The detection of IgE-secreting cells in the peripheral blood of patients with atopic dermatitis

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We report, for the first time, the identification of IgE-secreting cells in human peripheral blood with an ELISA plaque assay that detects the fingerprint of individual IgE-secreting cells. No IgE-secreting cells could be detected in the blood of normal individuals (IgE, <100 IU/ml) or atopic patients (IgE, <1000 IU/ml), but in patients with atopic dermatitis (AD) whose IgE was >2000 IU/ml, there was an average of 49 ± 9 IgE-secreting cells per 10^6 peripheral blood mononuclear cells (PBMNCs). The rate of IgE production per cell per day from the PBMNCs of patients with AD varied from 0.051 to 0.628 IU/ml, and the number of IgE-secreting cells was positively correlated with the serum-IgE levels of these subjects ($r = 0.74$; $p < 0.001$) and the amount of IgE detected in the culture supernatant ($r = 0.085$; $p < 0.02$). Secretion of IgE by these cells could be completely inhibited (96.2% ± 3%) by the addition of 75 μg of cyclohexamide to the cultures. Preformed intracellular IgE comprised 10% of the IgE detected in the supernatants of 7-day cultures. PBMNCs from patients with AD depleted of monocytes by adherence and T cells by E rosetting, all contained some detectable IgE-secreting cells, whereas isolated T cells and monocytes did not, supporting the view that cells secreting IgE that were detected were indeed B cells. (J ALLERGY CLIN IMMUNOL 1992;89:895-904.)

Key words: *IgE-secreting cell, ELISA plaque assay, atopic dermatitis, in vitro IgE production*

Recombinant cytokines have recently been demonstrated to determine the class and subclass of immunoglobulin produced by LPS-stimulated murine B cells.[1-4] Of particular interest is the observation that high concentrations of IL-4 cause large amounts of IgE production from these cells.[5] A number of groups have also reported that IL-4 enhances IgE production from human PBMNCs,[6-9] although the amounts produced are small. Epstein-Barr virus–stimulated purified human peripheral blood B cells have been demonstrated to switch on IgE production in the presence of IL-4, but even here the amounts of IgE produced do not exceed 40 to 50 ng/ml[10] compared with 3 to 4 μg/ml for LPS-stimulated murine spleen cells.[4] Furthermore, these quantities are only reached after prolonged culture (more than 14 days). Little is known about the numbers of B cells that can be stimulated to produce IgE in humans or about the amounts of IgE produced by such cells.

Abbreviations used

PBMNC:	Peripheral blood mononuclear cell
LPS:	Lipopolysaccaride
IL-4:	Interleukin-4
AP:	Alkaline-phosphatase
HBSS:	Hanks' balanced salt solution
SRBC:	Sheep red blood cell
FCS:	Fetal calf serum
AET:	2-Aminoethylisothiouronium hydrobromide
AMP:	2-Amino-2-methyl-1-propanol
5 BCIP:	5-Bromo-4-chloro-3-indolyl phosphate
PBS:	Phosphate-buffered saline
NA:	Nonadherent
FITC:	Fluorescein isothiocyanate
AD:	Atopic dermatitis

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TABLE I. The relationship between the level of serum IgE, the number of IgE-secreting cells, and the amount of IgE produced per cell

	Serum IgE (IU/ml)	Spots/10^6 cells	IU/ml IgE Spot/day
Cell line		5×10^5	0.023 ± 0.003
Patients			
H. Y.	9675	44 ± 5	0.055 ± 0.020
A. B.	3813	13 ± 3	0.628 ± 0.298
S. S.	2153	7 ± 3	0.229 ± 0.023
S. E.	4760	195 ± 41	0.051 ± 0.014
M. D.	2168	12 ± 4	0.080 ± 0.030
A. V.	2530	11 ± 5	0.141 ± 0.050
S. H.	2255	42 ± 8	ND
A. F.	27840	73 ± 9	ND
D. R.	7620	30 ± 7	ND
Mean	7260	49 ± 9	0.197 ± 0.075

ND, Not done.

Previous studies on IgE regulation in vitro have focused on the measurement, in the supernatants of PBMNC cultures, of spontaneously secreted IgE.[11-13] PBMNCs from atopic patients cultured in vitro have previously been demonstrated to secrete detectable quantities of IgE during a 7-day period, but spontaneously secreted IgE has not been detected in the cultures of PBMNCs from nonatopic patients. This study was undertaken to determine whether there were IgE-secreting cells in the peripheral blood of patients with atopic disease of differing severity and, if this were so, to identify the cells from which the IgE is derived and to determine the amounts produced.

