

Specific V β T Cell Subsets Mediate the Immediate Hypersensitivity Response to Ragweed Allergen¹

Harald Renz,* Joachim Saloga,* Katherine L. Bradley,* Joan E. Loader,*
Julia L. Greenstein,[†] Gary Larsen,* and Erwin W. Gelfand^{2*}

*Divisions of Basic Sciences and Pulmonary Medicine, Department of Pediatrics, National Jewish Center for Immunology and Respiratory Medicine, Denver, CO 80206, and [†]ImmuLogic Pharmaceutical Corporation, Cambridge, MA 02139

ABSTRACT. T and B cell responses after sensitization to ragweed (RW) were examined in a mouse model in which BALB/c mice were exposed to the allergen by ultrasonic nebulization. Sensitization resulted in the stimulation of an IgE anti-RW response and was paralleled by a rise in IgG1 anti-RW titers. Skin testing for immediate cutaneous hypersensitivity revealed the presence of allergic type I reactions to RW. Sensitization to RW in this way was also associated with the development of increased airways responsiveness as determined by electrical field stimulation of preparations of tracheal smooth muscle. Histologic examination of the airways and the lung indicated the presence of a mononuclear cell infiltrate in the mucosa and submucosa of the airways that was accompanied by an enlargement of local draining lymph nodes of the airways and the lung. T cell populations were analyzed for the frequency of V β -expressing T cells. Such analysis indicated that RW sensitization stimulated the expression of V β 8.1⁺, V β 8.2⁺, and V β 13⁺ T cells in the local lymphoid tissue and of V β 8.1⁺, V β 8.2⁺, V β 8.3⁺, V β 9⁺ and V β 14⁺ T cells in the spleen. Co-culture of these T cell populations with RW-primed B cells indicated that in the presence of RW, V β 8.2 T cells stimulated IgE and IgG1 production, whereas the other T cell populations showed a different stimulation profile for Ig isotypes and IgG subclasses. The transfer of V β 8.2 T cells from sensitized but not from nonsensitized control mice stimulated an allergen-specific IgE and IgG1 response and increased airways responsiveness in naive recipients. These data provide additional support for the pivotal role of specific V β -expressing T cell subpopulations in the stimulation of IgE/IgG1 production and increased airways responsiveness. *Journal of Immunology*, 1993, 151: 1907.

Immediate type allergic reactions are mediated by IgE. The regulation of IgE production is controlled by T cells. The stimulation of IgE production requires at least two distinct T cell signals or sets of signals that are

delivered by activated CD4⁺ T cells (1). One signal is IL-4, which is secreted by a subset of CD4⁺ T cells (Th 2 cells) and exerts a number of different effects on B cells including up-regulation of MHC class II molecules and stimulation of B cell proliferation (2, 3). Important for the stimulation of IgE production is the induction of ϵ -germline transcripts by IL-4 (4–7). The other signal for the stimulation of allergen-specific IgE production requires the engagement of the TCR with antigenic fragments (peptides) that are recognized on MHC class II molecules on APC (8, 9). The arrangement of TCR elements on the α - and β -chains, including the variable elements (V α and V β), determines the Ag specificity of the T cell.

The aim of our studies was to identify which TCR are stimulated after allergen sensitization and to analyze the role of different TCR-expressing murine T cells in the

Received for publication December 9, 1992. Accepted for publication May 18, 1993.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

¹ This work was supported by Grants PO-HL36577 (G.L.L., E.W.G.) and AI-26490, and AI-29704 (E.W.G.) from the National Institutes of Health. H.R. and J.S. are supported by Grants Sa-483/1-1 and Re 737/1-1 from the Deutsche Forschungsgemeinschaft. This work was funded in part by a grant from ImmuLogic Pharmaceutical Corporation, Cambridge, MA.

² Address correspondence and reprint requests to Dr. Erwin W. Gelfand, Department of Pediatrics, National Jewish Center for Immunology and Respiratory Medicine, 1400 Jackson Street, Denver, CO 80206.

pathogenesis of allergic diseases. In a recent series of studies, we identified a specific T cell population ($V\beta 8.1/8.2$ T cells) that was stimulated after sensitization of IgE high-responder BALB/c mice to OVA (10). In these experiments, mice were sensitized by allergen inhalation, which stimulated an allergen-specific IgE response and was associated with the development of increased AR³ as assessed by electrical field stimulation of trachea smooth muscle preparations (11). We found that $V\beta 8.1/8.2$ T cells from OVA-sensitized mice provided help for allergen-specific IgE production *in vitro* as well as *in vivo*. The transfer of $V\beta 8.1/8.2$ T cells from OVA-sensitized mice into nonsensitized recipients stimulated an allergen-specific IgE response, immediate cutaneous hypersensitivity to OVA, and increased AR (12).

These findings implied that $V\beta 8$ T cells may play a unique role in the regulation of murine IgE production and suggest the possibility that each allergen may stimulate different $V\beta$ TCR-expressing T cells with discrete effects on allergen-specific IgE production. The present study was undertaken to address this possibility to identify murine T cell populations that were stimulated by sensitization to a different allergen, RW. RW was selected since it is one of the most potent seasonal aeroallergens in North America (13–15). In these studies, BALB/c mice were sensitized to RW extract by ultrasonic nebulization of the allergen. The results of this form of sensitization were monitored by the measurement of allergen-specific IgE production and immediate cutaneous hypersensitivity. After completion of the sensitization course, AR was analyzed by electrical field stimulation. Specifically, we examined whether sensitization to RW resulted in the stimulation of selected $V\beta$ TCR T cell populations, different from those identified after sensitization to OVA. In the second part of the study, we delineated the function of these T cells in the regulation of antibody production and AR. The results provide additional insight into the understanding of lymphocyte function after exposure to a common allergen and may provide the basis for new immunotherapy strategies in allergic diseases.

Materials and Methods

Animals

Female BALB/cByJ mice between 8 and 12 wk of age were obtained from The Jackson Laboratory (Bar Harbor, ME).

Immunization

Mice were immunized by ultrasonic nebulization of a total amount of 500 μ g RW extract diluted in 7 ml of sterile PBS. A stock solution of allergen was prepared as described (13)

using 2 g pollen in 7 ml of buffer (no protease inhibitors were added). Up to eight mice were placed in a clear plastic box with a removable top (dimension 22 $\times$ 23 $\times$ 14 cm). The RW solution was aerosolized into one end of the box using an ultrasonic nebulizer (PulmoSonic model 25; The DeVilbiss Co., Somerset, PA) at a continuous pressure of 5 ψ . At the other end of the chamber were two small air holes to ensure a continuous cross-current flow of air. As defined by laser nephelometry (15), more than 90% of the Ag particles were in the 1 to 5 μ m range.

