

Comparison of the Allergenicity of Ovalbumin and Ovalbumin Peptide 323–339

Differential Expansion of V β -Expressing T Cell Populations¹

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ABSTRACT. We analyzed the effects of sensitization of BALB/c mice to the OVA peptide amino acids 323–339, on the development of an IgE response, immediate cutaneous hypersensitivity and airways responsiveness (AR). Daily aerosolization of OVA 323–339 for 20 min over a period of 10 days was as effective in the stimulation of a serum anti-OVA IgE antibody response as sensitization to native OVA by the same route. After sensitization to native OVA, the majority of the IgE anti-OVA response was directed against peptide 323–339. The antibody responses were paralleled by skin test responses in sensitized mice: 73% of OVA-sensitized mice developed immediate type reactions when tested with native OVA and 82% of the mice had positive immediate skin test responses to intradermal injection of peptide 323–339. After sensitization to the peptide, 69% of the mice had positive responses to native OVA and 77% responded to peptide 323–339. Aerosolization of OVA as well as OVA 323–339 led to a comparable increase in airway responsiveness as measured by electrical field stimulation of tracheal smooth muscle preparations. To characterize T cell subpopulations that were stimulated after allergen sensitization, the distribution of specific V β -expressing T cells was analyzed in local draining lymph nodes of the airways and the lungs. These lymph nodes were found to be enlarged after both OVA and OVA peptide sensitization. Sensitization to native OVA resulted in an increased percentage of V β 8.1 and V β 8.2 T cells whereas selective stimulation of V β 8.1 T cells was found after peptide sensitization. These data indicate that OVA peptide 323–339 represents a T and B cell epitope of OVA, which is important in the generation and development of immediate hypersensitivity responses in BALB/c mice. *Journal of Immunology*, 1993, 151: 7206.

OVA is a well recognized model Ag to study Ag-specific T and B cell-mediated immune functions. In addition, the regulation of responses to OVA is of clinical importance because it represents one of three major allergenic proteins of egg white (in addition to

ovomucoid and ovotransferrin), which is a common cause of food hypersensitivity (1–4). To study the role of T cells in the generation of immediate hypersensitivity, we have developed a murine system in which aerosolization of OVA to BALB/c mice, in the absence of adjuvants, resulted in a systemic allergen-specific IgE response and the appearance of increased AR.³ The development of an IgE response was associated with immediate-type skin test responses that were confirmed by passive cutaneous anaphylaxis (5–8).⁴

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³ Abbreviations used in this paper: AR, airway responsiveness; PBLN, peribronchial lymph nodes; EU, ELISA units; ES₅₀, Electrical stimulation resulting in 50% maximal contraction.

⁴ Renz, H., G. Lack, J. Saloga, R. Schwinzer, K. Bradley, J. Loader, A. Kupfer, G. L. Larsen, and E. W. Gelfand, 1992. Inhibition of IgE production and normalization of airways' responsiveness by sensitized CD8 T cells in a mouse model of allergen-induced sensitization. *J. Immunol. In Press*.

Characterization of the cellular components of the immune response indicated that distinct T cell subpopulations were expanded after OVA sensitization via the airways. In the local draining lymph nodes of the airways and the lung, (PBLN) an increase of V β 8.1/8.2-positive T cells was observed. In the spleen, OVA sensitization stimulated V β 8.1/8.2, V β 2, and V β 14 T cells (7). Transfer of V β 8.1/8.2 T cells from sensitized into non-sensitized mice triggered the development of increased AR and stimulated an allergen-specific IgE response (see footnote 4). In contrast, V β 2 T cells had an inhibitory effect on V β 8.1/8.2-mediated immune functions (7) (see footnote 4).

