Brief Definitive Report

T Cells Expressing Specific Vβ Elements Regulate Immunoglobulin E Production and Airways Responsiveness In Vivo

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Summary

The role of T cells expressing specific Vβ elements was examined in the regulation of allergen-specific immunoglobulin (IgE) production and airways responsiveness (AR). In BALB/c mice, inhalation of the allergen ovalbumin (OVA) induced an IgE anti-OVA response, immediate cutaneous reactivity, and increased AR. These results were associated with an expansion of Vβ8.1/8.2 T cells in local draining lymph nodes of the airways and the lung. Transfer of Vβ8.1/8.2 T cells from sensitized mice stimulated an IgE anti-OVA response, immediate cutaneous hypersensitivity, and increased AR in naive syngeneic recipients. In contrast, OVA-reactive Vβ2 T cells inhibited these effects. These data demonstrate for the first time that T cells with different Vβ specificities play a critical role in the in vivo regulation of allergen-specific IgE production and AR.

T lymphocytes play a central role in the regulation of IgE production in vitro and in vivo. In addition to cognate T-B cell interaction (1, 2), IL-4 is required for the generation of polyclonal and antigen-specific IgE responses (3-6), primarily by promoting Ig isotype switching (7). IgE levels are elevated in most allergic diseases, and in the lungs, changes in airways responsiveness (AR) are associated with allergic responses. A relationship between IgE and changes in AR is indicated by epidemiologic data linking asthma with IgE-related responses (8). To explore the interrelationship between T cells, IgE production, and the pathogenesis of AR, we have developed a murine model of allergen-induced airways hyperresponsiveness (9). After inhalation of allergen (OVA), BALB/c but not SJL/J mice develop OVA-specific IgE antibodies, immediate cutaneous reactivity to OVA, and an increase in AR, measured in tracheal smooth muscle preparations. Further, these responses could be passively transferred to naive, syngeneic recipients by lymphocytes from peribronchial lymph nodes (PBLN) of sensitized but not nonimmune mice (10). Sensitized PBLN are comprised of increased numbers of OVA-reactive T cells expressing Vβ8.1/8.2.

In in vitro studies of the functional properties of T cell subsets expressing different Vβ elements, we noted that Vβ8.1/8.2 T cells augmented IgE production whereas Vβ2+ T cells limited this increase (11). We now demonstrate that transfer of Vβ8.1/8.2 T cells from sensitized animals can passively transfer the capacity to develop allergen-specific IgE, immediate cutaneous hypersensitivity, and altered AR in naive recipients, and that cotransfer of Vβ2+ T cells prevents these responses. These data demonstrate that T cells bearing different Vβ elements are differentially involved in the in vitro and in vivo regulation of IgE production and AR.

Materials and Methods

Sensitization Protocol. BALB/c mice between 8 and 12 wk of age (The Jackson Laboratory, Bar Harbor, ME) were sensitized to OVA by inhalation as described (9). Briefly, animals were sensitized by ultrasonic nebulization of 1% OVA (chicken OVA, grade V; Sigma Chemical Co., St. Louis, MO) diluted in sterile PBS. For this purpose five mice at a time were placed in a plastic chamber (22 x 23 x 14 cm), and the OVA solution was aerosolized into one end of the box by using an ultrasonic nebulizer (PulmoSonic 25; DeVilbiss, Somerset, PA) at a continuous air flow of 5 psi (1 psi = 6.9 kPa). At the other end of the chamber were two small holes to ensure continuous air flow. With laser nephelometry, >90% of the chamber particles were found to be in the 1-2-μm range. Sensitization was achieved after a daily 20-min exposure for a consecutive 10 d. Nonimmune control mice were exposed to PBS under the same conditions. Care of the animals was in accordance with institutional guidelines.

