

Effect of antigen stimulation on antigen-specific IgE-plaque-forming cells from peripheral-blood lymphocytes of atopics

L. PERELMUTTER, L. POTVIN and J. HÉBERT*

*Division of Infectious Immunology, Laboratory Centre for Disease Control, Virus Building, Tunney's Pasture, Ottawa, Ontario K1A 0L2, and *Department of Immunology, S-308, C.H.U.L., 2705 boulevard Laurier, Quebec G1V 4G2, Canada*

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Summary

In the present study, it was shown that allergen challenge *in vitro* produced an increase in the number of antigen-specific IgE-plaque-forming cells of peripheral-blood lymphocytes from grass- or ragweed-allergic patients. Thus, the blood lymphocytes of all twelve (four rye grass I and eight AgE) sensitive donors responded whereas the blood lymphocytes of five non-atopic controls were unresponsive to antigen challenge. Allergen challenge doses of 10^{-10} – 10^{-12} g/ml were found to give the greatest number of plaque-forming cells whereas the number of plaque-forming cells at challenging doses between 10^{-9} and 10^{-7} g/ml were either the same or less than those obtained with unchallenged cells. The results are discussed as to whether this *in vitro* model system represents *in vivo* response to allergen of the allergic patient.

Introduction

In 1975, Geha, Colten and Merler reported that human T cells from patients with ragweed hay-fever when exposed to AgE *in vitro* formed a substance that, in the presence of antigen, induced B cells from AgE-sensitive donors to synthesize and secrete IgE and IgG antibodies. The response was measured using RID and P-K tests. In contrast, other investigators reported only suppression of IgE antibody response on addition of allergen to the peripheral-blood lymphocytes (PBL) (Del Prete *et al.*, 1980; Iwamoto *et al.*, 1980; Yamagihara, Yui & Shida, 1980). No doubt, part of the discrepancy in these results is due to the methods of detection IgE antibodies from the secreting cells as well as differences in allergen concentration used for challenge of such cells. In the present study, the very sensitive reverse haemolytic plaque assay for detection of antigen-specific IgE PFC, was utilized and a wide range of allergen concentration was employed to challenge the PBL from untreated ragweed- and grass-sensitive patients. This approach allowed measurement of an increasing number of antigen-specific IgE PFC on challenge with antigen compared to unchallenged cells.

This response was shown to be antigen concentration-dependent and differed from one patient to another.

Methods and materials

Blood was taken from twelve untreated ragweed ($n=8$) and grass ($n=4$) allergic rhinitis patients. All patients gave a positive history, skin test and demonstrated 3+ or greater by RAST. The whole blood from five non-atopics were also employed as controls. The study was performed between May 1981 and February 1982.

Allergen challenge protocol

Peripheral-blood lymphocytes (PBL) were separated from fresh heparinized blood by Ficoll-Paque density gradient centrifugation. After three washes in RPMI 1640, the PBL were resuspended at a concentration of 3×10^6 cells/ml. Aliquots of 0.2 ml of cell suspension were incubated with 30 μ l of different concentrations of AgE (NIH Lot No. A-601-902-185) or rye grass I (NIH Lot No. A-611-901-185) allergens (ranging from 10^{-12} to 10^{-7} g/ml) for 42 hr at 37°C in a humidified atmosphere of 5% CO₂ and 95% air. For responses of unchallenged cells, antigen was substituted by RPMI 1640. These challenged and unchallenged cells, were then washed 3 times with RPMI 1640, resuspended in enriched medium (2% glutamine (GIBCO), 100 U/ml penicillin, 1% streptomycin, 100 μ g/ml gentamicin and 10% fetal calf serum (Flow Labs), and then placed in microtitre plates for an additional 5 days at 0.2×10^6 cells/0.2 ml of media. The viability of the cells were assessed with ethidium bromide and acridine orange and cell counts performed. The suspension was then adjusted to 2×10^6 viable cells/ml. The RHPA for detection of antigen-specific IgE PFC was performed as outlined previously (Perelmutter, Potvin & Hébert, 1984).

