

Detection of specific IgE-secreting cells with an enzyme-linked immunospot assay

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Background: An *in vitro* assay system for monitoring the induction of human allergen-specific IgE synthesis with an enzyme-linked immunospot assay is described.

Methods: IgE-secreting cells, responding specifically to short ragweed pollen allergens. Amb a V and Amb a VI, were directly enumerated after the coculture of high-density, resting B cells with either autologous T cells or allergen-specific T-cell clones from the peripheral blood of two ragweed-allergic individuals. Both T-cell clones were type 2-like T helper cells (T_H2) as determined by reverse transcription polymerase chain reaction. The IgE-producing cells were detected on a nitrocellulose-based paper disc coated with antigen after treatment with biotinylated, anti-human IgE and avidin-peroxidase conjugate.

Results: We demonstrated the induction of Amb a V- or Amb a VI-specific IgE synthesis from either peripheral blood B cells or purified resting B cells. Specific IgE-secreting B cells could be detected only when T cells were present in the culture, providing that they were not separated by a membrane. The addition of interleukin-4 had an enhancing effect on the overall numbers of IgE-secreting cells and allergen-specific T cells and a synergistic effect in the coculture of B cells and an autoreactive T-cell line.

Conclusions: These results suggest that cognate interaction T and B cells is required for the *de novo* induction of IgE responses. This *in vitro* system could thus provide a useful model for analysis of the molecular and cellular mechanisms that regulate the allergen-specific IgE responses in human beings. (*J ALLERGY CLIN IMMUNOL* 1993;92:479-87.)

Key words: Allergen-specific IgE production, enzyme-linked immunospot assay, ragweed

Progress has been made in understanding the regulation of human IgE synthesis, but the precise molecular and cellular mechanisms involved in allergen-specific IgE production remain unclear. The main gaps in our understanding of the allergen-specific IgE secretion concern the molecular nature of the cognate signals between T and B

Abbreviations used

ELISPOT:	Enzyme-linked immunospot
GM-CSF:	Granulocyte-macrophage colony-stimulating factor
γ -IFN:	γ -Interferon
IL:	Interleukin
M_r :	Relative molecular mass
PBMCs:	Peripheral blood mononuclear cells
PCR:	Polymerase chain reaction

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cells and the precise functions of the various cytokines involved. In the murine system the regulation of IgE was found to be controlled essentially by the reciprocal activity of interleukin-4 (IL-4) and γ -interferon (γ -IFN).^{1, 2} More recent studies in human beings, in which alloreactive and autoreactive T cells were used,³⁻⁵ have demonstrated a positive correlation between the expres-

sion of IL-4 from the T cells and helper function for IgE; in contrast, γ -IFN (and also α -IFN and T-cell growth factor- β^6) downregulates the IL-4-induced IgE synthesis. These observations were further confirmed by the analysis of data obtained with a panel of phytohemagglutinin-induced T-cell clones and the finding that anti-IL-4 treatment consistently abolished synthesis of IgE, but not the other Ig isotypes in all of the activated T-cell clones and their supernatants that were tested.⁷⁻⁸ It was found that IgE production could be detected only when autologous T can B cells were cultured in the same well but not when they were separated by a membrane,⁹ demonstrating the need for cognate interaction between these cells for IL-4-dependent IgE synthesis in vitro. This finding was further supported by studies with either phytohemagglutinin-induced or alloreactive T-cell clones.¹⁰

The major difficulties in studying the molecular and cellular mechanisms of antigen-specific IgE synthesis are the low frequency of antigen-specific B cells and the accurate determination of the low levels of IgE synthesized in vitro. Although a variety of in vitro culture assays have been designed primarily for measuring spontaneous and/or total IgE protein levels in culture supernatants, the determination of de novo induction of antigen-specific IgE responses in human beings has yet to be established. Previously, an enzyme-linked immunospot (ELISPOT) assay¹¹⁻¹³ has been used primarily in the murine model to detect cytokine and antigen-specific IgE synthesis. We have adapted this technique to monitor the induction of specific human IgE antibody synthesis in vitro at the single cell level.

MATERIALS AND METHODS

Antigens

Ambrosia (ragweed) allergens, *Amb a V* (relative molecular mass [M_r] = 5000), *Amb a VI* (M_r = 11,500) and *Amb t V* (M_r = 4,400) were isolated and purified from short and giant ragweed pollens.^{14, 15}

Preparation of B and T cells

Peripheral blood mononuclear cells (PBMCs) were isolated from the blood of two ragweed-allergic individuals by Ficoll-Hypaque (Pharmacia Biosystems, Piscataway, N.J.) gradient centrifugation. The cells were separated into rosette-forming positive (E^+) and negative (E^- , or non-T) populations with the use of sheep erythrocytes treated with 2-aminoethylisothiuronium bromide hydrobromide. The E^+ T-cell population was used without further purification. More than 90% of the cells in this preparation were reactive with anti-

CD3 monoclonal antibody, as determined by flow cytometry analysis. To obtain highly purified B cells, the E^- cells were placed in a plastic dish (Falcon; Becton-Dickinson and Company, Franklin Lakes, N.J.) coated with inactivated fetal calf serum and incubated at 37° C for 90 minutes. Further depletion of T cells was performed by additional rosetting of the resultant nonadherent cell population. High-density resting cells were purified with the B cells by discontinuous Percoll (Pharmacia Biosystems) gradient centrifugation. This B-cell subpopulation was negative for cell-surface IgE and esterase.