In the present study we sought to further define the T cell response to OVA and to identify the allergenic peptides recognized by the subset of V β 8.1/8.2 T cells after OVA sensitization through the airways. The OVA peptide 323–339 is known to be responsible for about 25 to 35% of the BALB/c T cell response to native OVA (9). Binding studies of OVA peptides to MHC-class II molecules revealed that peptide 322–339 binds and stimulates I-A^d-restricted T cell hybridomas (10). This same peptide has also been shown to bind human specific IgE from a serum pool of egg-allergic patients (11). In contrast, there is little information on the role of peptide 323–339 in the stimulation and function of T cells in vivo. To specifically address this question, we sensitized BALB/c mice to native OVA or to OVA peptide 323–339 by ultrasonic nebulization of the proteins and analyzed the resulting T and B cell responses. Their effects on IgE production, cutaneous hypersensitivity, and AR were evaluated.

Materials and Methods

Sensitization protocol

Female BALB/c mice (10 wk of age; obtained from The Jackson Laboratory, Bar Harbor, ME) were sensitized to OVA (Sigma, St. Louis, MO) or to free OVA peptide 323–339 as described (5). Solutions of 1% OVA diluted in PBS or OVA 323–339 (500 μ g/ml) diluted in PBS were administered by aerosol to the mice. The aerosol was delivered by an ultrasonic nebulizer (PulmoSonic model 25; The DeVilbiss Corp., Somerset, PA). The nebulized particle size for both Ag was in the range of 1 to 5 μ m as determined by laser nephelometry (12). Sensitization was carried out for 20 min each day over a consecutive 10-day period. On each day, 7 ml of the allergen solution were nebulized to four mice in each group. Control mice received nebulizations of PBS alone. Two days after completion of the protocol, serum was collected and analyzed for anti-OVA specific Ig production. In parallel, immediate skin test responses and AR measured by electrical field stimulation of tracheal smooth muscle preparations were assayed. The proliferative responses and distribution of lymphocytes were studied in T cells prepared from various compart-

ments. All tests were carried out in a blinded fashion in that the evaluator was unaware of the sensitization status of the individual animals.

Peptide synthesis

Peptides were prepared by solid phase peptide synthesis (13) using an automated peptide synthesizer (Applied Biosystems model 430A, Foster City, CA). *N* $^{\alpha}$ -tert-Butyloxycarbonyl protection was employed for all peptide syntheses, and finished peptides were cleaved from the resin using standard procedures (14). Free peptides were purified by preparative reverse-phase HPLC. Amino acid analyses of pure peptides were within 5% of the values predicted from the OVA peptide sequence. Peptides were S-alkylated with excess iodoacetamide in Tris buffer (0.5 M, pH 8 to 8.5) and then repurified by preparative reverse-phase HPLC and lyophilized.

Measurement of allergen-specific serum Ig responses

Anti-OVA IgE and IgG1 antibody titers were measured in serum samples by ELISA as described (5, 7). Alkaline phosphatase-conjugated monoclonal rat anti-mouse IgE or IgG1 antibodies (Pharmingen, San Diego, CA) diluted in blocking buffer were used. Plates were read on an automated ELISA reader (Bio-Rad, Pleasanton, CA) at 410 nm. Anti-OVA antibody titers were calculated by comparison to allergen-specific serum standards that were prepared as described (5, 7). In order to calculate the portion of anti-OVA antibody titers that bind to peptide 323–339, samples were preincubated with 1 mg/ml OVA peptide 323–339 for 30 min at room temperature before the serum samples were added to the ELISA plates. The values were then compared with those from serum samples that were not preincubated. All samples were analyzed on the same ELISA plates.

Cell proliferation assay

To assess the proliferative response of lymphocytes to native OVA and peptide 323–339, mononuclear cells were prepared from the spleens of OVA and peptide-sensitized mice by lymphoprep gradient centrifugation. A total of 2×10^5 cells/well were incubated in round bottom microtiter plates for 5 days in the presence and absence of OVA or peptide 323–339. Six hours before cell harvesting, cells were pulsed with 1 μ Ci [^{3} H]TdR.

