Cooperation between Human Thymus-Derived and Bone Marrow-Derived Lymphocytes in the Antibody Response to Ragweed Antigen E in Vitro

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ABSTRACT Human T lymphocytes from patients with ragweed hay fever, when exposed to ragweed antigen E (AgE) in vitro, produced an activity that, in the presence of antigen, induced B cells from AgE-sensitive donors to synthesize and secrete IgE and IgG antibodies to AgE. Anti-AgE specificity was assessed both in vitro and in vivo. B lymphocytes from ragweed-sensitive individuals exposed in vitro to AgE alone failed to transform or to secrete antibody to AgE. The T cell activity had no effect on B cells of individuals not sensitive to AgE. The results of this study suggest that the human reaginic antibody response requires T and B cell cooperation. The experimental approach used may be a useful model for the investigation of the antibody responses of allergic individuals.

INTRODUCTION

Cell interactions between thymus-derived (T) and non-thymus-derived (B) lymphocytes are required for an antibody response to many antigens both in experimental animals (1) and man (2). In several species of experimental animals, T and B cell interaction is prerequisite for the production of reaginic antibody (IgE) (3). The role of this interaction in human IgE antibody response is not clear because experiments showing proliferation of lymphocytes exposed to allergens in vitro (4) with synthesis of macrophage migration inhibitory factor and mitogenic factor (5, 6) were performed with unfractionated suspensions of lymphocytes.

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In an attempt to study the role of isolated T and B cells in the antibody response to ragweed antigen E (AgE)[1] in vitro, T cells from AgE-sensitive donors were incubated with AgE, and culture fluid supernates were added to B cell cultures. The capacity of B cells to proliferate in culture and to synthesize and secrete specific antibodies to AgE was measured. Little mitogenic activity for B cells was detected from T cells stimulated with AgE, but synthesis and secretion of IgG and IgE immunoglobulins with antibody activity to AgE was demonstrated.

METHODS

Human subjects. Three ragweed-sensitive (H. C., S. S., and E. L.) and three control subjects (R. G., M. A., and C. L.) were studied. The ragweed-sensitive subjects consisted of a child and two adults with history and symptoms of seasonal allergic rhinitis and positive skin tests for immediate hypersensitivity to AgE; none had received immunotherapy. The controls were a normal adult and two children with neither a history of ragweed sensitivity nor positive skin tests with AgE. Leukocytes from the AgE-sensitive individuals released histamine when incubated with concentrations of AgE ranging between 1 and 10 ng/ml, whereas leukocytes from the controls at concentrations up to 10 μg/ml of AgE did not.

Human lymphocytes. Tonsils were obtained from E. L., M. A., and C. L. while undergoing tonsillectomy. Heparinized blood was collected from the other three subjects. Peripheral blood lymphocytes from each donor were studied on three different occasions. Suspensions of blood and tonsil

[1] *Abbreviations used in this paper:* AgE, ragweed antigen E; BSA, bovine serum albumin; E, sheep erythrocyte; MI, mitogenic index; P culture, experimental culture; PK, Prausnitz-Kustner; R culture, control culture; RID, radioimmunodiffusion; RIEP, radioimmunoelectrophoresis; TT, tetanus toxoid.

lymphocytes were prepared as described elsewhere (7). Cell suspensions were passed through a 50 × 3-cm column of glass beads (Superbrite, 3M Co., St. Paul, Minn.) at 37°C to remove adherent cells. Final suspensions always contained more than 90% viable lymphocytes. These were fractionated on gradients of bovine serum albumin (BSA) by layering 1 ml of the albumin solutions in 2% decrements, starting with 35% and ending with 19% BSA solutions (Sigma Chemical Co., St. Louis, Mo.) (8). Up to 5×10^8 cells were suspended in 1 ml of 17% BSA and layered on top of the gradient. Tubes were centrifuged at 10°C and 900 *g* for 45 min.

Preparation of T and B cell suspensions. T cells were derived from lymphocytes sedimenting in fraction 4 of the BSA gradient. 40–50% of the cells from this fraction formed rosettes with sheep red cells (E), and only 5–10% reacted with EAC1423 (EAC3) or with fluorescent antisera to human immunoglobulins (9). Lymphocytes derived from fractions 6 and 7 of the gradient were rosetted with E to obtain highly purified populations of B cells (98% reactivity with EAC3).

