559
8/7/78	5.3	71	13.7	184	4.7	63	5.0	67	77.7	1,041
12/13/78	6.0	134	11.3	253	4.4	99	3.0	67	68.3	1,530
Average Pra	5.9	111	12.8	241	6.4	121	6.0	113	73.8	1,390
Average	7.0	187	14.9	430	10.1	315	12.0	326	71.1	1,960
±SD	2.0	67	3.4	171	3.5	174	5.6	180	7.6	460
Patients Gre and Pra tested at time of herpes simplex infection										
Gre 7/5/78	1.3	17	9.7	130	6.0	80	5.7	76	80.0	1,072
Pra 2/5/79	1.0	24	15.0	354	5.6	132	6.6	156	69.6	1,643

NT, not tested.

numbers of Fc_ϵ^+ cells than before. Patient Gre had a low lymphocyte count; however, the Fc_ϵ^+ cells were proportionally much lower than the other lymphocyte subpopulations. Three months later, the Fc_ϵ^+ lymphocytes returned to preinfection levels in patient Gre. Patient Pra, despite having more lymphocytes, had ≅80% fewer Fc_ϵ^+ cells during the herpetic infection.

The lymphocyte subgroups of three patients with severe atopic dermatitis who were receiving corticosteroids systemically (group III) are shown in Table IV.

TABLE IV

Lymphocyte Subpopulations of Patients with Severe Atopic Disorders Receiving Systemic Steroid Treatment

Patient	Fc receptors				B cells				T cells	
	Fc_ϵ	Fc_ϵ	Fc_γ	Fc_γ	sIgM	sIgM	sIgD	sIgD	E	E
	%	mm^3	%	mm^3	%	mm^3	%	mm^3	%	mm^3
Bur										
7/7/78	0.2	5	33.3	865	1.3	34	1.3	71.3	71.3	1,854
9/5/78	0.3	14	13.3	604	4.6	209	NT	NT	83.0	3,768
Nic 10/19/78	0.3	17	31.0	1,705	5.0	275	12.0	660.0	78.7	4,329
Rus 10/25/78	0.2	NT	NT	NT	2.0	NT	2.6	NT	92.0	NT

NT, not tested.

They had 10,000–14,500 leukocytes/mm³ and high lymphocyte counts, observations that had already been made in these patients repeatedly before this study. They had statistically significantly lower percentages of Fc_ϵ^+ cells than in normals and the lowest percentage of B cells of all groups. The absolute number of B cells was within normal range. In contrast, the absolute numbers of T cells and Fc_γ cells were higher than in the normals. However, these differences were statistically not significant.

Effect of incubation on Fc_ϵ^+ lymphocytes. Because IgE bound to the lymphocytes in vivo could have inhibited a positive rosette assay, lymphocytes from two normal donors and two atopic patients were incubated for 2, 4, 6, and 18 h in RPMI-1640 medium containing 2.5% fetal calf serum, antibiotics and either no or 10, 100, or 500 μg/ml of IgE myeloma protein. The cells were washed with TH–2.5% fetal calf serum and analyzed for IgE rosettes. The percentage of Fc_ϵ^+ cells remained unchanged during the first 6 h of incubation and, as had previously been observed (6), decreased after 18 h incubation. This result was obtained with lymphocytes of both the normal and atopic donors. Addition of IgE to the culture medium had no effect on the percentage of IgE-rosetting cells.

Fc_ϵ^+ cells in sIg-depleted cells. To determine if the Fc_ϵ^+ lymphocytes in atopic patients were B cells as in normals (3, 6), the lymphocytes of four patients were depleted of anti-Ig-rosetting cells. As shown in Table V, no Fc_ϵ^+ cells were detected in sIg^+ cell-depleted lymphocyte preparations of two patients with low numbers of Fc_ϵ^+ cells (Pra during herpes infection and Rey). In contrast, the sIg^+ cell-depleted fraction of two patients (Neh, Kan) with 2.6 and 11.7 Fc_ϵ^+ cells in the unfractionated cells still had 0.6 and 0.5% Fc_ϵ^+ cells. These depleted cells were then analyzed by a mixed rosette assay with E_0'-IgE and neuraminidase-treated E. In various experiments, 15–38% of the Fc_ϵ^+ cells formed mixed rosettes, indicating that some of the Fc_ϵ^+ cells in the sIg-depleted cell population were presumably T cells. As expected, the sIg-enriched cell population had increased numbers of Fc_ϵ^+ cells. The cells of patient Kan were also depleted of and enriched for E-rosetting cells. E-depleted cells contained 38.3% Fc_ϵ^+ cells, whereas E-enriched cells had only 0.8–1.3%, a lower percentage than the remaining cells with sIg in these preparations. Patient Kan might have had Fc_ϵ^+ T cells that were lost by adhering to the colloidal iron used for isolating the lymphocytes. However, when an E-enriched cell population was prepared from cells that were not treated with colloidal iron, only 0.8% Fc_ϵ^+ cells were detected, a value that paralleled the smaller percentage of B cells found in this preparation as compared to the E-enriched cells obtained from iron-treated cells.

