

Increase of ovalbumin (OVA)-specific B cells in the peripheral blood of egg-allergic patients

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Allergen-specific B cells were detected by antigen-coated magnetic beads. The frequency of ovalbumin (OVA)-specific B cells was significantly higher in patients with egg white-allergy than in age-matched nonallergic individuals. These B cells, isolated by the immunomagnetic-beads method, produced anti-OVA antibodies of mostly IgM class when they were transformed by Epstein-Barr virus and cultured for about 2 weeks. Based on a chronologic analysis, the increase of OVA-specific B cells was found to precede the increase of IgG and IgE anti-OVA antibodies in the serum. These observations indicated that OVA-binding B cells in the peripheral blood are already committed to producing IgM antibody and probably are the precursors of antibody-forming cells of the IgG or IgE class. (J ALLERGY CLIN IMMUNOL 1991;87:729-36.)

Allergic individuals characteristically have higher levels of allergen-specific antibodies, especially of IgE, than nonatopic healthy individuals. Previous studies have demonstrated that there is a high incidence of detectable serum levels of antibodies specific to dietary antigen early in the natural history of allergic individuals, and the pathogenic role of food hypersensitivity in atopic disease was discussed.¹⁻³ In such cases, a significantly increased prevalence of IgE as well as IgG antibodies reactive with common dietary protein antigens, such as chicken OVA or bovine milk, has been demonstrated.⁴⁻⁶ An increased number of precursor B cells committed to the specific allergen and, as a result, increased specific immunoglobulin-secreting cells in the peripheral blood, may reflect elevated levels of allergen-specific serum antibodies observed in allergic individuals.

Although a previous article⁷ demonstrated no major difference between healthy and allergic individuals in the size of the pool of precursor B cells that differentiated with activation by EBV (to produce allergen-specific IgM or IgE), it would be possible to demonstrate that some differences may exist. This is because the method used in the earlier study was an indirect method to estimate the number of antigen-specific B cells, which exist in an extremely small

Abbreviations used

OVA:	Ovalbumin
EBV:	Epstein-Barr virus
PBMC:	Peripheral blood mononuclear cell
PBS:	Phosphate-buffered saline
BSA:	Bovine serum albumin
β -LG:	β -lactoglobulin

amount in the peripheral blood.^{8,9} The intent of this study was to determine the frequency of allergen-specific B cells by selecting the cells with magnetic beads coupled with allergen proteins. This method has been demonstrated to be simple and reliable for selecting specific lymphocytes.^{10,11}

The present study examined the specificity of lymphocytes detected by immunomagnetic beads coupled with OVA as an allergen protein. The study also investigated the frequency of OVA-specific lymphocytes in the peripheral blood of allergic patients, as well as the OVA-specific antibody production from B cells thus isolated and transformed by EBV.

MATERIAL AND METHODS

Patients and healthy blood donors

Peripheral blood was obtained from 24 infant patients allergic to egg white (3 to 36 months of age; mean age, 14 months), 11 age-matched allergic children who had symptoms of asthma or atopic dermatitis and elevated levels of serum IgE, but no sign of egg allergy (4 to 36 months of age; mean age, 12 months), 18 age-matched healthy children (4 to 48 months of age; mean age, 16 months), four adult patients allergic to egg white, and six healthy adults. The volume of blood samples was from 3 to 5 ml in each subject of the infants. The allergic patients (except the allergic adults) were studied at their first visit to our allergy clinic,

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when they had not yet received a diagnosis and treatment of food allergy. The subjects entered the allergic groups only after receiving a diagnosis as allergy by additional examinations. All patients with egg white-allergy displayed clinical symptoms of atopic dermatitis and hypersensitivity to egg white diagnosed on the basis of food elimination and challenge tests,¹² elevated serum total IgE levels, and positive scores in RAST for egg white ($>2+$). The control subjects were mainly selected from children in oral surgery wards at the same hospital waiting for minor surgery. The normal control children and adults had no allergic history, had serum total IgE levels <50 IU/ml, and were RAST negative for egg white-specific IgE. Total IgE (radioimmunosorbent test) and specific IgE (RAST) were determined with the Phedebas test (Pharmacia, Uppsala, Sweden).