Tissue culture. Lymphocytes were cultured in medium RPMI-1640 (Grand Island Biological Co., Grand Island, N. Y.), supplemented with 15% heat-inactivated human serum (type AB, Rh+). Penicillin G (50 U/ml) and streptomycin (50 μg/ml) were added to cultures of blood lymphocytes. Cultures of tonsil lymphocytes received kanamycin (50 μg/ml) and Mycostatin (E. R. Squibb & Sons, Princeton, N. J.) (50 μg/ml) in addition. Cultures of 10^6 lymphocytes/ml were placed in round-bottomed, stationary tubes in a humidified incubator at 37°C in an atmosphere of 5% CO_2 in air.

To assess responsiveness of cell donors, T cells were incubated for 6 days with AgE. The degree of incorporation of [^{3}H]thymidine into DNA was determined. Optimal stimulatory dose of AgE was determined in experiments that showed a biphasic proliferative T-cell response with maxima at AgE concentrations of 20–50 μg/ml and 10–100 ng/ml (10).

Antigen. A single preparation of ragweed AgE (mol wt 37,800), supplied by Abbott Laboratories (North Chicago, Ill.), was used. AgE was iodinated with carrier-free [^{125}I]sodium iodide to a sp act of 90,000 cpm/μg.

Generation and reactivity of T cell supernates. T cells were incubated for 3 days at 37°C with AgE (50 μg/ml) (P cultures). Control cultures were incubated without antigen but received AgE (50 μg/ml) at the end of the incubation period (R cultures). The ability of paired P and R supernates to stimulate DNA synthesis in B lymphocytes was assayed by incubating B cells with these supernates for 6 days. Results were expressed as the number of counts per minute of [^{3}H]thymidine incorporated by each culture. B cell mitogenic activity was considered present when the radioactivity incorporated by cultures stimulated with P supernates exceeded that incorporated by cultures stimulated with R supernates (mitogenic index [MI] greater than 1).

Electron microscopy of lymphocytes fixed in glutaraldehyde was done by counting at least 500 cells in each sample and estimating their degree of enlargement. Their size was roughly estimated at a primary magnification of 6,800 by observing whether their borders fell within the perimeter of a circle 4 cm in diameter inscribed on the fluorescent screen of the electron microscope.

Determination of protein synthesis by B cells. After a 6-day incubation in the presence of T-cell supernates and AgE, B cells were pulsed with 1 μCi of [L-^{14}C]leucine (260 mCi/mM), 0.5 μCi of [L-^{14}C]valine (220 mCi/mM), and 0.5 μCi of [L-^{14}C]isoleucine (250 mCi/mM) (New England Nuclear Corp., Boston, Mass.) per ml of culture. To quantitate protein synthesis, part of the supernate was precipitated with 10% trichloroacetic acid (TCA). Labeled supernates and supernatant fractions obtained after Sephadex G-200 fractionation were analyzed for the presence of secreted antibodies by radioimmunodiffusion (RID) and radioimmunoelectrophoresis (RIEP). Center wells (RID) and troughs (RIEP) contained the antisera. Peripheral wells contained supernates (concentrated fivefold), mixed with either IgG or IgE myelomas in amounts that would give optimal precipitation lines with the antisera. This one-step double immunoprecipitation was performed to increase the sensitivity of the autoradiographic method. Slides were exposed to Kodak X-ray film (Par Speed film, Eastman Kodak Co., Rochester, N. Y.) for 10 wk. RID and RIEP was also used to determine in vitro the specificity of the secreted antibodies. In these experiments, a mixture of anti-IgG or anti-IgE antiserum and radioiodinated [^{125}I]AgE was placed in the center wells (RID) and troughs (RIEP). Peripheral wells contained a mixture of supernates (concentrated fivefold) and myeloma IgG or IgE. Slides were exposed to the X-ray film for 2 wk.