Lymphocyte Preparation and Cell Transfer Protocol. Single cell suspensions were prepared from spleen on day 12, 2 d after completion of the sensitization protocol as described (10, 11). For the transfer experiments, Vβ8.1/8.2 and Vβ2 cells prepared from OVA-sensitized mice (Vβ8.1/8.2 and Vβ2 [OVA]) or from controls (Vβ8.1/8.2 and Vβ2 [PBS]) were isolated from spleen. T cells expressing desired Vβ elements were purified by panning on either KJ16 (Vβ8.1/8.2)- or B20.6 (Vβ2)-coated tissue culture flasks. After panning, the cells were expanded and subcultured with IL-2 (50 U/ml) and IL-4 (200 U/ml) containing culture medium (RPMI 1175 J. Exp. Med. © The Rockefeller University Press • 0022-1007/93/04/1175/06 $2.00
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supplemented with penicillin, streptomycin, and l-glutamine) for 3–4 d. Before cell transfer, the cell populations were analyzed by flow cytometry. The enrichment procedure resulted in a 96–99.5% population of T cells expressing the particular Vβ TCR. The distribution of CD4/CD8 cells was 4:1 in both the Vβ8 and Vβ2 subsets. The percentage of B cells was always <0.1%. Culture supernatants from expanded cells were free of total and anti-OVA IgG as analyzed by ELISA. 10 x 10^6 cells per mouse were transferred by intravenous (tail vein) infusion into syngeneic, nonimmunized mice. In cell mixture experiments, an equal number of 10 x 10^5 Vβ8 and 10 x 10^6 Vβ2 cells was injected. Immediately after transfer, the recipients were challenged with a single OVA nebulization for 20 min, which by itself did not induce anti-OVA Ig production (10).

**Determination of Anti-OVA Serum Antibody Levels.** Anti-OVA IgE and IgG serum antibody levels were measured by ELISA as described (9). Round-bottomed microtiter plates (96-well Immunolon II; Dynatech Laboratories, Burlingame, CA) were coated with 20 μg/ml OVA diluted in NaHCO₃ buffer, pH 8.2. After overnight incubation at 4°C, plates were washed three times and blocked with Trit-BSA (1%; wt/vol) buffer, pH 9.6, for 2 h at 37°C. Serum samples were diluted in 0.1 M Tris, BSA, 1% Tween buffer starting at a 1:20 dilution. These and additional twofold serial dilutions were added to the plates and incubated overnight at 4°C. After washing, either alkaline phosphatase–conjugated monoclonal rat anti-mouse IgE, IgG1 (Pharmingen Corp., San Diego, CA) or goat anti–mouse IgG (Sigma Chemical Co.) diluted in Tris, BSA, 1% Tween buffer was added for an additional 2 h. The reaction was developed with phosphatase substrate (Sigma Chemical Co.). Plates were read in a microplate autoreader (Bio-Rad Laboratories, Richmond, CA) at 410 nm.

The antibody levels of samples were compared with known standards (9). The standard serum was a pool of serum collected from five OVA-sensitized mice that demonstrated strongly positive skin reactions to OVA. The concentration of anti-OVA IgE was arbitrarily assigned 1,000 ELISA units (EU). For IgG, serum was pooled from mice immunized to OVA by intraperitoneal injection of 500 μg OVA/mouse. In this case, no positive skin reactions to OVA were found, and the concentration of IgG anti-OVA was arbitrarily assigned 1,000 EU. Analysis of ELISA data was performed with the Microplate Manager software for the Macintosh computer (Bio-Rad Laboratories).

**Determination of Immediate Cutaneous Hypersensitivity.** Skin tests were performed as described (12) at the same time the serum was collected and AR was analyzed. Mice were injected (intradermally) with 20 μl of an OVA solution (500 μg/ml in PBS) or, as a negative control, the same volume of PBS. The positive control was compound 48/80, an inducer of mast cell degranulation. Reactions collected and AR was analyzed. Mice were injected (intradermally) with 1 mg/ml compound 48/80. No wheals developed at the site of injection of the control solution. A positive reaction of a wheal reaction with a diameter of at least 0.3 cm. In positive reactions, the wheal reached a maximum between 15 and 20 min after injection and was approximately the same size as seen after injection of 1 mg/ml compound 48/80. No wheals developed at the site of injection of the control solution. A positive reaction to OVA was specific for this allergen, since injection of albumins (e.g., human serum albumin and BSA) from other species or an irrelevant allergen (e.g., ragweed) did not cause wheal formation in OVA-sensitized mice. Immediate skin tests were performed in a blinded fashion.