This study was conducted according to human subject review board, and all subjects and their parents were informed before participating.

Preparations and fractionation of mononuclear cell populations

PBMCs were isolated by Ficoll-Conray density centrifugation, and the cells were washed three times in PBS. At the same time, the serum was obtained, frozen, and stored until the assay for serum anti-OVA antibody.

PBMCs were fractionated into three cell populations, monocytes, B cells, and T cells, by adherent culture and erythrocyte rosette separation. The method is described elsewhere.^{13,14} Briefly, monocytes were isolated by adherence to a plastic culture plate being incubated for 1 hour at 37° C, and then T and B cells were fractionated by double rosette formation with sheep red blood cells pretreated with 2-aminoethylisothiuronium bromide hydrobromide and subsequent density gradient centrifugation. These procedures resulted in a T cell-enriched fraction that was $93.2\% \pm 3.6\%$ (mean $\pm$ SD) CD3⁺, $2.1\% \pm 1.3\%$ CD20⁺, $5.1\% \pm 3.7\%$ CD16⁺, and $0.8\% \pm 0.3\%$ CD14⁺, and a B cell-enriched fraction that was $79.3\% \pm 9.6\%$ CD20⁺, $3.2\% \pm 1.1\%$ CD3⁺, $7.2\% \pm 4.8\%$ CD14⁺, and $8.6\% \pm 2.2\%$ CD16⁺, as determined by cytofluorography with monoclonal antibodies to these human cell-surface antigens. Each enriched cell populations will be referred to as T or B cell fraction. Each cell population was resuspended in RPMI 1640 medium (GIBCO, Grand Island, N.Y.) and used for additional analyses.

Antigen coupling onto magnetic polystyrene beads

OVA (grade V, Sigma Chemical Co., St. Louis, Mo.) was dissolved in 0.5 mol/L of borate solution, pH 9.5, to a concentration of 150 μ g/ml. Paramagnetic polystyrene beads (Dyna beads, M-450 tosylactivated, Dynal, Oslo, Norway) were prepared as aqueous suspension at a concentration of 4×10^8 beads per milliliter (30 mg/ml). The beads were added to equal volumes of OVA solution and then incubated for 24 hours at 22° C by slow end-over-end rotation. Through this procedure, OVA proteins were chemically bound to the bead surface.¹⁵ Then, the beads were

washed three times, 10 minutes each time, and kept overnight at 4° C in 5 ml of PBS. After the beads had been washed, the tube containing the bead suspension was placed in a magnetic field (MPC-1, Dynal), which enabled the beads to be collected and the supernatant to be discarded, as described elsewhere.¹⁶ The beads were resuspended in PBS to a concentration of 4×10^8 beads per milliliter.

The beads, coupled with the other noncross-reactive antigen, β -LG (Sigma Chemical Co.), in the same way as OVA, were also prepared for the control experiment.

Rosetting and positive selection of the target cells by antigen-coupled beads

The cells binding to antigen-coupled particles were separated by the direct method for positive selection, as previously described.^{11,16} In brief, the fractionated cells (1×10^7 cells per milliliter) and OVA-coated beads were mixed in a beads-to-cells ratio of 3:1 and placed in an ice bath for 15 minutes with gentle shaking at intervals. The rosettes and free beads were separated from nonrosetting cells by means of the magnetic field. The rosetting cells were washed five times in PBS for sufficient removal of nonrosetting cells. Both rosetting and nonrosetting cell fractions were resuspended in RPMI 1640 with 10% fetal calf serum (Irvine Scientific, Santa Ana, Calif.). The number of rosettes was counted under a microscope, and the percentage of rosetting cells to total target cells was calculated.