Prausnitz-Kustner (PK) skin reaction. B cell supernates used in this experiment were generated as follows: after a 6-day incubation in the presence of T-cell supernates, B-cell pellets were washed and resuspended at 4×10^6 cells/ml in RPMI-1640 medium containing 15% human serum albumin (Hyland Div. Travenol Laboratories, Inc., Costa Mesa, Calif.). 48 h later, the supernates were collected and filtered through a 0.22-μm filter. All skin tests were performed on a single individual who had a negative history for hay fever and a negative skin test with AgE. Informed consent was obtained from this subject, who was an allergist and was aware of the nature and purpose of this experiment. At each forearm site, 0.05 ml of supernate at various dilutions was injected intradermally. 48 h later, 0.05 ml of AgE was injected at the same site at 15 and 30 min. The positive control consisted of an initial injection of 1:10 dilution of a serum from one of the AgE-sensitive subjects who gave no history of hepatitis and had no Australia antigen detectable in his serum. Negative control sites received an initial injection of RPMI-1640 medium containing 15% human serum albumin.

RESULTS

Proliferation of T and B lymphocytes in response to AgE. Only T cells of AgE-sensitive donors proliferated in response to AgE, as measured by [^{3}H]thymidine incorporation. AgE failed to cause proliferation of T cells obtained from donors not sensitive to AgE (controls) (Table I). B lymphocytes from both AgE-sensitive and control donors failed to transform in response to AgE alone (MI less than 2).

A small increase in DNA synthesis occurred only when B cells from AgE-sensitive donors were incubated in the presence of P supernates derived from the T cells of AgE-sensitive donors. With the above combination, MI ranged between 1.3 and 1.9 in nine different experiments. This lack of proliferation was confirmed by electron microscopy, where only 7–12% of the cells present in culture became larger. There was, however,

TABLE I

Stimulation of DNA Synthesis in T Lymphocytes by AgE

Source of peripheral blood lymphocytes	[^{3}H]Thymidine incorporated per culture		
	Control	AgE	MI
	cpm		
AgE-sensitive donors			
H. C.	2,077	43,812	21.1
S. S.	1,645	31,072	18.8
E. L.	1,883	19,668	10.5
Controls			
R. G.	1,266	1,024	0.9
M. A.	2,863	2,639	0.9
C. L.	1,785	2,341	1.3

Cultures contained 1×10^6 cells each. Values for H. C. and S. S. represent the average of three different experiments.

a consistent increase in the amount of radioactive TCA-precipitable material found in the culture fluid of AgE-sensitive B-cell cultures stimulated with P supernates derived from AgE-sensitive cells. When AgE-sensitive B-cell supernates were separated on Sephadex G-200, the increase in TCA-precipitable radioactive protein was found to be mainly in the 7S fraction.

Synthesis and secretion of IgE and IgG possessing anti-AgE specificity. AgE-sensitive B cells incubated in the presence of P supernates from AgE-sensitive T-cell donors secreted into their culture fluid both IgE and IgG immunoglobulins (Fig. 1). In a single experiment, IgE was also detected in the culture fluid of AgE-sensitive B cells stimulated with the R supernatant obtained from AgE-sensitive T cells (Fig. 1). In this particular case, the B cells were derived from the tonsil of a child who had allergic rhinitis and was presumably actively synthesizing IgE. No immunoglobulins were detected in the medium from control B-cell cultures exposed to AgE-sensitive T-cell supernates. The IgE and IgG secreted by AgE-sensitive B cells had specificity directed against AgE, the antigen used in the production of T-cell supernates. Only those supernates containing newly synthesized IgE (Fig. 1) exhibited anti-AgE specificity (Fig. 2).