**Analysis of AR.** AR was measured in vitro by electrical stimulation of tracheal smooth muscle preparations as described (10). Analysis of AR was performed in the same animals and at the same time point when the serum was collected for measurements of Ig titers. Tracheal smooth muscle segments of ~0.5 cm in length were prepared from each individual mouse and placed in muscle baths that were perfused with a Krebs-Henseleit solution, oxygenated, and maintained at 37°C. The tracheal segments were suspended by triangular supports transducing the force of contractions. Electrical field stimulation was delivered by a stimulator (S44; Grass Instrument Co., Quincy, MA) using an 8-nm, 2-ms pulse duration and increased frequencies (ranging from 0.5 to 40 Hz). Each stimulation was maintained until peak contractions were obtained. In all smooth muscle preparations, a current of 40 Hz resulted in maximal contraction. For each preparation, the electrical stimulation (Hz) causing 50% of the maximal contraction (ES₅₀) was calculated from linear plots. The mean ES₅₀ (± SD) of nonimmunized mice was 4.18 ± 0.24 Hz. This response was calculated at 100%. The values of the various experimental groups were compared with the control and were expressed as percent control ES₅₀. Analysis of AR and data calculations were performed in a blinded fashion. Statistical analysis was performed by two-tailed student’s t test. Depicted are mean ± SE for each group.

**Results and Discussion**

Vβ8.1/8.2 and Vβ2 T cells were isolated from OVA-sensitized and nonimmunized control mice, expanded in the presence of IL-2 and IL-4, and were infused into syngeneic, naive BALB/c mice. This combination of lymphokines was chosen to expand the T cell subsets since these factors play a central role in the growth and differentiation of different T cell subpopulations. Another advantage of this protocol is that at the end of the expansion period the T cells were activated, a requirement for the induction of Ig production by B cells. B cells were not present in the infusate since <0.1% of the cells were identified as B cells by flow cytometry. Further, in culture supernatants collected after expansion of the lymphocytes, neither allergen nonspecific nor anti-OVA IgE antibody was detectable.

Immediately after cell transfer the recipients were challenged with a single (20-min) exposure to OVA by nebulization. As shown earlier, this challenge by itself had no effect on IgE production and changes in AR (10). 5 d later serum was collected and IgE anti-OVA titers were measured by ELISA. As shown in Table 1, primary sensitization to OVA resulted in an increase in IgG anti-OVA antibody titers. Nonsensitized mice did not develop an IgE anti-OVA response. When Vβ8.1/8.2 T cells from primary, sensitized mice were transferred into naive recipients, an increase in serum IgE anti-OVA was detected. The transfer of 10 × 10⁶ cells resulted in a response that was about half of that observed in the serum of primary, donor mice. The transfer of the same number of Vβ8.1/8.2 T cells from nonimmunized mice was without effect. In contrast to the results with Vβ8.1/8.2 T cells, transfer of Vβ2 T cells from OVA-sensitized mice did not lead to any increase in IgE anti-OVA antibody. Cotransfer of a mixture of 10 x 10⁶ Vβ8.1/8.2 plus 10 x 10⁶ Vβ2 cells from sensitized (but nonimmune) mice markedly reduced the levels of OVA-specific IgE antibody when compared with the transfer of Vβ8.1/8.2 T cells alone.