Inhibition of rosette formation by free OVA

The specificity of the binding of antigen-coupled beads to target cells was determined by competitive inhibition. Fractionated cell preparations were tested before and after incubation for 15 minutes at 4° C with the following reagents at designated protein concentrations: OVA, BSA, and β -LG (Sigma Chemical Co.). The percent inhibition was calculated as follows: Inhibition % = $100 \times (1 - [\text{number of rosettes with inhibitor} / \text{number of rosettes without inhibitor}])$.

Transformation of the cells by EBV and cell culture

EBV-containing supernatant (from the B95-8 cell line) was used as the source of virus for transformation of the B cells.¹⁷ This supernatant was filtered (0.45 μ m pore size) before use. Both rosetting and nonrosetting cells from the B cell fraction were incubated overnight in 0.5 ml of medium and 0.5 ml of B95-8 supernatant at 37° C in an atmosphere of 5% CO₂ and 100% humidity. The next day, the suspension of the rosetting cells was pipetted and stirred to detach the binding particles from the cells, and the unbound cells were isolated from particles by use of the magnetic field. The unbound B cells from rosettes, and nonrosetting B cells that had been exposed to EBV-containing supernatant, were finally cultivated in flat-bottomed, 96-well plates (Sumitomo Bakelite Co., Tokyo, Japan) with a 200 μ l of culture medium per well. After about 2 weeks, when the number of cells was sufficiently increased for additional assay, the cells were transferred to 24-well plates

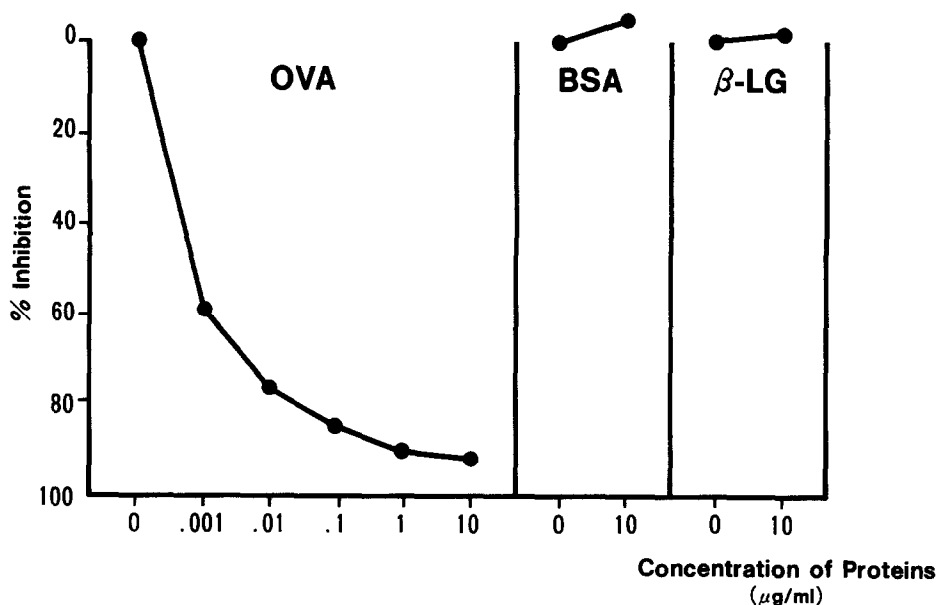


FIG. 1. Competitive inhibition of bead binding to B cells by antigen proteins. B cell fractions from a single patient with egg allergy were incubated with OVA, BSA, and β -LG, at indicated concentrations.

(Costar Corp., Cambridge, Mass.), and further cultured in a 1 ml medium at a concentration of 1×10^6 cells per milliliter for 1 week. Then, the supernatants were collected for ELISA.