TABLE V
Cell Surface Markers of sIg-Depleted Lymphocyte Populations from Atopic Patients

Patient	Lymphocytes	Rosetting cells				
		Fc_ϵ	Fc_γ	sIgD	sIgM	E
		%				
Pra	Unfractionated	1.0	15.0	6.6	NT	69.6
	sIg depleted	0.0	13.7	0.2	NT	66.5
Rey	Unfractionated	0.4	11.7	12.2	NT	87.8
	sIg depleted	0.0	11.0	0.2	NT	89.7
Neh	Unfractionated	2.6	21.4	28.8	NT	67.0
	sIg depleted	0.6	18.4	0.4	NT	82.7
Kan	Unfractionated	11.7	11.0	17.0	18.0	75.0
	sIg depleted	0.5	7.7	0.0	1.7	89.5
	E depleted	38.3	39.3	70.0	64.5	8.0
	sIg enriched	23.0	23.7	NT	57.3	33.7
	E enriched	1.3	7.3	8.0	8.7	82.0
	E enriched*	0.8	8.0	4.7	3.7	76.7

* E-enriched cells from mononuclear cells isolated by Ficoll-Hypaque centrifugation without prior incubation with colloidal iron.

Fc_ϵ^+ lymphocytes in monkeys injected with IgE. 10 Macaca monkeys (7 Rhesus, 2 pigtail, and 1 stump tail) were analyzed for lymphocyte subpopulations. The mean and SD of the cells with different lymphocyte markers were not significantly different from those of normal humans: Fc_ϵ^+, 1.0±0.9%; Fc_γ^+, 20.8±8.1%; $sIgM^+$, 9.8±5.8%; $sIgD^+$, 9.2±3.2%; E^+, 63.0±7.7%. Two sex- and age-matched pigtail monkeys with extreme high and low (3.3% and 0.3%) numbers of Fc_ϵ^+ cells were injected on 4 consecutive d with IgE myeloma protein Sha. The IgE levels rose from 0.29 μg/ml up to 125 μg/ml for 5 d and dropped thereafter to the pre-immune levels. Daily lymphocyte testing showed percentages of Fc_ϵ^+ cells similar to the preinjection values except on 1 d in one monkey, whose Fc_ϵ^+ cells rose to 7% on the 6th d after the first IgE injection. Except for this value, the Fc_ϵ^+ lymphocytes varied from 1.0 to 3.6% (average, 2.6%) and 0.0–1.4% (average, 0.6%), respectively, in the two monkeys during the 14-d observation period.

DISCUSSION

These studies demonstrate that patients with atopic disorders and severe hyperimmunoglobulinemia E have significantly more Fc_ϵ^+ lymphocytes than normal individuals. However, similarly afflicted patients treated systemically with corticosteroids generally have lower percentages and absolute numbers of Fc_ϵ^+

cells than normals. Atopic patients with relatively mild disease and normal or moderately elevated IgE levels had a slightly increased number of Fc_ϵ^+ cells that was no statistically significant from the normals. Nevertheless, this difference may acquire greater significance in the analysis of a larger number of patients. Antigen specific IgE levels rise and fall with seasonal antigen exposure (12). The Fc_ϵ^+ cells could, conceivably, increase in the circulation during a specific phase of exposure to a sensitizing agent and resultant IgE response, whereas these cells are found in lower numbers at other times. This phenomenon would explain why an increase of Fc_ϵ^+ cells is detectable only in the patients with severe and chronic disease. Serial analyses of patients during symptomatic and asymptomatic phases of atopic disease are necessary to test this assumption. Recently, Yodoi et al. (13) reported that Fc_ϵ^+ lymphocytes increased in lymph nodes of rats after infection with *Nippostrongylus brasiliensis*. This experimentally induced increase resembles the rise in Fc_ϵ^+ cells of severely atopic patients and both situations suggest that the Fc_ϵ^+ cells play an important role in the formation, regulation, and(or) metabolism of IgE antibodies.

