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Antigen-Specific T Cell Tolerance Down-Regulates Mast Cell Responses *in Vivo*

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Fel d I is the major cat allergen that induces asthma and allergic rhinitis in humans. To investigate the mechanism of allergic responses to this allergen, a mouse model was developed. Mice sensitized to chain 1 of Fel d I exhibited T cell responses, B cell responses, and mast cell responses when challenged with the protein. Subcutaneous injections of peptides containing the dominant T cell epitopes of the allergen induced T cell tolerance in presensitized mice. When challenged with the allergen intratracheally, these tolerized mice produced a decreased amount of histamine *in vivo*. The decrease in histamine release was not solely dependent on the reduction of allergen-specific IgE. These data show that mast cell activity in mice with an ongoing sensitivity to allergen can be regulated through peptide-induced T cell tolerance. © 2000 Academic Press

Key Words: **tolerance; mast cell; T lymphocyte; histamine.**

INTRODUCTION

Allergic reactions of the respiratory tract proceed in two distinct phases. The immediate phase occurs rapidly after inhalation of the inducing allergen as a result of activation and degranulation of tissue resident mast cells. The late phase is characterized by infiltration of airways by eosinophils. Mast cells arise from precursors in the bone marrow and complete their differentiation in resident tissues. They can be divided into connective tissue mast cells (CTMC) and mucosal mast cells (MMC) (1). MMC are present in the intestinal lamina propria and in the lung, whereas CTMC are distributed throughout connective tissues of the skin and peritoneal cavity. Compared to CTMC, MMC have been shown to be more sensitive to T cell regulation (2, 3).

The development of airway sensitivity to inhaled allergens is thought to be initiated by activation of allergen-specific Th2 lymphocytes (4). T cells help B cells to produce allergen-specific IgE which binds to FCcεRI on the surface of mast cells. Mast cells are triggered upon crosslinking of these cell-bound IgE molecules by incoming allergen to release a variety of mediators which are involved in the initiation of the allergic reaction (5). In addition to inducing immunoglobulin isotype switching to IgE, T lymphocytes also participate in the allergic reaction by secreting soluble mediators. IL-3, IL-4, IL-5, and IL-10 have been shown to play an important role in the initiation and regulation of allergic responses (6–8). There is abundant evidence that recruitment and activation of eosinophils is mediated by the secretion of cytokines from antigen-stimulated T lymphocytes and the role of T cell activity in regulation of late phase reaction is well established (8–11). However, the role of T cell activity in regulating the immediate phase reaction is more complex and less well understood. Although it is clear that IgE is central to allergen-specific activation of the secretory response of mature mast cells, recent experiments have indicated that other mediators, such as cytokines and chemokines, can play a role in regulation of the secretory function of mast cells (12–18).

Based on the central role played by T lymphocytes in the development of allergic reaction, modulation of allergen-specific T cell activity has been proposed as a therapeutic approach to controlling the severity of allergic inflammation. Mouse models have been developed to show that antigen-specific T cell responses can be down-regulated by the administration of peptide antigen in aqueous solution (19–23). We and others have shown that induction of allergen-specific T cell tolerance *in vivo* can inhibit allergen-induced recruitment of eosinophils into lungs (24–26). However, the effects of the modulation of allergen-specific T cell responses on mast cells *in vivo* are not known. We have developed a mouse model which allows us to monitor histamine release *in vivo* in response to bronchial allergen challenge. To generate an ongoing sensitivity to specific allergen, mice were immunized with cat allergen, chain 1 of Fel d 1 in alum. Two immunodominant peptides from this allergen (19) were used to down-

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regulate allergen-specific T cell responses *in vivo*. The degree of the allergen-induced immediate phase reaction was assessed by measuring the *in vivo* histamine release following bronchial challenge with the allergen. Data from these experiments demonstrate that antigen-specific T cell responses play an important role in the regulation of the mast cell-associated immediate phase of allergic responses in this model.

MATERIALS AND METHODS

Mice

Female B6CBAF1 (H-2^{bxk}) mice 4–8 weeks old were obtained from the Jackson Laboratory (Bar Harbor, ME).

