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Research Article

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Increase of Lymphocytes with Fc Receptors for IgE in Patients with Allergic Rhinitis during the Grass Pollen Season

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ABSTRACT Peripheral blood lymphocytes from 10 nonallergic donors and 7 patients suffering from seasonal allergic rhinitis and receiving desensitization therapy were analyzed by rosette assays for Fc receptors for IgE (FcεR) and IgG (FcγR) before, during and after the grass pollen season. Six of seven patients had moderately elevated IgE levels (330±268 IU/ml), all had high titers of skin sensitizing antibodies to grass pollens and serum IgE antibodies as measured by radioallergosorbent tests (RAST). Seven of the nonallergic donors had 2–30 IU/ml IgE and negative RAST, whereas three had 91–267 IU/ml IgE and two were RAST positive to the grass pollens. In March, when the patients were asymptomatic, the mean±SD of the FcεR$^+$ lymphocytes did not significantly differ from the nonallergic control group: nonallergic FcεR$^+$ 1.2±0.9% (29±20/mm^3), allergic FcεR$^+$ 2.0±3.1% (48±52/mm^3). In contrast, during the grass pollen season in May and June, when the patients developed symptoms of allergic rhinitis, they had significantly ($P < 0.01$) more FcεR$^+$ lymphocytes than the controls: nonallergic FcεR$^+$ 1.7±1.9% (40±46/mm^3), allergic FcεR$^+$ 4.7±1.2% (134±69/mm^3). In the postpollen period, August–October, most of the patients again had low numbers of FcεR$^+$ lymphocytes: nonallergic FcεR$^+$ 1.4±0.9% (26±13/mm^3), allergic FcεR$^+$ 2.1±1.9% (62±82/mm^3). The nonallergic control donors with elevated IgE levels and positive RAST always had low numbers of FcεR$^+$ lymphocytes. In contrast, two other nonallergic donors, who had 2–7 IU/ml IgE and negative RAST, showed significant increases of FcεR$^+$ lymphocytes over several weeks during the grass pollen season. No statistically significant changes in FcγR$^+$ lymphocytes occurred in both nonallergic and allergic donors. The total and specific IgE serum levels did not vary much in the nonallergic donors and patients during the period of study and any changes that did occur did not correlate with the changes in FcεR$^+$ lymphocytes.

The data demonstrate that FcεR$^+$ peripheral blood lymphocytes increase in allergic patients during natural antigen exposure and active disease in the absence of measurable increases of total and specific serum IgE. Because two nonallergic control donors also had temporary increases of FcεR$^+$ lymphocytes, an increase of peripheral blood FcεR$^+$ lymphocytes may be a sensitive indicator of an ongoing IgE immune response.

INTRODUCTION

Subpopulations of lymphocytes bear membrane receptors that specifically bind the Fc fragment of a given immunoglobulin class. Lymphocytes with Fc receptors for IgG (FcγR)[1] have been known for many years (1). Recently, we described a lymphocyte subpopulation that expresses Fc receptors for IgE (FcεR) (2, 3). The Fcε receptors on lymphocytes differ antigenically from those on mast cells (4) and bind IgE with an ~hundredfold lower affinity (5, 6). The FcεR$^+$ lymphocytes in normal persons are predominantly B cells that lack Fcγ receptors (2). Ragweed sensitive donors also have a small fraction of FcεR$^+$ T cells (7). In a previous study (8), we showed that severely atopic patients with highly elevated IgE levels have significantly more FcεR$^+$

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[1] *Abbreviations used in this paper*: E, sheep erythrocytes; Eo, Eo′, fresh, fixed ox erythrocytes; FcεR, FcγR, FcαR, Fc receptors for IgE, IgG, and IgA; RAST, radioallergosorbent test; sIg, cell membrane bound immunoglobulin.

lymphocytes than nonallergic controls. In contrast, allergic patients with moderately high IgE levels and no active disease had essentially the same number of these cells as the controls. To determine whether these patients show an increase of $Fc\epsilon R^+$ lymphocytes during active disease, we analyzed peripheral blood lymphocytes from patients known to suffer from severe seasonal allergic rhinitis before, during, and after the grass pollen season. We then compared the changes in the percentages of $Fc\epsilon R^+$ cells with the changes in the patient's total serum IgE and specific IgE as measured by radioallergosorbent test (RAST) to three common grass pollens to which they demonstrated skin sensitizing antibodies.

METHODS

Subjects. Controls were 10 healthy laboratory or clerical workers who had no history of allergies and had taken no medication at the time of blood testing. Donor 9 was also studied in March 1979 when she had severe generalized contact dermatitis, presumably because of exposure to an unidentified chemical.