At the same time that the IgE anti-OVA response was elevated in the primary, sensitized animals, only a relatively low IgG anti-OVA response was detected in the serum of...
Table 1. Transfer of Anti-OVA IgE Production by Vβ8.1/8.2 T Cells

<table>
<thead>
<tr>
<th>Donor exposure</th>
<th>Cells transferred to recipient</th>
<th>Anti-OVA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>IgE</td>
</tr>
<tr>
<td>OVA</td>
<td>-</td>
<td>1,348 ± 468</td>
</tr>
<tr>
<td>PBS</td>
<td>-</td>
<td>&lt;20</td>
</tr>
<tr>
<td>OVA</td>
<td>Vβ8.1/8.2</td>
<td>630 ± 54</td>
</tr>
<tr>
<td>PBS</td>
<td>Vβ8.1/8.2</td>
<td>120 ± 36</td>
</tr>
<tr>
<td>OVA</td>
<td>Vβ2</td>
<td>&lt;20</td>
</tr>
<tr>
<td>OVA</td>
<td>Vβ2 + Vβ8.1/8.2</td>
<td>125 ± 15</td>
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2 d after completion of the 10-d course (20 min each day) of OVA sensitization through the airways, serum was collected and analyzed for anti-OVA Ig production by ELISA as described (9). Nonimmune control mice were exposed to PBS for the same period of time. In the transfer experiments, Vβ8.1/8.2 and Vβ2 cells prepared from OVA-sensitized mice (Vβ8.1/8.2 and Vβ2 [OVA]) or from controls (Vβ8.1/8.2 and Vβ2 [PBS]) were obtained from spleen. Spleens were prepared on day 12, 2 d after completion of the sensitization protocol. 10 × 10^6 cells per mouse were transferred by intravenous (tail vein) infusion into syngeneic, nonimmunized mice. In cell mixture experiments, an equal number of 10 × 10^6 Vβ8 and 10 × 10^6 Vβ2 cells was injected. Immediately after transfer, the recipients were challenged with a single OVA nebulization for 20 min, which by itself did not induce anti-OVA ig production (10). 5 d later, serum was collected and analyzed for IgE, IgG, and IgG1 anti-OVA production by ELISA and expressed as units per milliliter serum. Serum from each animal was analyzed individually in a blinded fashion. The same mice were used for experiments depicted in Fig. 1 and Table 2. For each experimental group mean ± SD are depicted. Statistical analysis was performed by two-tailed student’s t test.

These mice. Particularly noteworthy is the fact that an IgG1 anti-OVA response was absent at this early time point, but increased over time (by day 28) (11). This pattern of antibody development was also seen after the transfer of Vβ8.1/8.2 cells, namely a relatively low IgG anti-OVA response, and no IgG1 anti-OVA antibodies were detected. The transfer of Vβ2 T cells did not result in an IgG response, and the cotransfer of both cell populations had no effect on IgG anti-OVA responses other than those observed with the transfer of Vβ8 T cells alone (Table 1).

The appearance of an IgE anti-OVA response in the recipients was accompanied by the acquisition of immediate cutaneous hypersensitivity responses to OVA, defined by the development of immediate wheal reactions 15–20 min after intradermal injection of OVA. Earlier studies demonstrated the reliability and sensitivity of this approach for screening IgE-mediated cutaneous reactivity (12). In addition, these responses were specific to the allergen used as injection of irrelevant allergens or control solutions did not generate any immediate responses. Positive reactions to OVA were elicited only when Vβ8.1/8.2 T cells from OVA-sensitized mice were transferred into recipient mice (Table 2). In recipients of the combination of Vβ8.1/8.2 and Vβ2 cells, the proportion of animals reacting to intradermal injection of OVA was markedly reduced. These data demonstrated that transfer of T cells expressing Vβ8.1/8.2, isolated from allergen-sensitized mice, can induce allergen-specific IgE production and immediate cutaneous hypersensitivity in recipients of these cells. Vβ2-expressing T cells from sensitized animals significantly reduced these responses when cotransferred with the Vβ8 T cells.

The response of tracheal smooth muscle preparations to electrical field stimulation represents one means for assessing AR in vitro (10). We assessed AR in this way to determine the role of transferred lymphocytes in altering airways function. At the same time that IgE was measured in the serum of sensitized animals, we demonstrated an increase in AR when tracheal smooth muscle preparations were exposed to these mice.