Immunization was carried out in one of the following ways: 1) a daily 20-min exposure to RW over a 10-day period was used; or 2) mice were exposed for 20 min once a week on four occasions. Age-matched control animals were exposed to PBS alone using the same protocols. Two days after the last exposure, venous blood was obtained from the tail vein under anesthesia (Avertin, 5 mg/g *i.p.*). Blood was allowed to clot at room temperature and serum was collected after centrifugation at 1500 rpm for 5 min at room temperature. Serum samples were stored at -20°C until analyzed for IgE and IgG anti-RW serum titers.

Determination of anti-RW serum antibody levels

IgE and IgG anti-RW serum antibody levels were measured by ELISA. Round bottomed microtiter plates (96-well Immulon II; Dynatech Laboratories) were coated with 20 μ g/ml RW diluted in NaHCO_3 buffer, pH 8.2.

The antibody levels in samples were compared with known standards. The IgE standard was obtained from a pool of serum collected from five mice sensitized to RW by *i.p.* injections of 50 μ g RW diluted in aluminium hydroxide. This procedure resulted in the development of strongly positive skin reactions to RW two wk after the injection. The concentration of IgE anti-RW was arbitrarily assigned to be 1000 ELISA units. For IgG, serum was pooled from mice immunized to RW by *i.p.* injection of 500 μ g RW/mouse. In this case, no positive skin reactions to RW were found and the concentration of IgG anti-RW was arbitrarily assigned to be 1000 ELISA units.

Expression of $V\beta$

Expression of $V\beta$ elements on freshly prepared T cells was analyzed by flow cytometry as described previously (10). Incubation of cells with biotinylated anti- $V\beta$ -antibodies ($V\beta 2$ (B20.6); $V\beta 3$ (KJ25a); $V\beta 5.1 + V\beta 5.2$ (MR9-4); $V\beta 6$ (RR4-7); $V\beta 9$ (TR130); $V\beta 8.1 + 8.2$ (KJ16); $V\beta 8.2$ (F23.2); $V\beta 8.1 + 8.2 + 8.3$ (F23.1), $V\beta 9$ (MR10-2); $V\beta 11$ (RR-15); $V\beta 14$ (14-2); $V\beta 17$ (KJ23a)) was carried out in staining buffer (PBS supplemented with 0.3% sodium azide and 2% heat-inactivated FCS for 30 min at 4°C). Stained cells were analyzed using an Epics cytofluorograph (Coulter Electronics, Hialeah, FL). Fluorescence intensity

³ Abbreviations used in this paper: AR, airways responsiveness; ICH, immediate cutaneous hypersensitivity; PBLN, peribronchial lymph nodes; ES₅₀, frequency causing 50% of maximal contraction; RW, ragweed.

was compared with negative controls, and cells were incubated with phycoerythrin-coupled streptavidin alone. Results were expressed as percent positive cells. For cell sorting experiments, cells were treated in a similar fashion but incubated in sterile PBS.

Purification and expansion of T cells

Two days after completion of the RW sensitization course, spleens were obtained and mononuclear cells were restimulated with RW (50 $\mu\text{g}/\text{ml}$) for 4 days. Viable cells were collected from the interface after Lymphoprep gradient centrifugation. The cells were then used for sequential panning. For this purpose, tissue culture flasks were coated with anti-V β 2 (B20.6), V β 8.2 (F23.2), V β 8.1 + V β 8.2 (KJ 16), V β 8.1 + 8.2 + 8.3 (F23.1), V β 9 (MR 10-2), or V β 14 (14.2) antibodies. The flasks were incubated first with the respective anti-isotype antibodies (goat anti-rat or goat anti-mouse Ig), which were diluted to a concentration of 100 $\mu\text{g}/\text{ml}$ in coating buffer. A second incubation was followed with the specific anti-V β antibodies. All incubations were carried out as described for coating of ELISA plates. For panning, lymphocytes were resuspended in complete tissue culture medium at a concentration of 10×10^6 cells/ml and incubated first on the anti-V β 8.2-coated plate for 1 h at 37°C. After completion of the incubation, nonadherent cells were removed gently by washing with PBS. The adherent cells were incubated further in complete tissue culture medium. The nonadherent cells were washed and resuspended in complete tissue culture medium. Analysis by flow cytometry indicated that these cells were depleted of V β 8.2 cells by more than 85%. This depleted population was then incubated in the V β 8.1/8.2-coated flask for 1 h. This procedure removed V β 8.1⁺ cells. To purify the V β 8.3⁺ cells, the procedure was repeated by incubation in a flask coated with anti-V β 8.1/8.2/8.3 antibody. This method of sequential panning was chosen since no antibody detecting solely V β 8.1 or V β 8.3 is currently available. The procedure was then repeated to select V β 2⁺, V β 9⁺, and V β 14⁺ cells.

For the B-T cell co-culture assay, the bound T lymphocytes were harvested from the panning flasks after 48 to 72 h and centrifuged over a Lymphoprep gradient as described, and the purity of the viable cells was determined by flow cytometry. The data indicated that the respective V β -expressing T cell populations reached a purity of at least 92%.

For in vivo transfer experiments, V β 8.2⁺ cells were expanded and activated as described (12). The purity of these cells was tested by flow cytometry and indicated a percentage of V β 8.2 T cells of greater than 95% in all experiments.

T- and B cell co-culture experiments

RW-primed B cells were purified from spleens 7 to 10 days after i.p. injection of 50 μg RW diluted in 300 μl PBS.

Spleens were removed and B cells were purified by incubation of mononuclear cells with anti-thymocyte serum (1:50 v/v; Accurate Chemical and Scientific Corporation, Westbury, NY) for 30 min at 4°C followed by addition of guinea pig C (1:15 v/v; Gibco, Grand Island, NY) for 30 min at 37°C. Cells were washed three times and monocytes/macrophages were removed by adherence on plastic Petri dishes for 2 h at 37°C. This preparation contained >98% B cells as judged by flow cytometry using mAb B220. B cells were then co-cultured with or without the different T cell populations (see above), and RW was added at a concentration of 10 $\mu\text{g}/\text{ml}$. Cells were cultured in 96-well flat bottomed culture plates (Falcon) in complete tissue culture medium for 9 days. At the end of the culture period, culture supernatants were removed and stored at -20°C until analysis.

IgG and IgE production

Culture supernatants were analyzed for IgE, IgG1, and total IgG by ELISA as described previously (10).

Lymphocyte transfer

Desired concentrations of T cells (between 5×10^6 and 5×10^7 /recipient) were resuspended in sterile PBS in a final volume not exceeding 0.25 ml. The cell suspension was injected i.v. into the tail vein of age- and sex-matched syngeneic recipients using a previously established protocol (12). Immediately after the transfer, the recipients received a single RW challenge delivered by ultrasonic nebulization as described above. This single challenge on its own was without effect when IgE levels and AR were monitored. Five days later, immunologic and physiologic parameters were analyzed.

Determination of ICH to RW

ICH was analyzed by skin testing as described recently (16). The test solutions were RW (15 $\mu\text{g}/\text{ml}$) diluted in PBS, PBS solution as the negative control, and compound 48/80 (Sigma) as the positive control. Skin tests were read by an investigator who was blinded to the treatment status of each animal.