12 patients who had a history of severe seasonal allergic rhinitis and were receiving maintenance immunotherapy with weekly subcutaneous injections (0.5 ml of a 1:20 wt/vol grass pollen mixture) volunteered to donate blood for the study. They were chosen because the immunotherapy did not control the hay fever symptoms. All but one of the 12 patients had low percentages of $Fc\epsilon R^+$ lymphocytes on initial examination. Serial blood samples before (March), during (May and June), and after (August through October) the 1980 grass pollen season were obtained from only seven patients. Five of them (1, 2, 4, 6 and 7) had received maintenance immunotherapy for 1 yr, patient 3 for 4 yr, and 5 for 5 yr. Pollen counts were determined daily by Donald Street, University of California at San Diego, School of Medicine, by utilizing the intermittent rotorod method. The mean grass pollen counts per month (spores/m^3) were March 1980 3.0, April 8.3, May 12.2, August 1.04, and September 1.02. The seven patients studied demonstrated high titers of skin sensitizing antibodies to grass pollens with 6–10 mm dermal wheal responses at $1{:}10^{-5}$ to $1{:}10^{-6}$ dilution of multiple grass pollen antigens. In addition to maintenance immunotherapy, patients were taking oral antihistamines and decongestants and patient 2 also topical nasal beclomethasone throughout the study, particularly when they had severe allergic rhinitis. None of the patients received systemic corticosteroid therapy.

Lymphocytes. Lymphocytes were isolated from 20 ml heparinized venous blood by a slightly modified method originally described by Perlmann et al. (2, 9). Briefly, the erythrocytes were sedimented in 3% dextran; the mononuclear cell-rich plasma was incubated with colloidal iron, and the iron removed with a magnet. The lymphocytes were then isolated by centrifugation over Ficoll-Hypaque. <1% of the cells in these preparations stained for nonspecific esterase (10). For rosette assays, the lymphocytes were suspended at 5×10^6 cells/ml in RPMI 1640 medium containing 2.5% fetal calf serum.

Rosette assays. $Fc\epsilon R^+$ lymphocytes were detected in a rosette assay using fixed ox erythrocytes (Eo′) coated with human IgE myeloma protein (P. Sha) (2). The rosette assays were performed in duplicate, in the presence and absence of 2 mg/ml IgE myeloma protein as inhibitor. The percentage of "spontaneously" formed rosettes which were not inhibitable with IgE, usually <0.3%, was subtracted from the number of rosettes formed without inhibitor. $Fc\gamma R^+$ lymphocytes were detected in a rosette assay using fresh ox erythrocytes (Eo) sensitized with a subagglutinating dose of rabbit IgG anti-Eo antibodies (2, 11). Cell surface IgM and IgD positive lymphocytes ($sIgM^+$, $sIgD^+$) were detected with Eo sensitized with conjugates consisting of rabbit Fab′ anti-Eo coupled with glutaraldehyde to purified goat $F(ab')_2$ antibodies to human IgM or IgD (12). T cells were detected as lymphocytes forming spontaneous rosettes with sheep erythrocytes (E) (12). At least 300, usually 400–800, lymphocytes were counted to obtain the percentages of rosetting cells. Values <0.125% are recorded as 0.0% in the Tables. Many donors were tested repeatedly within a 2-wk period before, during, and after the pollen season. Because no significant changes were found in such short time periods, the results were averaged for the data given in the Tables. The data were analyzed statistically with a Student's t test. P values above 0.05 were not considered statistically significant.

IgE determination. Total serum IgE was determined by paper radioimmunosorbent test (PRIST^R, Pharmacia, Inc., Piscataway, N. J.) and specific IgE to Timothy, Bermuda, and June grass pollen extract by RAST^R performed by the reference laboratory of Pharmacia, Inc. The serum samples were stored at −20°C and analyzed at the end of the study.

RESULTS

$Fc\epsilon R^+$ lymphocytes in nonallergic and allergic donors. The percentages and total numbers of $Fc\epsilon R^+$ lymphocytes of 10 nonallergic controls and 7 allergic patients before, during, and after the 1980 grass pollen season are summarized in Table I. In the prepollen season, all donors had low percentages of $Fc\epsilon R^+$ lymphocytes, except for one asymptomatic allergic patient who had 8.7%. The variation from 0–2.7% in the control donors was not considered significant, because it was occasionally observed in an individual donor tested repeatedly within a 2-wk span. By contrast, 2 of 10 nonallergic donors and 6 of 7 patients had significant increases of $Fc\epsilon R^+$ lymphocytes during the grass pollen season that clearly exceeded the variations of control donors 1 and 5 who showed the highest variations (Table II). During the pollen season, the mean±SD percentage and total number of $Fc\epsilon R^+$ lymphocytes of the patients were significantly higher ($P < 0.01$) than those of the 10 control donors. After the pollen season, one patient still had a high percentage and two a high total number of $Fc\epsilon R^+$ lymphocytes, whereas the others had low numbers of $Fc\epsilon R^+$ cells. The two control donors, 9 and 10, who had elevated $Fc\epsilon R^+$ cells during the grass pollen season, had again low numbers of $Fc\epsilon R^+$ cells in the postpollen season. The $Fc\epsilon R^+$ cells were repeatedly tested in patient 2 who had 8.7% $Fc\epsilon R^+$ cells in March; they steadily declined over the study period (Table I).