ELISA

Culture supernatants of EBV-transformed B cells were assayed by ELISA technique, as described elsewhere.^{18, 19} ELISA for the determination of OVA-specific IgG, IgA, IgM and IgE was performed on 96-well polyvinylchloride flat-bottom plates (Sumitomo Bakelite Co.), which were coated by incubation with 100 μ l of 10 μ g/ml solution of OVA in 0.1 mol/L of sodium carbonate buffer, pH 9.6, at 4° C overnight. The plates were then washed three times with 0.05% Tween 20 in PBS. Samples were added to the wells (50 μ l per well) and incubated at 4° C overnight. The plates were again washed three times with 0.05% Tween 20 in PBS. Antihuman IgG, IgA, IgM, or IgE antibody conjugated to peroxidase (Cappel Laboratories, Cochranville, Pa.) was added to the plates (50 μ l per well at a concentration of 2.5 μ g/ml in PBS containing 1% BSA).

After wells were incubated at room temperature for 2 hours and washed, a solution of azino-bis(3-ethylbenzoline-6-6-sulfonic acid, ABTS) containing H₂O₂ was added to each well and subsequent development of color was detected on a multichannel spectrophotometer (Immunoreader NJ-2000, Nippon InterMed, Tokyo, Japan). The antibody levels were expressed as optical density units at 414 nm, with background being deducted. The assays of the culture supernatants from rosetting and nonrosetting B cells that had been obtained from one individual and transformed by EBV at the same time were performed on the same plate, and the results were compared. Serum samples were diluted 1:1000

in PBS and tested for OVA-specific IgG by ELISA. The values were related to a standard serum rich in the anti-OVA IgG. One unit was defined as the amount of anti-OVA IgG in a 1:1000 dilution of the standard serum.

Estimation of total IgG, IgA, and IgM was performed by almost the same method with plates coated with anti-human IgG, IgA, or IgM (Cappel Laboratories), respectively. The optical density units were correlated to nanograms per milliliter of protein, with standard curves derived from dilutions of titrated reference human antibody. Values were determined in triplicate.

The purity of OVA-specific antibodies secreted by EBV-transformed B cells was determined by absorption with solid-phase antigens. Culture supernatants were added to the 96-well plate that had been coated with antigens, as described above, and after incubation at 4° C overnight, were collected for total immunoglobulin assay by ELISA.

Statistical evaluation

Differences in means were tested for significance with the Student's *t* test.

RESULTS

Specificity of the rosette formation with OVA-coupled particles

B cell fractions that had demonstrated high rosette formation with OVA-coated beads were used to test the specificity of the binding. An example of competitive inhibition of rosetting by OVA is illustrated in Fig. 1. The binding of OVA-coupled beads to cells was blocked by the preincubation of cells with OVA in a dose-dependent manner, and a mean of 92.3%

TABLE I. Percentage of B cells rosetting with OVA-coupled beads in B cell fraction from egg-allergic children, allergic children without egg allergy, nonallergic age-matched control subjects, egg-allergic adults, and healthy adult control subjects

Group	No.	% (Mean $\pm$ SD)
Egg-allergic children	24	4.17 $\pm$ 2.16
Allergic (nonegg) children	11	1.94 $\pm$ 0.46
Nonallergic children	18	0.98 $\pm$ 0.53
Egg-allergic adults	4	2.21 $\pm$ 0.83
Healthy adults	6	0.31 $\pm$ 0.20

(SD, 6.8%; $N = 5$) of the binding was inhibited by 10 $\mu\text{g}/\text{ml}$ of OVA. In contrast, no inhibition was observed with BSA or β -LG.

B cell fractions from the egg-allergic patient group also demonstrated rosette formation with the control beads coupled with β -LG, although the percentages ($2.77\% \pm 1.53\%$; $N = 8$) of cells rosetting with β -LG-coupled beads were significantly lower than percentages ($0.76\% \pm 0.42\%$) with OVA-coupled beads ($p < 0.05$). The rosette formation with β -LG-coupled beads was not blocked by OVA but by β -LG.

Rosette formation of T cells was also inhibited by the addition of OVA (data not presented).