We have previously reported that the Fc_ϵ^+ lymphocytes in normals are B cells (3, 5, 6). Our current analysis of sIg-depleted cells indicate that this is also true in patients with atopic illness. However, in two patients with increased numbers of Fc_ϵ^+ lymphocytes, Fc_ϵ^+ cells were found in sIg-depleted cell fractions that formed mixed Fc_ϵ–E rosettes and were presumably T cells. Because of the small number of such T cells, the Fc_ϵ rosette formation could have been nonspecific. This did, however, appear unlikely, since monomeric IgE at 2 mg/ml completely inhibited the IgE rosettes in these preparations. Yodoi et al. (13) also found Fc_ϵ^+ T cells in E-enriched cell populations of atopic patients and in rats infected with *N. brasiliensis*. However, as shown in four of our patients, the number of Fc_ϵ^+ T lymphocytes is small and we could not exclude that these cells were E-rosetting B cells (14, 15). Fc receptors for IgG (16), IgM (17), and recently IgA (18, 19) have been found in both T- and B-cell populations. It is likely, therefore, that all individuals have subsets of Fc_ϵ^+ B and T cells; however, because of the small number of Fc_ϵ^+ cells in most donors, the T cells are not as easily demonstrable.

The lymphocyte preparations were treated with colloidal iron because ≅25% of normal human monocytes form rosettes with Fc–IgE-coated indicator cells (20). These rosettes can only be inhibited with 10 mg/ml of IgE, whereas the rosettes formed by lymphocytes are completely inhibited by 1–2 mg/ml. The small percentage of uninhibitable rosettes in the present experiments were therefore most likely monocytes. If lymphocytes were isolated by Ficoll-Hypaque centrifugation omitting the iron treatment, the number of rosetting monocytes made it impossible to accurately determine the percent of Fc_ϵ-rosetting lymphocytes.

No significant differences in the percentages and absolute numbers of B, T, and Fc_γ^+ lymphocytes were found between the three groups of patients and normal humans. Interestingly, the patients with severe atopic disease and highly elevated Fc_ϵ^+ cell numbers (group II) had the lowest number of Fc_γ^+ cells. We have previously shown that normal lymphocytes with Fc_ϵ receptors do not have Fc_γ receptors (3). It is conceivable, therefore, that these patients had decreased levels of Fc_γ^+ B cells concomitant with increased Fc_ϵ^+ B cells.

Three patients with severe atopic dermatitis treated systemically with corticosteroid had lower numbers of Fc_ϵ^+ lymphocytes than normals. They also had the lowest percentage of B cells; however, because they had elevated lymphocyte counts, the absolute number of B cells was within normal range. Corticosteroids may have suppressed these cells and(or) their ability to form the Fc_ϵ receptors or caused a redistribution of the cells from the blood to lymphoid organs.

Notably, when two of the patients of group II developed a severe herpes simplex infection, the number of Fc_ϵ^+ cells decreased markedly. The mechanism for this is not understood. It could be the effect of endogenous corticosteroids or other active substances formed in response to the viral infection such as virally induced interferon.

The number of Fc_ϵ receptors on lymphocytes could be modulated by the serum IgE concentrations as has been suggested for basophils (21). At a high IgE concentration, the cells could form more Fc_ϵ receptors and are then detectable between Fc_ϵ^+ cell number and serum IgE level in patients with moderately increased IgE levels, all patients with very high IgE levels (group II) had high numbers of Fc_ϵ^+ cells. To test if a very high IgE concentration induces Fc_ϵ-rosetting cells, two Macaca monkeys were injected with a human IgE myeloma protein. This did not, however, result in an increase of Fc_ϵ^+ cells. Although human IgE was expected to affect monkey lymphocytes similarly to homologous IgE, because human and monkey IgE cross-react extensively (22), the lack of an increase in Fc_ϵ-rosetting cells in these monkeys does not rule out a possible effect of serum IgE on the expression of Fc_ϵ receptors on human cells. The affinity of the heterologous IgE to the monkeys' cell receptors could have been too low to increase the Fc_ϵ receptor synthesis. Yodoi et al. (13) reported that rat lymphocytes cultured with IgE, but not IgG-induced Fc_ϵ^+ lymphocytes. In contrast, incubation with IgE of lymphocytes from either normal or atopic humans did not result in an increase of Fc_ϵ receptor-positive

lymphocytes. Whether this is reflecting a species or methodological difference remains to be investigated.

