

IgE⁺ cells in the peripheral blood of atopic, nonatopic, and bee venom–hypersensitive individuals exhibit the phenotype of highly differentiated B cells

Peter J. Donohoe, PhD,^a Robert J. Heddle, MD, PhD,^a
Pamela J. Sykes, PhD,^b Michael Fusco, BSc,^a Loretta R. Flego, BSc,^a
and Hedly Zola, PhD^a Adelaide, South Australia

We have analyzed IgE⁺ cells in peripheral blood of atopic donors, donors hypersensitive to bee venom, and nonatopic control donors with two- and three-color flow cytometry. Although the percentage of IgE⁺ cells varied among these groups, the overall phenotypic patterns were similar. Most IgE⁺ cells do not display typical B-cell markers, such as CD19, CD20, and CD21. A significant proportion of these cells stain for CD38, indicating that they are more differentiated. IgE⁺ cells express FcγRII and CD45RO, an isoform associated with an advanced level of differentiation. The majority of IgE⁺ cells do not coexpress other surface immunoglobulin isotypes. In the case of bee venom–hypersensitive donors, we have been able to identify a small population of IgE⁺ cells with a specificity for phospholipase A2, a major immunogenic component of bee venom. The phospholipase A2⁺ cells display a phenotype similar to that of the IgE⁺ cells. (J ALLERGY CLIN IMMUNOL 1995;95:587-96.)

Key words: IgE cell phenotype, enumeration, bee venom hypersensitivity, desensitization, immunotherapy mechanism

Allergic diseases constitute the most frequent immunologic disorders. In normal nonatopic individuals the serum concentration of IgE is less than 500 ng/ml, increasing with the onset of certain parasitic infections.¹ IgE has a half-life of 2 to 3 days, favoring a return to normal serum levels quickly once the antigen or infection has been cleared.² In the case of allergy, serum levels of IgE specific to particular antigens may persist for many years even without antigenic challenge.

Given the central role of IgE in allergic disorders, the phenotype and frequency of IgE⁺ cells are of interest. The determination of the frequency of IgE-committed B cells in blood has yielded

Abbreviations used

DDAF:	FITC-labeled sheep anti-mouse anti-body
EBV:	Epstein-Barr virus
FITC:	Fluorescein isothiocyanate
IL:	Interleukin
PBLs:	Peripheral blood lymphocytes
PE:	Phycoerythrin
PLA-2:	Phospholipase A2

contradictory results. Some studies on the frequencies of peripheral blood lymphocytes (PBLs) secreting immunoglobulins of different classes and subclasses have ignored IgE cells because their numbers were too low to estimate.³ Studies have involved Epstein-Barr virus (EBV) transformation and culturing of the cells with interleukin (IL)-4.⁴⁻⁶ IL-4 is known to promote switching to C_ε in human lymphoid cells.^{7,8} It is unclear therefore whether the action of EBV or IL-4 may result in a biased selection of IgE⁺ cells and may not reflect the actual percentage of these cells in the blood. A more accurate determination of the frequency of

From ^athe Department of Clinical Immunology and the ^bDepartment of Haematology, Flinders Medical Centre and Flinders University, Bedford Park, South Australia.

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Reprint requests: Professor Hedly Zola, PhD, Department of Clinical Immunology, Flinders Medical Centre, Bedford Park, South Australia 5042.

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TABLE I. Monoclonal antibodies used in IgE cell analysis

Specificity	Antibody	Isotype	Study
CD19	FMC63	IgG2a	Zola et al. ²⁶
CD20	HB13d	IgG1	Kansas and Tedder ³⁷
CD21	HB135	IgG2a	ATCC Weis et al. ³⁸
CD22	29.110	IgG1	Barr et al. ³⁹
CD5	WM13	IgG1	K. Bradstock
p105	FMC7	IgM	Zola et al. ²⁴
CD45RO	UCHL1	IgG2a	Smith et al. ⁴⁰
CD45RA	FMC44	IgG1	Zola et al. ⁴¹
CD38	OKT10	IgG1	Ortho Terhorst et al. ⁴²
Plasma	PCA-1	IgG1	CE Anderson et al. ²⁷
CD32	41H.16	IgG2a	Zipf et al. ⁴³
CD23	B6	IgG2b	CE Thorley Lawson et al. ³⁰
IgM	HB57	IgG1	ATCC Kuritani et al. ⁴⁴
IgD	HB70	IgG3	ATCC Kuritani et al. ⁴⁴
IgG	HP 'Mix'	—	ATCC Reimer et al. ⁴⁵
IgG1Fc	HP6001	IgG2b	ATCC Reimer et al. ⁴⁵
IgG2Fc	HP6002	IgG1	ATCC Reimer et al. ⁴⁵
IgG2Fab	HP6014	IgG1	ATCC Reimer et al. ⁴⁵
IgG3Fc	HP6003	IgG1	ATCC Reimer et al. ⁴⁵
IgG3hinge	HP6050	IgG1	ATCC Reimer et al. ⁴⁵
IgG4Fab	HP6020	IgG2a	ATCC Reimer et al. ⁴⁵
IgG4Fc	HP6025	IgG1	ATCC Reimer et al. ⁴⁵
IgE	2-1-5	IgG1	K. Turner
IgE	HB121	IgG2a	ATCC Hook et al. ⁴⁶
CD14	FMC32	IgG1	Hancock et al. ⁴⁷
CD13	WM15	IgG1	Look et al. ¹³
CD33	WM53	IgG1	Favaloro et al. ⁴⁸
CD3	OKT3	IgG1	BD van Dongen et al. ⁴⁹
IgKappa	TB28-2	IgG1	BD Weinburg et al. ⁵⁰
IgLambda	1-155-2	IgG1	BD Weinburg et al. ⁵⁰
Neg.con.	X63	IgG1	Myeloma protein