Positive selection of OVA-specific cells and their frequencies in allergic patients and healthy individuals

B cells, T cells, or monocytes from allergic patients and healthy individuals were tested for positive selection by OVA-coupled particles. The B cell fraction contained substantial amounts of rosetting cells with the beads, and the percentage of rosetting B cells was calculated (Table I). The egg-allergic children group demonstrated a significantly higher score than did any of the other groups ($p < 0.01$), and the egg-allergic adult group had a higher percentage than the subjects with other allergy or the nonallergic group. Moreover, the allergic children without egg allergy also demonstrated a higher percentage than age-matched healthy children ($p < 0.05$), and the healthy children demonstrated a higher percentage than the healthy adults ($p < 0.05$).

No rosette formation was observed with the monocyte fraction in the above-mentioned procedure, but monocytes bound and phagocytosed the beads when they were incubated for more than 30 minutes or for 15 minutes at room temperature. The T cell fraction from each group contained a very small amount of

rosetting cells. The percentage of rosettes from T cells ranged from 1:10 to 1:50 of those from B cells (data not presented). Because of the extremely low number of rosetting cells, it is necessary to consider the contamination of B cells to a T cell fraction during separation with sheep red blood cells, although it is possible that OVA molecules fixed on the particles bind to T cell receptors on the surface of OVA-specific T cells. This point is now being investigated.

The percentage of contaminating nonrosetting cells among the isolated rosetting cell population was usually $<5\%$ in these experiments.

Correlation between the frequency of peripheral blood OVA-specific B cells and the titer of serum OVA-specific antibody

The percentages of rosetting B cells and the titers of serum OVA-specific antibody were compared for each group from younger than 5 months to 4 years of age. Anti-OVA IgG antibody was also detected in 21 egg-allergic patients (88%). The means and SD of the percentage of rosetting B cells and the titers of serum anti-OVA IgG and IgE antibody in each age group of the allergic patients are illustrated in Fig. 2, A. The anti-OVA IgG antibody concentration increased for subjects aged 6 months to 3 years, and the anti-OVA IgE antibody concentration elevated to some extent for subjects aged from 3 months, reaching a maximum for those aged 2 years. By comparison, the percentage of OVA-specific B cells increased for subjects as young as 3 months, reaching a maximum for subjects to the age of 1 year and then declining gradually for all subjects to the age of 3 years. There was no significant correlation between the percentage of rosetting B cells and OVA-specific IgE or IgG antibody. The means of the percentage of OVA-specific B cells in each group of nonallergic children is also illustrated in Fig. 2, B. The percentage of OVA-specific B cells was low and almost constant.

Secretion of OVA-specific antibody by lymphoblastoid cells established from B cells selected by OVA-coupled particles

Culture supernatants from the B cells that had been selected by rosetting with OVA-coupled particles and EBV-transformed were examined for OVA-specific antibody by ELISA. The representative results from several experiments in egg-allergic children and healthy control subjects are presented in Table II. In all experiments, OVA-specific IgM antibody levels were significantly higher in the supernatants from rosetting B cells than in supernatants from nonrosetting B cells. OVA-IgG antibody was also detected in most supernatants of rosetting B cells from each group. But

