

Pretreatment with Allergen Prevents Immediate Hypersensitivity and Airway Hyperresponsiveness

AKIHIRO OSHIBA, ECKARD HAMELMANN, KATHERINE L. BRADLEY, JOAN E. LOADER, HARALD RENZ, GARY L. LARSEN, and ERWIN W. GELFAND

Division of Basic Sciences and Pulmonary Medicine, Department of Pediatrics, National Jewish Center for Immunology and Respiratory Medicine, Denver, Colorado

The ability of subcutaneous pretreatment with an immunogenic peptide derived from Fel d I, the major cat protein, to suppress the development of allergic responses was examined in a mouse model of antigen-induced sensitization. BALB/c mice exposed to aerosolized Fel d I chain 1 peptide developed antigen-specific IgE responses, immediate cutaneous reactivity to the peptide, and increased airway responsiveness (AR). Both subcutaneous and intraperitoneal administration of the peptide prior to sensitization caused a 50% reduction in cutaneous reactivity which was associated with a decrease in serum anti-Fel d I chain 1 IgE and IgG1 antibody responses and an increase in specific IgG. Pretreatment with the peptide also suppressed spleen and lymph node proliferative responses to the peptide. However, only subcutaneous peptide injections could prevent the development of increased AR. Transfer of spleen cells from subcutaneously peptide-treated mice to sensitized recipients reduced serum antigen-specific IgE and IgG1 antibody responses and skin test reactivity, and prevented alterations in AR. These data suggest that IgE (and IgG1) responses and airway hyperresponsiveness induced by allergen sensitization via the airways can be modulated by subcutaneous administration of peptide. Further, the results define a model for investigating the modulatory effects of subcutaneous administration of immunogenic peptides or protein on an ongoing allergic response. **Oshiba A, Hamelmann E, Bradley KL, Loader JE, Renz H, Larsen GL, Gelfand EW. Pretreatment with allergen prevents immediate hypersensitivity and airway hyperresponsiveness.**

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It is generally accepted that allergic diseases such as asthma are associated with elevated levels of allergen-specific IgE in the serum and that immediate, IgE-mediated reactions in asthma are followed by chronic inflammation leading to increased airway responsiveness (AR) (1). Approaches to specifically control the state of hyperresponsiveness to a specific allergen have been considered useful from a clinical point of view. However, the mechanisms underlying immunologic tolerization or desensitization are still unclear with several possibilities proposed. It has been suggested that specific IgG induced by a given allergen may block the binding of the allergen to IgE or that parenteral injection of allergen may induce a reduction in IgE synthesis (2-4). Studies on T-cell responses during immunotherapy have shown a reduction in the lymphoproliferative responses to allergen (5). It has also been reported that immunotherapy results in a redistribution of T-cell populations, indicating that it might influence allergen-specific T cells and the cytokines they produce (6-8).

In vivo animal models of allergic diseases are useful for studying the immunologic mechanisms of sensitization and toleriza-

tion to specific allergens. In a number of animal studies, specific desensitization or immunologic tolerance to allergenic proteins has been demonstrated (9, 10). Chemically modified allergen extracts selectively inhibited IgE antibody production in sensitized mice (11). Desensitization to respiratory anaphylaxis was also observed in inbred guinea pigs (12) and to delayed-type hypersensitivity responses in sensitized mice (13). Administration of peptides prior to immunization induced peripheral T-cell tolerance which may closely correlate with suppression of allergen-specific T-cell responses (9). However, there has been no clear explanation of the mechanisms responsible for a reduction of IgE with immunologic tolerance or desensitization, or the prevention or normalization of altered AR.

We developed a murine model of allergic sensitization in which BALB/c mice exposed to an aerosolized antigen acquire antigen-specific IgE in the serum, immediate cutaneous hypersensitivity, and increased AR (14-16). This model provides an excellent means for investigating the immunomodulatory effects of antigen pretreatment on both IgE-mediated responses and airway hyperresponsiveness. The purpose of the present study was to determine if pretreatment with allergen could reduce immediate hypersensitivity and airway hyperresponsiveness which develop in mice sensitized by aerosolized antigen. For this purpose, we used a peptide from the major cat allergen, Fel d I chain 1, which has been shown to be immunogenic (9), to both pretreat and sensitize BALB/c mice. We found that nebulization with the peptide induced an elevation of antigen-specific IgE and increased AR and that pretreatment by subcutaneous injection with the same peptide modulated these responses.

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Correspondence and requests for reprints should be addressed to Dr. Erwin W. Gelfand, Department of Pediatrics, National Jewish Center for Immunology and Respiratory Medicine, 1400 Jackson Street, Denver, CO 80206.