Assessment of airway smooth muscle responsiveness

Airway smooth muscle responsiveness was assessed in vitro using a tracheal smooth muscle preparation as recently described (11). ES_{50} values were calculated from linear plots. All tissues were assessed in terms of the in vitro responsiveness by one investigator (J.E.L.), who was blinded to the immunologic treatment status of each airway preparation.

Table I
Nebulization of RW results in IgE and IgG1 anti-RW responses and immediate cutaneous hypersensitivity^a

Sensitization	Protocol	Anti-RW		Skin Test Response	
		IgE (EU ^b /ml)	IgG1 (EU/ml)	n	%
PBS	10 day	<40	<40	0/16	0
RW	10 day	606 ± 294	208 ± 72	14/15	93
RW	1/wk	1316 ± 213	193 ± 47	13/16	81

^a BALB/c mice were sensitized to RW for either 10 days ($n = 15$) or once a week for 3 wk ($n = 16$) or exposed to PBS alone ($n = 16$). Serum was collected 2 days after completion of the nebulization course and analyzed for IgE and IgG1 anti-RW antibody titers by ELISA as described in *Materials and Methods*. Results are expressed as mean ± SD. Immediate cutaneous hypersensitivity to RW was analyzed in parallel.

^b EU, ELISA units.

Statistical analysis

Different experimental conditions and groups were compared by the two-tailed Student's *t* test. Skin test results were compared by Fisher's exact test.

Results

Aerosolization of ragweed Ag results in the induction of an IgE and IgG1 response, ICH, and increased AR

BALB/c mice were sensitized to RW employing the short and long term exposure protocol. For the short term sensitization, animals were exposed to 500 µg RW for 20 min each day over 10 days. In the second protocol, the animals were exposed to the same amount of RW once a week over 3 wk (four nebulizations). The development of allergen-specific Ig titers was analyzed 2 days after the last exposure. The 10-day exposure as well as the long term sensitization protocol resulted in stimulation of RW-specific IgE antibody titers (Table I). Although both sensitization protocols elicited RW-specific IgE responses, higher antibody titers were detected after the long term protocol. In parallel to IgE, IgG1 anti-RW titers were also stimulated, but no differences were observed between short and long term sensitization.

As demonstrated recently utilizing a different allergen, OVA, increases in allergen-specific IgE (and IgG1) titers after sensitization of the skin were accompanied by the development of ICH as determined by intradermal skin testing (16). Positive immediate type skin reactions to RW, but not to the irrelevant allergen OVA, were detected in 14 of 15 mice sensitized to RW over 10 days (93%). A similar result was observed in mice sensitized over 3 wk; in this case, 13 of 16 mice had positive reactions (81%) (Table I). In contrast, none of the PBS-exposed control mice showed any reaction to the allergen, but all responded to compound 48/80.

Sensitization by allergen (OVA) inhalation triggers not only increased IgE production but altered AR (11, 17).

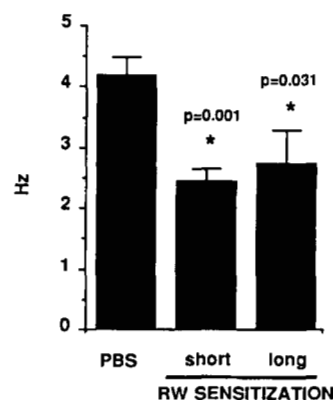


FIGURE 1. Determination of airways responsiveness by electrical field stimulation. Two days after the last aerosolization of allergen or PBS, tracheas were prepared as described in *Materials and Methods* and exposed to increased frequencies of electrical stimulation. Tracheas were prepared from PBS-exposed control mice and from mice sensitized to RW employing either a short term sensitization protocol (10 consecutive days) or a long term protocol (3 wk, four exposures). For each trachea, a dose-response curve was established and the ES₅₀ was calculated. Expressed are mean ± SE of ES₅₀ for each group. *, Significant differences; *p* values are shown.

We determined whether similar increases in AR developed in mice sensitized to RW. For this purpose, tracheal smooth muscle was prepared, and the degree of contractility was measured by electrical field stimulation. The ES₅₀ values were calculated for each tracheal preparation from a dose-response curve performed with increasing electrical frequencies (ranging from 0.5 to 40 Hz). Increases in AR were observed after short term (10 day) as well as long term (3 wk) sensitization (Fig. 1). Under both conditions, a significant decrease in ES₅₀ was observed. The sensitization over a period of 10 days resulted in a decrease in the ES₅₀ levels from 4.18 ± 0.3 to 2.45 ± 0.2 Hz ($p < 0.001$). Similar data were found after long term sensitization to RW, during which the ES₅₀ was also significantly ($p < 0.03$) lower in RW-sensitized mice than in the control group.

These data demonstrate that short as well as long term sensitization to RW stimulated formation of allergen-specific IgE and IgG1 antibodies, which were associated with the development of ICH to the allergen but not to irrelevant allergens. Furthermore, these changes were associated with the development of increased AR after sensitization using both protocols.

Local lymphocytes are increased in numbers and stimulated IgE production

Histologic analysis of the airways and lungs of sensitized mice was performed 2 days after the last exposure to allergen (Fig. 2, A and B). Diapedesis of predominantly