Control donor 9, a 24-year-old female, had previously been serving as a control donor. She had 0.3–1.9% $Fc\epsilon R^+$ lymphocytes twice in August 1978 and three

TABLE I

Fcε⁺ Lymphocytes of 10 Nonallergic and 7 Allergic Donors before, during and after the 1980 Grass Pollen Season

	Age/Sex	Prepollen FcεR⁺		Pollen FcεR⁺		Postpollen FcεR⁺	
		%	per m³	%	per m³	%	per m³
Nonallergic donor							
1 (H.S.)	47 M	2.7	70	1.3	29	0.5	13
2 (P.S.)	39 F	0.7	26	0.0	0	1.3	29
3 (G.B.)	38 M	1.0	20	1.6	33	1.5	27
4 (E.B.)	38 M	1.3	22	0.4	5	2.0	34
5 (C.B.)	28 F	3.7	78	1.2	23	2.8	33
6 (M.S.)	50 F	0.7	20	1.0	32	2.5	52
7 (W.G.)	28 M	0.8	22	1.0	7	2.0	30
8 (S.H.)	32 F	0.3	18	0.5	17	0.5	19
9 (T.K.)	24 F	1.4	38	5.8	142	0.6	15
10 (S.G.)	28 M	0.0	0	4.6	101	0.6	8
Mean±SD		1.2±0.9	29±20	1.7±1.9	40±46	1.4±0.9	26±13
Allergic patient							
1 (J.J.)	63 M	0.5	12	5.0	92	2.8	35
2 (M.S.)	38 M	8.7	130	4.6	71	1.1	17
3 (J.C.)	31 M	1.5	55	4.3	87	0.7	14
4 (C.B.)	33 M	1.7	102	6.7	207	5.8	232
5 (C.W.)	42 F	1.6	40	2.7	71	2.8	108
6 (G.G.)	42 F	0.0	0	4.3	223	0.9	15
7 (R.M.)	32 M	0.0	0	5.3	189	0.5	10
Mean±SD		2.0±3.1	48±52	4.7±1.2*	134±69*	2.1±1.9	62±82

* $P < 0.01$.

times in February 1979. She was tested for lymphocyte subpopulations in March 1979 when she had severe systemic contact dermatitis (Fig. 1). At the height of the disease, she had a marked decrease of E rosetting T cells from 78 to 33%, and an increase of sIgM⁺ B cells from 6 to 37% and of sIgD⁺ B cells from 8 to 31%. In contrast, neither the percentages nor the absolute numbers of FcεR⁺ and FcγR⁺ lymphocytes changed significantly from values before and after the episode of contact dermatitis. The absolute number of lymphocytes varied from 2,800/mm³ to 3,400/mm³ over the study period, indicating that the change of the T:B cell ratio occurred also in the total number of cells. The B cells were atypical in that most of them did not express FcγR.

Total and specific IgE serum levels in normal donors and allergic patients. The total serum IgE levels and RAST scores (counts per minute) to Timothy, Bermuda, and June grass for the 10 nonallergic donors and 7 allergic patients is shown in Table III. Seven of the control donors had low IgE levels (2–30 IU/ml) and negative RAST scores (<1000 cpm). Unexpectedly, three control donors (3, 4, 8) had elevated IgE levels and donors 3 and 4 also had positive RAST scores to all three grass pollens tested. None of these control donors had a history of allergies; in particular, they never had symptoms suggestive of allergic rhinitis and itching or atopic dermatitis resulting from contact with grasses. Normal donors 9 and 10, who had elevated FcεR⁺ lymphocytes during the grass pollen season, both had low IgE levels and negative RAST scores. Donor 9 was skin test negative and donor 10 was skin test positive at a $1:10^{-3}$ grass pollen dilution. Neither the total IgE nor RAST scores changed much in the nonallergic controls during the study period.