MATERIAL AND METHODS

Materials

Mouse antihuman IgE clone 7.12 was a kind gift from Dr. A. Saxon (University of California-Los Angeles, Los Angeles, Calif.). The CB 3524 plasmacytoma cell line secreting human/mouse IgE anti-NP was obtained from the European Collection of Animal Cell Cultures (Division of Biologics, Public Health Laboratory Service Center for Applied Microbiology and Research, Porton Down, Salisbury, U.K.). Pokeweed mitogen, 5 BCIP, P-nitrophenyl phosphate tablets, Triton X-405, AMP and AET were purchased from Sigma Ltd. (Poole, Dorset, U.K.). AMPAK AP-amplifier kits were a kind gift from Novo Bio Laboratories (Cambridge, U.K.). AP rabbit antihuman IgE was prepared as described previously.[14] Fab' anti-IgE–conjugated AP was prepared by A. Johannsson, Novo Bio Laboratories. Nunc, sterile 96-microwell, flat-bottomed plates, maxisorb ELISA plates, tissue-culture tubes (10 ml), and flat-bottomed flasks (250 ml) were purchased from Gibco Ltd. (Paisley, Scotland). All other reagents were purchased from British Drug Houses Ltd. (Dagenham, Essex, U.K.). The purity of all the different cell populations was determined with FITC-labeled monoclonal antibodies specific for CD3 (Leu-4, pan-T cell) and CD14 (Leu-M3, monocyte) (Beckton Dickinson, Cowley, U.K.) with the fluorescein-activated cell scanner (Beckton Dickinson).

Culture reagents

Ficoll-Hypaque was purchased from Nycomed (Copenhagen, Denmark). Percoll and heparin were purchased from Pharmacia/LKB, Plc. (Milton Keynes, U.K.). RPMI 1640, containing L-glutamine, Dulbecco's minimal essential medium, HBSS, and SRBCs were purchased from Gibco Ltd. FCS was purchased from Sera Laboratories (Crawley, Sussex, U.K.). Penicillin and streptomycin were purchased from Gibco Ltd.

Culture of IgE-secreting cell line CB 3524

The CB 3524 plasmacytoma cell line secreting human/mouse IgE anti-NP was cultured in Dulbecco's minimal essential medium supplemented with 10% heat-inactivated FCS in flat-bottomed flasks in a 5% CO_2-humidified atmosphere at 37° C. Cells were maintained at a concentration of 3 to 9 $\times$ 10^5/ml, fed every 2 to 3 days, and split as necessary.

Subjects studied

Blood was collected from six nonatopic control subjects, seven atopic subjects, and nine patients with AD. Serum-IgE levels were <100 IU/ml in the nonatopic control subjects, <1000 IU/ml in the atopic subjects, and >2000 IU/ml in the patients with AD. Atopy was defined as a positive skin prick test to grass pollen, dust mite, or cat (3 mm diameter wheal greater than wheal of saline control) and history of one or more of the following: rhinitis, asthma, and conjunctivitis. The patients with AD have been described in detail previously.[15] None of the subjects had received oral steroids or other immunomodulating drugs for 2 weeks before the study.

Preparation of PBMNCs

Venous blood (50 ml) was collected in preservative-free heparin (20 U/ml), diluted 1:1 with HBSS, and divided

into 20 ml lots, and the PBMNCs were isolated by centrifugation over 10 ml of Ficoll-Hypaque at 1000 *g* for 20 minutes at 18° to 25° C. The PBMNCs collected from the interface were washed at 4° C and centrifuged at 450 *g* twice in HBSS and once in RPMI 1640 containing 5% FCS. The number of viable cells was determined by trypan blue exclusion, and the cells were resuspended at the appropriate concentration in RPMI 1640 containing 10% FCS and 2 U/ml of penicillin/streptomycin.

Separation of T and non-T cells

AET-treated SRBCs were prepared by washing 2 ml of SRBCs with HBSS buffer at 300 *g*, resuspended in 2 ml of HBSS, and activated with freshly prepared sterile-filtered AET (0.5 gm/12 ml H_2O, pH 9.0, adjusted with 10 mol/L of NaOH) for 30 minutes at 37° C. The AET-treated SRBCs were washed three times as before and stored as a 5% suspension for up to 18 hours at 4° C. To 5 × 10^6/ml of PBMNCs in RPMI/10% FCS, 1 ml of a 5% suspension of AET-treated SRBCs was added. The cells were then centrifuged at 200 *g* for 7 minutes and incubated at 4° C overnight or for 1 hour at 37° C. The rosettes were resuspended by gentle rotation, washed at 4° C three times with RPMI/10% FCS at 450 *g*, resuspended in 5 ml of RPMI/10% FCS, and counted. Viability and the percentage of rosettes were determined, and the rosettes were separated by centrifugation at 650 *g* for 20 minutes at 18° to 20° C over 10 ml of Ficoll-Hypaque. T cell rosettes were recovered after flash lysis (20 seconds) of the SRBCs with 2 ml of sterile water and washed three times at 4° C with RPMI/10% FCS at 450 *g*. The T cells were then resuspended in 5 ml of RPMI/10% FCS and counted. The T cell-enriched fraction contained >97% T cells with FITC-labeled anti-CD3 (Leu-4, Beckton Dickinson) by fluorescein-activated cell scanner analysis. In the experiment described in Table II and Fig. 5, T cells were prepared by rosetting followed by adherence to a plastic Petri dish as described below.

Monocyte depletion

Monocytes were isolated from PBMNCs by adherence to plastic Petri dishes. Briefly, 10 ml of the PBMNCs at 2.5 × 10^6 cells per milliliter were added to Petri dishes and incubated for 1 hour at 37° C. The NA cells were gently aspirated with HBSS (NA cells would contain T cells), and the adherence cells were rinsed and eluted by incubation with PBS (Ca^{++} and Mg^{++} free) containing 5 mmol/L of glucose for 15 minutes at 37° C. The cells were then removed with a rubber policeman (the rubber-tipped plunger of a 1 ml sterile syringe). The adherent cells were adjudged to contain >80% monocytes with FITC-labeled monocyte-specific marker CD14 (Leu-M3, Becton Dickinson).