Analysis of immediate cutaneous hypersensitivity

Development of immediate cutaneous hypersensitivity to whole OVA or peptide 323–339 was assessed by intradermal skin testing (8). For each skin test, 20 μ l of the test solution were injected intradermally on the ventral surface of the abdomen using a 30-gauge needle. The concentrations of the OVA and OVA peptide 323–339 solutions were

the same as in the solutions used for sensitization. In addition, PBS was injected as a negative control and compound 48/80 (Sigma) was used at a concentration of 1 μ g/ml as a positive control. At least 1.5-cm distances were kept between the sites of injection in order to avoid confluence of the test solutions. Resultant wheal formations were scored after 15 to 20 min. A reaction was scored as positive if the wheal reaction was greater than 0.3 cm in diameter.

Analysis of AR

Airways smooth muscle responsiveness was determined in vitro as described (6). Briefly, after killing the animals, tracheal rings of about 0.5 cm in length were prepared and placed in Krebs-Henseleit baths suspended by triangular supports transducing the force of contractions. Electrical field stimulations were delivered by a Grass S44 stimulator (Grass SIV 5) using 8 V, 2-ms pulse durations and 0.5 to 40 Hz frequencies. Each challenge was maintained until a peak contractile response was obtained. A frequency of 40 Hz was established to result in maximal contraction. ES_{50} , the frequency that caused half-maximal contraction, was calculated from linear plots.

Flow cytometry

To analyze the frequency of certain V β -expressing T cell populations, local draining lymph nodes from the airways and the lung were prepared and single cell preparations were obtained by dispersing small tissue pieces through stainless steel mesh. Mononuclear cell suspensions were prepared by density gradient centrifugation (Lymphoprep, Organon Teknica Corp., Durham NC) for 15 min at 1500 rpm at room temperature. Cells from the interface were washed three times in PBS and cell aliquots were transferred to 96-well microtiter plates and washed with staining buffer (PBS supplemented with 2% heat-inactivated FCS and 0.1% sodium azide). Then 20 μ l of 1/10 diluted normal mouse serum were added for blocking of nonspecific binding sites followed by 20 μ l of biotinylated mAb, which were diluted in staining buffer, and staining was performed as described (7). The following antibodies were used: anti-CD3 (2C11), anti-V β 8.1/8.2/8.3 (F23.1), anti-V β 8.1/8.2 (KJ16), anti-V β 8.2 (F23.2), anti-V β 14 (14.2), and anti-V β 2 (B20.6), all provided by Drs. J. Kappler and P. Marrack, National Jewish Center, Denver, CO. Plates were incubated for 30 min on ice followed by washing in staining buffer. Isotype-specific control antibodies were incubated in parallel. Cells were then incubated with 20 μ l of diluted phycoerythrin-streptavidin (Tago, Burlingame, CA) for 30 min on ice in the dark. After the second incubation, cells were washed and resuspended in staining buffer and analyzed on an EPICS analyzer (Coulter Electronics, Hialeah, FL) using a gate for lymphocytes. The frequency of V β

Table I
Stimulation of anti-OVA IgE by OVA (323–339) sensitization^a

Sensitization	Serum Anti-OVA (EU/ml)	
	IgE	IgG1
OVA	1835 $\pm$ 396	<20
OVA (323–339)	1810 $\pm$ 637	35 $\pm$ 2

^a BALB/c mice were sensitized to either native OVA or to OVA peptide 323–339 by ultrasonic nebulization of the allergens for 20 min during a consecutive 10-day period. On day 12 serum samples were taken and analyzed for anti-OVA IgE and IgG1 production by ELISA. Expressed are means $\pm$ SD from 15 animals in each group. Sera from PBS-exposed control mice contained <20 EU/ml of anti-OVA IgE or IgG1 antibody.

elements expressing T cells was calculated as a percentage of CD3-positive T cells.