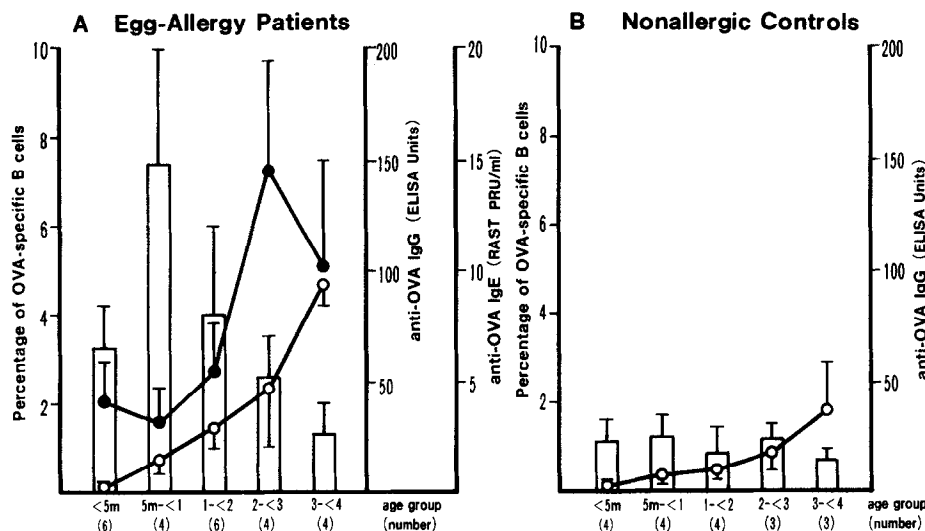


FIG. 2. Chronologic changes in OVA-specific B cell frequency and serum anti-OVA antibody level. **A**, Egg-allergic children. **B**, Nonallergic children. Means and SD values for the percentage of OVA-specific B cells (open bars) and the serum level of IgE (●) and IgG (○) anti-OVA antibody are illustrated for each age group. IgE antibody was not detected in any of the nonallergic children.

OVA-IgE antibody was detected in supernatants of rosetting cells only from two patients allergic to egg. As for the total immunoglobulin production, nonrosetting B cells fully secreted all classes of immunoglobulin, whereas rosetting B cells characteristically produced a comparable amount of IgM, but very small amounts of IgG and IgA in each group.

Specificity of antibody production of lymphoblastoid cells from selected B cells

The culture supernatants were tested by absorption with solid-phase antigens to determine what fraction of total immunoglobulin in the supernatants was represented by OVA-specific antibodies. Typical results for two experiments with selected B cells from the egg-allergic patients are presented in Table III. When the supernatants from rosetting B cells were tested, 65% to 88% of the total IgG and IgM were absorbed by solid-phase OVA, but less by the other antigen, β -LG. However, when the supernatants from nonrosetting B cells of the same donors were tested, the total immunoglobulin was hardly absorbed by any antigens.

DISCUSSION

This study confirmed the usefulness of antigen-coupled magnetic particles for selecting antigen-specific cells from human PBMCs. The study also demonstrated that B cells isolated in this way are actually committed to produce the specific antibody. Methods, such as this one, have recently become widespread with the development of new particle

products. The principle is based on specific binding of the beads to the receptors or antigens on the surface of the cell.

The positive selection of the wanted lymphocytes by magnetic beads coated with specific antigens or monoclonal antibodies has been reported previously.^{11, 16, 20, 21} In this study, the lymphocytes specific to OVA that is a popular allergen in food hypersensitivity were isolated by use of the magnetic polystyrene beads coupled with OVA molecules. The rosette formation was confirmed to be specific to OVA by inhibition assay.

It was also confirmed that B cells isolated by the OVA rosettes were actually committed to producing anti-OVA antibody by the observation that these B cells secreted more OVA-specific antibodies, compared with the controlled B cells, when they were transformed by EBV and cultured for more than 2 weeks. Rosetting B cells produced amounts of total IgM antibodies as large as nonrosetting B cells, and considering these data of the raw ELISA absorbance for anti-OVA antibodies, IgM secreted by OVA-binding populations appeared to contain a fair amount of OVA-specific IgM. Moreover, the absorption test ascertained that rosetting B cells mainly produced OVA-specific antibodies. B cells probably formed rosettes with antigen-coupled beads via OVA-specific immunoglobulins on their surface. Some rosettes were also obtained in T cell fraction. This finding could be due to a small number of contaminating B cells, since T cell receptors generally recognize the spe-

TABLE II. Levels of total immunoglobulin and OVA-specific antibodies in the culture supernatants of EBV-transformed B cells from egg-allergic patients and nonallergic control subjects