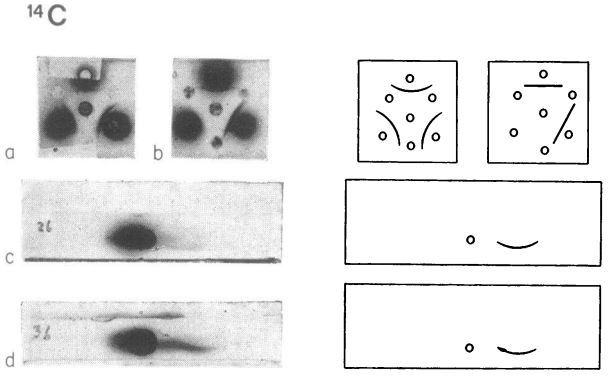

FIGURE 1 RID and RIEP of supernates from B lymphocytes incubated in the presence of P and R supernates from AgE-sensitive T cells; exposure time 10 wk. *a.* Center well: anti-human IgE. Peripheral wells contain supernates from: top, P-stimulated B cells of H. C.; right, P-stimulated B cells of E. C.; left, R-stimulated B cells of E. C. Peripheral wells received myeloma IgE in addition. *b.* Center well: anti-human IgG. Peripheral wells contain B-cell supernates distributed as in *a* and to which myeloma IgG was added. *c.* Trough: anti-human IgE. Well: supernate of P-stimulated B cells from H. C. mixed with myeloma IgE. *d.* Trough: anti-human IgG. Well: supernate of P-stimulated B cells from H. C. mixed with myeloma IgG.

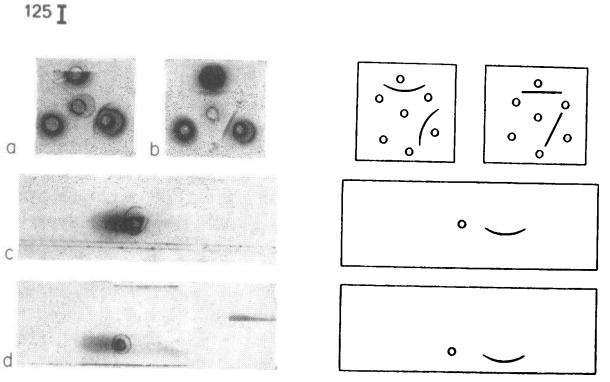

FIGURE 2 ^{125}I-RID and -RIEP; exposure time 2 wk: *a, b, c,* and *d* are exactly as in Fig. 1, but [^{125}I]AgE was added to the center wells of the RID and to the troughs of the RIEP.

Antibody activity of supernates measured in vivo. On two different occasions, a total of 12 sites were prepared for PK testing in the forearm of an AgE-insensitive volunteer. These included three different supernates shown in vitro to contain newly synthesized IgE with antibody activity against AgE. One of these three supernates was tested at two different concentrations and, once diluted, was heat-inactivated at 56°C for 1 h. The remaining seven sites were prepared with control preparations, three of which were R supernates from AgE-sensitive T- and AgE-sensitive B-cell cultures, one from an AgE-negative B exposed to an AgE-sensitive T-cell supernate, two from AgE-sensitive B exposed to AgE-negative T supernates, and one a medium control. In all cases except one, T cells were stimulated with AgE, the exception being one AgE-negative but tetanus toxoid (TT)-sensitive T-cell preparation used as a control. The latter was incubated with TT and assayed on AgE-sensitive B cells. When ragweed antigen was injected 48 h after preparation of the site, the sites with supernates from AgE-sensitive T and AgE-sensitive B-cell cultures produced a wheal-and-flare reaction within 15 min of antigen injection (Table II).

This activity was abolished by heating. Two concentrations of AgE (0.2 and 50 μg/ml) were used on T cells to produce P supernates capable of IgE anti-AgE antibody synthesis.