Results

Sensitization of BALB/c mice to OVA peptide 323–339 induced an Ag-specific IgE response

BALB/c mice were sensitized to whole OVA or to OVA peptide 323–339 by aerosolization for 20 min/day over a period of 10 consecutive days. Two days later, serum samples from sensitized mice were assayed for anti-OVA IgE and IgG1 antibody titers. Sensitization to the native OVA was associated with a rise in anti-OVA IgE antibody titers, but not IgG1 antibody titers (Table I), as previously described (7). Similarly, after sensitization to OVA 323–339, an anti-OVA IgE antibody response was measured and the levels were in the same range as those measured after sensitization to the whole Ag. In addition, sensitization to OVA 323–339 stimulated a small, but reproducible, IgG1 antibody response that ranged just above the detection limit of the ELISA system.

In order to evaluate the portion of anti-OVA IgE antibodies that recognized OVA peptide 323–339, serum samples from sensitized animals were preincubated with peptide 323–339 before the measurement of anti-OVA IgE antibody titers by ELISA. In serum samples from mice sensitized to native OVA this resulted in a 50% reduction in anti-OVA IgE titers (Fig. 1A). In contrast, in serum samples from mice sensitized to OVA peptide, an 80% reduction was observed after preincubation with the peptide (Fig. 1B). These data indicate that the anti-OVA IgE antibody response after OVA sensitization is comprised, to a large extent, of IgE antibodies recognizing peptide 323–339. Furthermore, in mice sensitized to peptide 323–339 the IgE response is directed almost exclusively to the peptide.

The presence of functional IgE antibodies was confirmed by immediate hypersensitivity skin testing. In OVA-sensitized mice, 73% of the animals developed immediate cutaneous responses to native OVA and 82% showed positive skin test results to peptide 323–339. Similar results were found after sensitization to peptide 323–339; in 69%

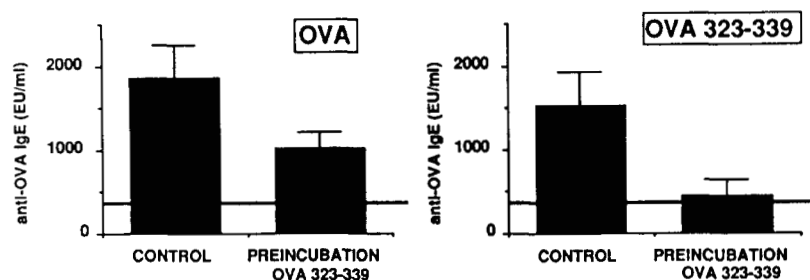


FIGURE 1. Inhibition of anti-OVA IgE titers by preincubation of serum samples with peptide 323–339. Serum samples from OVA-sensitized mice ($n = 8$; Fig. 1A) or OVA peptide 323–339-sensitized mice ($n = 8$; Fig. 1B) were assessed for anti-OVA IgE antibody titers by ELISA as described. Before adding the samples to the ELISA plates, samples were preincubated with OVA peptide 323–339 (1 mg/ml) for 30 min at room temperature. Preincubated or non-preincubated samples were analyzed in parallel on the same ELISA plates. Plates were developed as described in *Material and Methods* and anti-OVA IgE titers were calculated by comparison with the standard as described. Expressed are mean $\pm$ SD for each group.

of these mice, skin tests were found to be positive after intradermal injection of OVA, and injection of peptide 323–339 elicited reactions in 77% of the animals (Table II).

Sensitization to OVA peptide 323–339 increased AR

As described previously, sensitization of BALB/c mice to OVA via the airways led to an increase in AR, monitored by electrical field stimulation of tracheal smooth muscle preparations. Since sensitization to OVA peptide 323–339 stimulated a similar immediate cutaneous hypersensitivity response as sensitization to the whole protein, we determined whether sensitization to the peptide would also alter the AR of those mice. The results are illustrated in Figure 2, in which AR is expressed as a percentage of control ES_{50} . The control ES_{50} was calculated from mice exposed to PBS alone. The ES_{50} values in this group (4.13 ± 0.22 Hz) were assigned 100% and the effects of OVA and OVA peptide sensitization were compared with these controls. After sensitization to native OVA, a 34% decrease in the ES_{50} was observed (2.72 ± 0.16 Hz), consistent with the development of increased AR (6). A similar decrease in ES_{50} was measured in OVA peptide-sensitized mice; the ES_{50} decreased by 33% to 2.77 ± 0.25 Hz. The degree of change was not significantly different from results in OVA-sensitized mice, and in both groups reflected a significant ($p < 0.001$) decrease compared with PBS-exposed, control animals.