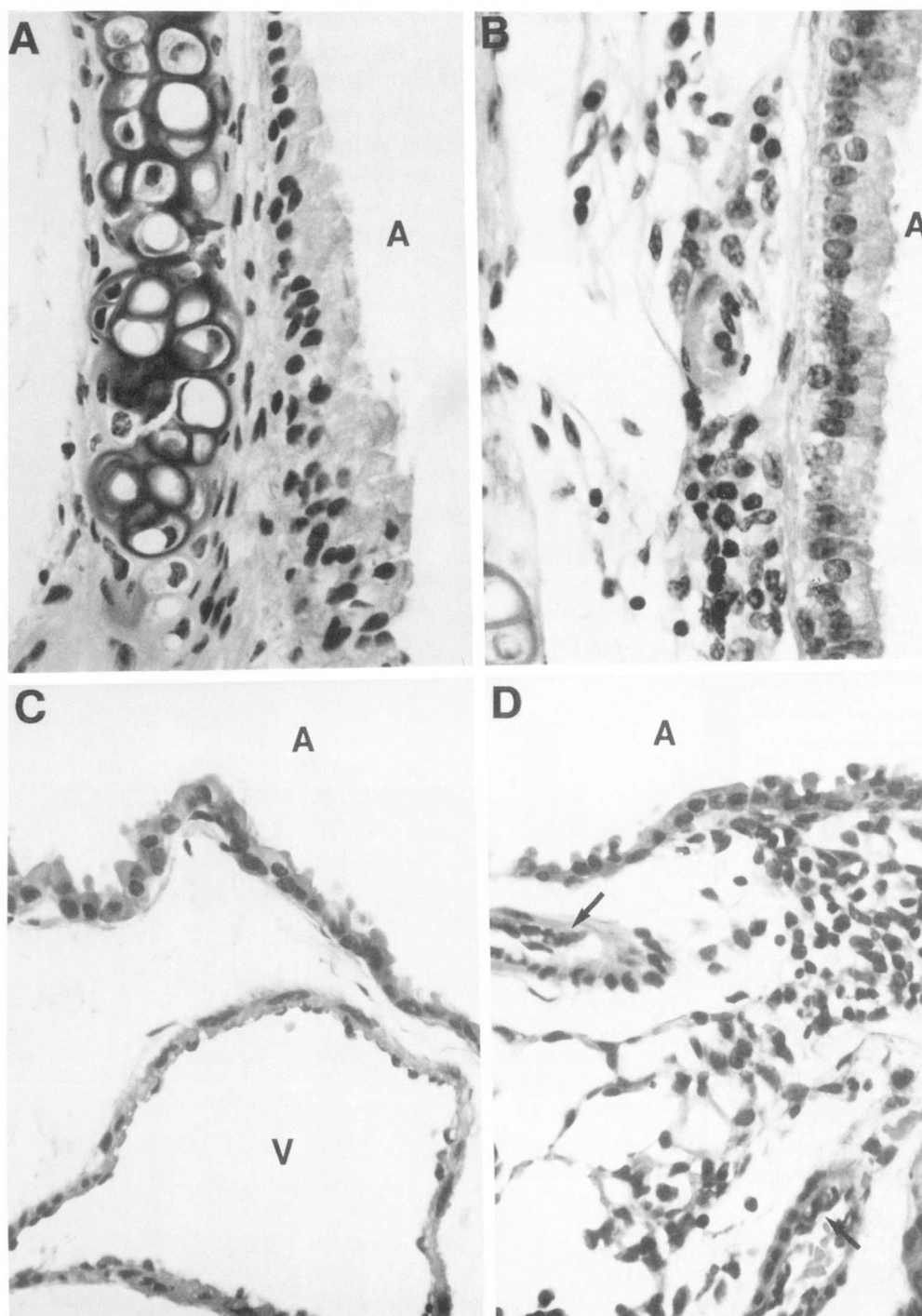


FIGURE 2. Histologic analysis of small airways and the trachea. Histologic sections were prepared from tissue 2 days after the last nebulization. Tissue was processed as described in *Materials and Methods*. Sections were prepared from small airways (A and B) and the trachea (C and D) of RW-sensitized mice (B and D) and PBS-exposed control animals (A and C). V, small blood vessel; A, airways, arrows point to small blood vessel where adherence and diapedesis of predominantly mononuclear cells occurred. Magnifications are $\times 125$.

mononuclear cells was found from small blood vessels that were located in close anatomical association with smaller and larger airways. This resulted in an influx of these cells into the submucosa and mucosa of the airways. The trachea displayed similar pathologic changes, but the influx of mononuclear cells was more evident than in the periphery

of the lung (Fig. 2, C and D). This increase in mononuclear cells was accompanied by an enlargement of local draining lymph nodes of the airways and the lung (PBLN). An approximately fivefold increase in total lymphocyte numbers was found in PBLN from RW-sensitized compared to PBS-exposed mice.

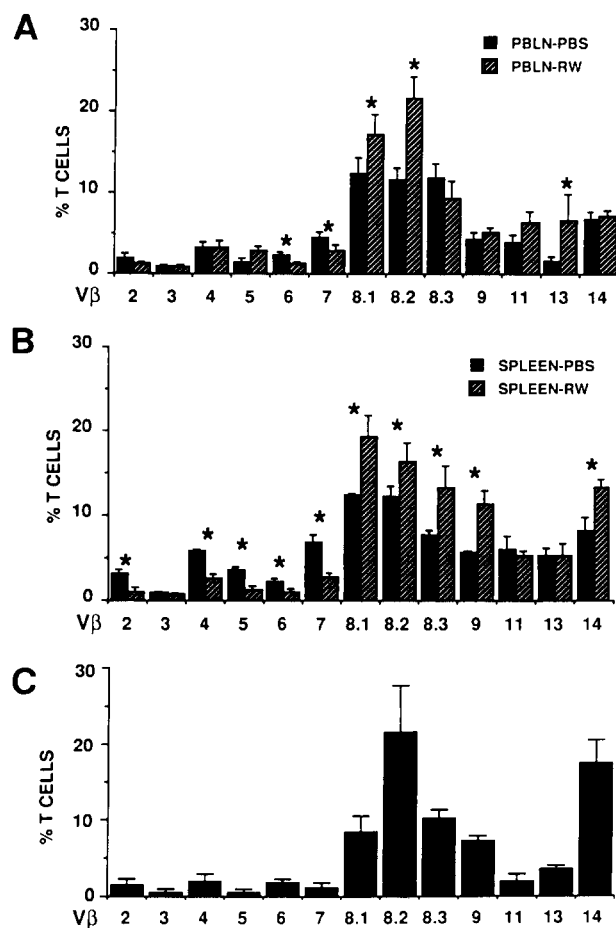


FIGURE 3. Analysis of the frequency of V β -positive T cell populations. Two days after the last allergen/PBS aerosolization, local draining lymph nodes of the airways and the lungs (PBLN) (A) and spleens (B) were prepared. Mononuclear cells were stained with anti-V β and anti-TCR/CD3 antibodies and analyzed by flow cytometry using a gate for lymphocytes as described in *Materials and Methods*. Based on the frequency of TCR/CD3 $^{+}$ cells, the percentage of V β -expressing T cells was calculated. Cells from three to four mice per experiment were pooled and analyzed together. Expressed are the mean $\pm$ SD of five independently performed experiments. C, Results of the analysis of cells from sensitized mice that were restimulated with the Ag for several days as described in *Materials and Methods*. These cells were analyzed on a gate for lymphoblasts. Expressed are mean $\pm$ SD of three independently performed experiments. *, Significant ($p < 0.05$) differences between the groups.

Sensitization to RW increases the frequency of T cells bearing specific V β elements on the TCR

The frequency of T cell subsets expressing different V β elements was examined in fresh lymphocytes prepared from PBLN and spleens of RW-sensitized mice. As demonstrated in Figure 3A, sensitization to RW resulted in a significant increase in the proportions of V β 8.1-, V β 8.2-, and V β 13-bearing T cells in PBLN. In parallel, the frequency of other V β -expressing T cells decreased (e.g.,

V β 6- and V β 7-bearing T cells). In addition to V β 8.1 $^{+}$ and V β 8.2 $^{+}$ T cells, an expansion of V β 8.3 $^{+}$, V β 9 $^{+}$, and V β 14 $^{+}$ cells was detected in the spleen. This was paralleled by decreases among subpopulations bearing V β 2, V β 4, V β 5, V β 6, and V β 7 (Fig. 3B). The data in Figure 3 accounted for more than 95% of the V β -expressing T cells in the respective tissues.