An *Amb a VI*-specific T-cell clone (TS309) was derived from the peripheral blood T cells of a ragweed-allergic subject after periodic stimulation with *Amb a VI* and rIL-2 and by using the limiting dilution cloning method as described previously.¹⁶ This T-cell clone responded to only *Amb a VI* and not to the control antigens, *Amb t V* and *Amb a V* (from giant and short ragweed pollens, respectively). A proliferation assay, which was run in parallel with the analysis of specific IgE induction (as described below), showed a stimulation index of 10 in response to *Amb a VI*. An "autoreactive" T-cell line obtained from the PBMCs showed a high degree of proliferation in the presence of irradiated autologous PBMCs without addition of exogenous antigen. An *Amb a V*-specific T-cell clone (AP1.2), which was used for certain experiments, was derived from the PBMCs of an additional ragweed-allergic individual (AP) as described.¹⁶

Cell cultures and measurement of specific IgE and total IgE synthesis

Measurement of specific and total IgE synthesis was performed by an ELISPOT assay.¹¹⁻¹³ For measurements of *Amb a VI*-specific IgE, highly purified B cells (2×10^5) or high-density B cells (2.5×10^5) were cultured with autologous peripheral blood T cells (1×10^5), or the *Amb a VI*-specific T-cell clone (5×10^5 ; TS309), or an autoreactive T-cell line (5×10^5 cells) in the presence or absence of *Amb a VI* ($2 \mu\text{g/ml}$) in 1 ml of culture medium containing RPMI-1640 (Gibco, Grand Island, N.Y.) supplemented with 10% fetal calf serum (Hyclone Laboratories, Inc., Logan, Utah), streptomycin (100 mg/ml), penicillin G (100 U/ml), 5×10^{-5} mol/L 2-mercaptoethanol, and 10 mmol/L N-2-hydroxyethylpiperazine-N-2-ethanesulfonic acid (HEPES) buffer (pH 7.4). In some experiments, rIL-4 (200 U/ml; Genzyme, Boston, Mass.) was added to the cultures. In other experiments, T and B cells were cocultured in compartments separated by a 0.4 μm membrane (Millipore, Bedford, Mass.). For analysis of *Amb a V*-specific IgE synthesis, autologous non-T cells (5×10^5), isolated as described above, were cultured with *Amb a V*-specific T-cell clone (AP1.2; 5×10^5 cells) in the presence or absence of *Amb a V* ($2 \mu\text{g/ml}$) in 1 ml of culture medium.

After 7, 9, or 12 days of culture at 37° C in a 5%

CO₂-humidified incubator, the cells were washed and resuspended in fresh culture medium. The cell suspension (4×10^5 cells or 4×10^5 B cells alone) was then added in duplicate to 96-well plates with nitrocellulose bottoms (Milliliter HA, Millipore) coated with anti-human IgE, *Amb a* V (5 µg/ml in 0.1 mol/L sodium carbonate buffer, pH 9.6), *Amb a* VI (5 µg/ml), or control antigen *Amb ± V* (5 µg/ml) and incubated for an additional 24 or 48 hours to allow IgE secretion from the cells. The plates were washed with phosphate-buffered saline containing 0.05% Tween 20 and incubated overnight at 4° C with biotinylated, goat anti-human IgE (5 µg/ml; Organon Teknika Corp., Durham, N.C.) followed by incubation with an avidin-peroxidase conjugate (1:1000 dilution; Organon Teknika Corp.). Spots representing IgE-producing B cells were developed with the substrate 3-amino-9-ethylcarbazole in 0.1 mol/L citrate, pH 5.0. The spots were enumerated with the aid of a dissecting microscope.

Culture supernatant preparation from activated T cells

Peripheral blood T cells (1×10^6 /ml), separated as described above, were stimulated with *Amb a* VI (2 µg/ml) in the presence of irradiated PBMCs and cultured for 4 days in medium containing 2% fetal calf serum. The cell-free culture supernatants were then separated by centrifugation at 400 g for 5 minutes, and concentrated five times by lyophilization.