ATCC, American Type Culture Collection (Rockville, MD.); Ortho, Ortho Diagnostic Systems Inc. (Raritan, N.J.); CE, Coulter Electronics (Hialeah, Fla.); BD, Becton Dickinson (San Jose, Calif.)

IgE cells is likely to be obtained by fluorescence labeling and direct analysis by flow cytometry.

In this report we enumerate IgE⁺ cells in blood and determine their phenotype.

METHODS

Twenty individuals served as donors of PBLs. Five had no history of atopic disorders and tested negative by multiple allergen disc immunoassay (Phadiatop; Pharmacia Diagnostics AB, Uppsala, Sweden), five were atopic, and 10 were hypersensitive to honeybee (*Apis mellifera*) venom. Bee venom-hypersensitive donors were selected on the basis of having experienced a life-threatening reaction to bee venom and a positive RAST or skin test response to honeybee venom. PBLs were prepared from heparinized blood by separation on Ficoll-Hypaque (Pharmacia). The cells were washed twice in phosphate-buffered saline azide and resus-

pended at 10⁷ cells/ml. Before analysis with the test monoclonal antibodies, the cells were incubated with normal sheep serum at room temperature for 30 minutes. This step was included to displace any adsorbed human immunoglobulin.

Cells were stained by two-color labeling as described previously.^{9,10} Briefly, 50 µl aliquots of cells were stained as follows. First, a monoclonal antibody was added for the particular marker, listed in Table I, followed by fluorescein isothiocyanate (FITC)-labeled sheep anti-mouse immunoglobulin (DDAF), (Silenus Laboratories, Melbourne, Australia). Mouse immunoglobulin binding sites were blocked with the addition of normal mouse serum at a concentration of 1 mg/ml. The second reagent, biotinylated anti-IgE antibody or biotinylated PLA-2 was added and stained with phycoerythrin-streptavidin (PE-SA) (Caltag Laboratories, San Francisco, Calif.). Reagents were incubated for 30 min-

utes each, and the cells were maintained on ice throughout the experiments. Concentrations of reagents and incubation conditions were determined in advance.

Cells were stained by three-color fluorescence with the following combinations: (1) Kappa: FITC/Lambda: PE (Simultest; Becton-Dickinson Immunocytometric Systems, San Jose, Calif.) + biotinylated anti-IgE antibody/streptavidin-conjugated Tricolor (Caltag), (2) 41H.16 (CD32):FITC + CD3:PE (Becton Dickinson) + biotinylated anti-IgE antibody/streptavidin-conjugated Tricolor, or (3) (OKT10 [CD38] + DDAF) + CD3:PE + biotinylated anti-IgE antibody/streptavidin-conjugated Tricolor. For combinations 1 and 2, all three reagents were added together. The cells were then washed, and streptavidin-conjugated Tricolor was added. For combination 3, OKT10 was added first and stained with DDAF. CD3:PE and biotinylated anti-IgE antibody were then added and stained with streptavidin-conjugated Tricolor. For combination 3, OKT10 was added first and stained with DDAF. CD3:PE and biotinylated anti-IgE antibody were then added and stained with streptavidin-conjugated Tricolor. Three-color staining with biotinylated PLA-2 in place of biotinylated anti-IgE antibody was also carried out.

Biotin and FITC-labeled reagents were prepared by using standard methods.^{11,12}

Fluorescence was analyzed with a FACScan flow cytometer (Becton Dickinson and Company, Franklin Lakes, N.J.) with standard settings. For each antibody, 10,000 cells were accumulated, and a dual-contour plot was generated. The samples were re-run by "live gating" (real-time) to select only IgE⁺ or PLA-2⁺ cells with the physical parameters of lymphocytes. Real-time gating was included to allow a more precise analysis of these rare cells. Approximately 2 to 3 × 10⁵ ungated cells were scanned to obtain sufficient numbers of cells for analysis as fluorescence histograms.