Peptides/Proteins

Peptides IPC-1 (residues 17–33) and IPC-2 (residues 29–55), which are located on the N-terminal of the cat allergen Fel d 1, were synthesized using conventional Merrifield solid-phase t-Boc or Fmoc chemistry. The peptides were purified by reverse-phase HPLC and were greater than 90% pure by HPLC. The lyophilized peptides were resuspended in phosphate-buffered saline (PBS) and sterilized by γ irradiation (10,000 rad) or by passage through a 0.2-μm filter. Chain 1 of Fel d 1 (27) and Der p II (28) were expressed and purified essentially as described (29). Chain 1 of Fel d I was thrombin cleaved and HPLC purified to remove nonrelevant amino acids at the N terminus and was greater than 90% pure. Recombinant Der p II included the His_6 tag and was purified by metal-ion chromatography and was 80% pure as determined by SDS–PAGE and Coomassie blue staining.

Injections

Tolerization in all experiments was induced by sc injection of 300 μg of IPC-1 and IPC-2 in 200 μl PBS on days 1, 4, and 7. Control mice were injected with 200 μl of PBS according to the same schedule. For sensitization, injections of Fel d I and Der p II were given ip in a volume of 200 μl of protein suspension in alum (Pierce, Rockford, IL).

Intratracheal Challenge

Mice were anesthetized by injecting 0.3 ml of a mixture of ketamine (8.88 mg/ml, Fort Dodge Labs, Iowa) and xylazine (0.67 mg/ml, Fermenta Animal Health Co., Kansas City, MO) intraperitonealy and immobilized with jaws open using rubber bands. A 25-gauge needle with its tip filed off was inserted past the epiglottis into the trachea guided by a fiber optic probe to light the area. A syringe containing 100 μg of the Fel d 1 in 0.1 ml PBS was attached to the inserted needle and the protein solution was delivered into the trachea. With a fresh syringe 0.2 ml of air was then delivered and the mice were allowed to recover from the anesthesia.

In Vitro Cultures

Spleens were homogenized to a single-cell suspension by pressing through a steel mesh screen. Spleen cells (4×10^5) were cultured in a 96-well plate with various concentrations of Fel d 1 in a final volume of 200 μl. Cultures were maintained in RPMI 1640 (GIBCO, Grand Island, NY) supplemented with 10% FCS (Sigma, St. Louis, MO), 10 mM glutamine, penicillin (100 U/ml), streptomycin (100 U/ml), and 2×10^{-5} M 2-ME, at 37°C and 5% CO_2, for various periods of time and pulsed with 1 μCi (50 μl) of [^{3}H]thymidine (ICN Pharmaceuticals, Inc., Irvine, CA) for 12–16 h before harvest. After harvest of cells onto glass fiber mats, [^{3}H]thymidine incorporation was measured by scintillation counting. Culture supernatants (50 μl) were removed from a parallel set of cultures at 24 h and stored at −20°C for IL-2 measurements.

Cytokine Assays

IL-2 was detected by bioassay using the cytokine-sensitive cell line CTLL3. All assay cultures were performed with 50 μl of culture supernatant and 50 μl of cells (5×10^3 CTLL3) and maintained for 48 h, the final 24 h in the presence of [^{3}H]thymidine. After harvest of cells onto glass fiber mats, [^{3}H]thymidine incorporation was measured by scintillation counting. Standard curves were generated using known amounts of purified murine rIL-2 (Biosource Int., Camarillo, CA). Data were generated as cpm and converted to Units per milliliter by logit transformation using the standard curve as a reference.

Histamine Assay

Histamine levels in serum samples were determined by either glass fiber assay or radioimmunoassay. The quantitation by glass fiber assay was performed using 96-well plates containing glass microfibers essentially as described previously (30, 31). Briefly, serum samples diluted in PACM (Pipes, albumin, calcium, magnesium) buffer were incubated in the plates overnight at 4°C, allowing histamine to bind the glass fibers. The plates were washed with distilled water, followed by 1 h of incubation at 37°C with 0.4% SDS. The histamine was eluted from the wells with sodium hydroxide and incubated for 10 min at room temperature with *O*-phthaldialdehyde (OPT; Sigma) to yield a fluorescent product. The reaction was terminated with 0.59% perchloric acid, and fluorescence quantitated using an auto sampler and fluorometer (Gilson fluorometer, Model 121). Histamine levels were quantitated by comparison to a standard curve generated by coupling his-

tamine dihydrochloride (Sigma) to OPT. The assay was sensitive to 2 ng/ml histamine. Quantitation of serum histamine by RIA was performed using a kit from Immunotech (Marseille, France) according to the instructions recommended by the vendor (AMAC, Westbrook, ME). RIA was sensitive to 0.02 ng/ml histamine.