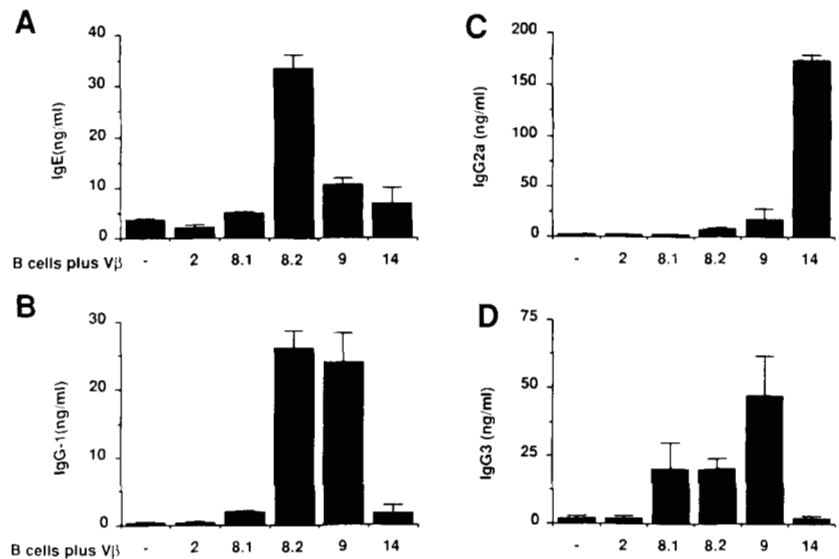
The distribution of V β -bearing T cell subsets was also examined after culture of lymphocytes with RW. For this purpose, spleen mononuclear cells from RW-sensitized mice were cultured with RW (50 μ g/ml) for 5 days, and the V β repertoire of surviving T cells was then studied by flow cytometry using a forward/sideward scatter for T cell blasts (Fig. 3C). Approximately 60% of all T cell blasts expressed either V β 8.1, V β 8.2, V β 8.3, V β 9, or V β 14. These data support the results obtained by the V β analysis on freshly prepared lymphocytes and indicated the expansion of V β 8.1-, V β 8.2-, V β 8.3-, V β 9-, and V β 14-expressing T cells in response to sensitization to RW.

Specific V β -expressing T cells differentially stimulate IgE and IgG subclass production

To analyze the capacity of the different T cell subpopulations to stimulate Ig production, V β 8.1 $^{+}$, V β 8.2 $^{+}$, V β 9 $^{+}$, and V β 14 $^{+}$ T cells from RW-sensitized mice were purified by panning (12). As a negative control, V β 2 T cells that were not found in higher frequency after RW sensitization were also purified. These T cells were co-cultured with RW-primed splenic B cells. The co-culture was carried out in the presence of RW (10 μ g/ml) for 9 days, and culture supernatants were assayed for total IgE and IgG subclass production.

Analysis of IgE production (Fig. 4A) indicated that the highest amounts of IgE were stimulated by V β 8.2 cells. The other V β -bearing T cells contributed to IgE production to a significantly lesser extent. The stimulation of IgG subclass production showed a different pattern. V β 9 T cells provided help for IgG1 production to a similar degree as V β 8.2 cells, whereas the other V β -bearing cells showed only limited ability to stimulate IgG1 production (Fig. 4B). The V β 14 $^{+}$ cells, which did not stimulate IgG1 and demonstrated minimal capacity to induce IgE, showed a strong capacity to induce total IgG2a production (Fig. 4C). IgG3 was stimulated by V β 9 T cells and to a lesser degree also by V β 8.1 and 8.2 T cells (Fig. 4D). V β 2 cells from sensitized mice did not stimulate IgE or IgG production in this system. In addition to the analysis of splenic V β 8.2 T cells, V β 8.2-expressing cells were also purified from PBLN and co-cultured with sensitized B cells. It was found that PBLN V β 8.2 cells also stimulated IgE production (data not shown). Because of the limited number of PBLN lymphocytes in these experiments, only the role of V β 8.2 T cells was studied, inasmuch as they were present in the highest

FIGURE 4. Analysis of IgE and IgG subclass production. After completion of the RW-aerosolization course, different V β -expressing T cell populations were purified by sequential panning as described in *Materials and Methods*. B cells were prepared from spleens of mice that were immunized to RW by i.p. injections of RW as described. An equal number (50,000 cells each/well) of B and T cells were co-cultured for 9 days in the presence of RW (10 μ g/ml). Cell-free culture supernatants were harvested and analyzed for IgE (A), IgG1 (B), IgG2a (C), and IgG3 (D) production by ELISA as described. Shown are the mean $\pm$ SD from one of three experiments.



frequency in the lymph nodes. These data indicate that the different V β -bearing T cells stimulated a different profile of Ig isotypes and IgG subclass production.

Transfer of IgE production, immediate skin test reactivity, and AR by V β 8.2 T cells

Since the *in vitro* experiments indicated that V β 8.2 T cells stimulated the highest IgE response compared to other T cell subsets, we investigated their capacity to stimulate IgE production *in vivo*. For this purpose, V β 8.2 cells were purified from RW-sensitized and nonsensitized mice and expanded as described. These cells were then transferred by i.v. injection into nonsensitized recipients that received a single RW challenge via the airways immediately after the transfer. Five days later, the recipients were analyzed for RW-specific Ig production, ICH, and AR.

The results, summarized in Table II, indicate that splenic V β 8.2 T cells stimulated allergen-specific IgE and IgG1 production, and the amounts measured 5 days after cell transfer were in the ranges observed after sensitization over a 3-wk period in primary animals. Six of eight recipients (75%) of V β 8.2 T cells from sensitized donors had positive immediate-type skin test responses to the allergen, whereas none of the control mice (receiving V β 8.2 T cells from nonsensitized donors) demonstrated these responses. Similar to results obtained in primary sensitized mice, serum from animals receiving cells from sensitized mice elicited short latency (performed with heat-inactivated serum) as well as long latency passive cutaneous anaphylaxis tests, indicating that the IgE and the IgG1 antibodies were able to trigger immediate type I allergic responses to RW in the skin (18–20). In parallel to Ig production and ICH, increased AR was found in recipients after transfer of V β 8.2 cells from RW-sensitized but not from nonsensitized mice. The decrease in ES₅₀ of about 40% was in the range ob-

served after primary RW sensitization. Kinetic experiments indicated that at day 2 after transfer of V β 8.2 T cells, AR was normal in recipients, and only 25% of the recipients developed ICH (data not shown). These data demonstrated that V β 8.2 T cells from RW-sensitized mice can transfer the capacity to stimulate IgE and IgG1 production, induce allergen-specific ICH, and trigger increased AR.