DISCUSSION

In man, immediate hypersensitivity reactions are generally mediated by homocytotropic reaginic (IgE) antibody. Frequently, these reactions occur in the absence of cutaneous delayed hypersensitivity to the allergens (5). Recently, a number of studies have established the presence of lymphocyte reactivity to allergens in vitro (4-6). However, the relation between this cellular response and the in vivo allergic reaction remains to be studied. The present study demonstrates that cooperation between human T and B lymphocytes is necessary for the in vitro antibody response to ragweed antigen E. To induce antibody synthesis, this reaction required T and B cells from AgE-sensitive donors and the presence of the antigen. Human B lymphocytes neither proliferated nor engaged in antibody synthesis in response to AgE alone. However, culture fluid supernates from AgE-stimulated T lymphocytes contained an activity that caused B lymphocytes to synthesize and secrete antibody. This activity was recovered only from T cells of AgE-sensitive donors and also required for its detection B cells from AgE-sensitive subjects. The activity did not depend on transplantation antigens since T-cell supernates were active on autologous B lymphocytes. It was detected only in T-cell cultures but not in B-cell cultures from either peripheral blood or tonsil. It had previously been shown (2) that supernates of antigen-stimulated T cells induced B cells to transform only when the B cells were incubated alone without T cells. The reason for this requirement is not known, but it was assumed to apply to the IgE, AgE system described here. Moreover, these experiments fail to indicate whether the activity in stimulated T cell supernates is specific for immunoglobulin class or antigen. Kishimoto and Ishizaka (11) have also described a soluble activity in cultures of rabbit T cells that stimulates IgE antibody production by B cells. Supernates of AgE-stimulated T cells contained little, if any, mitogenic activity when tested on B cells. Mitogenic indices of P- versus R-stimulated B-cell cultures never exceeded 2. By electron microscopy, there was no significant difference in the percent of large cells found in P- and R-stimulated B-cell cultures. These results differ from those obtained previously with TT antigen (2). Supernates of TT-stimulated T cells caused vigorous blast transformation of B-cell cultures (MI ranging from 7.5 to 32.6) with the percent of large cells in these cultures reaching 50–60%. The difference between the mitogenic activity of supernates from T cells stimulated with TT and AgE remains to be explained: release of mediator by T cells in response to different antigens may be qualitatively and quantitatively different. The number of B cells responding to cell mediators may depend on the number of antigen-sensitive cells which may differ for each antigen. Thus, the number of AgE-sensitive B cells may be much smaller than that of TT-sensitive cells. Our data on the mitogenic activity of AgE-stimulated T-cell supernates cannot be compared to those of Rocklin, Pence, Kaplan, and Evans (5), and Maini, Dumonde, Faux, Hargreave, and Pepys (6), who used for their system unfractionated blood lymphocytes, a mixture of T and B cells, and obtained moderately good mitogenic activity (positive Δ P-R cpm, and MI of approximately 3) attributable to T cell proliferation. The IgE and IgG secreted by B cells after stimulation with the T-cell supernate contained antibodies to AgE, the antigen used to stimulate the T-cell suspensions. This AgE specificity was demonstrated in vitro by RID and RIEP. Evidence for IgE anti-AgE activity was obtained by passive sensitization of human skin with stimulated B-cell supernates (PK reaction, Table II). The antibody activity was destroyed by heating and was, therefore, probably due to

TABLE II

PK Reactions Induced by Supernates of Peripheral Blood B Cells from AgE-Sensitive Donors

Source of cells			
T cell* donor	B cell target	B cell supn dilution	Wheal size
			mm
P culture			
H. C.	H. C.	Undil	6 × 6
S. S.	S. S.	Undil	10 × 4
E. L.	H. C.	Undil	23 × 23
E. C.	H. C.	1:10	8 × 14
E. C.	H. C.	1:10 heated	0
H. C.	R. G.‡	Undil	0
R. G.‡	H. C.	Undil	0
R. G. (TT)§	H. C.	Undil	0
R culture			
H. C.	H. C.	Undil	0
S. S.	S. S.	Undil	0
E. C.	H. C.	Undil	0
—	—	Medium	0

Undil, undiluted; supn, supernate.

* T cells from peripheral blood were incubated with 50 μg/ml AgE except in S. S., where T cells were incubated with 0.20 μg/ml AgE.

‡ R. G. is AgE-negative donor. All other donors are AgE sensitive.

§ T cells were incubated with TT antigen.

IgE. Neither RID nor the PK reaction was performed quantitatively, so that direct comparison of the amount of IgE antibody detected by these methods was not possible. Although lymphocytes from only six individuals were studied, the findings establish the helper role in man of T lymphocytes in IgE antibody response to antigens in vitro. A similar T-B cell cooperation in the IgE response has been described in experimental animals (12, 13) with the observation, comparable to the one discussed here, that the formation of IgE antibody requires carrier-specific T helper cells (11, 12). The methods described in the present study may be useful in the investigation of the in vitro immune response of allergic patients and in the assessment of the value of various modalities of treatment.

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