IgE Assays

Allergen-specific IgE levels in the sera were measured by ELISA as described (28). Corning ELISA plate (Costar, Cambridge, MA) wells were coated by incubation of 50 μl/well of Fel d I or Der pII at a concentration of 0.01 mg/ml in PBS overnight at 4°C. The wells were washed with PBS containing 0.05% Tween 20 and blocked with 0.5% gelatin in PBS for 1 h at room temperature. Plates were washed three times with PBS–Tween. Sera were diluted in PBS and 100 μl/well was added to the washed plates. Plates were kept at room temperature for 1 h and washed three times with PBS–Tween. After incubation (1 h at RT) and washing, the antigen-bound mouse IgE was detected by incubation (RT, 1 h) with 100 μl/well of rat anti-mouse IgE (EM95, Southern Biotechnology, Birmingham, AL) at 1:2500 dilution. Biotinylated goat anti-rat IgG (H + L) (Southern Biotechnology Associates) was used as an additional signal amplification step in this assay. Plates were washed three times with PBS–Tween and 100 μl of streptavidin-HRP (Southern Biotechnology Associates) was added to the wells to detect antigen-bound biotinylated antibody complexes. TMB substrate (Kirkegaard and Perry Laboratories, Gaithersburg, MD) was used according to manufacturer's recommendations. Plates were incubated for 5 min, at the end of which time 100 μl/well of 1 M phosphoric acid was added to stop the developing reaction and OD (450 nm) values were determined using a Biokinetics reader (EL 312e microplate, Bio-Tek Instruments). OD values were converted into units/ml of IgE by assigning an arbitrary unit value to a serum sample containing allergen-specific IgE.

IgE Passive Transfer

Purified mouse monoclonal IgE anti-DNP antibodies (50 μg/injection in PBS) (Sigma, St. Louis, MO) were administered iv in a volume of 200 μl/injection by two tail vein injections given at 24-h intervals. Eighteen hours after the second IgE injection, the mice were intratracheally challenged with 100 μg of dinitrophenyl–human serum–albumin (Sigma) in PBS in a volume of 100 μl. Thirty minutes after the intratracheal challenge, mice were sacrificed and serum samples were collected for the determination of histamine levels.

RESULTS

Sensitization of Mice to Cat Allergen for an Immediate Phase Reaction

To investigate the regulation of the immediate phase of allergic reaction *in vivo*, a mouse model was developed. B6CBAF1 mice which mount a strong immune response to cat allergen Fel d 1 were sensitized by ip injection with chain 1 of Fel d 1 (which from here on will be referred to as Fel d I) in alum. These mice and a separate group of untreated, control mice were challenged 1 month later with either Fel d I in PBS or PBS alone by an it instillation. Histamine levels were measured in the serum samples collected from all groups of mice 30 min after the challenge. As shown in Fig. 1A, the Fel-d-I-primed mice responded to the bronchial challenge with Fel d I and produced substantial amounts of histamine in their serum. The release of histamine was allergen-induced, as only a negligible amount of histamine was detected in the serum of allergen-primed mice receiving it instillation of PBS alone. Unprimed, naive mice failed to produce histamine upon challenge with either PBS or Fel d I. These data confirm that sensitization of mice by allergen is a necessary step in inducing allergen-specific histamine release *in vivo*. Furthermore, these data demonstrate that in this model, sensitization and challenge with a single, specific allergen is sufficient to produce histamine levels that can reach detectable levels in the serum. To determine whether the glass fiber assay was specific for histamine in the serum, 11 serum samples with a wide range of histamine levels were also assayed by radioimmunoassay. As shown in Fig. 1B, serum levels of histamine obtained using glass fiber assay agreed well with the values obtained by the radioimmunoassay. All other data shown for the quantitation of histamine in this study were obtained by using glass fiber assay.

To further confirm that allergen-induced histamine release was dependent on the presence of allergen-specific IgE, mice were injected with either Fel d I in alum or a control allergen, Der p II, in alum. Three weeks after the sensitization IgE and histamine levels were measured in each set of mice following the it challenge with Fel d I. As shown in Fig. 1C, Fel-d-I-specific IgE, but not Der-p-II-specific IgE, was detected in mice sensitized with Fel d I. Similarly, Der-p-II-sensitized mice contained IgE specific for Der p II but not for Fel d I. Histamine was produced in response to it challenge with Fel d I only in the mice containing Fel-d-I-specific IgE, whereas mice containing Der-p-II-specific IgE failed to produced histamine in response to Fel d I challenge (Fig. 1D). These data confirm that *in vivo* histamine production in this system is allergen-specific and depends on the presence of allergen-specific IgE.