Lymphocytes from OVA- and OVA 323–339-sensitized mice respond to peptide 323–339 in vitro

In order to analyze the responsiveness of lymphocytes to OVA after sensitization, cell proliferation experiments were performed in the presence of OVA or OVA peptide (Fig. 3). After sensitization to OVA, splenic mononuclear cells, obtained 2 days after the sensitization protocol was completed, proliferated in a dose-dependent fashion to OVA. Maximal proliferation was observed at concentra-

Table II
Sensitization to OVA (323–339) induced immediate cutaneous hypersensitivity to OVA and OVA 323–339^a

Sensitization	% Positive Responses	
	OVA	OVA 323–339
PBS	0/12 (0%)	0/12 (0%)
OVA	8/11 (73%)	9/11 (82%)
OVA 323–339	9/13 (69%)	10/13 (77%)

^a BALB/c mice were exposed to PBS, OVA, or OVA peptide 323–339 by ultrasonic nebulization as described in *Materials and Methods*. On day 12, skin tests were performed in order to assess the presence of immediate hypersensitivity responses to OVA or OVA peptide 323–339. The development of wheal reactions was assessed 15 to 20 min after the intradermal injection of OVA, OVA peptide, and positive and negative control solutions. Wheal reactions greater than 3 mm in diameter were considered positive. Expressed are the number and percentage of mice showing positive reactions.

tions greater than 10^{-6} M. The dose response to OVA after OVA 323–339 sensitization was shifted slightly to the left; that is, lower concentrations of allergen were required to stimulate maximal response. With splenic cells from mice sensitized to OVA peptide, a half-maximal response was stimulated at about 10^{-9} M; the same concentration of OVA induced only minimal increases in the response of cells from OVA-sensitized mice. In contrast, the proliferative response to OVA 323–339 was similar regardless of whether the mice were sensitized to OVA or the peptide.

Stimulation of specific V β -expressing T cell populations after peptide sensitization

We previously showed that following OVA sensitization via the airways, there was an enlargement of the local draining lymph nodes, PBLN. Enlargement was attributable to the expansion of CD4⁺ T cells and B lymphocytes. The T cell response to OVA in PBLN was also marked by an increase in V β 8.1/8.2-positive cells (as well as V β 2 and V β 14 in the spleen) (5, 7). As shown in Figure 4, after sensitization to OVA the selective increase in V β 8.1 and V β 8.2-positive T cells was confirmed using flow-cytometric cell analysis with specific antibodies; the percentage of V β 2, V β 8.3, and V β 14 T cells was relatively