As shown in Table III, six of seven patients had moderately elevated IgE levels and all had positive RAST scores, usually to all three grass pollens. The changes in total IgE and RAST scores during the study period were minor and did not correlate with the changes in the percentages of FcεR⁺ lymphocytes, except for patient 2, who showed a concomitant decline in FcεR⁺ lymphocytes and RAST scores during the study period.

FcγR⁺ and sIg⁺ cells in nonallergic donors and allergic patients. The lymphocytes of all normal donors and all patients were also analyzed for FcγR⁺ lymphocytes and cell membrane bound immunoglobulin (sIg⁺) B cells. As shown in Table IV, no statistically significant differences in the mean±SD of FcγR⁺ lymphocytes and B cells were observed between nonallergic donors and patients before, during, and after the grass pollen season. However, the pa-

TABLE II
Percent FcεR⁺, FcγR⁺ and sIg⁺ Lymphocytes in Nonallergic Donors 9, 10, 1 and 5

Donor	Date	FcεR⁺		FcγR⁺	sIg⁺
		%	*per m³*	%	%
9 (T.K.)	3/20	1.4	38	11.0	16.7
	5/6	6.5	121	14.0	19.8
	5/9	5.2	90	25.3	21.3
	5/14	5.0	215	13.0	9.0
	6/13	2.0	85	14.7	11.0
	6/18	1.6	42	22.6	13.2
	8/7	0.0	0	11.3	4.5
10 (S.G.)	4/1	0.0	0	31.2	8.0
	5/8	3.4	91	23.0	17.3
	5/19	5.5	139	30.3	13.0
	5/27	5.0	75	33.7	12.2
	6/12	3.7	102	37.7	18.8
	8/20	0.6	8	14.6	7.0
	9/19	2.7	40	13.0	11.5
1 (H.S.)	3/19	2.3	64	8.6	4.3
	3/24	3.1	78	10.3	3.0
	5/1	1.3	29	9.5	3.6
	8/13	0.5	13	8.5	5.0
5 (C.B.)	3/25	3.1	78	6.3	8.8
	4/2	2.3	27	16.0	6.3
	5/7	1.2	23	15.3	10.3
	6/18	0.8	35	16.6	16.3
	8/20	2.8	33	6.8	11.7

tients showed the lowest percentages of FcγR⁺ cells at the time when their percentages of FcεR⁺ cells were increased during the grass pollen season, perhaps because a portion of the FcγR⁺ cells became FcεR⁺ cells. We have previously shown that lymphocytes are either FcγR⁺ or FcεR⁺, but rarely both (2).

DISCUSSION

The subpopulation of FcεR⁺ peripheral blood lymphocytes increases in atopic patients sensitive to the grass pollens when they are exposed to antigen and have allergic rhinitis. The increase of FcεR⁺ lymphocytes was not associated with significant increases of total serum IgE or specific IgE, as determined by RAST to Bermuda, Timothy, and June grass pollens. Most patients showed relatively small changes in total and specific serum IgE levels and, except for one patient (2), the changes that did occur did not correlate with the changes in FcεR⁺ lymphocytes. In a previous study, we showed that mildly atopic patients with moderately elevated IgE levels had normal numbers of FcεR⁺ lymphocytes, whereas severely atopic patients with IgE levels > 10,000 IU/ml had elevated percentages of FcεR⁺ lymphocytes. We had assumed that the very high levels of IgE were responsible for the large numbers of FcεR⁺ lymphocytes. However, the present observations indicate that mildly atopic patients with moderately increased IgE levels also have high percentages of FcεR⁺ lymphocytes when they are exposed to antigen and have active allergic disease.

The increase of FcεR⁺ cells appears to correlate with exposure to antigen rather than allergic disease; in fact, it may be an indicator of an ongoing IgE response. Two healthy nonallergic donors showed significant increases of FcεR⁺ lymphocytes during the grass pollen season. The two nonallergic donors had no history of allergic disease, low IgE levels and negative RAST; one was skin test negative to grass pollens and the other only moderately positive. The increase of FcεR⁺ lymphocytes may be unrelated to an IgE response in these donors. However, this seems unlikely in view of the finding of Yodoi and Ishizaka (7) demonstrating that FcεR⁺ lymphocytes increase in rats during an IgE response resulting from infection with the parasite *Nippostrongylus brasiliensis*. Since immunoglobulin serum levels do not change appreciably during normally occurring immune responses, both donors may have had small IgE responses at the time of the in-