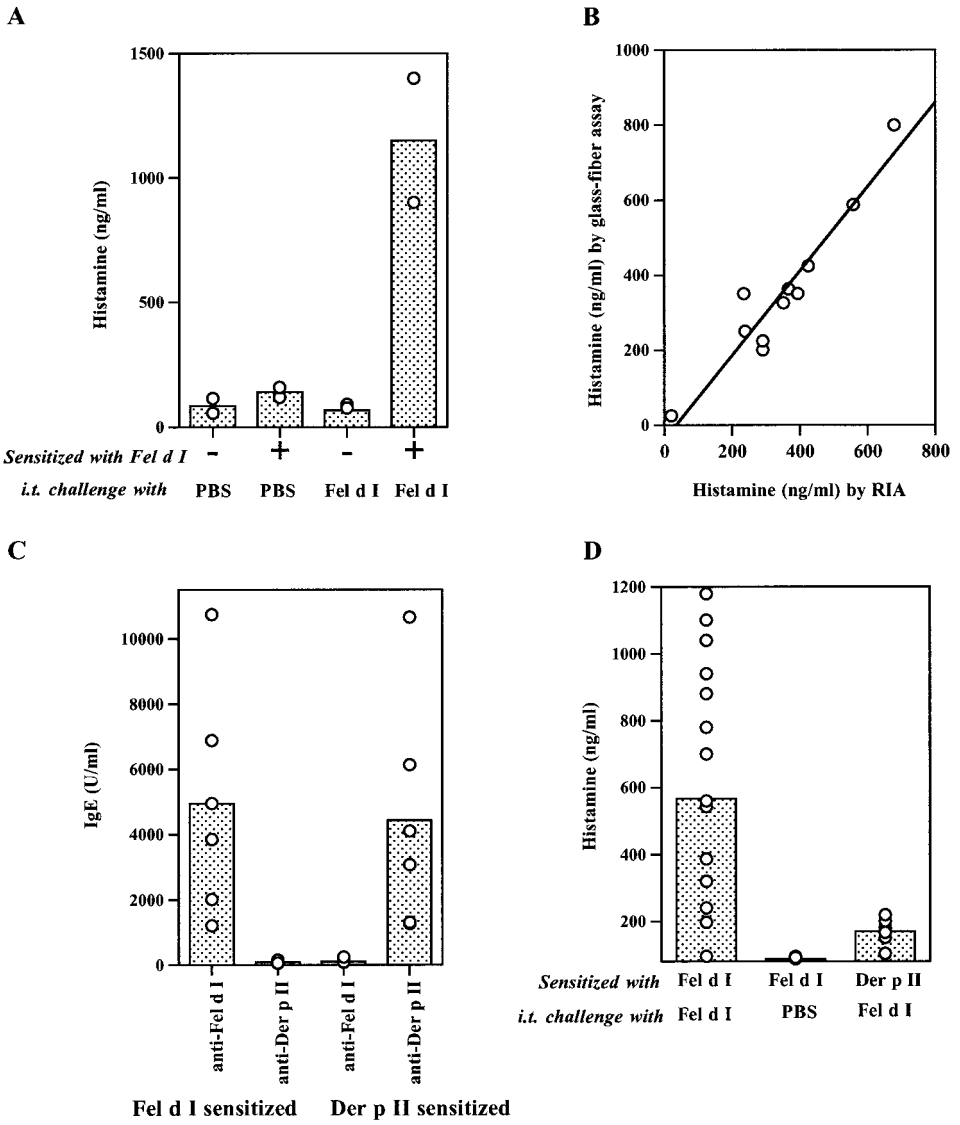


FIG. 1. (A) Immunization with Fel d I in alum sensitizes mice to histamine release upon bronchial challenge with Fel d I. Mice were injected ip with either PBS or Fel d I in alum as indicated. Four weeks later, both groups of mice were challenged intratracheally with either Fel d I in PBS or PBS alone. Histamine levels were measured in the serum 30 min after the challenge. Each dot represents the value for an individual mouse and the average value in each group is represented by a bar. (B) Comparison of histamine quantitation by glass fiber assay and by RIA. Eleven serum samples containing a wide range of histamine amounts as measured by glass fiber assay were selected for quantitation of histamine by RIA. All serum samples were obtained from Fel-d-I-sensitized mice 30 min after it challenge. (C) Fel-d-I-specific IgE is detected in the serum of only the Fel-d-I-sensitized mice. Mice were injected ip with either Fel d I in alum or Der p II in alum. Three weeks later, Fel-d-I-specific and Der-p-II-specific IgE levels were determined in the sera of both groups of mice by ELISA. For each allergen, optical density values were converted into units by assigning an arbitrary value to a single serum sample containing allergen-specific IgE. Each dot represents the value for an individual mouse; the average value for each group of mice is represented by a bar. (D) Specificity of histamine release. Mice were injected ip with either Fel d I in alum or Der p II in alum. Three weeks later, both groups of mice were challenged intratracheally with either Fel d I or PBS. Histamine levels were determined in the serum 30 min after the it challenge. Each dot represents the value for an individual mouse and the average value in each group is represented by a bar.

Subcutaneous Peptide Injections Tolerize Allergen-Specific T Cells in Sensitized Mice