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MATERIALS AND METHODS

Animals

Female BALB/c mice from 8 to 12 wk of age were obtained from Jackson Laboratories (Bar Harbor, ME). All experimental animals used in this study were under a protocol approved by the Institutional Animal Care and Use Committee of the National Jewish Center for Immunology and Respiratory Medicine.

Antigens

Recombinant Fel d I chain 1 (kindly provided by Dr. G. Sunshine, ImmuLogic Pharmaceutical Corp., Waltham, MA) was used for pretreatment by subcutaneous or intraperitoneal injection and for sensitization by nebulization. The peptide was expressed and purified as described previously (9, 17) and contained the complete 70-amino acid Fel d I chain 1 sequence (18).

Injections

For pretreatment, mice (3 to 4 mice/group/experiment) were injected intraperitoneally or subcutaneously at a dorsal site, with 150 μ g of peptide in 150 μ l of phosphate-buffered saline (PBS), 10 d and 5 d before the initial nebulization of peptide for sensitization. We adopted this pretreatment protocol based on our preliminary experiments determining concentrations and intervals for subcutaneous peptide administration and because of a previous report in which the same pretreatment schedule of subcutaneous injections of Fel d I was demonstrated to effectively tolerate T cell-dependent immune responses to the peptide following intraperitoneal injection of the peptide in complete Freund's adjuvant (9). Control groups were pretreated according to the same schedule with 150 μ l of either PBS alone or bovine serum albumin (BSA) at the same concentrations.

Sensitization

Mice (3 to 4 mice/group/experiment) were sensitized to aerosolized antigen as described (14). Briefly, groups of mice were exposed to 100 μ g of Fel d I chain 1 diluted in 7 ml PBS by ultrasonic nebulization once a week for 3 wk. Preliminary experiments with BALB/c mice showed that, for Fel d I peptide, weekly nebulization for a 3-wk period was a more effective schedule for the selective induction of allergen-specific IgE than daily exposure to antigen over a 10-d period as used with other allergens (14, 15). Age-matched control mice were exposed to PBS alone, following the same protocol. One to two d after the last exposure, serum was obtained from the animals and skin testing and AR were performed and evaluated by an investigator who was blinded to the sensitization and treatment status of the animals.

Analysis of Immediate Cutaneous Hypersensitivity

Development of immediate cutaneous hypersensitivity to Fel d I was assessed by intradermal skin testing as described previously (16). The abdomen of the mouse was shaved, and 20 μ l of test solution were injected intradermally with a 30-gauge needle. Test solutions were Fel d I diluted in PBS at a concentration of 100 μ g/ml, PBS as the negative control, and 100 μ g/ml of BSA in some experiments. Subsequent wheal reactions were assessed after 15 min and were scored as positive if the wheal diameter was greater than 3 mm in any direction. The actual size of the wheal response was measured.

Determination of AR

Airway smooth muscle responsiveness was determined *in vitro* as described (19). Briefly, after killing the animals, mouse tracheal rings of about 0.5 cm in length were sectioned and placed in Krebs-Henseleit baths suspended by triangular supports transducing the force of contractions. Electrical field stimulation was delivered by a Grass S44 stimulator (Grass SIV 5) using 8 V, 2 ms pulse duration and 0.5 to 40 Hz frequencies. Each stimulation was maintained until peak contractile responses were obtained. The 40 Hz frequency was established earlier to result in maximal contractions. ES_{50} , the frequency that caused 50% of maximal contractions, was calculated from linear plots.

Cell Preparation and Culture Conditions

Two days after completion of the peptide nebulization protocol, peribronchial lymph nodes (PBLN) and spleens were obtained. Mononuclear

cells (MNC) were purified by dispersing small tissue pieces through stainless steel mesh followed by density gradient centrifugation (Lymphocyte Separation Medium; Organon Teknica, Durham, NC). The cells were washed three times with PBS and resuspended in RPMI 1640 medium (GIBCO, Grand Island, NY) supplemented with 10% heat-inactivated fetal calf serum (HyClone Laboratories, Logan, UT), 5 mM L-glutamine, 0.05 mM 2-mercaptoethanol, 100 U/ml penicillin, and 100 μ g/ml streptomycin (all from GIBCO). All cultures were incubated at 37° C in a humidified atmosphere containing 5% CO₂.