RESULTS

Validation of analyses

Because IgE⁺ cell populations are rare in peripheral blood, careful preparation of the cells was needed to eliminate false-positive results caused by absorption of immunoglobulin to the cells mediated by Fc receptors. This was accomplished by washing the cells twice with phosphate-buffered saline after separation on Ficoll-Hypaque and then preincubating the cells with normal sheep serum for 30 minutes at room temperature. The effectiveness of this step is shown in Fig. 1. In all tests cells were triple-stained with kappa:FITC, lambda:PE, and biotinylated anti-IgE antibody/Tricolor. Cells were real-time gated on the IgE⁺ population and analyzed for kappa and lambda expression. Because individual B cells express only one light chain type, expression of both kappa and lambda suggests FcR-mediated uptake of serum immuno-

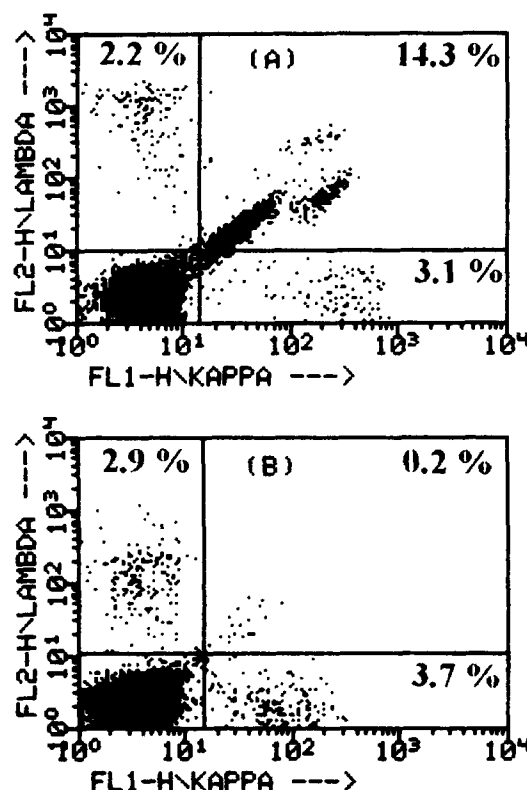


FIG. 1. Reduction of adsorption of immunoglobulin to lymphocytes. Cells were incubated at room temperature for 30 minutes with neat normal sheep serum. Data are presented on PBLs from an atopic donor (A) without and (B) with preincubation.

globulin. Cells stained with either kappa or lambda, but not both, indicate that the antibody has been produced by the cells. A kappa/lambda test was included in each experiment. Data from experiments showing a characteristic "diagonal" dual-stained population (Fig. 1, A) were excluded.

The gating selections for collection of cells and data analysis are shown in Fig. 2. Triple-staining of cells for kappa/lambda and IgE expression is used to illustrate the choice of gating parameters. Parameters were selected such that the maximum number of IgE⁺ cells expressed either kappa or lambda light chains, but not both. The kappa/lambda ratio of 2.6 in Fig. 2 is close to the range of 1.0 to 2.3 (Becton Dickinson, *Monoclonal Antibody Source Book*). In Fig. 2, C the brightly fluorescent cells in R3 were assumed to be the IgE⁺ population. The less intensely stained populations in R4 and R5, when analyzed for kappa/lambda expression, have increasing numbers of double-negative populations. These cells may have taken up the anti-IgE antibody nonspecifically. The anti-IgE antibody is a direct biotin conjugate, and the lower