The next set of experiments was conducted to test if modulation of allergen-specific T cell responses would affect the immediate allergic response. It has been previously shown that sc administration of the immunodominant peptides IPC-1 and IPC-2 of Fel d I can down-regulate Fel-d-I-specific T cell responses in mice (19). We have used a modification of this system to down regulate allergen-specific T cell responses.

To model the situation that would exist in humans, animals were presensitized with allergen to assure the presence of an ongoing response at the time of T cell tolerization. B6CBAF1 mice were sensitized with Fel d

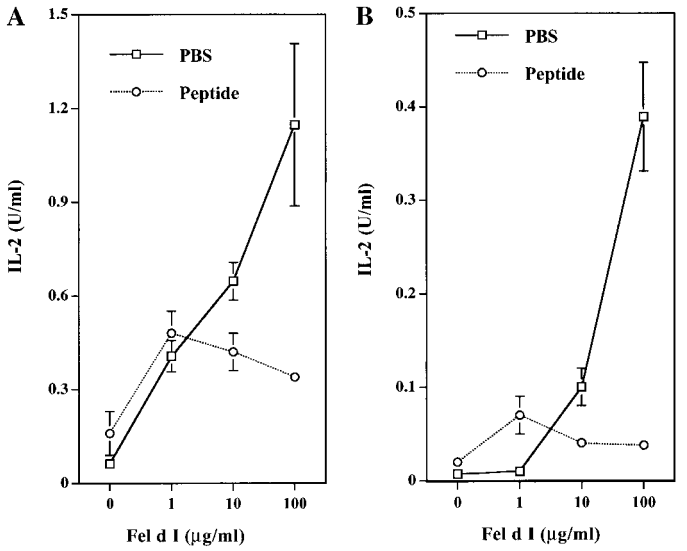


FIG. 2. Injection of IPC-1 and IPC-2 causes a decrease in Fel-d-I-specific T cell responses in presensitized mice. B6CBAF1 mice presensitized to Fel d I were injected with IPC-1 and IPC-2 to induce T cell tolerance. LN (B) and spleen (A) cells from control and peptide-injected groups of mice (5 mice/group) were cultured with varying concentrations of Fel d I for 24 h. IL-2 levels in the supernatant were determined by their ability to support the growth of an indicator cell line (CTLL). Error bars represent the standard error of the mean for triplicate measurements. Some error bars may be too small to see on the scale of this graph. Results are representative of three experiments.

I by giving them a single ip injection of 100 μg Fel d I in alum. Four weeks later, T cell tolerance was induced with three sc injections of peptides in PBS. To confirm that the T cells were tolerized, mice were challenged 7 days later with 100 μg Fel d I in alum and the responsiveness of splenocytes and LN cells to Fel d I was determined *in vitro* 12 days after challenge. As shown in Fig. 2, cells from a control group of mice injected with PBS responded to allergen stimulation *in vitro,* as evidenced by IL-2 production. In contrast, splenocytes and LN cells isolated from mice injected with soluble peptides produced substantially reduced amounts of IL-2, demonstrating that T cells were tolerant. These data confirm previous findings and extend them to the system used here to show that the administration of peptide antigens inhibits T cell responses to subsequent allergen challenge in mice with preexisting immune responses to the allergen.