Fractionation of non-T cells

Non-T cells were further separated by centrifugation on a discontinuous Percoll gradient in 10 ml tissue-culture tubes as described previously.[16] Non-T cells were mixed with 20% Percoll and layered onto the Percoll gradient and centrifuged at 1000 *g* at 18° to 20° C for 20 minutes. Four non-T cell fractions were collected. Those fractions >45% Percoll were designated B_1; those fractions from the 45% to 50% and 50% to 55% interfaces, B_2 and B_3; and those fractions from the bottom of the tube, B_4. Cells were washed three times in RPMI/5% FCS before use.

TABLE II. The identity of the IgE-secreting cells

Patient	No. spots/10^6 cells added			
	Non-T	T	NA	Adherent
H. Y.	116 ± 7	0	ND	ND
S. S.	20 ± 10	0	ND	ND
M. D.	21 ± 7	0	14 ± 5	0
S. H.	152 ± 14	0	45 ± 9	Faint blueing of wells
A. F.	230 ± 17	0	69 ± 12	0

ND, Not done.
Non-T cells represented PBMNCs depleted of T cells by rosetting with AET SRBCs. T cells were prepared from PBMNCs by rosetting with AET SRBCs followed by removal of adherent cells to plastic Petri dishes. Adherent cells were prepared from PBMNCs by adherence to plastic Petri dishes. NA cells were cells that did not bind.

Culture conditions

Cells were prepared at concentrations of 1, 2, and 4 × 10^5 ml in RPMI containing 10% FCS and 2 U/ml of penicillin/streptomycin at a final volume of 1 ml in tissue-culture tubes, or at 200 μl per well in 96-microwell plates and cultured in a 5% CO_2-humidified atmosphere at 37° C. Unless it is otherwise indicated, all cell cultures were performed in duplicate. Some cultures were supplemented with the protein synthesis–inhibitor, cyclohexamide (75 μg/ml), to distinguish IgE that was synthesized de novo from preformed IgE released from the cultured cells. Acid extraction was used to evaluate the amount of cell-associated IgE before and after culture as described previously.[17]

ELISA plaque assay

IgE-secreting cells were detected with the ELISA plaque assay, as described previously, with modifications.[18] Optimal conditions were determined with the CB 3524 human/mouse cell line. All substrate reagents were sterile filtered. The principle of the assay is illustrated in Fig. 1. Briefly, microtiter plates were coated with mouse monoclonal antihuman IgE clone 7.12 at 10 μg/ml in 0.1 mol/L, pH 9.6, sodium bicarbonate buffer at 50 μl per well overnight at 4° C or at 37° C for 1 hour and washed three times in ELISA wash (PBS, pH 7.4, 0.05 mol/L/0.05% Tween 20). The cells were washed and added to the anti-IgE–coated plate. Cell-free wells and wells to which a known concentration of human IgE had been added were included as controls. The cells were incubated overnight at 37° C with the anti-IgE–coated plates and washed under nonphysiologic conditions with ELISA wash buffer that lysed the cells; 50 μl of AP rabbit antihuman IgE (1/300 in ELISA assay diluent, PBS/0.5% Tween 20/1% horse serum) was added to the plates and incubated overnight at