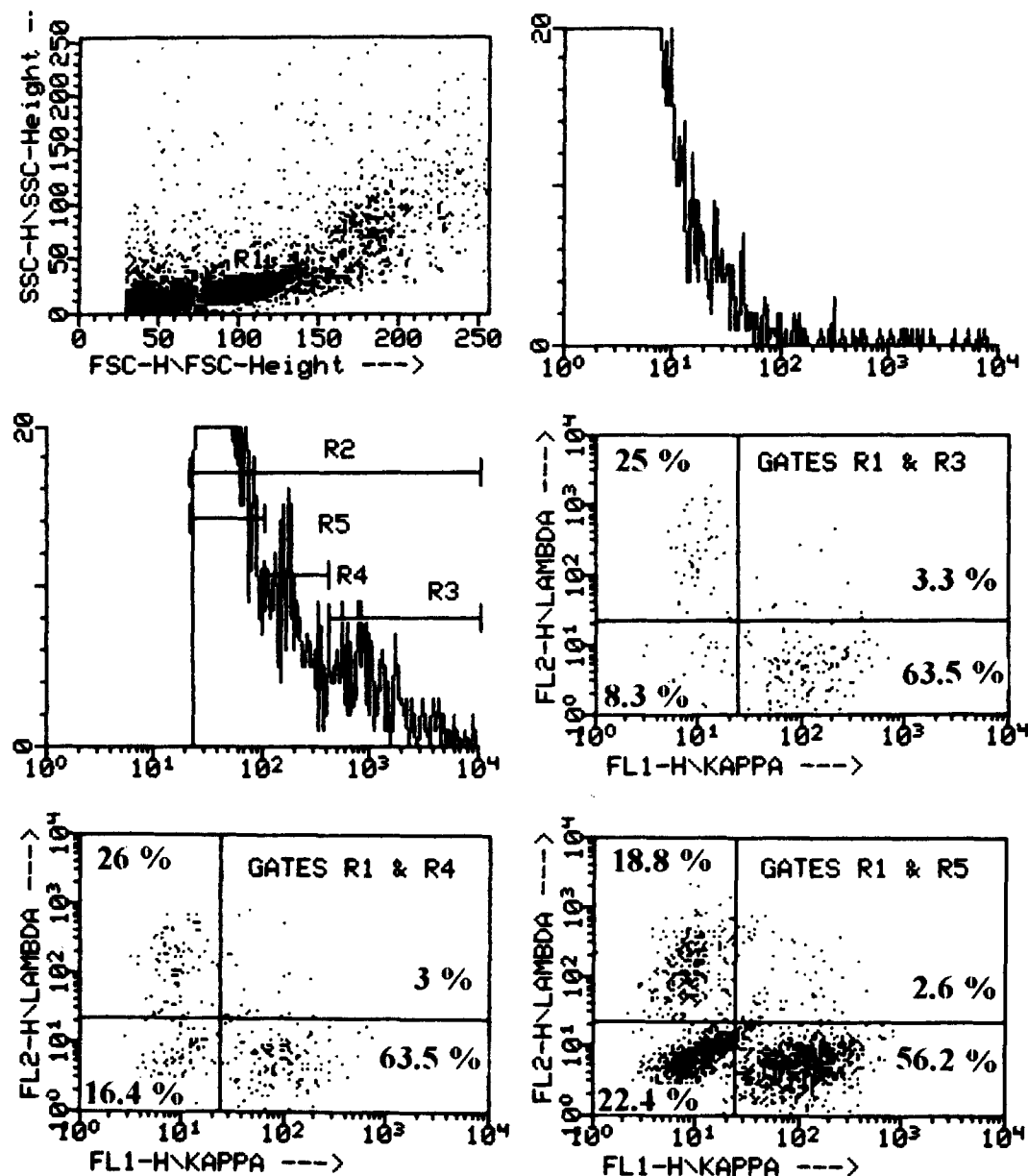


FIG. 2. Gating parameters used to analyze lymphocytes (R1) from 10,000 PBLs (upper left). IgE fluorescence distribution in the lymphocyte population (R1) (upper right). Real-time gating histogram showing the range for data collection (R2) and the gating (R3) used for data analysis (center left). Dot plot of expression of kappa and lambda light chains on IgE real-time-gated cells with gate R1 and the brightly fluorescent population R3 (center right). At lower left and right are dot plots showing kappa and lambda expression on the less brightly stained populations in R4 and R5.

sensitivity normally associated with direct conjugates may account for the failure to detect light chains on cells that express IgE. As an additional control, IgE⁺ cells were analyzed for expression of the basophil markers CD13 and CD33. No difference between the expression of these markers and that of the negative control X63 (see Table I) was found.

In a control experiment the percentage of IgE⁺ cells was reduced from 0.23% to 0.01% by incubating the cells with IgE (culture supernatant of the IgE myeloma line, U266BL) at the same time as the biotinylated anti-IgE. Similarly, addition of unbiotinylated PLA-2 (final concentration, 50 ng/ml) inhibited the biotinylated PLA-2. The percent-

TABLE II. Mean expression ($\pm$ SD) of markers on IgE⁺ and PLA-2⁺ cells

	Nonatopic IgE ⁺ (n = 5)	Atopic IgE ⁺ (n = 5)	Bee venom-hypersensitive	
			IgE ⁺ (n = 10)	PLA-2 ⁺ (n = 6)
CD19	11.9 $\pm$ 2.7	16.4 $\pm$ 5.4	9.3 $\pm$ 6.8	12.8 $\pm$ 5.7
CD20	14.0 $\pm$ 4.4	12.9 $\pm$ 7.4	11.3 $\pm$ 4.6	14.1 $\pm$ 5.5
CD21	11.4 $\pm$ 7.1	15.3 $\pm$ 6.3	9.5 $\pm$ 6.2	12.2 $\pm$ 3.6
CD22	11.6 $\pm$ 8.4	11.6 $\pm$ 8.0	9.2 $\pm$ 8.6	7.1 $\pm$ 4.2
CD5	33.2 $\pm$ 10.4	20.7 $\pm$ 16.1	35.5 $\pm$ 16.5	27.2 $\pm$ 14.7
p105	6.5 $\pm$ 1.3	9.3 $\pm$ 3.0	10.9 $\pm$ 9.3	8.5 $\pm$ 5.7
CD45RO	77.4 $\pm$ 4.4	76.1 $\pm$ 7.1	76.1 $\pm$ 6.8	78.1 $\pm$ 7.5
CD45RA	19.4 $\pm$ 7.1	18.6 $\pm$ 6.0	17.1 $\pm$ 4.4	14.3 $\pm$ 7.4
CD38	79.8 $\pm$ 6.5	82.4 $\pm$ 11.4	81.9 $\pm$ 13.8	84.9 $\pm$ 13.5
PCA-1	14.0 $\pm$ 5.2	7.2 $\pm$ 5.4	6.4 $\pm$ 5.7	11.0 $\pm$ 8.3
CD23	9.9 $\pm$ 4.2	7.8 $\pm$ 5.3	8.9 $\pm$ 8.1	10.1 $\pm$ 7.4
CD32	84.4 $\pm$ 9.4	86.1 $\pm$ 10.8	81.6 $\pm$ 10.8	79.0 $\pm$ 11.6
IgM	15.2 $\pm$ 4.9	8.5 $\pm$ 7.0	10.7 $\pm$ 8.5	14.3 $\pm$ 8.8
IgD	14.4 $\pm$ 5.7	8.1 $\pm$ 6.3	9.8 $\pm$ 3.2	15.7 $\pm$ 9.5
IgG	14.6 $\pm$ 5.6	5.5 $\pm$ 1.7	7.3 $\pm$ 3.5	14.3 $\pm$ 7.7
IgE	—	—	—	59.6 $\pm$ 18.9
CD14	13.9 $\pm$ 5.7	8.7 $\pm$ 6.4	5.2 $\pm$ 6.6	6.1 $\pm$ 4.5