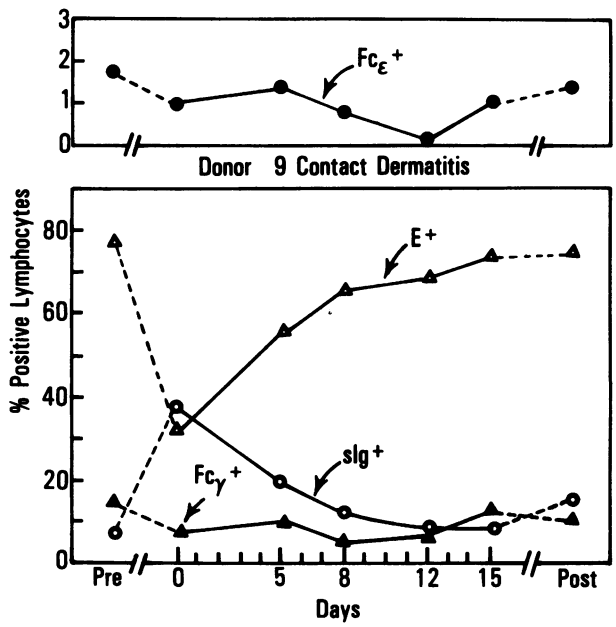

FIGURE 1 Lymphocyte subpopulations of nonallergic donor 9 during generalized contact dermatitis (day 0 to day 15). E⁺ = T cells forming spontaneous rosettes with sheep erythrocytes; sIg⁺ = sIgM⁺/sIgD⁺ cells; Fcγ⁺, Fcε⁺ = lymphocytes forming rosettes with rabbit IgG or human IgE coated ox red cells. Pre, Post = values obtained ~1 mo before or after the contact dermatitis.

TABLE III

IgE Serum Concentrations and RAST Scores to Timothy, Bermuda and June Grass Pollen of 10 Nonallergic Donors and 7 Allergic Patients before, during and after the 1980 Grass Pollen Season

			RAST score		
		IgE	Timothy	Bermuda	June
		IU/ml		*cpm**	
Nonallergic donor					
1 (H.S.)	Pre	16	367	503	565
	Pollen	18	NT	NT	NT
	Post	22	488	492	570
2 (P.S.)	Pre	2	307	254	249
	Pollen	4	337	366	234
	Post	8	280	316	322
3 (G.B.)	Pre	165	1,623	2,461	2,063
	Pollen	156	1,109	2,127	1,513
	Post	179	806	1,591	1,369
4 (E.B.)	Pre	91	7,077	769	7,636
	Pollen	139	12,344	1,832	13,223
	Post	140	11,529	1,837	13,404
5 (C.B.)	Pre	16	511	364	314
	Pollen	23	367	352	349
	Post	17	563	362	711
6 (M.S.)	Pre	2	237	357	401
	Pollen	5	276	282	290
	Post	5	NT	NT	NT
7 (W.G.)	Pre	22	351	282	366
	Pollen	23	244	309	191
	Post	30	255	225	260
8 (S.H.)	Pre	265	322	479	311
	Pollen	267	294	351	356
	Post	197	269	298	197
9 (T.K.)	Pre	2	351	352	354
	Pollen	2	233	264	347
	Post	2	276	268	342
10 (S.G.)	Pre	6	412	292	276
	Pollen	7	316	303	291
	Post	7	322	373	352
Allergic patient					
1 (J.J.)	Pre	132	7,976	20,029	11,571
	Pollen	100	7,645	17,302	13,025
	Post	120	6,610	14,681	3,735
2 (M.S.)	Pre	316	11,788	13,408	15,739
	Pollen	362	9,548	10,027	13,594
	Post	168	5,333	6,825	6,834
3 (J.C.)	Pre	76	1,492	523	1,477
	Pollen	85	1,367	457	1,315
	Post	74	1,171	497	407
4 (C.B.)	Pre	375	1,213	2,953	1,233
	Pollen	301	1,062	3,375	992
	Post	241	1,135	3,811	824
5 (C.W.)	Pre	880	23,814	17,197	27,554
	Pollen	969	24,614	18,031	27,691
	Post	1,350	24,493	18,548	29,633
6 (G.G.)	Pre	192	13,197	10,295	16,660
	Pollen	163	13,960	9,842	16,579
	Post	130	13,230	9,571	14,766
7 (R.M.)	Pre	323	23,634	27,659	28,685
	Pollen	389	21,968	25,157	27,957
	Post	468	26,087	28,118	29,434

*< 1,000 cpm is considered negative. NT, not tested.

crease of FcεR$^+$ lymphocytes. Whether they made IgE antibodies to grass pollens in amounts too small for detection by RAST, or whether they responded to another antigen, is unknown. We will study these two donors during future grass pollen seasons to determine whether their increases of FcεR$^+$ lymphocytes were related to the grass pollen season or simply coincidental. Assuming that the increase of FcεR$^+$ lymphocytes is an indication of IgE antibody formation, the observations made in the two nonallergic donors indicate that an increase of FcεR$^+$ lymphocytes is not restricted to atopic individuals and can occur in the absence of allergic symptoms and high IgE serum levels.