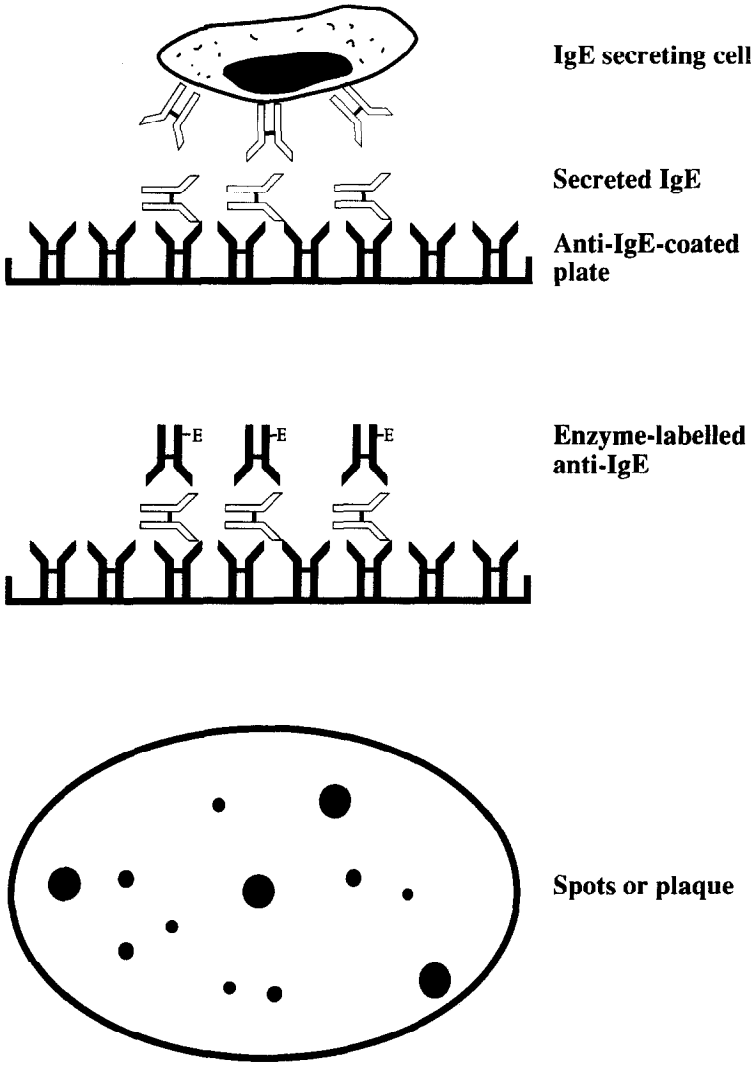

FIG. 1. Principle of the ELISA plaque assay.

4° C or for 3 hours at room temperature, after which they were washed three times as before. No difference was observed in the number of spots elicited when these different incubation times/temperatures were used.

AMP substrate buffer was then prepared by dissolving 100 μl of Triton X-405 (at 30° C), 150 mg of MgCl $6H_2O$, and 1 gm of NaN_3 in 500 ml of distilled water, 98.5 ml of AMP at 30° C was added, and the mixture was stirred for 1 hour and made up to 900 ml with distilled water. The pH was adjusted to 10.25 with concentrated HCl and, after an overnight incubation at room temperature, was checked. The volume made up to 1 L with distilled water. The plates were washed as before, and 200 μl of 5 BCIP/agarose was added. This was prepared by mixing 4 ml of 5 BCIP (1 mg/ml in AMP buffer) at 40° C plus 16 ml of 3% wt/vol agarose (in distilled water) at 60° to 70° C. Within 15 to 20 minutes the "plaques" began to appear as macroscopic blue spots easily visible to the naked eye. The plates were left covered at 4° C overnight to allow the color to develop and were then counted with the aid of a hand lens, original magnification × 10. Because the number of cells that could be added to each well was limited to around 4×10^5 and the frequency of the cells in PBMNCs was low, cells were cultured in replicates of 25, and the values were corrected to IgE plaques per 10^6 PBMNCs.

The assay was fully optimized. The concentration of anti-IgE–coating antibody and the type and concentration of AP-labeled anti-IgE–detector antibody were found to be important. The use of the Fab′ fragment of the anti-IgE AP conjugate reduced the background color but also caused the spots to be fainter and difficult to observe. No additional spots were detected after the first 3 hours of incubation of the cells with the anti-IgE–coated plate, but after 24 hours, the plates became infected with bacteria because incubation of the cells with the plates was not performed under sterile conditions. The intensity of the spots was greater at 24 than

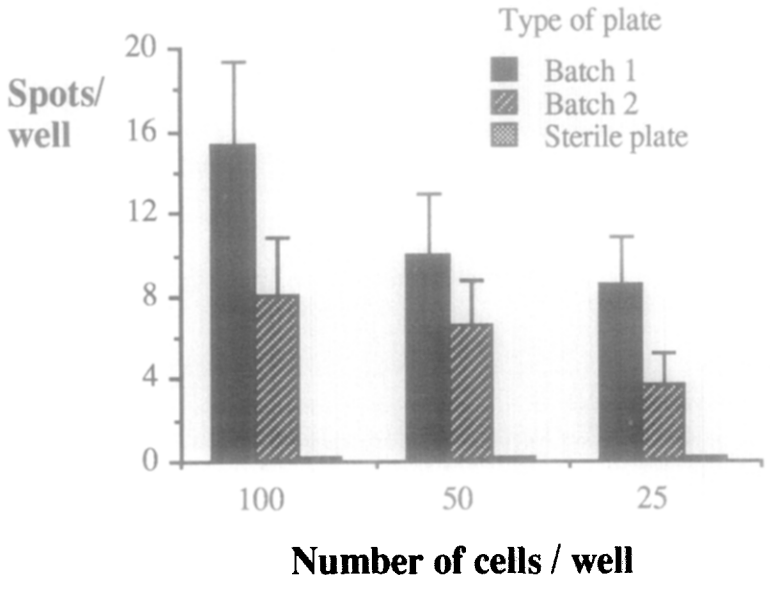

FIG. 2. Number of IgE plaques formed by IgE-secreting B cell line, CB 3524, with two batches of Nunc Maxisorb ELISA microtiter plate with the Nunc sterile microtiter plate.

at 3 hours. Variation between replicate wells did not exceed 25% except when numbers of IgE-secreting cells were less than five per well. No spots were observed in the cell-free wells, although the occasional nongranular, intensely stained artifact was observed. These artifacts were not difficult to distinguish from the IgE plaques. The frequency with which IgE-secreting cells could be detected was determined with the plasmacytoma cell line (CB 3524) and was found to be 47%. Failure to detect some spots might be explained because some of the cells would probably be in the process of dividing or dying. Indeed, when the cell line was first used, nearly all added cells yielded spots.

IgE ELISA

Total serum IgE levels were determined by a previously described two-site ELISA.[14] The interassay coefficient of variation was 6.1%. An ultrasensitive ELISA was used to measure IgE in the culture supernatants.[19] The interassay coefficient of variation was 9.2%.