Discussion

This study demonstrates that sensitization of BALB/c mice to the common allergen ragweed induced a systemic RW-specific IgE response. This response was achieved by ultrasonic nebulization of the allergen and was accompanied by evidence of sensitization of the mice through the local immune system of the airways and the lungs. The development of an allergic immune response was associated with acquisition of immediate cutaneous reactivity and the development of increased AR, as measured by electrical field stimulation of isolated tracheal smooth muscle preparations. Functional studies including lymphocyte transfer experiments indicated not only that T cells regulated the IgE and IgG1 responses, but also that they are part of the regulatory system that is involved in the control of AR.

It is well established that the production of IgE is under the regulatory control of T cells (21–23). A number of investigations focusing on the regulatory events governing polyclonal IgE production demonstrated that several sets of signals are required to stimulate IgE secretion. Activated but not resting CD4 T cells are able to induce IgE production (24). The process of cognate interaction between T and B cells is involved in this regulatory pathway (25). One mode of T-B cell interaction links the TCR and MHC class II molecules on B cells. This interaction leads to the activation of resting B cells and enhances the responsiveness

Table II
Transfer of IgE responsiveness and increased AR by V β 8.2 cells^a

T Cells	Donor	Anti-RW		Skin Test Response	ES ₅₀ (Hz)
		IgE (EU ^b /ml)	IgG1 (EU/ml)		
V β 8.2 (n = 16)	PBS	<40	<40	0/16 (0%)	3.0 $\pm$ 0.45
V β 8.2 (n = 8)	RW	1111 $\pm$ 82 ^c	237 $\pm$ 24 ^c	6/8 (75%) ^c	2.13 $\pm$ 0.23 ^c

^a V β 8.2 T cells were prepared from spleens of RW-sensitized and nonsensitized (PBS-exposed) controls and purified as described in *Materials and Methods*. Cells (10 $\times$ 10⁶/recipient) were transferred i.v. into nonsensitized animals that received one RW challenge immediately after the transfer. Five days later, serum samples were taken and analyzed for IgE and IgG1 anti-RW antibody titers by ELISA (means $\pm$ SD, ELISA units/ml). ICH was determined in parallel, and AR was analyzed by electrical field stimulation. Results are expressed as ES₅₀ (means $\pm$ SE).

^b EU, ELISA units.

^c Significant differences between the groups of mice.

of B cells to soluble T cell factors, the IL. One IL, IL-4, plays a critical role in the regulation of IgE by inducing the switch to IgE at the DNA level (26–28), resulting in the production of functional and active germline- ϵ transcripts (29–31). In addition to IgE, IL-4 also regulates the production of IgG1 (32). Since the sensitization to RW resulted in the stimulation of both IgE and IgG1 antibodies, it appears likely that IL-4 produced by activated T cells is involved in this system as well.

Several elements of the data support the pivotal role of T cells in the sensitizing response to RW and, subsequently, to the regulation of IgE production and development of increased AR. Histologic examination of the airways and the lungs at the end of either sensitization protocol demonstrated an influx of mononuclear cells in the local tissues. Adherence, predominantly of mononuclear cells to the inner wall of small blood vessels, diapedesis of these cells from the vessels into the submucosal and mucosal layers of small and large airways, and the enlargement of local draining lymph nodes of the airways and the lung characterized the response in RW-sensitized animals. The role of T cells in the regulation of allergic diseases in humans has been supported by data from a number of groups. Analysis of cells prepared from bronchial lavages before and after allergen challenge and of cells in bronchial biopsies from asthmatic patients (33–36) have indicated an increase in T cell numbers in the local environment during active disease. Additional analysis of the functional properties of these T cells showed that there was an increased number of IL-4⁺ and IL-5⁺ cells (37). These findings were paralleled by low numbers of IFN- γ -producing T cells.

The present study focused on the changes in the distribution of certain T cell populations that accompanied sensitization to RW, when allergen was administered via the airways. One obvious change involved the distribution of T cell subsets expressing different TCR-V β elements. This analysis indicated that different populations of V β -expressing T cells were selectively expanded in the PBLN and spleens of RW-sensitized mice. In PBLN, the major increase was in V β 8.2 T cells followed by an increased frequency of V β 13 and V β 8.1 T cells. In addition to V β 8.1

and V β 8.2 T cells, an increase in frequency of V β 8.3-, V β 9-, and V β 14-expressing cells was found in the spleens of sensitized animals. After in vitro stimulation of cells from sensitized animals with RW, a similar pattern of V β expression was observed in responding T cell lymphoblasts, namely V β 8.1, V β 8.2, V β 8.3, V β 9, and V β 14 cells.

Ragweed contains a number of different allergenic epitopes (9, 14). It is possible that each of the T cell epitopes stimulates a different V β -expressing T cell subset, accounting for the number of subsets that increased after Ag stimulation. This does not account for the differences in the pattern of V β -bearing T cells found to be stimulated in PBLN and spleen. One explanation for such differences may be that different populations of APC located in PBLN and spleen have different properties for Ag processing, leading to the presentation of different T cell epitopes to the TCR. The presence of specialized APC in different locations has been suggested by other investigators (38).

More importantly, it appeared that each of the T cell subpopulations expanded after sensitization exhibited a unique pattern of help for the production of Ig by primed B cells in vitro. The functional properties of the T cell subpopulation were examined in an allergen-driven system in which the T cells originated from RW-sensitized mice, the B cells were primed to the allergen, and RW was present in the culture. Under these conditions, V β 8.2 T cells provided help for IgE and IgG1 production, and V β 9 T cells stimulated IgE to a lesser degree but stimulated IgG1 to a degree similar to that of V β 8.2 cells. V β 14 T cells stimulated IgG2a, but not IgG1, and V β 8.1 T cells did not stimulate IgE or IgG1. T cells from nonsensitized animals showed little capacity to provide help for Ig synthesis under these conditions. These data indicated that IgE production was primarily stimulated by sensitized V β 8.2 T cells. This was the subset that also demonstrated the highest degree of expansion in PBLN after sensitization to RW. The pathways that may be used by these T cells to regulate Ig production by B cells are still unclear. Since IgE and IgG1 production are known to be dependent on the production of IL-4 while other IgG subclasses may be regulated by other IL, it is possible that different T cell subsets differ in their pattern

of cytokine production. It is unlikely, however, that a restricted profile of IL production is strictly aligned with a certain V β -bearing T cell population.

Based on our findings that V β 8.2 T cells stimulated IgE and IgG1 production to the highest degree and showed the greatest expansion in local draining lymph nodes, this T cell population was selected for in vivo transfer experiments. The results of these transfer experiments indicated that V β 8.2 T cells from RW-sensitized mice, but not from non-sensitized controls, transferred the capacity to induce RW-specific IgE/IgG1 responses and increases in AR in non-sensitized recipients. V β 8.2 T cells from sensitized and non-sensitized mice were treated in an identical fashion. The purification was accomplished by panning, and hence activation with anti-V β 8.2 antibodies, followed by an expansion in IL-4- and IL-2-containing medium. One advantage of this method is that the T cells are activated at the end of the purification period, and this activation may be one of the requirements for providing help for B cells (24). Although all recipients received a single RW challenge immediately after the cell transfer, anti-RW antibody titers were stimulated only after transfer of V β 8.2 T cells from sensitized mice, whereas these responses were not observed after transfer of cells from control, non-sensitized animals. These data imply that the stimulation of IgE and increased AR is not a general functional capacity of all activated V β 8.2 T cells, but is dependent on T cells that encountered the allergen previously.