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REFERENCES

1. Ishizaka, K. 1970. Human reaginic antibodies. *Annu. Rev. Med.* **21:** 187–200.
2. Lawrence, D. A., W. O. Weigle, and H. L. Spiegelberg. 1975. Immunoglobulins cytophilic for human lymphocytes, monocytes, and neutrophils. *J. Clin. Invest.* **55:** 368–376.
3. Gonzalez-Molina, A., and H. L. Spiegelberg. 1977. A subpopulation of normal human peripheral B lymphocytes that bind IgE. *J. Clin. Invest.* **59:** 616–624.
4. Gonzalez-Molina, A., and H. L. Spiegelberg. 1976. Binding of IgE myeloma proteins to human cultured lymphoblastoid cells. *J. Immunol.* **117:** 1838–1845.
5. Spiegelberg, H. L., and P. M. Dainer. 1979. Fc receptors for IgG, IgM and IgE on human leukemic lymphocytes. *Clin. Exp. Immunol.* **35:** 286–295.
6. Hellström, U., and H. L. Spiegelberg. 1979. Characterization of human lymphocytes bearing Fc receptors for IgE from blood and lymphoid organs. *Scand. J. Immunol.* **9:** 75–86.
7. Meinke, G. C., A. M. Magro, D. A. Lawrence, and H. L. Spiegelberg. 1978. Characterization of an IgE receptor isolated from cultured B-type lymphoblastoid cells. *J. Immunol.* **121:** 1321–1328.
8. Perlmann, H., P. Perlmann, G. R. Pape, and G. Hallden. 1976. Purification, fractionation and assay of antibody-dependent lymphocyte effector cells (K cells) in human blood. *Scand. J. Immunol.* **5**(Suppl.): 57–68.
9. Gleich, G. J., A. K. Averbeck, and H. A. Swedlund. 1971. Measurement of IgE in normal and allergic serum by radioimmunoassay. *J. Lab. Clin. Med.* **77:** 690–698.
10. Bazaral, M., and R. N. Hamburger. 1972. Standardization and stability of immunoglobulin E (IgE). *J. Allergy.* **49:** 189–191.
11. Spiegelberg, H. L., and W. O. Weigle. 1968. The production of antisera to human γG subclasses in rabbits using immunological unresponsiveness. *J. Immunol.* **101:** 377–380.
12. Berg, T., and S. G. O. Johansson. 1969. IgE concentrations in children with atopic diseases. A clinical study. *Int. Arch. Allergy Appl. Immunol.* **30:** 219–232.
13. Yodoi, J., T. Ishizaka, and K. Ishizaka. 1979. Presence of T cells bearing IgE receptors. *Fed. Proc.* **38:** 1088. (Abstr.)
14. Bentwich, Z., S. D. Douglas, F. P. Siegal, and H. G. Kunkel. 1973. Human lymphocyte-sheep erythrocyte rosette formation: some characteristics of the interaction. *Clin. Immunol. Immunopathol.* **1:** 511–522.
15. Dickler, H. B., N. F. Adkinson, Jr., and W. D. Terry. 1974. Evidence for individual human peripheral blood lymphocytes bearing both B and T cell markers. *Nature (Lond.).* **247:** 213–215.
16. Dickler, H. B. 1976. Lymphocyte receptors for immunoglobulin. *Adv. Immunol.* **24:** 167–214.
17. Moretta, L., S. R. Webb, C. E. Grossi, P. M. Lydyard, and M. D. Cooper. 1977. Functional analysis of two human subpopulations: help and suppression of B cell responses by T cells bearing receptors for IgM (T_M) or IgG (T_G). *J. Exp. Med.* **146:** 184–200.
18. Strober, W., N. E. Hague, L. G. Lum, and P. A. Henkart. 1978. IgA-Fc receptors on mouse lymphoid cells. *J. Immunol.* **121:** 2440–2445.
19. Lum, L. B., A. V. Muchmore, D. Kerren, J. Decker, I. Koski, W. Strobert, and R. M. Blaese. 1979. A receptor for human IgA on human T lymphocytes. *J. Immunol.* **22:** 65–69.
20. Melewicz, F. M., and H. L. Spiegelberg. 1979. IgE mediated cytotoxicity by human monocytes. *Fed. Proc.* **38:** 925. (Abstr.)
21. Malveaux, F. J., M. C. Conroy, N. F. Adkinson, and L. M. Lichtenstein. 1978. IgE receptors on human basophils. Relationship to serum IgE concentration. *J. Clin. Invest.* **62:** 176–181.
22. Ishizaka, K., T. Ishizaka, and T. Tada. 1969. Immunoglobulin E in the monkey. *J. Immunol.* **103:** 445–453.