Determination of carryover of AgE in cells supernatants and cells during AgE-stimulation experiments

For the purpose of determination of AgE carryover into cell supernatants and cells during the antigen challenge experiments, AgE was radiolabelled with ¹²⁵I using the chloramine-T method such that the final specific activity was 30 μ l/g AgE and radioactive counts were 5×10^6 for 10^{-7} g/ml of AgE. This material was substituted for unradiolabelled AgE in the antigen challenge experiment. After counting the cell supernatants and cell pellets, the amount of AgE in these materials was determined (Table 1). It can be seen that the supernatants and cells from those cells challenged with 10^{-7} g/ml AgE had 3 pg of AgE carried over prior to examining the PBL by RHPA. No detectable AgE could be found in the cell supernatants and cells when AgE challenge doses were used at concentrations below 10^{-7} g/ml.

Results

Table 2 summarizes the results of allergen challenge of PBL from untreated AgE, rye grass I-sensitive patients as well as non-atopic controls. It is clear that *in vitro* allergen exposure of these cells caused an increase in the number of antigen-specific IgE PFC compared to the unchallenged cells indicating stimulation of formation of IgE antibody from these cells for all eight AgE and four rye grass I patients. Maximum number of PFC occurred with challenging doses between 10^{-10} and 10^{-12} g/ml of AgE or rye grass I whereas challenging doses of 10^{-7} – 10^{-9} induced either no response or a suppression of response. None of the non-atopic controls produced specific PFC. All

Table 1. Carryover of AgE in cell supernatants and cells during AgE stimulation experiments

Absolute quantity of AgE added to 0.2 ml of cell suspension (pg)	Radioactivity added (counts)	Radioactivity in		Amount of AgE detected prior to RHPA (pg)	
		Supernatants	Cells	Supernatants	Cells
3000	1.5×10^6	230	200	3	3
300	1.5×10^5	<150	<150	<3	<3
30	1.5×10^4	<150	<150	<3	<3
3	1.5×10^3	<150	<150	<3	<3
0.3	1.5×10^2	<150	<150	<3	<3
0.03	150	<150	<150	<3	<3

Table 2. Antigen-specific IgE PFC on allergen challenge

Patient	Antigen		No. of antigen-specific IgE PFC/ 10^6 viable cells* challenging dose of antigen (g/ml)						
	AgE	Rye grass I	0	10^{-7}	10^{-8}	10^{-9}	10^{-10}	10^{-11}	10^{-12}
1	+	-	33	20	20	30	67	40	50
2	+	-	50	40	40	120	150	350	180
3	+	-	40	60	60	80	80	120	150
4	+	-	50	30	60	60	80	208	120
5	+	-	94	40	70	90	240	110	40
6	+	-	130	80	100	100	110	200	120
7	+	-	400	80	200	220	320	400	400
8	+	-	120	40	150	180	220	400	320
9	-	+	296	120	320	380	400	480	310
10	-	+	20	40	80	80	120	240	160
11	-	+	100	60	80	80	180	240	120
12	-	+	50	30	50	120	200	120	60
13	-	-	<20	<20	<20	<20	<20	<20	<20
14	-	-	<20	<20	<20	<20	<20	<20	<20
15	-	-	20	40	<20	<20	<20	<20	<20
16	-	-	<20	<20	<20	<20	<20	<20	<20
17	-	-	<20	<20	<20	<20	<20	<20	<20

* Each response represents duplicates of RHPA.

of the PBL were collected during the height of the grass and ragweed seasons; it is not surprising to find unchallenged PBL forming antigen-specific IgE PFC, termed spontaneous IgE antibody-producing cells. It can be seen from Table 3, that 6 months after the season, a definite decrease in the production of spontaneous PFC occurred. However, such cells, taken well after the pollen season, maintained their ability to become stimulated on allergen challenge. In a separate experiment, it was shown that

the addition of cycloheximide to the RHPA reagents, 18 hr prior to observing PFC response, prevented the formation of PFC on allergen challenge. It can also be seen from Table 4 that cycloheximide caused a decrease in spontaneous antigen-specific IgE-PFC response (patients 3 and 4).

Table 3. Ability of PBL from grass and ragweed patients to produce antigen-specific IgE PFC during and 6 months after pollen season

Patient	AgE	No. of antigen-specific IgE-PFC/10 ⁶ viable cells	
		During season*	Six months after season*
1	—	120	40
	+	400	280
2	—	130	20
	+	200	180
3	—	94	20
	+	240	200
4	—	400	60
	+	400	320

* Responses in the RHPA represent responses obtained at optimal antigen challenge concentrations.