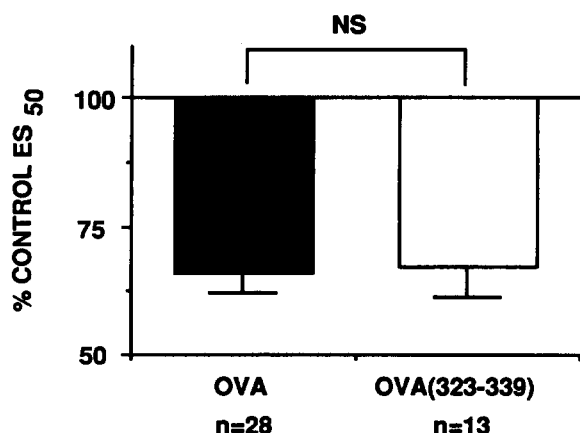


FIGURE 2. Sensitization to peptide 323–339 induced increased AR. Mice were sensitized to whole OVA ($n = 28$) or to OVA peptide 323–339 ($n = 13$) by ultrasonic nebulization of the allergens that were diluted in PBS. Control mice ($n = 18$) received PBS aerosolizations alone. Two days after completion of a 10-day course of daily nebulizations for 20 min each day, AR was analyzed by electrical field stimulation of tracheal smooth muscle preparations. As described in *Materials and Methods*, tracheal ring preparations were stimulated with increased electrical frequencies (ranging from 0.5 to 40 Hz) and smooth muscle constriction was expressed as isometric tension. For each trachea, a dose response curve was established which was used to calculate the ES₅₀. The mean ES₅₀ of PBS control mice was calculated as 100% (4.13 Hz) and the effects of OVA and OVA peptide 323–339 sensitization were compared with this control and expressed as % control ES₅₀. Sensitization to OVA caused a decrease in ES₅₀ to 2.72 ± 0.16 Hz and nebulization of peptide 323–339 resulted in a similar decrease in ES₅₀ to 2.77 ± 0.25 Hz.

unchanged. After sensitization to OVA 323–339 a similar enlargement of PBLN was found and frequency analysis of V β -expressing T cells revealed a selective expansion of V β 8.1 positive T cells. After peptide aerosolization, the frequency of V β 2, V β 8.2, V β 8.3, and V β 14 T cells was unchanged. Thus, there appeared to be a selective stimulation of V β 8.1 positive T cells in local draining lymph nodes of the airways after sensitization to OVA peptide.

Discussion

Ag-specific stimulation of T lymphocytes requires the formation of a trimolecular complex consisting of class II MHC molecules, processed Ag or peptide and the TCR. The nature of the immune response elicited by this complex may in some way be determined by the peptides and APC involved in interactions with the TCR. In recent years increased understanding of the processing mechanisms and the binding of defined peptides with class II molecules has been obtained. In addition, characterization of immunogenic peptides that can bypass the requirements of processing of native Ag has permitted a direct study of the

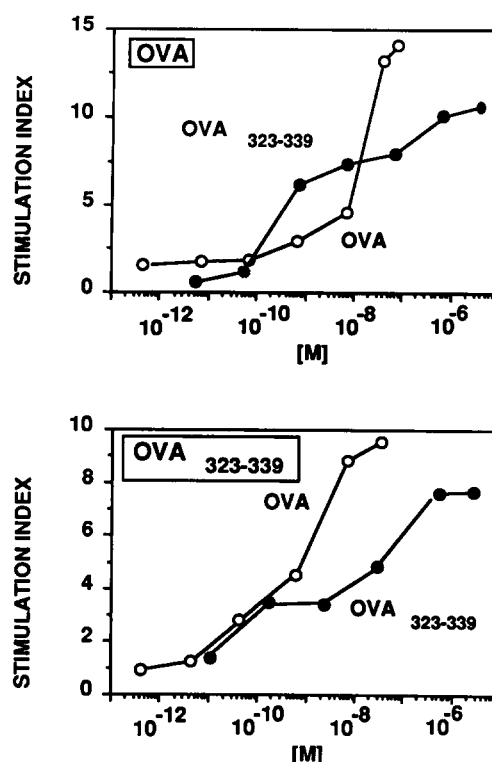


FIGURE 3. Proliferative response of lymphocytes from OVA and OVA peptide-sensitized mice to OVA and peptide 323–339. Mononuclear cells from OVA and OVA peptide 323–339-sensitized mice were prepared from the spleen and incubated with indicated concentrations of either native OVA or OVA peptide 323–339. After 5 days the proliferative response was measured by [³H]TdR incorporation, which was added to the culture in a concentration of 1 μ Ci 6 h before cell harvesting. The responses to the proteins were compared with the medium control (stimulation index of 1; mean cpm in OVA-sensitized mice was 1035 cpm, and 2180 cpm in peptide-sensitized mice) and the results were expressed as a relative increase (stimulation index) compared with this control. Expressed are mean values from three representative experiments. SD was always <15%.