age of PLA-2-binding cells detected was reduced from 0.26% to 0.01% in this experiment.

Initial analysis was carried out on 10,000 cells (no gate) to generate a dual-parameter dot plot. Similar results were obtained by gating on either PLA-2⁺ or IgE⁺ cells. Real-time-gated data were collected and analyzed on cells from 10 of the 20 donors. Other controls included in each experiment were X63, as a negative control, and the monocyte marker CD14. Mean staining of IgE⁺ cells with CD14 was 12.8% for the nonatopic group, 8.7% for the atopic group, and 5.2% for the bee venom-hypersensitive group.

Enumeration of IgE⁺ cells

PBLs were analyzed from three groups of donors, (1) nonatopic control subjects, (2) atopic subjects, and (3) bee venom-hypersensitive patients by means of two- and three-color flow cytometry. A compilation of all the results from each of the donor groups is given in Table II and shows the percentages of IgE⁺ and PLA-2⁺ cells expressing the markers tested. The percentage of IgE⁺ cells in PBLs was determined by analysis of cells staining with biotinylated anti-IgE antibody in each of the tests. In each experiment dual-staining of cells for IgE with 20 different markers was carried out. A histogram of IgE expression for each of these sets of data was generated, and the percent-

age of IgE⁺ cells was determined with the gating illustrated in Fig. 2. Because IgE⁺ cells are infrequent in peripheral blood, data on 100,000 cells were also collected from nine of the donors to obtain more precise results (Fig. 3). The percentage of IgE⁺ lymphocytes ranged from 0.02% to 0.08% (mean = 0.05%) in nonatopic donors, 0.23% to 0.46% (mean = 0.33%) in the atopic donors, and 0.18% to 0.43% (mean = 0.29%) in the bee venom-hypersensitive group (Fig. 4). The percentage of IgE⁺ cells in the nonatopic group differed significantly from the percentages in the atopic group ($p < 0.005$) and the bee venom-hypersensitive group ($p < 0.001$) as determined by the Mann Whitney U test. No significant difference in IgE⁺ cell numbers was found between the latter two groups ($\alpha = 0.10$).

Phenotype of IgE⁺ cells

Ficoll-Hypaque-isolated PBLs were dual-stained with B-cell markers CD19, CD20, CD21, CD22, and FMC7 and with anti-IgE. The majority of IgE⁺ cells (84% to 98%) did not express any of these markers (Fig. 5 and Table II). There is considerable variation in the percentages of dual-stained cells among the donors. However, there is no significant difference in expression of these markers among the three donor groups.