Culture	Total Ig (ng/ml)			OVA-specific antibodies (absorbance at 414 nm)			
	IgG	IgA	IgM	IgG	IgA	IgM	IgE
Pat 1							
Rosette (+)	6*	12	1514	0.101	0.009	0.702	0.031
Rosette (-)	<u>93</u>	<u>103</u>	1623	<u>0.000</u>	0.012	<u>0.010</u>	<u>0.002</u>
Pat 2							
Rosette (+)	5	2	1728	0.104	0.013	0.601	0.027
Rosette (-)	<u>868</u>	<u>715</u>	2228	<u>0.016</u>	0.011	<u>0.001</u>	<u>0.001</u>
Cont 1							
Rosette (+)	2	23	2235	0.030	0.005	0.210	0.003
Rosette (-)	<u>342</u>	<u>1866</u>	2065	<u>0.003</u>	0.002	<u>0.001</u>	0.001
Cont 2							
Rosette (+)	3	21	2284	0.050	0.012	0.572	0.006
Rosette (-)	<u>718</u>	<u>2007</u>	2562	<u>0.000</u>	0.016	<u>0.011</u>	0.003

Ig, Immunoglobulin; *pat*, patient; *cont*, control subject; (+), rosetting; (-), nonrosetting.

Both rosetting and nonrosetting B cells were transformed by EBV and cultured after selection by OVA-coupled beads.

Values represent the means of triplicate determinations for single experiments.

The significance of differences in specific antibody titers between rosetting and nonrosetting cells was determined with paired Student's *t* test analyses.

*The significant differences from nonrosetting formation ($p < 0.05$) are underlined.

cific antigen associated with major histocompatibility molecules^{22, 23} and since the beads were coated with OVA alone. It remains to be determined whether T cells actually bind to the antigen-coated beads.

Monocytes made no formation of rosettes under the conditions used in the present study, but they non-specifically bound and phagocytosed the beads as time progressed. From these observations, this method appeared excellent for the positive selection of antigen-specific B cells.

The proportion of OVA-specific B cells in the peripheral B cell population was determined, and comparisons were made between allergic patients and healthy individuals. The percentage of OVA-specific B cells was significantly higher in patients allergic to egg than in age-matched allergic patients without egg allergy or healthy control subjects. The allergic children without egg allergy also had some higher scores than the healthy children. Healthy adults had the lowest percentage.

The means of the percentages of OVA-specific B cells were compared among age groups of egg-allergic patients. Interestingly, the increase in the OVA-specific B cell percentage preceded the increase in serum OVA-specific IgE and IgG antibodies. It reached a maximum for subjects to the age of 1 year and then gradually decreased. This fact indicates that

a population of allergen-specific precursors increases with exposure of dietary proteins during the first year of life. These B cells were demonstrated to secrete OVA-specific antibodies of mostly IgM by the study of cultured, EBV-transformed cells. These cells therefore actually appeared to be young B cells before developing a commitment to produce IgG or IgE. Moreover, the increased precursor population may further differentiate the IgG- or IgE-producing B cells that no longer circulate in large numbers. Most of the rosetting B cells appeared to be resting precursor B cells bearing IgM on their surface, since OVA-specific IgM antibodies can hardly be detected, even in the serum of egg-allergic patients. Other possible explanations for the predominant production of IgM isotype are that the switching of isotypes of OVA-specific antibodies that the EBV-transformed cells produce may be disproportionate originally in the peripheral blood and that specific B cells bearing surface IgM may bind more firmly with the antigen-coupled beads than specific B cells bearing other isotypes on their surface. A recent study demonstrated that the cells that secrete IgE are triple isotype-positive cells bearing IgE, IgM, and IgD and that they secrete all three isotypes after EBV activation.²⁴ It is therefore possible that a small percentage of OVA-IgM secreting cells (from rosetting cells) may also secrete OVA-IgE or IgG antibody.