Statistical analysis

All analyses were performed on an Apple Macintosh computer (Apple Computer, Inc., Cupertino, Calif.) with Statview+ (BrainPower, Inc., Calabasas, Calif.). Comparison of IgE and the number of IgE-secreting cells were by linear regression. Because the absolute detection rate for IgE-secreting cells is not known, we have assumed it to be 100%. These data, obtained with the IgE-secreting plasmacytoma cell line (CB 3524), suggested that approximately 50% of these cells could be detected. This finding is likely to be an underestimate since some of these cells were dividing. Indeed, in some of the first experiments with this cell line, as many as 80% of the cells yielded spots.

RESULTS

ELISA plaque assay

The assay was developed with the mouse/human plasmacytoma IgE-secreting cell line, CB 3524. A variety of plastic tissue-culture plates were tested for suitability in this assay. No spots could be detected with NUNC 24-well square plates or Sterilin 25-well plates (Sterilin Ltd., Stone, England) described by other investigators.[18, 20] The best results were obtained with the NUNC 96-well maxisorb microtiter plates that we routinely use for ELISA (Fig. 2), although some batch-to-batch variation was noted. Pretreatment of plates with gluteraldehyde did not increase sensitivity (results not presented). The formation of spots could be completely inhibited when 100 IU of highly purified human myeloma IgE (patient W. T.), but not a similar quantity (250 ng) of IgG, was added to the wells (results not presented), confirming the specificity of the assay.

Detection of IgE antibody-secreting cells in human peripheral blood

IgE spots were detected in the PBMNCs of patients with AD. These spots and the spots from the cell line (Fig. 3, *A* and *B*) had a granular appearance (Fig. 3, *C*). The spots obtained with both the cell line and the PBMNCs from patients with AD were not all the same size, indicating that different amounts of IgE were secreted by some cells. This finding was probably caused by differences in the age of individual

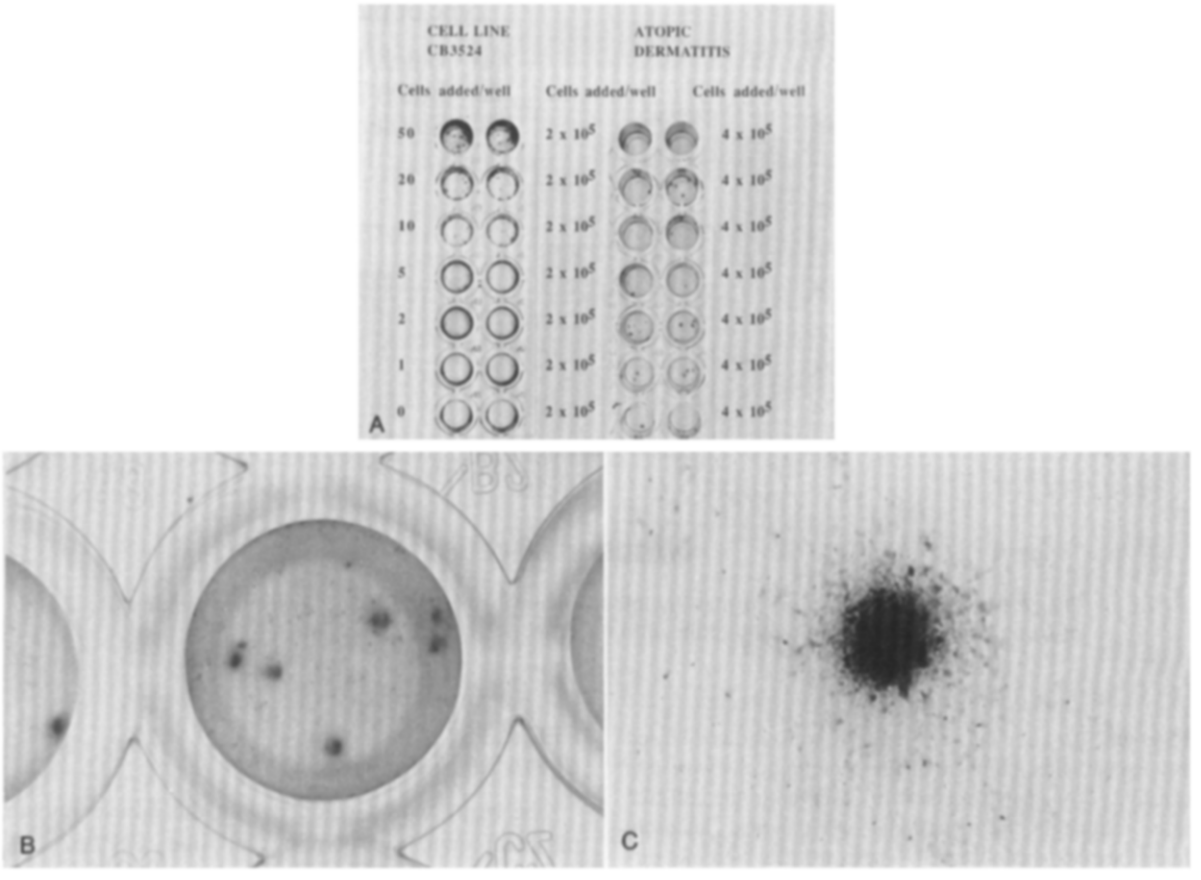

FIG. 3. A, Typical appearance of two different types of spots obtained from cell line and PBMNCs of patient with AD revealing large definite spots and numerous smaller spots. **B,** Original magnification ×15. **C,** Original magnification ×100; 200 μl of cell suspension was added to the wells.

cells. The number of IgE-secreting cells from the PBMNCs of nonatopic and atopic subjects and patients with AD was determined with the ELISA plaque technique (Fig. 4). Cells actively secreting IgE were only detected in the blood of patients with AD. No such cells were observed with PBMNCs from blood of atopic or nonatopic subjects.