The mean coexpression of CD5 on IgE⁺ cells is

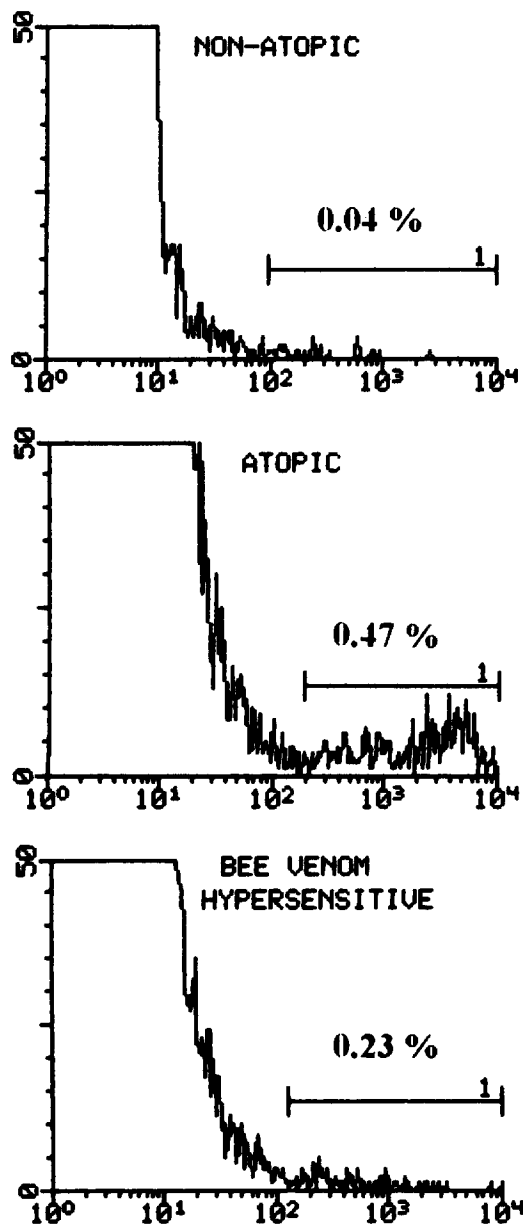


FIG. 3. Histograms of IgE⁺ cells after gating on the lymphocyte population. Examples from each of the donor groups are shown with data from 100,000 cells. IgE population was selected with the criteria outlined in the legend to Fig. 2.

33.2% (± 10.4) for nonatopic donors, 20.7% (± 16.1) for atopic donors, and 35.5% (± 16.5) for bee venom-hypersensitive donors (Table II). CD5 is expressed on 27.2% (± 14.7) of PLA-2⁺ cells.

The mean coexpression, from each of the donor groups, of CD38 on IgE⁺ cells ranges from 79% to 84% (Fig. 5 and Table II). To ensure that the T-cell population was not included, three-color

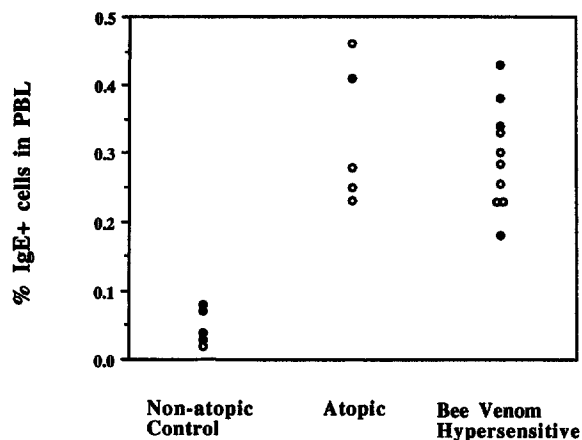


FIG. 4. Direct enumeration of IgE⁺ cells in PBLs from each of the three donor groups. Filled circles, Cell count carried out on 100,000 cells, $n = 3$; open circles, cell count carried out on 10,000 cells, $n = 20$ (see text).

fluorescence was carried out with PE-labeled CD3. The T-cell population was then gated out, and the remaining cells were analyzed for dual expression of CD38 and IgE cells (Fig. 6). This showed that all IgE⁺ cells in the CD3-negative population expressed CD38.

Mean expression of PCA-1 on IgE⁺ cells in the donor groups was 14%, 7.2%, and 6.4%, respectively; and the expression of PCA-1 on PLA-2⁺ cells was 11% (Table II).

The expression of the low-affinity Fc ϵ RII marker (CD23) on IgE⁺ cells was low, with a maximum of 17% in the bee venom-hypersensitive group. Mean expression was 9.9%, 7.8%, and 8.9% from groups 1, 2, and 3, respectively. A mean expression of 10.1% was found for PLA-2⁺ cells (Table II).

The majority (80%) of IgE cells in all three groups expressed CD32 (Fig. 5 and Table II). Cells were triple-stained as in the case of CD38; the CD3-negative population still showed dual expression of IgE and Fc γ RII (Table II).

The expression of the leukocyte common antigen on IgE⁺ cells was 76% (± 6.1) CD45RO and 18.4% (± 5.8) CD45RA (Fig. 5 and Table II). A similar pattern emerges for PLA-2⁺ cells with 78.1% (± 7.5) CD45RO and 14.3% (± 7.4) CD45RA.