RNA isolation, cDNA synthesis, and polymerase chain reaction

For analysis of steady-state cytokine transcripts in allergen-specific T-cell clones, AP1.2 and TS309, cytoplasmic RNAs were isolated from cells stimulated with or without relevant antigen for 16 hours as described by Sambrook et al.¹⁷ After pelleting, the cells were lysed with lysis buffer (containing 0.5% Nonidet P-40 [Sigma Chemical Co., St. Louis, Mo.], 100 mmol/L NaCl, 50 mmol/L Tris-HCl, and 5 mmol/L MgCl₂) in the presence of RNasin (1 U/µl; RNase inhibitor) and dithiothreitol (1 mmol/L), extracted with phenol/chloroform isoamyl alcohol followed by ethanol precipitation. The first-strand cDNA synthesis was performed with avian myeloblastosis virus reverse transcriptase (2.5 U/µl; Promega Corp., Madison, Wis.), oligo-(dT)₁₆ primer (2.5 µmol/L), and deoxyribonucleoside triphosphate (0.5 mmol/L each) in the presence of RNasin (1 U/µl), 5 mmol/L MgCl₂, in a 20 µl reaction at 42° C for 20 minutes.

Polymerase chain reaction (PCR) amplifications¹⁸ were performed with an aliquot (10%) of the reverse transcriptase product in the presence of a "master mix" containing: PCR buffer, MgCl₂ (final concentration, 2 mmol/L), deoxyribonucleoside triphosphates (final concentration, 0.4 mmol/L each), AmpliTaq polymerase (Perkin-Elmer Corp., Norwalk, Conn.; 1 U/50 µl reaction) and paired primers for the various cyto-

kines (0.5 nmol/L of each primer) to a total volume of 50 µl. The cytokine-specific primer pairs were either purchased (Clontech, Palo Alto, Calif.) or synthesized (based on the known cDNA sequences for the cytokines) at the Johns Hopkins DNA Synthesis Laboratory. PCR was carried out for 45 cycles under the following conditions: for IL-2, denaturation at 94° C for 60 seconds, annealing and extension at 60° C for 1 minute; granulocyte-macrophage colony-stimulating factor (GM-CSF), IFN-γ, IL-4, IL-5, IL-6, and β-actin, denaturation at 95° C for 45 seconds, annealing at 60° C for 45 seconds, and extension at 72° C for 90 seconds. The primers used are as follows: IL-2 5' primer, 5'-GAATGGAAT TAATAATTACAAGAATCCC-3', 3' primer, 5'-TGTTTCAGATCCCT TTAGTTCCAG-3'; IL-4 5' primer, 5'-ATGGGTC TCACCTCCCAACT-GCT-3', 3' primer, 5'-CGAACACTTT GAATATTTCT-CTCTCAT-3'; IL-5 5' primer, 5'-GCTTCTGCATTT-GAGTTTGCTAGCT-3', 3' primer, 5'-TGGCCGTCA ATGTATTTC TTTATTAAG-3'; IL-6 5' primer, 5'-ATGAACTC CTCTCCACAAGCGC-3', 3' primer, GAAGAGCCCTCAGGCTGGACTG-3'; γ-IFN 5' primer, 5'-ATGAAATATACAAGTTATATCTT GGC-TTT-3', 3' primer, 5'-GATGCTCTTCG ACCTCG-AAACAGCAT-3'; GM-CSF, 5' primer, 5'-GGCTGCA-GAGCCTGCTGCTCTTGGG CACTG-3', 3' primer, 5'-CTGGAGGTCAAACATTTCTG AGATGACTTC-3'; β-actin 5' primer, 5'-TGACGGGGTCAACCCACACT-GTGCCCATCTA-3', 3' primer, 5'-CTAGAAGCA TTT-GCGGTGGACGATGGAGGG-3'. The reaction product was visualized by subjecting to electrophoresis in a 2% agarose gel (1% Nusieve GTG, FMC, and 1% agarose; Sigma) in 1× triborate ethylenediaminetetraacetic acid buffer containing 0.5 µg/ml ethidium bromide. Verification of the specific cytokine gene amplification was established by the fragments of the predicted size. Their identities were confirmed by restriction enzyme digests, which provided appropriately sized fragments, or by Southern blotting with cytokine-specific riboprobes.

RESULTS

Total IgE and specific IgE synthesis from peripheral blood B cells

To quantitate the IgE-secreting cells with the ELISPOT assay, we first examined the kinetics of total IgE and *Amb a* VI-specific IgE synthesis from the peripheral blood B cells of a ragweed-allergic subject. Highly purified peripheral blood B cells were cultured in vitro with *Amb a* VI for different time periods (7, 9, or 12 days) either alone or with autologous peripheral blood T cells, followed by an additional 1 or 2 days in plates with nitrocellulose based coated with either anti-human IgE or *Amb a* VI to allow for the secretion and detection of total and specific IgE antibodies, respectively. We found that an initial culture for