Most of the allergic patients did not show a significant rise in total IgE during the grass pollen season. This is consistent with previous reports demonstrating that patients receiving immunotherapy do not show the increase of IgE seen in untreated hay fever patients (13, 14). The lack of an increase in specific IgE as measured by RAST during the grass pollen season may also have been the result of immunotherapy. Patients receiving long term maintenance immunotherapy usually have high titers of IgG blocking antibody that may be responsible for falsely low IgE RAST scores (15, 16). It is likely that our patients made at least a small IgE response when they were exposed to the grass pollens. Therefore, enumerating FcεR$^+$ lymphocytes may prove to be the more sensitive indicator of an IgE host response than measuring total or specific IgE serum levels.

The mechanism of generation of FcεR$^+$ lymphocytes is not understood. In man, an increase of the IgE serum

TABLE IV

FcγR⁺ and sIg⁺ Lymphocytes in Nonallergic and Allergic Donors before, during and after the Grass Pollen Season

	Prepollen	Pollen	Postpollen
		FcγR*	
		%	
Nonallergic (10)	15.7±7.8	18.0±8.1	14.8±7.5
Allergic (7)	17.6±12.6	11.9±6.9‡	14.4±4.7
		% sIg⁺	
Nonallergic (10)	10.2±4.7	11.6±4.2	8.3±3.0
Allergic (7)	12.4±6.4	12.8±5.4	9.9±4.2

* Mean±SD

‡ 0.3 > 0.2

level per se, does not appear to cause an increase of FcεR⁺ lymphocytes. The atopic donors had higher IgE levels than the nonallergic controls but only slightly higher numbers of FcεR⁺ cells before and after the grass pollen season. Three nonallergic donors also had elevated IgE levels, yet their numbers of FcεR⁺ lymphocytes were always low. Previously, we injected two monkeys with an IgE myeloma protein; neither developed an increase of FcεR⁺ cells despite their high levels of circulating IgE (8). Similarly, culturing lymphocytes from normal (6) or allergic patients (17) in the presence of IgE did not induce FcεR⁺ cells. In contrast, FcεR⁺ cells formed when lymphocytes from ragweed sensitive patients were cultured with both ragweed antigen E and an IgE myeloma protein (17). Furthermore, lymphocytes activated by mixed lymphocyte culturing became FcεR⁺ when IgE was added to the culture medium (18). These in vitro studies suggest that the generation of FcεR⁺ lymphocytes may first involve activation of the cells and then modulation by IgE. The activation may result from antigen exposure or from interaction of the lymphocytes with accessory cells such as macrophages. IgE secreted in the microenvironment of the lymphocytes may then modulate the activated cells to express FcεR.

In contrast to human cells, rat lymphocytes become FcεR⁺-bearing cells when the IgE concentration increases in the cells' environment. Yodoi and Ishizaka (18, 19) demonstrated that culturing normal rat mesenteric lymph node lymphocytes in the presence of IgE induces generation of FcεR⁺ cells. We injected purified IgE myeloma proteins into normal rats which resulted in an increase of FcεR⁺ lymphocytes in the blood, spleen and nodes.[2] These in vitro and in vivo observations do not necessarily indicate that FcεR⁺ lymphocytes are generated via a different mechanism in rats and humans. As discussed above, the generation of FcεR⁺ lymphocytes may initially involve a cell activation step after which IgE modulates the activated cell. Rats may have a more responsive IgE system than humans, since rats normally have higher proportions of FcεR⁺ lymphocytes in their blood and spleen (20) than man (3). This higher value for FcεR⁺ cells may reflect a larger percentage of activated lymphocytes, which become induced to form Fcε receptors by the normally low concentrations of IgE.

Lymphocytes with Fc receptors for IgA (FcαR) are increased in mice bearing IgA secreting plasmacytomas (21) and patients with IgG or IgA multiple myeloma also have increases in FcγR⁺ or FcαR⁺ lymphocytes, respectively (22). The IgA serum concentrations in normal individuals are much higher than those of IgE; however, the subpopulation of FcαR⁺ lymphocytes is numerically similar to the FcεR⁺ subpopulation (23). Most likely, the serum concentrations of all immunoglobulin classes influence the number of the respective Fc receptor-positive lymphocytes, yet the threshold concentrations necessary to induce Fc receptor positive cells appear to be different.