Coexpression of IgE with other serum immunoglobulins

Very few IgE⁺ cells isolated from PBLs coexpress other immunoglobulin isotypes (Table II). It was noted, however, that a slightly higher proportion of nonatopic donors (14.7%) compared with

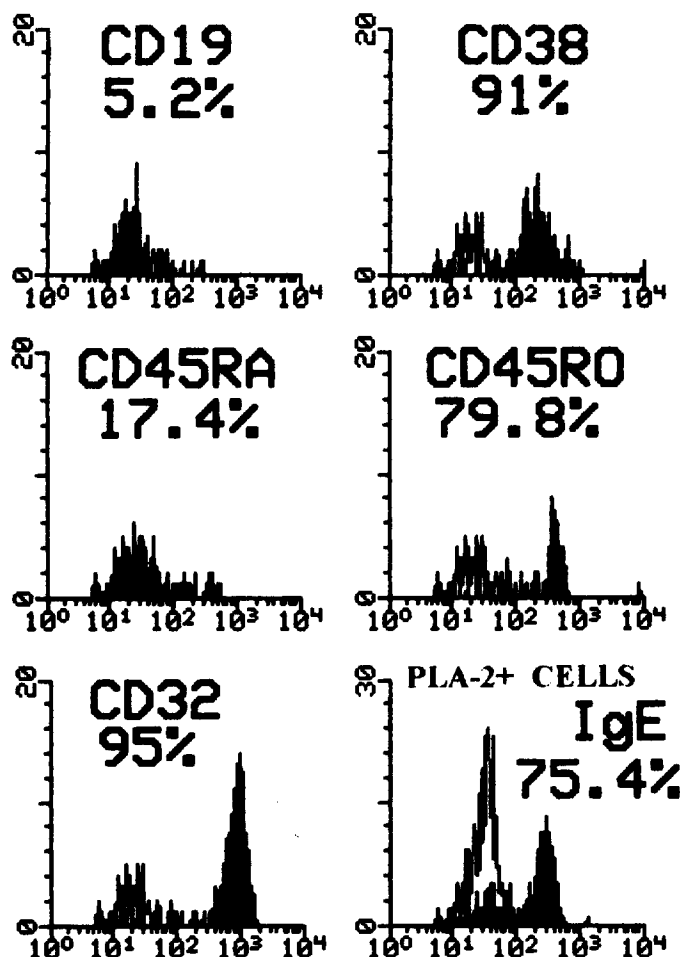


FIG. 5. Histograms showing the percent expression of CD19, CD38, CD32, CD45RO, and CD45RA on cells collected by real-time gating on the IgE⁺ lymphocyte population. The lower right histogram shows expression of IgE on PLA-2⁺ population. The negative control (X63) is superimposed on each graph (unshaded area).

atopic and bee venom-hypersensitive donors (7.4%) coexpressed other immunoglobulin isotypes. On PLA-2⁺ cells isolated from bee venom-hypersensitive donor PBLs, 59.6% expressed IgE class antibody, and an average of 14.8% coexpressed other isotypes.

DISCUSSION

The data described here show that IgE⁺ cells in both atopic and nonatopic individuals differ in their phenotype from typical CD19⁺/CD20⁺/CD21⁺ circulating B cells. The majority of IgE⁺ cells expressed CD38 (79% to 85%), CD32 (79% to 86%), and CD45RO (76% to 78%) but lacked the typical B-cell markers CD19, CD20, CD21, and CD22 (7% to 15%). The presence in the IgE population of up to 14% of cells staining with CD14 suggests that populations of up to 15% of the IgE⁺ (or PLA-2⁺) subset should be inter-

preted with caution. Because basophils have a high-affinity IgE receptor, the possibility arises that IgE⁺ cells analyzed in blood are in fact basophils. Although it is likely that some of the IgE⁺ cells seen in the absence of the stringent precautions described to reduce Fc-receptor-mediated interference are actually basophils, the IgE⁺ cells that are the subject of this article are not basophils because: (1) they do not coexpress kappa and lambda light chains (IgE taken up by Fc-receptor would show a mixture of light chains); and (2) they do not express CD13 and CD33, both of which are expressed by basophils.¹³⁻¹⁷ In the case of bee venom-hypersensitive donors, there is a strong correlation between PLA-2⁺ cells and IgE⁺ cells, suggesting that in the early stages of immunotherapy, these two populations are the same. In all of these donors a small population of IgE⁺ cells with specificity for PLA-2 was found in peripheral blood.