Relationship between IgE-secreting cells and serum and culture supernatant IgE

The number of spots detected from the cell line, and from all the patients with AD tested, closely paralleled the amount of IgE secreted in replicate sterile cultures (Fig. 5). The level of serum IgE of the donor, the number of IgE-secreting cells, and the amount produced by their cultured PBMNCs were compared (Table I). The number of IgE-secreting cells was closely correlated with both the serum-IgE levels of the patient ($r = 0.74$; $p < 0.01$) and the amount of IgE detected in the culture supernatant ($r = 0.85$; $p < 0.01$).

IgE synthesis compared with secretion of preformed IgE

To determine whether the IgE secreted was newly synthesized, a protein synthesis inhibitor, cyclohexamide (75 μg/ml), was added to the culture. Much less IgE (96.2% ± 3%) was measured in the supernatants of cultures to which cyclohexamide had been added than that in parallel cultures (results not presented). Acid extraction of the cell pellets of cultures on day 0 and day 7 revealed that approximately 10% of the IgE detected in the culture supernatant after 7 days was present in the pellet on day 0 (results not presented). This finding suggests that most of the IgE in the culture supernatant is the result of de novo synthesis.

Identity and heterogeneity of IgE-secreting cells

As mentioned above, even with the IgE cell line, CB 3524, the spots detected varied in size and inten-

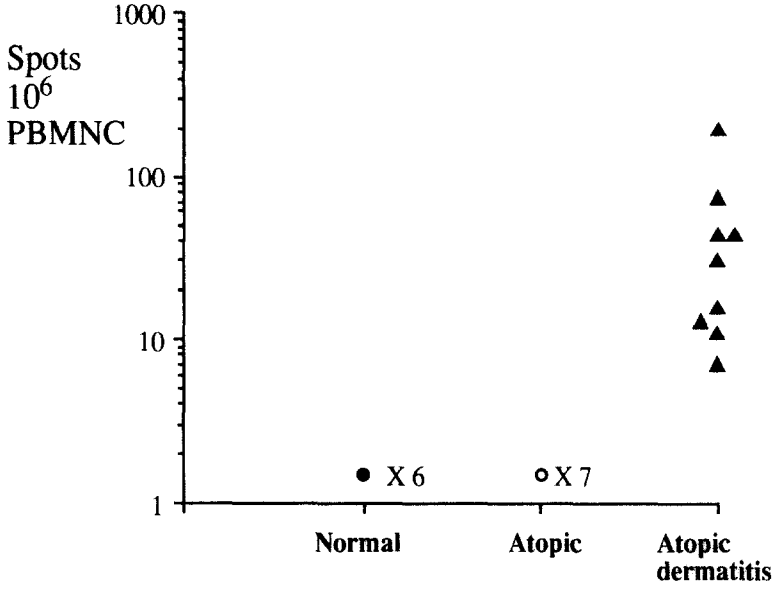

FIG. 4. Number of IgE spots detected in the PBMNCs of normal subjects (●), of atopic subjects (○), and atopic patients with AD (▲).

sity. A more clear-cut difference in spot size was observed in the PBMNCs of patients with AD. Two distinct sizes of spots were observed: large spots, easily visible to the naked eye that are the spots recorded in this article, and smaller spots that were more numerous but were not present in cell-free wells.

To determine the identity of these seemingly different populations of IgE-secreting cells, the number of spots in different lymphocyte populations was determined (Table II and Fig. 5). Non-T cells were PBMNCs depleted of T cells by rosetting with AET SRBCs. T cells were prepared from PBMNCs by rosetting with AET SRBCs, followed by removal of adherent cells to plastic Petri dishes. Adherent cells were prepared from PBMNCs by adherence to plastic Petri dishes; NA cells represented those PBMNCs that did not bind. The number of IgE-secreting cells was determined by the ELISA plaque assay at a concentration of 4×10^5 per well, and the distribution of spot size was compared in the different fractions. That the IgE antibody-secreting cells are restricted to the non-T fraction is presented in Table II, although some faint blueing of the wells was observed with one adherent cell population. Removal of monocytes by adherence did not reduce the proportion of spots detected (NA cells), and no spots, faint or large, were observed from wells containing adherent cells alone. The non-T cells were further fractionated by density on a Percoll gradient, and the different fractions were assayed for IgE secreting cells. The B_1 and B_2 fractions contained, between them, 87% of the IgE-secreting cells,

TABLE III

Cell line CB 3524	AD	
Cells added/well	Cells added/well	Cells added/well
50	2×10^5	4×10^5
20	2×10^5	4×10^5
10	2×10^5	4×10^5
5	2×10^5	4×10^5
2	2×10^5	4×10^5
1	2×10^5	4×10^5
0	2×10^5	4×10^5

and the faint spots were also restricted to these fractions (Fig. 6). A greater density of faint spots was observed in the B_2 fraction. These results support the view that the IgE antibody-secreting cells detected by the ELISA plaque assay are indeed B cells. Despite removal of 85% of the monocytes, the small spots were still found, and although their identity remains unknown, it is probable that they are B cells.