One of the nonallergic control donors was studied at the time when she had severe, generalized, contact dermatitis. She showed a marked increase in the percentage and total number of sIg⁺ B cells and a decrease of E rosetting T cells, particularly at the height of disease. In contrast, her percentage and total numbers of FcεR⁺ and FcγR⁺ lymphocytes remained within normal limits. Contact dermatitis is believed to be a T lymphocyte-mediated, delayed hypersensitivity reaction, and a decrease of T cells has also been reported by others (24). It was reported that patients with contact dermatitis have an increase of sIgD⁺ but not sIgM⁺ lymphocytes (25). Our donor had a high number of both sIgD⁺ and sIgM⁺ lymphocytes, suggesting that she had an increase of sIgM⁺/sIgD⁺ peripheral blood B lymphocytes, which neither expressed FcεR nor FcγR. The majority of FcεR⁺ lymphocytes in normal donors (2, 3) and in atopic patients (7, 8) are B cells. The fact that the numbers of lymphocytes with Fcε receptors remained normal in the donor during the acute contact dermatitis, despite the overall B cell increase, supports our assumption that an increase of FcεR⁺ cells is most likely related to an IgE immune response and does not represent some nonspecific phenomenon.

The function of the Fcε receptors on lymphocytes is unknown. The significantly larger population of FcεR⁺ lymphocytes in allergic patients and in rats infected with *N. brasiliensis* parasites (7), conditions associated with IgE responses, suggests that Fcε receptors may play a regulatory role by either enhancing or

[2] Spiegelberg, H. L. Manuscript in preparation.

suppressing IgE antibody formation. Evidence was obtained suggesting that interactions between IgE antibody-antigen complexes and $Fc\gamma R^+$ B cells inhibit the differentiation of B cells into plasma cells (26–29). Analogously, IgE antibody-antigen complexes may react with $Fc\epsilon R^+$ B cells and prevent them from differentiating into $sIgE^+$ lymphocytes and subsequently into IgE producing plasma cells. Rat T lymphocytes with Fcε receptors were recently identified as the source of IgE binding factors which, depending on their state of glycosylation, have either a potentiating or suppressing effect on the in vitro generation of IgE forming cells (30–32). These IgE binding factors may be Fcε receptors or Fcε receptor fragments released from the cells. Although both the clinical and the experimental observations strongly suggest that Fcε receptors on B and T lymphocytes are involved in the regulation of IgE antibody formation, their definitive role remains to be shown.