Subcutaneous Peptide Injections Tolerize Allergen-Induced Histamine Release

The next set of experiments was conducted to determine if a reduction in antigen-specific T cell responses by peptide administration could affect the magnitude of the immediate phase allergic reaction, as evidenced by histamine release. Mice sensitized to Fel d I were injected with peptides to induce T cell tolerance and then boosted once with Fel d I in alum. The *in vivo* mast cell response was measured by challenging the mice with Fel d I intratracheally. Thirty minutes later, serum samples were collected and assayed for the levels of histamine produced. As shown in Fig. 3, substantial amounts of histamine were detected in the serum of control, untolerized mice in response to intratracheal challenge with Fel d I. In contrast, mice tolerized by peptide treatment had significantly reduced levels of serum histamine.

As shown in Fig. 4, tolerization with peptide resulted in decreased levels of Fel-d-I-specific IgE in peptide-treated mice but reached statistical significance in only one of two experiments ($P = 0.07$ for A and 0.05 for B). However, the level of allergen-specific IgE in the serum of an individual animal did not correlate with the level of histamine released upon intratracheal challenge ($r^2 = 0.017$ for A and 0.11 for B). These data indicate that factors other than IgE are likely to play a role in regulating mast cell secretory functions.

In Addition to IgE, Other Components of Immune Response Regulate Histamine Release in Vivo

To more closely examine the effects of lymphocyte mediators on mast cell function, a system was devised in which two groups of mice, differing in T cell responsiveness, maintained equivalent levels of Ag-specific

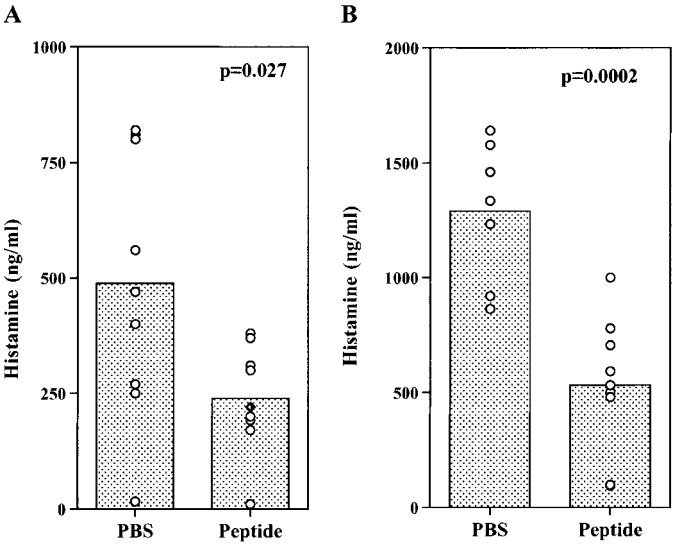


FIG. 3. Injection of IPC-1 and IPC-2 causes a decrease in Fel d I-induced histamine release *in vivo* in presensitized mice. B6CBAF1 mice presensitized to Fel d I were injected sc with IPC-1 and IPC-2 to induce T cell tolerance. Both control and peptide-treated groups of mice were intratracheally challenged with 100 μg Fel d I in PBS. Histamine levels in sera were measured 30 min following the intratracheal challenge. Each dot indicates the histamine level in the serum of an individual mouse. The average histamine level for each group is represented by the bar. Statistical significance was determined by using the two-tailed, unpaired *t* test. A and B represent two independent sets of experiments.