Table 4. Effect of cycloheximide on specific PFC response

Patient	AgE	Cycloheximide	IgE PFC/10 ⁶ viable cells (AgE)*
1	—	—	20
	+	—	180
	+	+	20
	—	+	20
2	—	—	< 20
	+	—	230
	+	+	< 20
	—	—	< 20
3	—	—	80
	+	—	150
	+	+	20
	—	+	20
4	—	—	60
	+	—	210
	+	+	< 20
	—	+	< 20

* These responses represent optimal responses obtained with allergen challenge of the PBL.

Discussion

It was previously shown (Perelmutter, 1983) that the RHPA can be used to detect antigen-specific PFC from PBL of grass- and ragweed-sensitive patients. In the present study this assay was capable of detecting an increase in the number of PFC on challenge with antigen. These results confirm the original observation (Geha *et al.*, 1975) that B cells from ragweed-atopic patients can be stimulated by AgE to produce IgE antibodies against AgE. More recently, this assay system has been utilized to detect antigen-specific IgE PFC with timothy (Beaudoin *et al.*, 1982), mountain cedar (T. Knicker, J. Dolan & L. Perelmutter, unpublished observations), and β -lactoglobulin (Parish, 1982) antigens. The antigen-driven response observed in the present study was found to be dose-dependent. Thus, maximum production of PFC were observed at antigen challenging doses between 10^{-10} and 10^{-12} g/ml antigen whereas the number of PFC obtained at challenging doses between 10^{-9} and 10^{-7} g/ml was either the same or less than that seen with the unchallenged cells. The failure of Del Prete *et al.* (1980), Iwamoto *et al.* (1980) and Yamagihara *et al.* (1980) to observe an increase in IgE antibody response on challenge with specific antigen can be accounted for by the concentration of allergen used to challenge the PBL *in vitro*. Thus the allergen-challenging doses of 10^{-4} – 10^{-6} g/ml used by these investigators would cause sufficient antigen carryover to produce antigen inhibition of RAST to detect IgE antibodies in the cell supernatants. In the present study, using challenging doses of 10^{-7} g/ml of AgE caused 3 pg of this material to be carried over prior to testing the PBL for PFC. This level of carryover at that concentration probably accounts for some of the decrease in the number of PFC compared to unchallenged cells seen in the present study. This is particularly true since it was previously shown (Perelmutter *et al.*, 1984) that when 4 pg of AgE was added to the RHPA an inhibition of 26% antigen-specific IgE PFC was observed.

What causes the increase in antigen-specific IgE PFC on challenge with antigen? Is the allergen exposure of the PBL from atopics causing an active *in vitro* synthesis or driving out IgE from the cytoplasm to cell surface? The RHPA cannot distinguish between these two possibilities. However, the fact that cycloheximide blocks this antigen-driven response favours the concept of active synthesis. Most probably the increase in the antigen-driven PFC represents a secondary response because of previous aero-exposure of allergen. Also, it cannot be determined at the time whether the increase in number of PFC is due to antigen reacting with memory B cells to produce new PFC or whether antigen causes polycloning of existing PFC.

Does the antigen-driven response of PBL model represent an *in vivo* response? Challenge of PBL from twelve atopics produced an increase of PFC whereas none of the PBL of the five non-atopics could be stimulated to produce PFC. In addition, in a preliminary study (Perelmutter, 1983), patients on immunotherapy were less responsive to produce antigen-specific IgE PFC on allergen stimulation. Moreover, those PBLs from the treated patients required higher antigen levels for PFC production (Perelmutter, 1983). Whether PBL contains the principle B cell that produces IgE antibody, and T cells that modulate this production, remains to be determined. Nevertheless, the *in vitro* model system described in this paper has been useful for studying the responsiveness of PBL of atopics during and after the pollen seasons and thus has the potential of distinguishing between the T-cell-independent, spontaneous IgE antibody-producing B cells and those B cells which are T-dependent and form IgE antibody on allergen challenge in culture. This model system can be useful for

determining the allergenic properties of modified allergens and in a preliminary study, it was found that challenge of PBL from untreated ragweed-sensitive patients with polyethylene glycol (PEG)-modified ragweed was incapable of stimulating the PBL to produce PFC whereas the native ragweed preparations were capable of response. The supernatants of the PEG-modified challenged PBLs are being investigated for antigen-specific suppressor factor for IgE and IgG ragweed antibodies. Finally, our laboratory (Konowalchuk, Spiers & Perelmutter, 1982) is presently producing Epstein-Barr virus-transformed cell lines for IgE immunoglobulin production. By achieving the conditions of allergen-stimulating specific IgE PFC, it is conceivable, in the near future, to have continual cell lines making antigen-specific IgE antibodies.

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