Table 2. Transfer of Immediate Cutaneous Hypersensitivity with Vβ8.1/8.2 T Cells

<table>
<thead>
<tr>
<th>Donor exposure</th>
<th>Cells transferred to recipient</th>
<th>Positive skin tests %</th>
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<tbody>
<tr>
<td>OVA</td>
<td>-</td>
<td>31/27</td>
</tr>
<tr>
<td>PBS</td>
<td>-</td>
<td>0/22</td>
</tr>
<tr>
<td>OVA</td>
<td>Vβ8.1/8.2</td>
<td>8/9</td>
</tr>
<tr>
<td>PBS</td>
<td>Vβ8.1/8.2</td>
<td>0/9</td>
</tr>
<tr>
<td>OVA</td>
<td>Vβ2</td>
<td>2/10</td>
</tr>
<tr>
<td>OVA</td>
<td>Vβ2 + Vβ8.1/8.2</td>
<td>2/10</td>
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Immediate cutaneous hypersensitivity was determined at the same time AR was measured and serum was taken for antibody titer analysis. Expressed are the number and percentage of mice with positive reactions to OVA. In all mice, the positive control solution compound 48/80 yielded positive reactions, and tests with PBS were negative.
Figure 1. Analysis of AR in OVA-sensitized mice and recipients of Vβ8.1/8.2 and Vβ2 T cells. AR was measured in vitro by electrical field stimulation of tracheal smooth muscle preparations. Analysis of AR was performed in the same animals and at the same time point when the serum was collected for measurements of Ig titers. Each stimulation was maintained until peak contractions were obtained. In all smooth muscle preparations, a current of 40 Hz resulted in maximal contraction. For each preparation, the ES50 was calculated from linear plots. The mean ES50 (± SE) of nonimmunized mice was 4.18 ± 0.24 Hz. This response was calculated as 100%. The values of the various experimental groups were compared with the control and were expressed as percent control ES50. Analysis of AR and data calculations were performed in a blinded fashion. Statistical analysis was performed by two-tailed student's t test. Depicted are mean ± SE for each group.

electrical field stimulation. In contrast to nonimmunized controls, OVA-sensitized mice developed increases in AR, indicated by a decrease in the mean ES50 from 4.18 to 2.73 Hz. This drop in ES50 of 30–40% was significant (Fig. 1) and was in the range found by other investigators using similar methodology in a variety of different animal systems (13, 14).

In parallel to the data obtained for IgE production, the transfer of Vβ8.1/8.2 T cells from sensitized animals into naive, syngeneic recipients resulted in a similar increase in AR in these animals, in the same range observed with the primary, sensitized mice (Fig. 1). The lowest effective number of T cells capable of transferring altered AR was 10 × 10⁶ Vβ8.1/8.2 T cells. Transfer of 5 × 10⁶ cells did not lead to changes in AR (data not shown). Control mice that received Vβ8.1/8.2 T cells from nonimmune mice (up to 30 × 10⁶) developed a decrease rather than any increase in AR. A similar effect was observed after the transfer of Vβ2 T cells from OVA-sensitized mice. This decrease in AR is unexplained at present. When Vβ8.1/8.2 T cells from OVA-sensitized mice were mixed with an equal number of Vβ2 T cells (10 × 10⁶) from sensitized (but not from nonimmune) animals and transferred, the increases in AR observed with transfer of Vβ8.1/8.2 T cells alone were abolished. For these effects as well as for the induction of the IgE response, the single OVA challenge was required after the cell infusion, since only marginal changes were observed when the single OVA nebulization was omitted (data not shown).

To further demonstrate the specificity of these T cell effects, the isolated lymphocytes were preincubated with anti-Vβ8.1/8.2 (KJ16) antibody before cell transfer. Table 3 summarizes the effect of this procedure on IgE production, immediate cutaneous hypersensitivity, and AR. Anti-OVA IgE production was inhibited by >80% when compared with animals receiving Vβ8.1/8.2 cells treated with an anti-Vβ2 antibody of the same isotype. Analysis of immediate cutaneous responses revealed that only 2 of 10 recipients receiving cells treated with KJ16 antibody had a positive reaction to OVA compared with six of six animals who received cells treated with the anti-Vβ2 antibody. Also, animals receiving Vβ8.1/8.2 T cells treated with KJ16 antibody did not develop any appreciable increases in AR. The mechanism whereby pretreatment of cells with KJ16 antibody abolishes these responses is unclear, but binding of KJ16 antibody may prevent TCR-MHC interaction in the recipients.