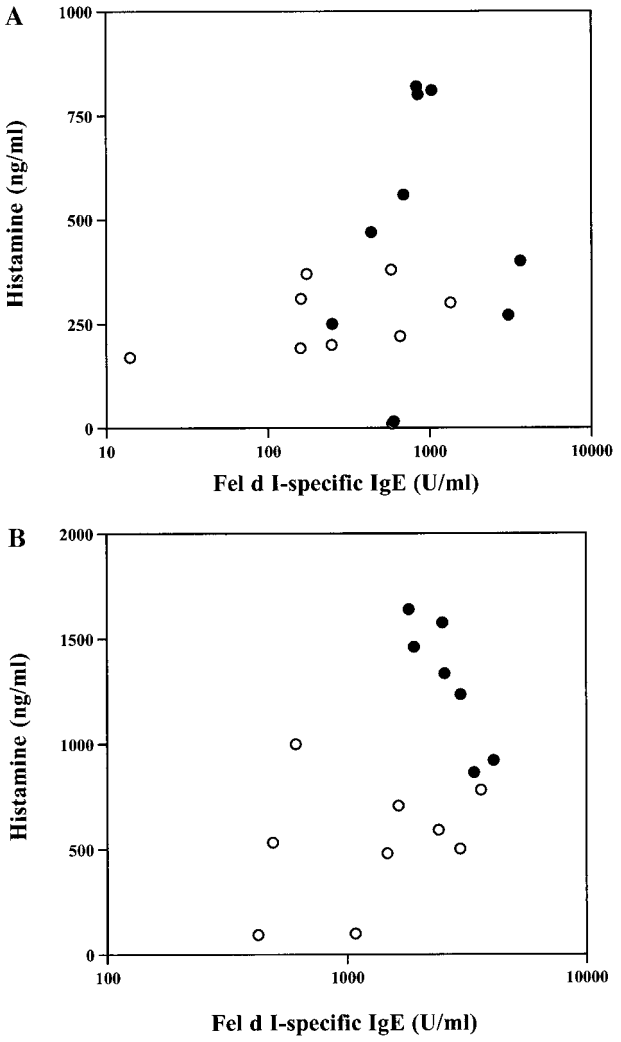


FIG. 4. Relationship of IgE levels and histamine release. The levels of Fel-d-I-specific IgE were measured in serum samples for which histamine levels are shown in Fig. 3. Each circle represents the histamine levels and Fel-d-I-specific IgE levels for an individual mouse. Filled circles represent the values for an individual mouse in the control group and open circles represent the values for an individual mouse in the peptide-treated group. A and B represent data from two independent sets of experiments and correspond to A and B in Fig. 3.

IgE. DNP-specific IgE was passively transferred either into naïve mice with no ongoing immune response or into mice previously immunized with an unrelated Ag, Fel d I. Both groups of mice were intratracheally challenged with DNP-HSA 18 h after the passive transfer of IgE. Thirty minutes after the challenge, sera were collected and assayed for histamine levels. As shown in Fig. 5, significantly ($P = 0.03$) higher levels of histamine were detected in the serum from mice with an ongoing immune response to Fel d I compared to the levels produced by naïve mice. These data indicate that mediators derived from other ongoing immune responses can influence the degree of the immediate phase allergic reaction.

Taken together these data show that peptide injections can cause a reduction in allergen-induced histamine release *in vivo*. Furthermore, these data also indicate that the reduction in histamine release may not be solely due to the reduction in the level of allergen-specific IgE, suggesting that other mediators of immune response may be involved in mast cell secretory function.

DISCUSSION

The aim of the present study was to set up an experimental model in which the effect of allergen-specific T cell modulation on the immediate phase of allergic reaction could be evaluated. The approach we have taken is to develop a mouse model in which mast cell response to allergen challenge can be monitored *in vivo*. Fel d I is the major cat allergen that induces asthma and allergic rhinitis in humans. B6CBAF1 mice can be sensitized to this allergen for Fel-d-I-specific T and B lymphocyte responses. We have developed this model to demonstrate that Fel-d-I-sensitized mice

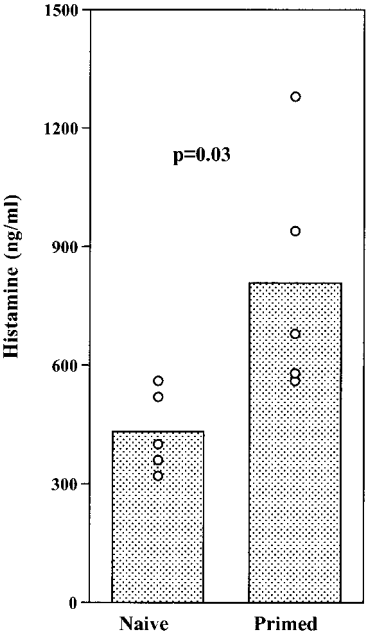


FIG. 5. Histamine release *in vivo* can be modulated in an IgE-independent fashion. B6CBAF1 mice were sensitized by two weekly ip injections of Fel d I in alum intraperitoneally. One week after the second injection, the sensitized mice and a group of naïve, control mice were given two doses of anti-DNP IgE (50 μg) iv at 24-h intervals. Eighteen hours later, both groups of mice were challenged it with 100 μg DNP-HSA. Serum histamine was measured 30 min after the it challenge. Each dot represents the histamine level for an individual mouse and average values in each group are represented by bars. Statistical significance was determined by using the two-tailed unpaired test. Results are representative of two experiments.

upon intratracheal challenge with Fel d I can produce serum histamine levels that can easily be detected by simple glass fiber assay. The histamine release is specific to allergen challenge because exposure to PBS or an irrelevant allergen did not produce a significant amount of histamine in the serum. The *in vivo* histamine response to the allergen was rapid; peak responses were detected 30 min after the challenge (data not shown). Although it cannot be definitely established in this study that the histamine release is solely due to mast cell degranulation, the kinetics of this release is consistent with its being a mast cell-mediated immediate type hypersensitive response.