DISCUSSION

Since the first study determined that IgE was secreted from the PBMNCs of patients with AD,[11] many researchers have reported that the PBMNCs of patients with elevated levels of serum IgE secrete more IgE than PBMNCs of their nonatopic counterparts.[17, 21-23]

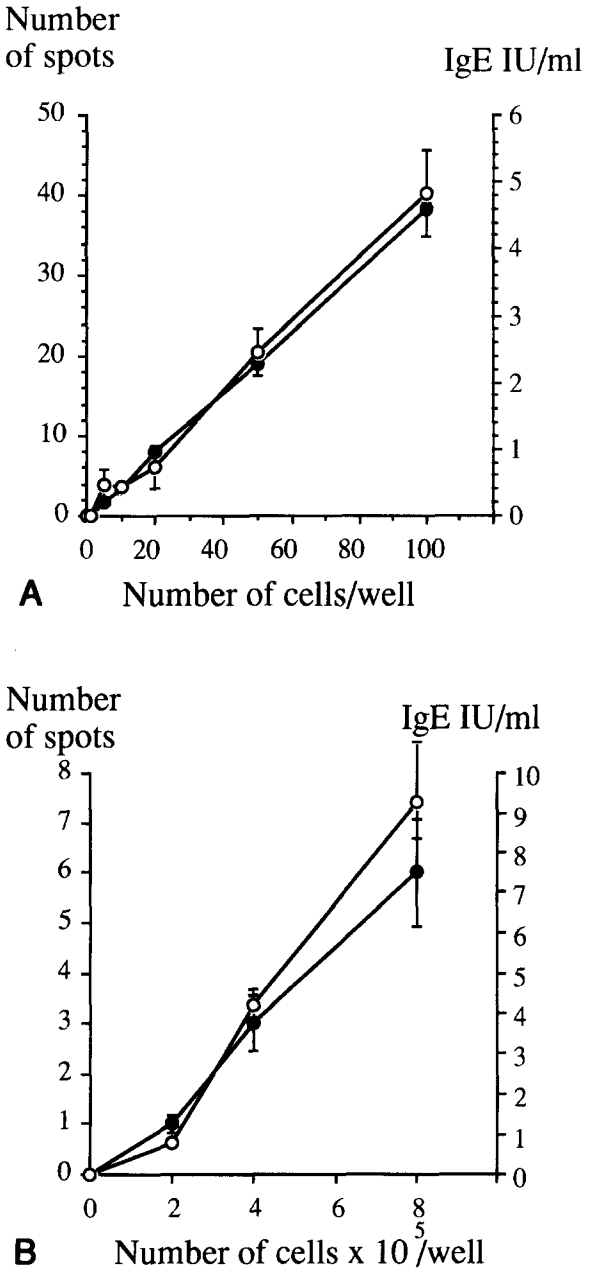

FIG. 5. Relationship between number of IgE-secreting cells (●) and the amount of IgE (○) secreted in parallel cultures. **A**, Cell line of PBMNCs from patients with AD. **B**, IgE cell line from patients with AD.

From previous studies, it is not, however, clear if the number of IgE-secreting cells in human peripheral blood is low or whether these cells produce small amounts of IgE. Indeed, at a recent workshop on B cell regulation in Paris, it was suggested that there might not be any IgE-synthesizing B cells present in the culture at all. Certainly, the amount of IgE detected in vitro is at the lower limits of the assays used.[24] We have previously described an ultrasensitive ELISA capable of reliably determining small quantities of IgE.[19] This study was undertaken to determine whether IgE-secreting cells could be detected in human peripheral blood, with the ELISA plaque assay,[18, 25] to determine the identity of such cells, and to determine how much IgE they produced.

The IgE-secreting cell line CB 3524 was used to develop and optimize the ELISA plaque assay. This assay detects the fingerprint of cells secreting a range of different proteins, in this case, IgE. With optimal conditions, 47% of CB 3524 cells were found to be actively secreting IgE. When the assay was first set up with fresh plasmacytoma cells, close to 100% of those added could be detected. Similar figures have been reported for the U266 cell line[20] and with a number of other myelomas and hybridomas.[25, 27, 28] It is likely that the remaining cells consisted of newly activated, dying, or dividing cells, which would be expected not to secrete much IgE. The assay performed poorly with sterile culture plates, and best results were obtained with the same nonsterile high protein-binding capacity microtiter plates that we use for other ELISAs. It is well-known that different plastics vary considerably in their capacity to bind protein.[29]

The number of IgE-secreting cells in the peripheral blood of nonatopic control subjects, atopic subjects, and patients with AD was determined. No spots were detected in the PBMNCs of any of the nonatopic control subjects or in the PBMNCs of atopic patients without AD studied. However, IgE-secreting cells were found in the peripheral blood of all the patients with AD (IgE >2000 IU/ml), with a mean of 49 ± 9 spots per 10^6 PBMNCs. We have not studied atopic patients whose serum IgE level is <2000 IU/ml and cannot verify if the increased number of IgE-secreting cells is simply a function of high serum IgE, the disease, or a combination of the two. An average of 0.20 IU of IgE was secreted by each IgE-secreting cell per day from the patients with AD. Ten times less, 0.023 IU/ml, was produced from the cell line daily.