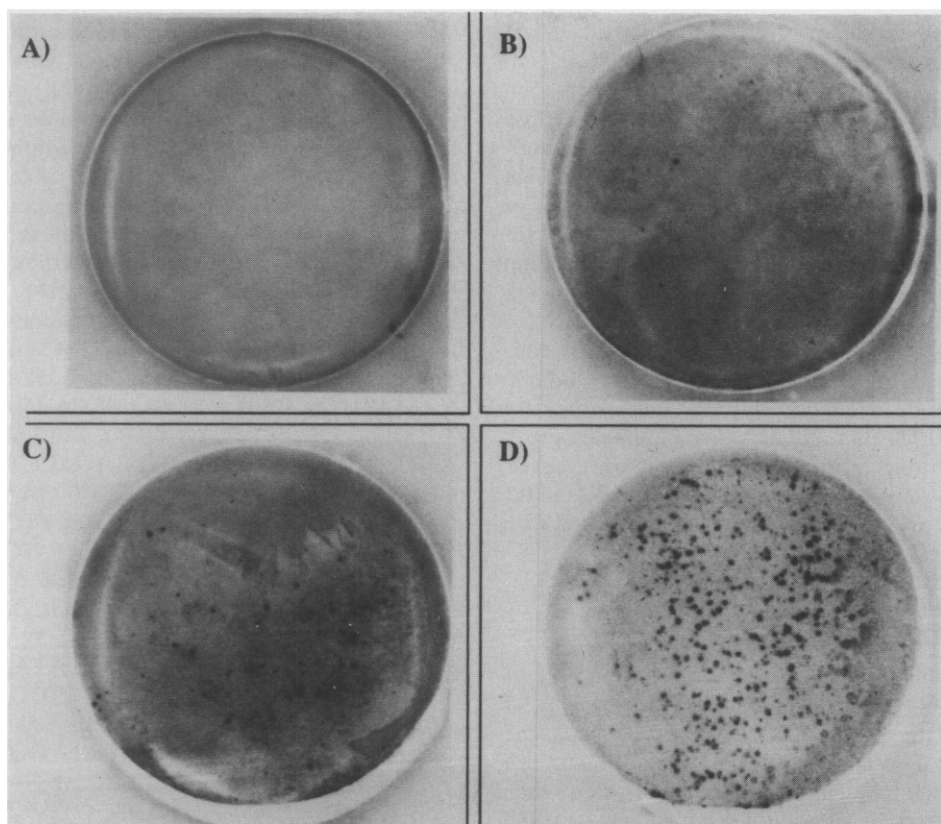


FIG. 1. Detection of IgE synthesis with the ELISPOT assay. Highly purified B and T cells were derived from PBMCs from a ragweed-allergic individual as described in Materials and Methods. T cells (5×10^5) were cultured alone or with autologous B cells (2×10^5) in the presence or absence of *Amb a VI* ($2 \mu\text{g/ml}$). After 12 days in culture, the cells were transferred to 96-well nitrocellulose-based plates coated with anti-human IgE or *Amb a VI* ($5 \mu\text{g/ml}$) and incubated for an additional 48 hours. The IgE secretion from the cells was determined by an assay with biotinylated, goat anti-human IgE ($5 \mu\text{g/ml}$) followed by avidin-peroxidase conjugate. Spots representing IgE-producing B cells were developed with the substrate 3-amino-9-ethylcarbazole. **A**, Culture with T cells alone; **B**, B cells only; **C**, culture with T and B cells plus *Amb a VI*; **D**, T and B cells without antigen.

either 7 or 9 days, followed by a 2-day incubation, was not sufficient to demonstrate detectable IgE-secreting cells. However, a 12-day culture followed by an additional 2-day incubation gave rise to the IgE-secreting cells that could readily be identified as colored spots on the nitrocellulose bases of the wells after the addition of biotinylated anti-human IgE and avidin-peroxidase conjugate (Fig. 1). The cells remained viable during this initial 12-day culture period. An additional 2-day seeding and incubation period in the wells was optimal, when compared with a 24-hour incubation, in allowing differentiation of B cells into Ig-secreting cells. Neither T cells nor B cells that were cultured alone showed any sign of colored spots (Fig. 1, *A* and *B*, respectively), but spots representing *Amb a VI*-specific IgE-secreting cells

were clearly visible after the coculture of B cells with autologous peripheral blood T cells and antigen (Fig. 1, *C*). There was also a spontaneous production of IgE in the cultures containing a mixture of peripheral blood B and T cells (Fig. 1, *D*). The induction of anti-*Amb a VI* IgE synthesis was antigen-specific, because no spots could be observed either in *Amb t V*-stimulated cultures or in cultures stimulated with *Amb a VI* where the paper discs were coated with *Amb t V*.