Cell Proliferation Assay

To assay the proliferative response of lymphocytes to Fel d I, MNCs were cultured in the presence or absence of the peptide. Cells (2×10^5 per well) were incubated in triplicate, in 96-well tissue-culture plates for 3 d. During the last 6 h of culture, cells were pulsed with 1 μ Ci of [³H]thymidine (ICN Biomedicals Inc., Costa Mesa, CA) and the plates were harvested with a Cambridge cell harvester (Cambridge Technology Inc., Watertown, MA). [³H]thymidine incorporation was measured in a scintillation counter.

Lymphocyte Transfer Protocol

For cell transfer experiments, spleen cells were prepared from mice injected subcutaneously with Fel d I or PBS, 10 and 5 d earlier. Cells (1×10^7) were resuspended in 200 μ l of PBS and injected into the tail vein of age- and sex-matched syngeneic recipients on the same day. Following cell transfer, recipients were exposed to the same sensitization protocol as previously described over 3 wk. Immunologic and physiologic parameters were analyzed 1 to 2 d after the last exposure to antigen.

Measurement of Serum Ig

Serum IgE, IgG1, and IgG anti-Fel d I chain 1 antibody levels were assayed by ELISA. Flat-bottom microtiter plates (Immulon II; Dynatech Laboratories, Chantilly, VA) were coated by incubation of 5 μ g/ml of Fel d I chain 1 in NaHCO₃ buffer, pH 8.2, overnight at 4° C. The wells were washed with PBS and blocked with 0.2% gelatin in PBS. Serum samples were diluted 1:10 in gelatin buffer and incubated in duplicate on the plates overnight at 4° C. After washing, alkaline phosphatase-conjugated rat anti-mouse IgE or IgG1 (PharMingen, San Diego, CA), or sheep anti-mouse IgG (The Binding Site Inc., San Diego, CA) were added and incubated for 2 h at 37° C. After development with phosphatase substrate (Sigma 104; Sigma Chemical Co., St. Louis, MO), plates were read in a microplate autoreader (Bio-Rad Laboratories, Pleasanton, CA). The antibody titers of the samples were measured in optical density units at 410 nm, with background values for the binding of unimmunized mouse serum being subtracted and the values were related to pooled standards that were generated in the laboratory and deliberately assigned to be 100 ELISA units (EU)/ml. Anti-Fel d I chain 1 serum standards were obtained by intraperitoneal sensitization of mice with 50 μ g of Fel d I chain 1 in 0.1 ml of alum (Imject Alum; Pierce, Rockford, IL). The lower limits of detection for the specific antibodies were 1 EU/ml as a function of the standard curve for IgE and IgG isotypes and the IgG1 subclass. Total serum IgE and IgG levels were determined using the same method in plates coated with sheep anti-mouse IgE or IgG antibodies (The Binding Site Inc.), and levels were calculated by comparison with known mouse IgE and IgG standards (PharMingen). The limit of detection for total Ig was 100 pg/ml of IgE and 1 ng/ml of IgG.

Statistical Analysis

Student's two-tailed unpaired *t* test was used to determine the level of difference between experimental groups and control conditions.

RESULTS

Sensitized Mice Develop Antigen-induced Immediate Cutaneous Reactivity and Pretreatment with Injected Fel d I Chain 1 Reduces Skin Reactivity

As described previously (15, 20), sensitization of BALB/c mice to aerosolized antigens such as ragweed and ovalbumin via the airways led to the induction of immediate cutaneous hypersensitivity associated with increased AR. We determined whether mice nebulized with Fel d I chain 1 would also develop immediate skin

TABLE 1
Fel d I CHAIN 1-SPECIFIC IMMEDIATE
CUTANEOUS HYPERSENSITIVITY*

Pretreatment	Nebulization	Wheal Diameter† (mm)	Positive Responders (%)
—	PBS	0.0 ± 0.0	0/12 0
—	FC1	11.5 ± 1.7	12/12 100
SC PBS × 2	FC1	10.0 ± 2.5	16/16 100
SC BSA × 2	FC1	10.3 ± 1.3	7/7 100
SC FC1 × 2	FC1	4.9 ± 2.0‡	11/13 85
SC FC1 × 1	FC1	9.8 ± 4.1	7/7 100
IP FC1 × 2	FC1	5.3 ± 1.5‡	8/8 100

* Cutaneous reactivity to Fel d I chain 1 (FC1) was determined in the same mice described in Figure 1: nonsensitized (PBS-exposed) control mice, FC1-nebulized groups: nonpretreated mice, mice after two subcutaneous injections of PBS, mice after two subcutaneous injections of BSA, mice after two subcutaneous injections of FC1, mice after a single subcutaneous injection of FC1, or mice after two intraperitoneal injections of FC1. Skin tests were performed 1 d after completion of the nebulization protocol. The size of wheal responses was assessed 15 min after intradermal injection of FC1 solution. A positive skin test response was defined by active development of a wheal ≥ 3 mm in diameter.