interaction between Ag and MHC class II molecules. Much of this latter work has characterized the immunogenic peptides of OVA. Buus et al. demonstrated the immunogenic peptide, OVA 323–339, could be bound by I-A^d, but not I-E^d, I-A^R, or I-E^k (15). In BALB/c mice, which express I-A^d, peptide 323–339 elicited roughly 25–35% of the T cell proliferative response to native OVA (9, 10). In contrast, characterization of OVA T cell responses in I-A^S-restricted mice revealed two different peptides (225–240 and 233–248), which resulted in roughly 50% of the OVA-specific T cell proliferative response in these mice (16).

Although the bulk of the known MHC restriction patterns associated with the immune response to defined peptides have been based on T cell proliferative responses of IL-2 production (10, 17), little is known

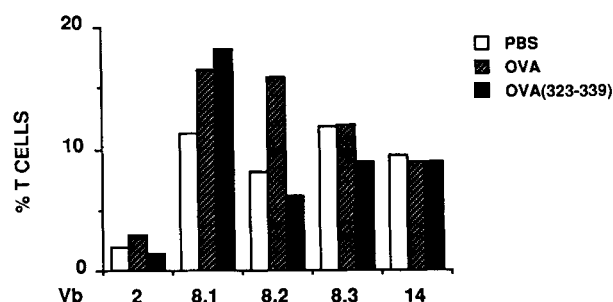


FIGURE 4. Frequencies of V β -expressing T cells in local draining lymph nodes of the airways and the lungs (PBLN) before and after sensitization. Lymph nodes from PBS ($n = 5$), OVA ($n = 8$) and OVA peptide 323–339 ($n = 8$) sensitized mice were prepared and mononuclear cell preparations were purified as described in *Materials and Methods*. Cells were stained with biotinylated anti-CD3 and anti-V β antibodies followed by staining with phycoerythrin-streptavidin. Cells were analyzed by flow cytometry gating on lymphocytes as described. Based on the frequency of CD3-positive T cells, the percentage of V β -expressing T cells was calculated. Expressed are the mean of the frequency for each V β expressing T cell population. The SD was always less than 10%. * indicates significant ($p < 0.01$) differences compared with PBS-exposed control mice.

whether these same restriction patterns hold for other immune responses, T cell dependent or T cell independent. We have now compared the capacity of OVA peptide 323–339 to elicit immediate hypersensitivity responses in BALB/c mice sensitized via the airways. This mode of sensitization to native OVA was shown to elicit Ag-specific IgE antibodies, immediate cutaneous hypersensitivity, and an increase in AR. In animals sensitized to OVA, T cells obtained from both PBLN and spleen proliferated in response to specific Ag in a dose-dependent fashion.

In the present study sensitization to OVA peptide 323–339 stimulated a similar set of responses. After 10 days of daily exposure to aerosolized peptide, mice developed serum IgE antibody levels that were not distinguishable from those observed in animals sensitized to intact OVA. In sera from animals sensitized to OVA, the levels of OVA-specific Ig were reduced by 50% when absorbed with peptide before ELISA. Levels of OVA-specific IgE from animals sensitized to the peptide were reduced by more than 80% under similar circumstances. These results indicate that, similar to T cells, the major determinant recognized by OVA-sensitized B cells was peptide 323–339. These findings in BALB/c mice are similar to what has been described for human (17, 18) or rabbit OVA-specific IgE (18). The studies of serum IgE antibody responses were paralleled by the studies of immediate cutaneous reactivity. Sensitization to OVA or to peptide 323–339 resulted in a similar percentage of skin test responders to the peptide or OVA.

The stimulation of an IgE response to OVA has always

been associated with the development of increased AR, measured by electrical field stimulation of tracheal smooth muscle preparations. The presence of IgE antibodies alone, however, are not sufficient, because local challenge with allergen is also required for developing increased AR (19). Sensitization to the peptide via the airways similarly resulted in increased AR. In fact, the changes in AR were not distinguishable from the changes elicited by the native protein. These data clearly indicate that OVA peptide 323–339 is an effective allergen, eliciting the full spectrum of immediate hypersensitivity response observed after sensitization to OVA itself.