The results of this study confirm that subcutaneous injection of peptide antigen can induce T cell tolerance in presensitized mice and therefore can be used therapeutically to reduce unwanted T cell responses to specific allergen. This study has focused on monitoring T cell responses by measuring antigen-specific IL-2 production. However, we have previously shown that both Th1 and Th2 responses are reduced under this tolerance regimen (24). It has been previously shown that allergen-induced T cell tolerance can inhibit eosinophil recruitment to the lung in response to allergen challenge (24–26). The data presented here demonstrate that mice sensitized to producing Fel-d-I-specific histamine can be treated with a high dose of Fel d I peptides to produce decreased levels of histamine. Thus, reduction of allergen-specific T cell responses by peptide treatment not only inhibits allergen-induced late phase allergic reaction but can also inhibit immediate phase response as measured by *in vivo* histamine release. The two Fel d I peptides used in the present study have also been used in human clinical trials. Initial data from these trials suggest that subcutaneous injections of Fel d I peptides resulted in decreasing allergic rhinitis and lung functions in a dose-dependent manner (32, 33). Immunological analysis showed reduced IL-4 production in response to Fel d I by peripheral T cells of treated patients (34). Interestingly, at the day of peptide injection some patients transiently experienced early late phase allergic reaction-like symptoms. These symptoms could be related to T cell cytokine release during the tolerance induction phase which will be consistent with the *in vivo* mouse model of T cell tolerance where T cell tolerance induction is preceded by transient T cell hyperactivation (35).

Slightly lower levels of allergen-specific IgE were detected in the sera of peptide-tolerized mice, but there was no direct correlation between the levels of histamine release and serum levels of IgE in individual mice. Despite the absence of correlation with IgE levels, peptide-treated mice produced decreased amounts of histamine. Thus it seems that mast cell responses to the Fel d I allergen were also affected by lymphocyte-derived factors in an IgE-independent fashion. Although others have suggested that T cell cytokines play a role in mast cell activation (36–38), the data presented here represent, to the best of our knowledge, the first *in vivo* demonstration that allergen-specific T cell tolerance can down-regulate mast cell secretion and thereby the immediate phase of an allergic response. The peptides used to induce T cell tolerance in this study were selected because in B6CBAF1 mice they represent dominant Fel d I T cell epitopes (19). It is expected that *in vivo* they will form MHC/peptide complexes and modulate T cell activity (35). However, the effects of these peptides on other cell types can not be completely ruled out.

The most surprising finding of this study stems from the fact that two sets of mice infused with equal amounts of specific IgE produced different amounts of histamine when challenged with allergen; mice with an ongoing immune response to an unrelated antigen (Fel d I) produced higher levels of histamine than naive mice with no ongoing immune response. Specifically, the data show that despite administration of equal amounts of DNP-specific IgE in both sets of mice, higher levels of histamine were produced in response to DNP-HSA challenge in the mice with ongoing lymphocyte activity to Fel d I. These data further support the conclusion that soluble lymphocyte mediators may play a role in regulating the immediate phase of the allergic reaction in both B-cell-dependent and B-cell-independent manners.

The mechanism by which mice with ongoing immune responses produce enhanced levels of histamine *in vivo* is not clear. Several T cell cytokines, including IL-3, IL-4, IL-9, and IL-10, are known to play a role in the proliferation and differentiation of mucosal mast cells (1, 36). Repeated injections of specific antigen can induce mucosal mast cell hyperplasia (39). Therefore, it is possible that enhanced histamine release in sensitized mice is due to an increase in mast cell number. More recent studies have suggested that the secretory function of mast cells can be affected *in vitro* by T-cell-derived cytokines (37, 38). T cell cytokines such as IL-4 have been reported to increase FcεRI density on mast cells (40, 41). It is possible, that in mice with ongoing immune responses, mast cells express higher levels of FcεRI, resulting in an increased number of DNP-specific IgE molecules displayed on the mast cell surface. Investigation of these possibilities will be the focus of our future studies.

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