We obtained quantitative data concerning the requirement for B and T cells and the influence of IL-4 on IgE biosynthesis by directly counting IgE-producing cells with the use of a dissecting microscope. There was a significant threefold increase in the total number of non-antigen-specific IgE-secreting cells when rIL-4 was added to the

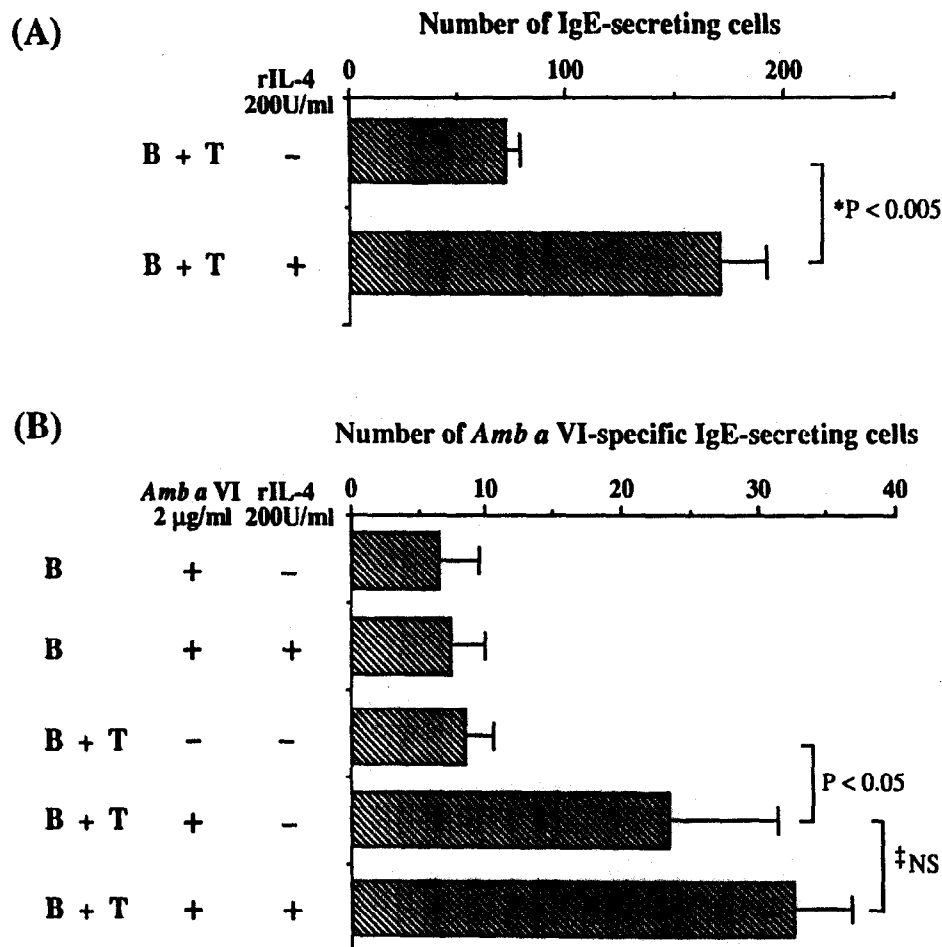


FIG. 2. Enumeration of spots representing IgE-secreting cells. Cell cultures and IgE measurements were performed as described in the legend to Fig. 1, except for the addition of rIL-4 in the cultures where indicated. The number of spots was enumerated with the aid of a dissecting microscope. *Statistical analysis with Student's *t* test; ‡ no statistical significance.

culture that contained peripheral blood B and T cells (Fig. 2, A). To determine the number of cells secreting *Amb a* VI-specific IgE, several cultures were established in parallel with either B cells alone or a mixture of T and B cells in the presence or absence of rIL-4 (Fig. 2, B). Our results showed that the number of specific IgE-secreting cells increased (on average, 15 spots above background) when autologous T cells and *Amb a* VI were present in the culture; however, the addition of rIL-4 did not significantly enhance the *Amb a* VI-specific IgE response. These results indicate that T cells are required for the induction of *Amb a* VI-specific IgE synthesis.

To validate the ELISPOT assay further, we also analyzed *Amb a* V-specific IgE synthesis in the presence of an *Amb a* V-specific T-cell clone, AP1.2. Fig. 3, A shows that there is a significant increase in the number of specific IgE-secreting

cells in the presence of specific T cells and *Amb a* V. Although the addition of rIL-4 enhanced the number of IgE-secreting cells when autologous peripheral blood T cells and antigen were added (Fig. 3, A), the addition of rIL-4 had no additive effect when specific T cells and antigen were present in the culture. This may reflect the maximum responses of specific B cells in the culture, or endogenous IL-4 may be sufficient to provide optimal responses. In addition, no spots could be detected either in the presence of a control antigen, *Amb t* V, or in cultures stimulated with *Amb a* V where the paper discs were coated with *Amb t* V. With the use of PCR techniques, the *Amb a* V-specific T-cell clone, after activation, was found to express IL-3, IL-4, IL-5, IL-6, GM-CSF, and β -actin genes, demonstrating the T_H2 -like cytokine profile (Fig. 3, B). The expression of IL-4 proteins in clone AP1.2 was also demonstrated by