These data are very similar to what we have reported after sensitization to OVA via the airways (11), and to some degree after sensitization through the skin (J. Saloga, H. Renz, G. L. Larsen, and E. W. Gelfand, manuscript in preparation). In these studies, we identified a T cell population that also expressed the V β 8.2 receptor and that stimulated IgE production and transferred increased AR in vivo. The RW system is another example of the specific role V β -expressing T cells play in the regulation of AR. In both allergen-dependent systems, T cells from the same V β family played a central role in the stimulation of IgE production. This finding raises the question as to whether the appearance of this population in two independent systems was coincidental or whether V β 8 T cells carry specific functional properties that enable them to direct the immune system into similar pathways, in this case the production of IgE. At the present time, there is no evidence that the expression of the TCR, particularly of specific TCR-V β elements, is coupled to the functional capacity or the cytokine profile secreted by these cells. It is possible, however, that the recognition of Ag or allergen by the TCR provides a signal or sets of signals that drive the T cells in a certain direction, e.g., to produce IL-4 or alternatively, IFN- γ . These possibilities are the focus of current experiments in the laboratory.

It is unclear what role T cells have in the modulation of

AR. This could be via a direct interaction of the T cells with the neuroimmunologic control system of airway smooth muscle or that increases in AR are linked to the presence of IgE or IgG1 in an indirect fashion. Studies in humans suggest a link between IgE and airways hyperreactivity. A recent study demonstrated the significant correlation between serum IgE titers and the degree of airway hyperreactivity (39). In addition, a number of different animal studies utilizing in vitro as well as in vivo systems suggested a positive interrelationship between development of reaginic antibodies and the development of increased AR (40–44). However, the mechanisms through which these antibodies regulate AR remain to be delineated.

In conclusion, this study further documents the role of certain V β -expressing T cells in the regulation of allergen-specific IgE and IgG1 production and the development of increased AR. Furthermore, the data indicate that different T cell populations, activated by allergen sensitization, differentially regulate Ig isotype production. Studies are currently underway to delineate the functional pathways through which these T cell populations exhibit their differential activities.

Acknowledgments

We would like to thank Jan Henson for technical help in the preparation of the lung histology, and Mrs. Jane Watkins for excellent secretarial assistance in the preparation of this manuscript.

References

1. Gascan, H., J.-F. Gauchat, M.-G. Roncarolo, H. Yssel, H. Spits, and J. E. de Vries. 1991. Human B cell clones can be induced to proliferate and to switch to IgE and IgG4 synthesis by interleukin 4 and a signal provided by activated CD4⁺ T cell clones. *J. Exp. Med.* 173:747.
2. Howard, M., J. Farrar, M. Hilfiker, B. Johnson, K. Takatsu, T. Hamaoka, and W. E. Paul. 1982. Identification of a T-cell derived B cell growth factor distinct from interleukin 2. *J. Exp. Med.* 155:914.
3. Stein, P., P. DuBois, D. Greenblatt, and M. Howard. 1986. Induction of antigen-specific proliferation in affinity-purified small B lymphocytes: requirements for BSF-1 by type 2 but not type 1 thymus-independent antigens. *J. Immunol.* 136:2080.
4. Lowenstein, H., and D. G. Marsh. 1983. Antigens of *Ambrosia elatior* (short ragweed) pollen III. Crossed radio-immunoelectrophoresis of ragweed-allergic patients' sera with special attention to quantification of IgE response. *J. Immunol.* 130:727.
5. Stavnezer, J., G. Radcliffe, Y.-C. Lin, J. Nietupski, L. Berggren, R. Sita, and E. Severinson. 1988. Immunoglobulin heavy-chain switching may be directed by prior induction of transcripts from constant-region genes. *Proc. Natl. Acad. Sci. USA* 85:7704.
6. Rothman, P., S. Lutzker, W. Cook, R. Coffman, and F. W. Alt. 1988. Mitogen plus interleukin-4 induction of C ϵ transcripts in B-lymphoid cells. *J. Exp. Med.* 168:2385.