As previously reported,[22, 30] most IgE measured in the cell cultures was the result of de novo synthesis, and production was completely abolished by cyclohexamide. Preformed IgE released from cultured PBMNCs of the patients with AD constituted 10% of the IgE present in the supernatant after 7 days of culture. It has recently been demonstrated[31] that monocytes have the capacity to bind IgE and store IgE in intracellular compartments from which it can be released by an antigen-mediated stimulus. In addition, many B cells and monocytes bear CD23 on their surface[32] that binds IgE and therefore provides another

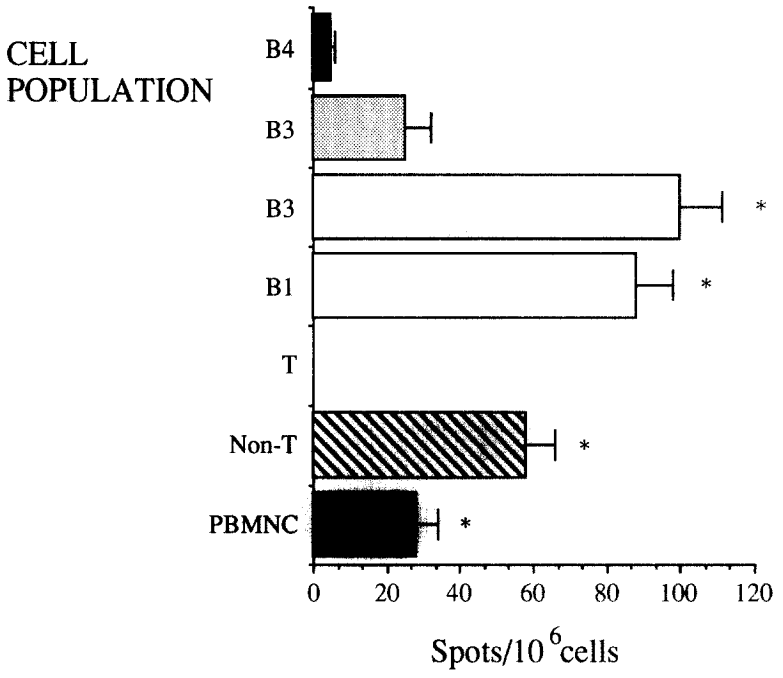

FIG. 6. Number of IgE-secreting cells in PBMNCs, T cells, non-T cells, and in non-T cells separated on Percoll-density gradient. Non-T cells represented PBMNCs depleted of T cells by rosetting with AET SRBC. T cells were prepared from PBMNCs by rosetting with AET SRBC, followed by removal of adherent cells to plastic Petri dishes; $B_{1\text{ to }4}$ fractions were non-T cells prepared as above, applied to a Percoll-density gradient, detailed in MATERIAL AND METHODS; *faint spots.

route through which it can be carried into the culture. Some cell-surface CD23 is shed after activation of B cells, and this finding may contribute to the IgE detected in the culture supernatant. Indeed, the appearance of IgE in the culture supernatants parallel the release of soluble CD23 from the surface of IL-4–stimulated B cells.[8]

The identity of IgE-secreting cells was determined by separation of PBMNCs into T cells or by E rosetting, and into adherent and NA cells by adherence to plastic. The results indicated that the IgE-secreting cells were restricted to the non-T and NA fractions, supporting the view that these cells were B cells. Two populations of spot size were observed: the large definite type of spot enumerated in all experiments and numerous small spots too faint to count. With Percoll[33] to separate the non-T, NA cells, we found that fractions B_1 and B_2 contained >85% of the large IgE-secreting cells and all the faint spots. These fractions are reported to contain activated B cells, fractions B_3 and B_4 containing mainly resting cells. We speculate that the faint spots observed in these fractions may be either dying or newly activated B cells.

What remains unclear is whether the presence of IgE-secreting cells in the peripheral blood of patients with AD simply reflects the fact that they have high serum IgE levels or if it reflects a more extreme form of immunologic abnormality. Studies of patients who have very high serum IgE levels but do not have AD will be needed to resolve this question. T cells of patients with AD do, however, have a reduced capacity to secrete interferon-γ,[34] and PBMNCs from patients with AD, administered exogenous interferon-γ reveal reduced spontaneous IgE synthesis, although serum IgE levels are unchanged.[35, 36]

In summary, IgE-secreting cells could be found only in the peripheral blood of patients with AD (IgE >2000 IU/ml), not in that of atopic patients or normal subjects. The cells secreting IgE were not T cells, were not adherent, were hypodense, and are believed to be plasma cells. A linear relationship exists between the number of IgE-secreting cells and the amount of IgE generated during culture, the amount secreted by individual cells ranging from 0.055 to 0.628 IU of IgE per day. In the supernatants of cultures in which the amount of IgE detected after 7 days was 1 to 2 IU of IgE, we were unable to find a single IgE-secreting cell by the method used.

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