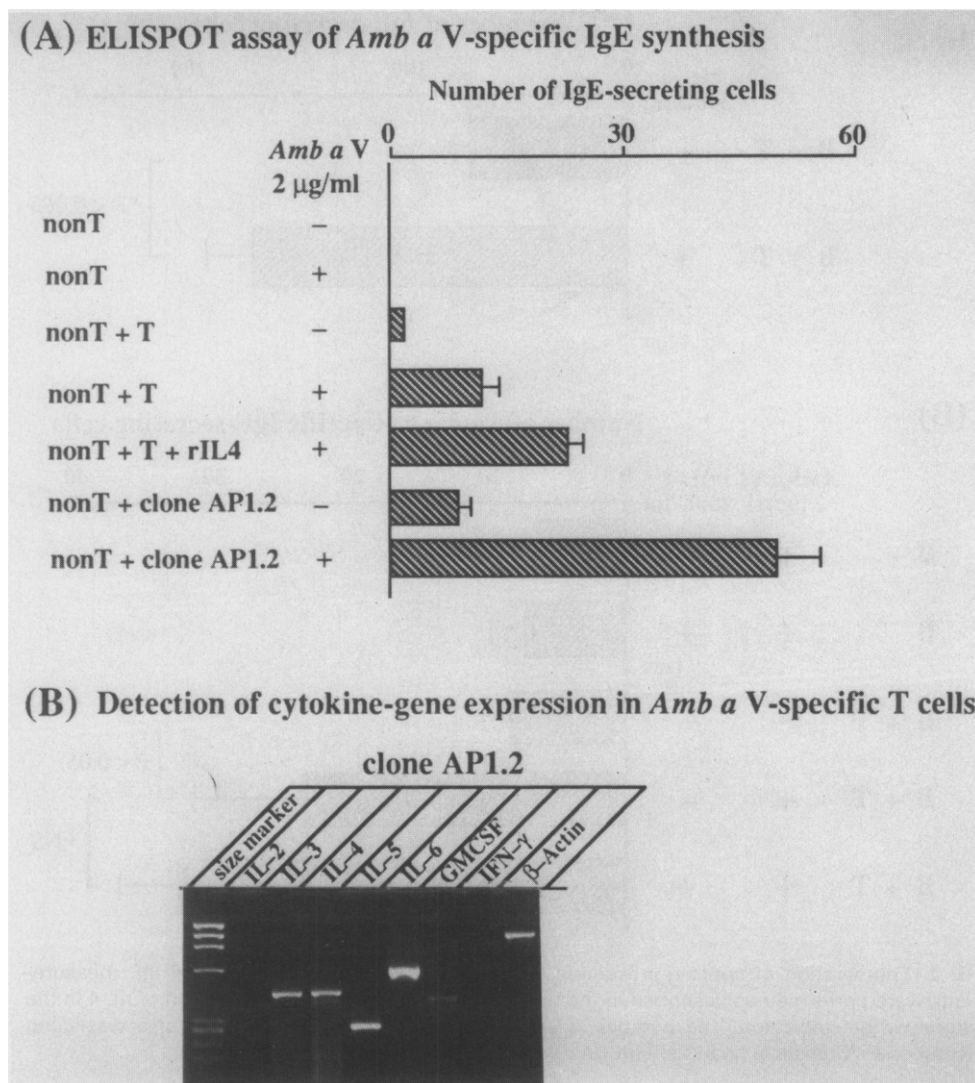


FIG. 3. A, Detection of *Amb a* V-specific IgE synthesis with the ELISPOT assay. Autologous PBMCs were separated into rosette-forming positive (*T*) and negative (*non-T*) populations with sheep erythrocytes treated with 2-amino-ethylisothiuronium bromide. In some experiments, rIL-4 (200 U/ml) was added to the cultures. **B,** PCR analysis of cytokine-gene expression in a *Amb a* V-specific T-cell clone (AP1.2).

a bioassay in which a mouse cell line transfected with human IL-4 receptor cDNA was used (Kaiser et al., unpublished data). A similar T_{H2} -like cytokine profile was also observed in an *Amb a* VI-specific T-cell clone, TS309. These results are consistent with results of other studies,⁴⁻⁶ demonstrating that T_{H2} -like cells play a functional role in IgE regulation in human beings.