7. Rothman, P., Y.-Y. Chen, S. Lutzker, S. C. Li, V. Stewart, R. Coffman, and F. W. Alt. 1990. Structure and expression of germ line immunoglobulin heavy chain ϵ transcripts: interleukin-4 plus lipopolysaccharide-directed switching to C ϵ . *Mol. Cell. Biol.* 10:1672.
8. Moller, G. 1987. Antigen requirements for activation of MHC restricted responses. *Immunol. Rev.* 98:1.
9. O'Heir, R. E., R. D. Garman, J. L. Greenstein, and J. R. Lamb. 1991. The specificity and regulation of T cell responsiveness to allergen. *Ann. Rev. Immunol.* 9:76.
10. Renz, H., K. L. Bradley, P. Marrack, and E. W. Gelfand. 1992. T cells expressing V β 8 and V β 2 regulate murine IgE production. *Proc. Natl. Acad. Sci. USA* 89:6438.
11. Larsen, G. L., H. Renz, J. E. Loader, K. L. Bradley, and E. W. Gelfand. 1992. Airway response to electrical field stimulation in sensitized inbred mice. Passive transfer of increased responsiveness with peribronchial lymph nodes. *J. Clin. Invest.* 89:747.
12. Renz, H., K. Bradley, J. Saloga, J. Loader, G. L. Larsen, and E. W. Gelfand. 1993. T cells expressing specific V β elements regulate IgE production and airways responsiveness in vivo. *J. Exp. Med.* 177:1175.
13. Rafnar, T., I. G. Griffith, M.-C. Kuo, J. F. Bond, B. L. Rogers, and D. G. Klapper. 1991. Cloning of *Amb a* I (antigen E), the major allergen family of short ragweed pollen. *J. Biol. Chem.* 266:1229.
14. Bond, J. F., R. D. Garman, K. M. Keating, T. J. Briner, T. Rafnar, D. G. Klapper, and B. L. Rogers. 1991. Multiple *Amb a* I allergens demonstrate specific reactivity with IgE and T cells from ragweed allergic patients. *J. Immunol.* 146:3380.
15. Clay, M. M., D. Pavia, S. P. Newman, and S. W. Clarke. 1983. Factors influencing the size distribution of aerosols from jet nebulization. *Thorax* 38:755.
16. Saloga, J., H. Renz, G. Lack, K. Bradley, G. Larsen, and E. W. Gelfand. Development and transfer of immediate cutaneous hypersensitivity in mice exposed to aerosolized antigen. *J. Clin. Invest.* 91:133.
17. Renz, H., H. R. Smith, J. E. Henson, B. S. Ray, C. G. Irvin, and E. W. Gelfand. 1992. Aerosolized antigen exposure without adjuvant causes increased IgE production and increased airways responsiveness in the mouse. *J. Allergy Clin. Immunol.* 89:1127.
18. Ovary, Z. 1958. Immediate reactions in the skin of experimental animals provoked by antibody-antigen interaction. *Progr. Allergy* 5:459.
19. Ovary, Z. 1958. Passive cutaneous anaphylaxis in the mouse. *J. Immunol.* 81:355.
20. Watanabe, N., and Ovary, Z. 1977. Antigen and antibody detection by in vivo methods; a reevaluation of passive cutaneous anaphylactic reactions. *J. Immunol. Methods* 14:381.
21. Hodgkin, P. D., L. C. Yamashita, B. Seymour, R. L. Coffman, and M. R. Kehry. 1991. Membranes from both Th1 and Th2 T cell clones stimulate B cell proliferation and prepare B cells for lymphokine-induced differentiation to secrete Ig. *J. Immunol.* 147:3696.
22. Croft, M., and S. L. Swain. 1991. B cell response to T helper cell subsets. II. Both the stage of T cell differentiation and the cytokines secreted determine the extent and nature of helper activity. *J. Immunol.* 147:3679.
23. Clement, L. T., M. K. Dagg, and G. L. Gartland. 1984. Small, resting B cells can be induced to proliferate by direct signals from activated helper T cells. *J. Immunol.* 132:740.
24. Gascan, H., J.-F. Gauchat, M.-G. Roncarolo, H. Yssel, H. Spits, and J. E. de Vries. 1991. B cell clones can be induced to proliferate and to switch to IgE and IgG4 synthesis by interleukin 4 and a signal provided by activated CD4⁺ T cell clones. *J. Exp. Med.* 173:747.
25. Vercelli, D., H. H. Jabara, K.-I. Arai, and R. S. Geha. 1989. Induction of human IgE synthesis requires interleukin 4 and T/B cell interactions involving the T cell receptor/CD23 complex and MHC class II antigens. *J. Exp. Med.* 169:1295.
26. Leberman, D. A., A. Leberman, and R. L. Coffman. 1988. Interleukin 4 causes isotype switching to IgE in T cell-stimulated clonal B cell cultures. *J. Exp. Med.* 168:853.
27. Gerondakis, S. 1990. Structure and expression of murine germ-line immunoglobulin heavy chain transcripts induced by interleukin 4. *Proc. Natl. Acad. Sci. USA* 87:1581.
28. Gauchat, J.-F., D. A. Leberman, R. L. Coffman, H. Bascan, and J. E. de Vries. 1990. Structure and expression of germline ϵ transcripts in human B cells induced by interleukin 4 to switch to IgE production. *J. Exp. Med.* 172:463.
29. Stavnezer, J., G. Radcliffe, Y.-C. Lin, J. Nietupski, L. Berggren, R. Sita, and E. Severinson. 1988. Immunoglobulin heavy-chain switching may be directed by prior induction of transcripts from constant-region genes. *Proc. Natl. Acad. Sci. USA* 85:7704.
30. Rothman, P., S. Lutzker, W. Cook, R. Coffman, and F. W. Alt. 1988. Mitogen plus interleukin-4 induction of C ϵ transcripts in B-lymphoid cells. *J. Exp. Med.* 168:2385.
31. Rothman, P., Y.-Y. Chen, S. Lutzker, C. S. Li, V. Stewart, R. Coffman, and F. W. Alt. 1990. Structure and expression of germ line immunoglobulin heavy-chain ϵ transcripts: interleukin-4 plus lipopolysaccharide-directed switching to C ϵ . *Mol. Cell. Biol.* 10:1672.
32. Vitetta, E. S., J. Ohara, C. D. Myers, J. E. Layton, P. H. Krammer, and W. E. Paul. 1985. Serological, biochemical, and functional identity of B cell stimulatory factor 1 and B cell differentiation factor for IgG1. *J. Exp. Med.* 162:1726.
33. Azzawi, M., B. Bradley, P. K. Jeffrey, A. J. Frew, A. J. Wardlaw, G. Knowles, B. Assoufi, J. Collins, S. Durham, and A. B. Kay. 1990. Activated T lymphocytes and eosinophils in bronchial biopsies in stable atopic asthma. *Am. Rev. Respir. Dis.* 142:1407.
34. Kay, A. B. 1991. Asthma and inflammation. *J. Allergy Clin. Immunol.* 87:893.
35. Mattoli, S., V. L. Mattoso, M. Soloperto, L. Allegra, and A. Fasoli. 1991. Cellular and biochemical characteristics of bronchoalveolar lavage fluid in symptomatic non-allergic asthma. *J. Allergy Clin. Immunol.* 87:794.
36. Corrigan, C. J., A. Hartnell, and A. B. Kay. 1988. T lymphocyte activation in acute severe asthma. *Lancet* 1:1129.
37. Durham, S. R., S. Ying, V. A. Varney, M. R. Jacobson, R. M. Sudderick, I. S. Mackay, A. B. Kay, and Q. A. Hamid. 1992. Cytokine messenger RNA expression for IL-3, IL-4, IL-5 and granulocyte/macrophage-colony-stimulating factor in the nasal mucosa after local allergen provocation: relationship to tissue eosinophilia. *J. Immunol.* 148:2390.
38. Vidard, L., K. L. Rock, and B. Benacerraf. 1992. Diversity in MHC class II ovalbumin T cell epitopes generated by distinct proteases. *J. Immunol.* 149:498.
39. Sears, M. R., B. Burrows, E. M. Flannery, G. P. Herbison,

- C. J. Hewitt, and M. D. Holdaway. 1991. Relation between airway responsiveness and serum IgE in children with asthma and in apparently normal children. *N. Engl. J. Med.* 325:1067.
40. Regal, J. F. 1984. Immunoglobulin G- and immunoglobulin E-mediated airway smooth muscle contraction in the guinea pig. *J. Pharmacol. Exp. Ther.* 228:116.
41. Yamauchi, N., K. Ito, M. Suko, A. Ishii, and T. Miyamoto. 1986. IgG2 antibodies block IgE antibody-induced asthma in guinea pigs. *Int. Arch. Allergy Appl. Immun.* 80:76.
42. Sorkness, R., S. Blythe, and R. F. Lemanske, Jr. 1988. Pulmonary antigen challenge in rats passively sensitized with a monoclonal IgE antibody induces immediate but not late changes in airway mechanics. *Am. Rev. Respir. Dis.* 138:1152.
43. Patterson, R., and K. E. Harris. 1992. IgE-mediated rhesus monkey asthma: natural history and individual animal variation. *Int. Arch. Allergy Immunol.* 97:154.
44. Souhrada, M., and J. F. Souhrada. 1988. Specific reaginic antibody IgG1-induced changes in airway smooth muscle cells. *J. Appl. Physiol.* 65:767.