TABLE III. Results from absorption of OVA-specific antibodies with solid-phase antigens

Culture	Total Ig (ng/ml) (percentage of Ig absorbed by solid-phase antigens)					
	Untreated		Treated by OVA plate		Treated by β -LG plate	
	IgG	IgM	IgG	IgM	IgG	IgM
Exp 1						
Rosette (+)	7.7	2562	0.9 (88.3)	713 (72.2)	5.6 (27.3)	2433 (5.4)
Rosette (–)	588	2306	595 (0.0)	2062 (10.6)	630 (0.0)	2883 (0.0)
Exp 2						
Rosette (+)	5.4	1574	1.9 (64.8)	390 (75.2)	9.3 (0.0)	1700 (0.0)
Rosette (–)	512	2228	502 (2.0)	1972 (11.5)	550 (0.0)	2417 (0.0)

Ig, Immunoglobulin; exp, experiment.
Supernatants from B cells selected by rosette formation with OVA-coupled beads and EBV-transformed were tested by ELISA for total immunoglobulin before and after incubation in antigen-coated plates.
Values represent the means of triplicate determinations for single experiments.

Additional characterization of rosetting B cells should be performed by means of cloning.

In this experiment, the bias of isotype production by the B cells rosetted with the beads, which had been EBV-transformed, was observed in the nonallergic subjects as well as in the egg-allergic patients. One possibility as regards this finding is that allergens like OVA may differ from other antigens in their ability to stimulate B cell populations or to select their isotypes. But considering that serum levels of OVA-specific antibodies are much lower in the nonallergic subjects than in the allergic patients, there may be some mechanisms that control production of allergen-specific antibodies and isotype switching, predominantly in the nonallergic condition.

Egg-allergic patients have high serum levels of both OVA-specific IgE and IgG antibody, but it has been reported that the IgG antibody to food antigens is also increased in nonallergic children, although the level is lower.²⁵ The difference in the percentage of rosetting B cells may reflect the different amounts of OVA-specific antibodies in each group. In the present study, it was observed that quantitative differences existed between allergic and normal individuals. However, no statistically significant correlation was found between the percentage of OVA-specific B cells and the serum levels of anti-OVA IgE or IgG antibodies in each patient. This finding may be explained by a time lag in the development between the specific cell frequency and the serum-specific antibody level, as discussed above. In a previous study,⁷ no significant differences

in EBV-driven, B-cell precursor frequencies for total IgE and IgM or pollen-specific IgE and IgM (by limiting dilution culture and after ELISA assay) were observed between normal and allergic individuals. This finding is in contrast to the present finding that the frequency of total or IgM class OVA-specific B cells is increased in allergic patients. The discrepancy between these two results may depend on the difference in sensitivity of the methods⁷⁻⁹ or kinds of allergens. In fact, a mean of 4.2% for OVA-binding B cell frequencies from the peripheral blood of the egg-allergic patients appears to be unusually high. Egeland et al.¹¹ reported the positive selection of rheumatoid factor-positive B cells by means of magnetic beads coated with immune complex composed of human serum albumin and IgG antihuman serum albumin. In that study, there was a significantly higher percentage of rosetting cells in the patients with Waaler-Rose-positive rheumatoid arthritis. The percentages ranged from 0.13% to 0.83%. In a recent study,²⁶ the circulating B cells committed to the production of antibodies to rabies virus were quantitated. It was reported that up to 2% of the circulating B cells were committed to the production of IgM antibodies that bound the virus and that, after immunization, their percentages consistently increased threefold. Thus, the human B cell repertoire in the peripheral blood can appear highly biased toward specific antigens in the individuals who have been somewhat hyperimmunized. The higher frequencies of OVA-specific B cells in egg-allergic patients and, to some extent, of

their frequencies in control individuals, might be explained by the fact that OVA is a common environmental protein that passes through the intestinal mucosa more easily, especially in the infantile period, and constantly stimulates the immune system.

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