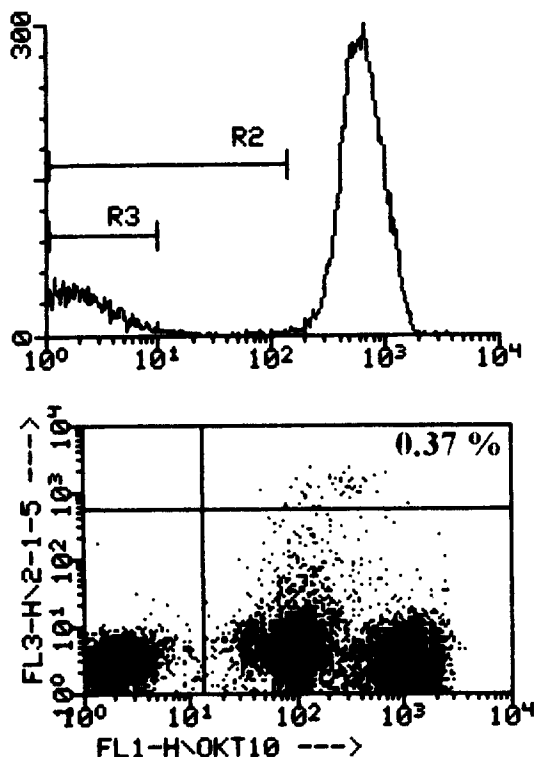


FIG. 6. Expression of CD38 on IgE⁺ cells with three-color staining. **Top.** Histogram of CD3 expression on lymphocytes triple-stained with anti-IgE, CD38, and CD3. Region R2 shows the gating for collection of cells, and R3 shows the gating used to generate the dot plot in the bottom panel. **Bottom.** Dot plot of cells stained with OKT10 and IgE after gating out the CD3-negative population. Quadrants set for IgE⁺ parameters were selected by the generation of a histogram as described in the legend to Fig. 2.

A direct count of the numbers of IgE cells in PBLs was made from data on 10,000 and 100,000 cells. By directly counting IgE⁺ populations in peripheral blood, any bias was eliminated, which may be encountered with other enumeration methods that utilize EBV transformation and culturing in the presence of IL-4.⁴⁻⁶ King et al.^{18, 19} enumerated IgE B cells by an IgE-specific immunoplaque assay (ELISPOT). Although the lower end of the range (0.19% to 1.6%) of IgE⁺ cells obtained for individuals with hyperimmunoglobulinemia E recurrent infection syndrome is comparable to our estimation of 0.3% IgE⁺ cells in PBLs from atopic donors, our estimations for IgE⁺ cells in nonatopic subjects is higher than their finding of up to 0.002%. The studies of King et al.^{18, 19} involve an estimation of the numbers of B cells by flow cytometry with anti-CD19. A direct comparison with this study is therefore difficult because we

have shown that most IgE⁺ cells do not express CD19.

CD19 and CD20 are expressed at all stages of B-cell differentiation,^{20, 21} whereas CD21 and CD22 are expressed only on mature B cells.^{22, 23} FMC7 antibody, which reacts with a 105 kd glycoprotein (p105) expressed on all mature B cells, has previously been shown to be concordant with the expression of CD22,²⁴ and this is reflected in our results (Table II). Because B cells differentiate toward the plasma cell stage, expression of these B-cell markers is lost. From the data presented here, showing a lack of typical B-cell markers, it would appear that IgE⁺ cells in peripheral blood are more highly differentiated. Indirect evidence that the IgE⁺ cells here are of B-cell lineage is presented by demonstrating the expression of kappa or lambda light chains, but not both, on real-time-gated IgE⁺ cells. This is further supported by the expression of CD38 on the non-T-cell population of lymphocytes. CD38 is expressed on T cells²⁵ and plasma cells and on more than 80% of B cells.²⁶ Few IgE⁺ cells express PCA-1, which has been shown to be expressed on cells only after surface immunoglobulin has been lost.²⁷ This would suggest that the IgE⁺ cells are of B-cell lineage but are only seen at a differentiation stage between B-cell and plasma-cell stages.

The expression of CD5 on B cells is complex,²⁸ and several B-cell subpopulations have been identified.²⁶ CD5 is expressed on 30% of IgE⁺ cells; however, no indication of the particular subpopulation was determined.

The expression of CD45 isoforms on IgE⁺ cells is in contrast to the expression on other surface immunoglobulin B cells.⁶ Jensen et al.²⁹ have demonstrated CD45RO expression on highly differentiated normal and malignant B cells, and the presence of CD45RO on IgE⁺ cells is therefore consistent with our other data showing IgE⁺ cells in blood to be a highly differentiated population.

The low-affinity FcεRII (CD23)³⁰ is expressed on 70% to 80% of PBLs,²⁶ as compared with a mean expression of 8% on IgE⁺ cells. This is interesting in view of the fact that IL-4, which is known to upregulate expression of CD23 on B cells,³¹ is a requirement for IgE production. Other investigators have shown that expression of CD23 on PBLs is elevated in atopic individuals.³² The lack of CD23 expression shown here is not contradictory because the earlier studies have not specifically selected IgE⁺ cells.

CD32, the 40 kd (FcγRII),³³ is expressed on IgE⁺ cells. The monoclonal antibody 41H.16 de-

fects this molecule on more than 95% of B cells and between 19% and 42% of T cells.³⁴

Class switching involves deletion of upstream heavy chain genes, so that IgE⁺ cells would be expected to have lost the ability to produce IgM, IgD, and IgG. However, B cells can produce multiple isotypes by differential splicing of long mRNA transcripts. Coexpression of IgE with other isotypes has been described for human cell lines.^{5,35} More recently, it has been shown that class switching must occur for IgE to be produced and also that this class switching occurs in a sequential fashion.³⁶ Our observation that the majority of IgE⁺ cells do not coexpress other isotypes indicates that most IgE cells are irreversibly switched and pertains to the mechanism of immunotherapy, because it implies that switching of IgE cells back to IgG production is an unlikely mechanism.

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