This study provides evidence that IgE production and development of immediate cutaneous hypersensitivity reactions

<table>
<thead>
<tr>
<th>Table 3. Effect of Pretreatment of Transferred T cells with Anti-Vβ8.1/8.2 or Anti-Vβ2 Antibody</th>
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<tr>
<td>Treatment</td>
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<tr>
<td>--------------------------------</td>
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<tr>
<td>Anti-Vβ2 antibody (n = 6)</td>
</tr>
<tr>
<td>Anti-Vβ8.1/8.2 antibody (n = 8)</td>
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</table>

Before cell transfer, Vβ8.1/8.2 T cells were incubated with mAb KJ16 (10 μg/ml) for 30 min at 4°C. Flow cytometry (staining with biotinylated KJ16) indicated that this procedure resulted in binding of this antibody to all Vβ8.1/8.2 T cells. As a control Vβ8.1/8.2 were also incubated with anti-Vβ2 antibody (10 μg/ml) for 30 min at 4°C. This antibody is of the same isotype as the KJ16 antibody. Cells were then transferred as described in Fig. 1. 5 d later, serum was collected and analyzed for IgE anti-OVA responses. At the same time, skin tests were performed and AR was determined using tracheal smooth muscle preparations. Analyses of ELISA titers, skin tests, and AR were performed as described in Materials and Methods in a blinded fashion.

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in response to specific antigen challenge, as well as the development of increased AR, can be regulated by T cell subpopulations that are only distinguished by the pattern of Vβ elements expressed on the TCR. The same T cell subset that can transfer allergen-specific IgE production also transfers the increase in AR. It is presently unclear if the increases in AR are causally linked to the presence of IgE or whether both events are independent of each other. However, both events are regulated by the same T cells in a positive (Vβ8.1/8.2) as well as negative (Vβ2) direction. This emphasizes the close relationship between the presence of certain antigen-specific T cell subsets, the regulation of IgE production, and changes in AR. This relationship between distinct Vβ-expressing T cell subsets is further supported by the findings in SJL/J mice. This strain of mice, which lacks Vβ8 T cells, failed to respond to antigen challenge with specific IgE responses, cutaneous reactivity, or changes in AR (10, 12). At the present time it is unclear how these responses are linked to Vβ8-expressing T cells or their regulation by Vβ2 T cells. Although Vβ8 cells from both sensitized as well as control mice were similarly activated by the panning and expansion procedure, only Vβ8 cells from antigen-exposed mice demonstrated stimulatory effects on IgE production and AR.

The effects of the different Vβ-expressing T cell subsets may be related to different patterns of cytokine production. It is conceivable that different Vβ-expressing T cell subsets exhibit specific patterns of cytokine production. IL-4 is essential for the stimulation of IgE production, whereas the T cell product IFN-γ is known to inhibit IL-4-induced IgE production (6). Vβ8 and Vβ2 T cells may thus differ in the pattern of secreted cytokines. In freshly isolated CD4 T cells, such differences among T cell subsets have not been observed but they have been demonstrated in T cell clones (Th1 and Th2 clones) (15). It would be surprising that Vβ8 T cells in general are characterized as high IL-4 producers. A more likely possibility is that the differential effects of Vβ8 and Vβ2 T cells are related to antigen specificity and/or antigen presentation. Similar preferential usage of a particular Vβ region gene segment has been reported for human T cells reactive with a specific tetanus toxin-derived peptide. The same Vβ segment was used irrespective of the expression of different MHC class II antigens on APC (16). The presentation of specific immunogenic epitopes on different APC may drive subsets of T cells along different pathways with Th2- or Th1-like activities. Thus, certain cytokine patterns may be assigned to specific Vβ-bearing T cell subsets. It now becomes important to examine whether sensitization to other allergens also leads to a selective expansion of distinct Vβ-bearing T cell subsets and whether such subpopulations also play a differential role in the regulation of IgE production and AR.

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