It therefore appeared that the same peptide acted as a potent T and B cell immunogen. Peptide 323–339 is an important T cell epitope responsible for up to 30% of the T cell response to OVA in BALB/c mice. It was also shown that the same peptide binds to human (17, 18) and rabbit (18) Ig. Several possibilities may explain these findings. In the three-dimensional structure of OVA, peptide 323–339 may be located on the surface of the molecule. This would prevent binding of anti-OVA Ig to this region. Furthermore, proteins such as OVA are subjected to denaturation in vivo, resulting in unfolding of the protein. This may expose region 323–339, resulting in recognition by antibodies. Denatured OVA still retains antibody-binding activity (18).

It is not clear why an immunogen on one hand may elicit an immune response (e.g., IgG and IgM) and under other circumstances elicit an IgE response. Some of the factors governing the type of response include route of sensitization, the concentration of Ag used for immunization, and the use of adjuvants. For example, parenteral injection of OVA together with Freund's adjuvant favors development of an anti-OVA IgG response. This contrasts with our approach to sensitization in which, in the absence of adjuvant, sensitization through the airways results in a predominant IgE response. It is conceivable that APC residing in different tissue compartments have a differential ability to stimulate an IL-4-mediated immune (IgE) response. OVA peptide 323–339 is a potent immunogen and may be readily presented on the surface of alveolar or lung macrophages, which promote the selective expansion of certain T cell subsets, resulting in the development of an allergic response.

The development of Ag-specific IgE requires the presence of a signal derived from cognate interaction between T and B cells (20) and a second signal, provided by IL-4 (21–24). Although the first signal may be bypassed by other stimuli (e.g., ligation of CD40, EBV, hydrocortisone (25–27)), it emphasizes the role of MHC-TCR interaction at this level. Similar to the in vitro studies identifying peptide 323–339 as a major T cell immunogen, we found that sensitization to the peptide, in vivo, via the airways also triggered an Ag-specific T cell proliferative response. The extent of the response was similar to that observed in spleens

derived from OVA-sensitized mice.

Surprisingly, the response to peptide 323–339 triggered a selective expansion of T cells expressing V β 8.1. Earlier studies demonstrated that in PBLN, OVA triggered the selective expansion of the subfamily of V β 8.1/8.2 T cells (7) (see footnote 4). These results were confirmed here and, now using specific reagents to distinguish individual members of the family, sensitization to OVA was found to result in the expansion of both V β 8.1 and V β 8.2 (but not V β 8.3) T cell subsets. The finding that the peptide triggered the expansion of only V β 8.1 T cells may be in keeping with it serving as a selective T cell immunogenic peptide for this subset of T cells. The data therefore also suggest that OVA-reactive V β 8.2 T cells recognize a different immunogenic peptide or epitope.

For the allergen OVA, these data are in keeping with the following model: aerosolization of OVA in BALB/c mice sensitizes V β 8.1/8.2 T cells in the local draining lymph nodes of the airways and the lung (PBLN). Selective stimulation of V β 8.1 T cells may be caused by the presentation of peptide 323–339 by MHC class II Ag to this subset of T cells, whereas other peptides stimulated V β 8.2 T cells. These activated V β 8.1/8.2 T cells were then able to stimulate B cells to produce IgE (likely through cognate interaction with B cells and production of IL-4) (our unpublished observations). As a result, the anti-OVA IgE response was in part directed against the peptide 323–339 and led to the development of immediate cutaneous hypersensitivity to this peptide and to OVA. Local exposure to the allergen together with the systemic IgE response resulted in the increase in AR. These data suggest an important role of OVA peptide 323–339 in the development of immediate hypersensitivity responses in BALB/c mice to OVA.

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