Induction of *Amb a* VI-specific IgE synthesis from resting B cells

To rule out the possibility that the induction of *Amb a* VI-specific IgE synthesis in vitro may result

primarily from the expansion of activated *Amb a* VI-specific B cells that have undergone a class switch in vivo, highly purified resting B cells (surface IgE⁻) were analyzed. As demonstrated in Fig. 4, *A*, *Amb a* VI-specific IgE responses were observed only in the presence of autologous peripheral blood T cells plus antigen, and the addition of rIL-4 significantly increased the number of specific IgE-secreting cells. Furthermore, the necessity for direct interaction between T and B cells for the generation of specific IgE responses was demonstrated by the fact that concentrated supernatants from *Amb a* VI-activated T-cell cul-

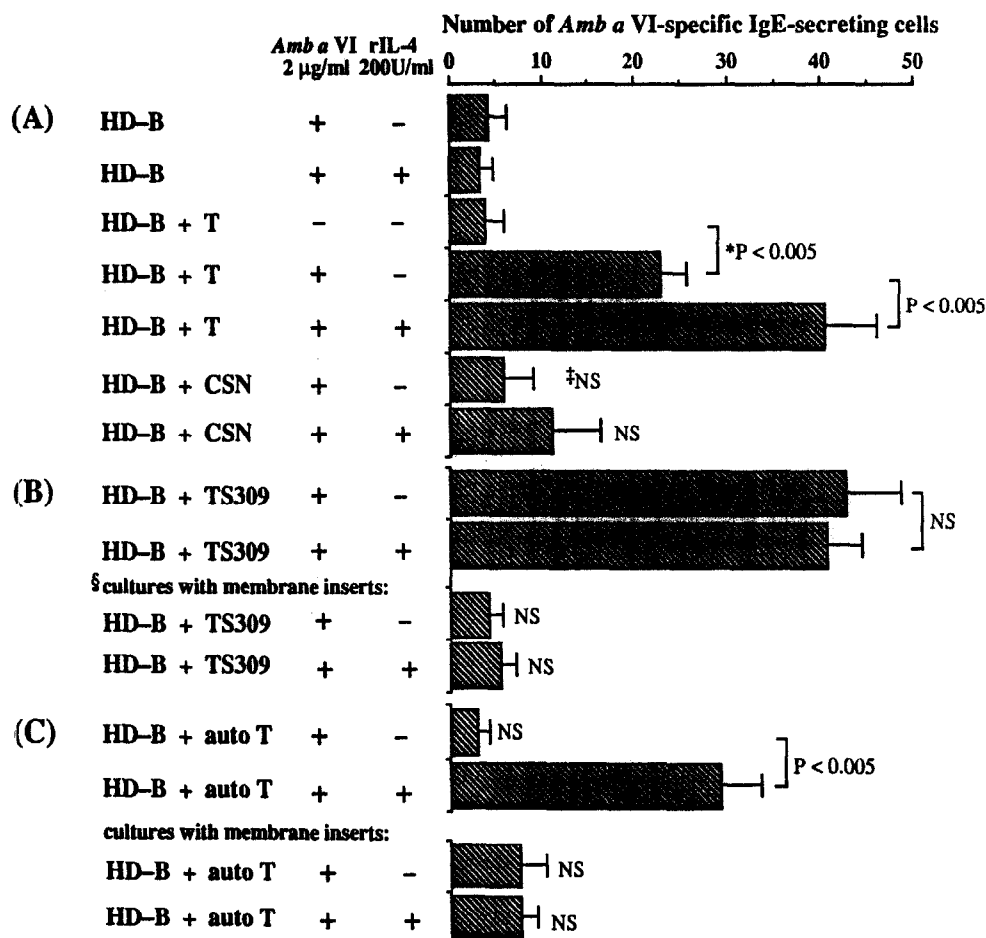


FIG. 4. Quantitation of *Amb a* VI-specific IgE-secreting cells. The high-density, resting B cells (HD-B) were purified as described in Materials and Methods. For the induction of *Amb a* VI-specific IgE synthesis, B cells (2×10^5) were cultured alone or with autologous T cells (5×10^5), an *Amb a* VI-specific T-cell clone (TS 309), an autoreactive T-cell line (auto T), or concentrated activated T-cell culture supernatants (CSN) in the presence or absence of *Amb a* VI. rIL-4 was added in the cultures as indicated. After 12 days in culture, the cells were transferred to 96-well nitrocellulose-based plates coated with *Amb a* VI (5 µg/ml) and incubated for an additional 48 hours. For measurement of IgE, assays were run as described in the legend to Fig. 1. *Student's *t* test; † no statistical significance when compared with the culture containing only high-density B cells, or as otherwise indicated; §T and B cells were cocultured in compartments separated by a 0.4 µm membrane.

tures had no effect on IgE synthesis, even with the addition of rIL-4 (Fig. 4, A).