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REFERENCES

1. Dickler, H. B. 1976. Lymphocyte receptors for immunoglobulin. *Adv. Immunol.* **24:** 167–207.
2. 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.
3. Hellström, U., and H. L. Spiegelberg. 1979. Characterization of human lymphocytes bearing Fc receptors for IgE isolated from blood and lymphoid organs. *Scand. J. Immunol.* **9:** 75–86.
4. 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.
5. Ishizaka, T. 1980. IgE receptors and their interference. *In* Adv. Allergology and Clin. Immunol. A. Ochling, I. Glazer, E. Methor and C. Arkesman, editors. Pergamon Press, Inc., New York. 586–588.
6. Spiegelberg, H. L., and F. M. Melewicz. 1980. Fc receptors for IgE on subpopulations of human lymphocytes and monocytes. *Clin. Immunol. Immunopathol.* **15:** 424–433.
7. Yodoi, J., and K. Ishizaka. 1979. Lymphocytes bearing Fc receptors for IgE. I. Presence of human and rat T lymphocytes with Fcε receptors. *J. Immunol.* **122:** 2577–2583.
8. Spiegelberg, H. L., R. D. O'Connor, R. A. Simon, and D. A. Mathison. 1979. Lymphocytes with immunoglobulin E Fc receptors in patients with atopic disorders. *J. Clin Invest.* **64:** 714–720.
9. Perlmann, H., P. Perlmann, G. R. Pape, and G. Hållden. 1976. Purification, fractionation, and assay of antibody-dependent lymphocyte effector cells (K cells) in human blood. *Scand. J. Immunol.* **5**(Suppl.): 57–68.
10. Horwitz, D. A., A. C. Allison, P. Ward, and N. Knight. 1977. Identification of human mononuclear leucocyte populations by esterase staining. *Clin. Exp. Immunol.* **30:** 289–298.
11. Hallberg, T., B. W. Gurner, and R. R. A. Coombs. 1973. Opsonic adherence of sensitized ox red cells to human lymphocytes as measured by rosette formation. *Int. Arch. Allergy Appl. Immunol.* **44:** 500–513.
12. 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.
13. Levy, D. A., L. M. Lichtenstein, E. O. Goldstein, and K. Ishizaka. 1971. Immunologic and cellular changes accompanying the therapy of pollen allergy. *J. Clin. Invest.* **50:** 360–369.
14. Gleich, G. J., G. L. Jacob, J. W. Yuninger, and L. L. Henderson. 1977. Measurement of the absolute levels of IgE antibodies in patients with ragweed hay fever. *J. Allergy Clin. Immunol.* **60:** 188–198.
15. Zeiss, C. R., L. C. Grammer, and O. Levitz. 1981. Comparison of the radioallergosorbent test and a quantitative solid phase radioimmunoassay for the detection of ragweed specific immunoglobulin E antibody in patients undergoing immunotherapy. *J. Allergy Clin. Immunol.* **67:** 105–110.
16. Zimmerman, E. M., J. W. Yuninger, and G. J. Gleich. 1980. Interference in ragweed pollen and honeybee venom radioallergosorbent tests. *J. Allergy Clin. Immunol.* **66:** 386–393.
17. Yodoi, J., and K. Ishizaka. 1980. Induction of Fcε-receptor bearing cells in vitro in human peripheral lymphocytes. *J. Immunol.* **124:** 934–938.
18. Yodoi, J., T. Ishizaka, and K. Ishizaka. 1979. Lymphocytes bearing Fc receptors for IgE. II. Induction of Fcε-receptor bearing rat lymphocytes by IgE. *J. Immunol.* **123:** 455–462.
19. Yodoi, J., and K. Ishizaka. 1979. Lymphocytes bearing receptors for IgE. III. Transition of $Fc\gamma R(+)$ cells to $Fc\epsilon R(+)$ cells by IgE. *J. Immunol.* **123:** 2004–2010.
20. Fritsche, R., and H. L. Spiegelberg. 1978. Fc receptors for IgE on normal rat lymphocytes. *J. Immunol.* **121:** 471–478.
21. Hoover, R. G., and R. G. Lynch. 1980. Lymphocyte surface membrane immunoglobulin in myeloma. II. T cells with IgA-Fc receptors are markedly increased in mice with IgA plasmacytoma. *J. Immunol.* **125:** 1280–1285.
22. Hoover, R. G., S. Hickman, H. M. Gebel, N. Rebbe, and R. G. Lynch. 1981. Expansion of Fc receptor-bearing T lymphocytes in patients with immunoglobulin G and immunoglobulin A myeloma. *J. Clin. Invest.* **67:** 308–311.
23. Lum, L. G., A. V. Muchmore, N. O'Connor, W. Strober, and R. M. Blaese. 1979. Fc receptors for IgA on human B and human non-B non-T lymphocytes. *J. Immunol.* **123:** 714–719.
24. Carpeto, F. J., R. K. Winkelmann, and R. E. Jordon. 1976. T and B lymphocytes in contact and atopic dermatitis. *Arch. Dermatol.* **112:** 1095–1100.
25. Cormane, R. H., S. Husz, and F. Hamerlinck. 1974. Immunoglobulin-complement-bearing lymphocytes in allergic contact dermatitis and atopic dermatitis (eczema). *Br. J. Dermatol.* **90:** 597–605.

26. Sinclair, N. R. St. C. 1969. Regulation of the immune response. I. Reduction in ability of specific antibody to inhibit long-lasting IgG immunological priming after removal of the Fc fragment. *J. Exp. Med.* **129:** 1183–1201.
27. Ryan, J. L., R. D. Arbeit, H. B. Dickler, and P. A. Henkart. 1975. Inhibition of lymphocyte mitogenesis by immobilized antigen-antibody complexes. *J. Exp. Med.* **142:** 814–826.
28. Sidman, C. L., and E. R. Unanue. 1976. Control of β-lymphocyte function. I. Inactivation of mitogenesis by interaction with surface immunoglobulin and Fc-receptor molecules. *J. Exp. Med.* **144:** 882–896.
29. Kölsch, E., J. Oberbarnscheidt, K. Brüner, and J. Heuer. 1980. The Fc-receptor: its role in the transmission of differentiation signals. *Immunol. Rev.* **49:** 61–78.
30. Yodoi, J., and K. Ishizaka. 1980. Lymphocytes bearing Fc receptors for IgE. IV. Formation of IgE-binding factor by rat T lymphocytes. *J. Immunol.* **124:** 1322–1329.
31. Suemura, M., J. Yodoi, M. Hirashima, and K. Ishizaka. 1980. Regulatory role of IgE-binding factors from rat T lymphocytes. I. Mechanism of enhancement of IgE response by IgE-potentiating factor. *J. Immunol.* **125:** 148–154.
32. Yodoi, J., M. Hirashima, and K. Ishizaka. 1980. Regulatory role of IgE-binding factors from rat T lymphocytes. II. Glycoprotein nature and source of IgE-potentiating factor. *J. Immunol.* **125:** 1436–1441.