The importance of T cells and IL-4 on the induction of *Amb a* VI-specific IgE class switching and protein synthesis was further demonstrated by the finding that the *Amb a* VI-specific T-cell clone, TS309, was able to induce *Amb a* VI-specific IgE synthesis from autologous resting B cells (surface IgE⁻) at the initiation of the cultures (Fig. 4, B). The addition of rIL-4 produced no enhancement of specific IgE synthesis, suggesting that the response of the resting B cells was clearly maximal in the presence of cloned T cells

and *Amb a* VI. The ability of clone TS309 to induce specific IgE responses was abolished by separating the T cells from B cells with a membrane (Fig. 4, B), suggesting again that the direct physical interaction between T and B cells is required. Furthermore, the addition of rIL-4 had no effect on *Amb a* VI-specific IgE synthesis. The direct interaction was an antigen-specific event because an irrelevant allergen, *Amb t* V, was not able to induce specific IgE synthesis (data not shown). In further experiments (Fig. 4, C) it was noted that the induction of *Amb a* VI-specific IgE synthesis could also be achieved by an autoreac-

tive T-cell line without apparent antigen specificity, provided that rIL-4 was added to the culture. This T-cell line showed significant proliferative responses in the presence of irradiated, autologous PBMCs alone and could induce specific IgE synthesis only when the T cells were in direct contact with the B cells (Fig. 4, C).

DISCUSSION

The role of IgE in immediate hypersensitivity has been appreciated for some time, but the molecular and cellular mechanisms that regulate antigen-specific IgE synthesis have yet to be defined. In human beings, it has been difficult to detect specific IgE synthesis in vitro, particularly for the minor allergens, such as *Amb a V* and *Amb a VI*. In this study we have shown that the ELISPOT assay can be used to monitor both allergen-specific and nonspecific IgE synthesis de novo at the single-cell level, both qualitatively and quantitatively. We have also provided evidence that direct interaction of T cells and B cells is required for the induction of specific IgE responses when both an *Amb a VI*-specific T-cell clone and an autoreactive T-cell line are used.

The precursor frequency of IgE-secreting B cells is known to be low, which has hindered progress in elucidating the regulatory mechanisms of specific IgE biosynthesis. Furthermore, the specificity and sensitivity of various conventional immunoassays sometimes vary. We have circumvented these problems by using the ELISPOT assay. On the basis of data from two experiments, we found that of 2.5×10^5 input B cells, there were between 23 and 42 spots under our experimental conditions, which represent cells secreting *Amb a VI*-specific IgE after the coculture of B cells with either peripheral blood T cells or an *Amb a VI*-specific T-cell clone in the presence of antigen. Thus the precursor frequency of specific B cells may be well below 10^{-5} , which may explain our failure to measure specific IgE to *Amb a VI* consistently and accurately in the culture supernatants which conventional ELISA (unpublished data). The ELISPOT technique thus offers a better approach to provide quantitative data on specific IgE responses, especially to minor allergens like *Amb a V* and *Amb a VI*.

It has previously been demonstrated that an activation signal generated after interaction of T cells and B cells is able to synergize with IL-4, allowing IgE synthesis from the B cells.^{5, 10, 19} The

signal thus generated in either allogenic or autologous mixed lymphocyte reaction,⁹ or in the case of anti-CD40, treatment is synergistic with IL-4 in inducing productive IgE class switching.²⁰ After coculture with autologous resting B cells, we found that an *Amb a VI*-specific T-cell clone (TS309) was able to induce specific IgE synthesis from the resting B cells; however, this "helper" function could be inhibited by separating the T and B cells with a membrane, suggesting that *Amb a VI*-dependent T-B *cognate* interaction is essential for the induction of *Amb a VI*-specific IgE synthesis in our culture system. In addition, the effect of IL-4 and T-B *cognate* interaction on the induction of specific IgE synthesis was clearly shown by use of an autoreactive T-cell line where the physical separation of T and B cells abolished the synergistic effect of IL-4. Our study therefore further strengthens the idea that both *cognate* help (class II major histocompatibility complex-restricted T-B interaction) and *noncognate* help (activation-differentiation cytokines, particularly IL-4) are normally required for the induction of IgE synthesis.

Studies^{10, 21-23} in both humans and murine systems have shown that B cells can be activated and differentiated into Ig-secreting cells in an antigen unrelated and major histocompatibility complex-independent manner by activated T cells or by membranes from activated T cells, provided that IL-4 is added to the culture. These studies suggest that although *cognate* help is clearly involved, an additional costimulatory factor(s) (independent of the major histocompatibility complex), associated with the activated T-cell membrane, is required. Recent murine studies have also suggested that direct T-B interactions provide an activation signal that allows B cells to enter the cell cycle. Subsequent elaboration of T-cell-derived cytokines is required for the progression of B cells into S phase and ultimate differentiation into Ig-secreting cells.²⁴ Our in vitro system provides a useful model for definitive analysis of the molecular nature of the *cognate* signals between T and B cells and the precise functions of the various cytokines in allergen-specific IgE responses in human beings. Furthermore, this assay system could be used to explore the molecular mechanisms of allergen-induced immunoglobulin-gene class switching and IgE expression.

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