† Results are expressed as mean ± SD.

‡ p < 0.01 for wheal diameter compared with sensitized, nonpretreated mice.

test reactivity and whether preinjection with the peptide could alter skin reactivity. Although no wheal reactions were observed in nonsensitized mice (nebulized with PBS alone), mice sensitized to Fel d I chain 1 developed positive skin test reactions to intradermal injections of peptide (Table 1). These mice showed no response to intradermal injections of PBS or the irrelevant antigen BSA. Pretreatment with subcutaneous injections of PBS or BSA on Days -10 and -5 did not affect cutaneous reactivity. In contrast, injection of Fel d I chain 1 subcutaneously or intraperitoneally on Days -10 and -5 resulted in a 50% reduc-

tion in the size of the wheal response compared with PBS- or BSA-pretreated mice; using 3 mm as the indicator of a positive response, there was little change in the overall frequency of positive responders. Those mice that received only a single injection of Fel d I chain 1 administered subcutaneously on Day -10 or -5 were not different from controls. Pretreatment alone never elicited positive skin test responses at any time point unless followed by sensitization (data not shown).

Subcutaneous Injections of Fel d I Chain 1 Prevents Altered AR in Sensitized Mice

We monitored ES_{50} values to compare the effects of pretreatment and sensitization on AR among the different groups. A decrease in the ES_{50} represents an increase in AR (19). Control mice exposed to aerosolized PBS had a mean ($\pm$ SEM) ES_{50} of 4.1 ± 0.2 Hz. Sensitization to nebulized Fel d I chain 1 caused a significant decrease in the ES_{50} values to 2.9 ± 0.3 Hz ($p < 0.02$) (Figure 1). These results were identical in mice that received pretreatment with PBS or that were pretreated with BSA. Pretreatment with two subcutaneous injections of Fel d I chain 1 prior to sensitization resulted in normal ES_{50} values with a mean of 3.8 ± 0.3 Hz (significantly different, $p < 0.03$, compared with sensitized mice pretreated with PBS or BSA). The ES_{50} values in these pretreated mice were not statistically different from the values in the nonsensitized control mice. A single dose of subcutaneous Fel d I chain 1 was ineffective. Surprisingly, two intraperitoneal injections of Fel d I chain 1 failed to significantly alter AR in sensitized mice ($p > 0.05$), even though cutaneous reactivity was reduced after the pretreatment.

Sensitization to Fel d I Chain 1 Induces an Antigen-specific Proliferative Response and Pretreatment with the Peptide Prior to Sensitization Prevents the Response to Subsequent Sensitization

To analyze the responsiveness of lymphocytes to Fel d I chain 1 following sensitization, cell proliferation assays were performed in cultures containing the peptide (Figure 2). PBLN and spleen cells from Fel d I chain 1-sensitized mice showed proliferative responses to Fel d I chain 1 in a dose-dependent manner. The antigen-induced proliferation of PBLN and spleen cells was significantly decreased ($p < 0.05$) in response to the peptide in mice pretreated with two subcutaneous or intraperitoneal injections of the peptide compared with the control mice (Figure 2A, B). A single subcutaneous injection of the peptide or two subcutaneous injections of BSA did not have any effect on the proliferative responses (Figure 2C, D). PBLN or spleen cells from each group showed comparable responses to mitogenic stimulation with PHA.

Pretreatment with Fel d I Chain 1 Induces a Reciprocal Change in the *In Vivo* IgE and IgG Antigen-specific Antibody Responses

We have shown that in BALB/c mice exposed to aerosolized antigens, the development of immediate cutaneous hypersensitivity and airway hyperresponsiveness was associated with antigen-specific IgE production (14, 16, 19). To determine whether the changes demonstrated above for cutaneous reactivity and AR were associated with changes in antigen-specific antibody concentrations, we examined the effect of these treatments on *in vivo* antibody responses. Anti-Fel d I IgE, IgG1, and IgG antibody concentrations were detected in the serum of sensitized mice who were not pretreated: the relative concentrations ($\pm$ SD) were IgE, 11.0 ± 2.6 ; IgG1, 7.7 ± 2.5 ; and IgG, 78.9 ± 13.1 EU/ml, respectively. These values were taken as 100% and the percentage of control antibody titers for each pretreatment group was calculated (Figure 3). Two days of either subcutaneous or intraperitoneal injection with Fel d I chain 1 prevented the increases in

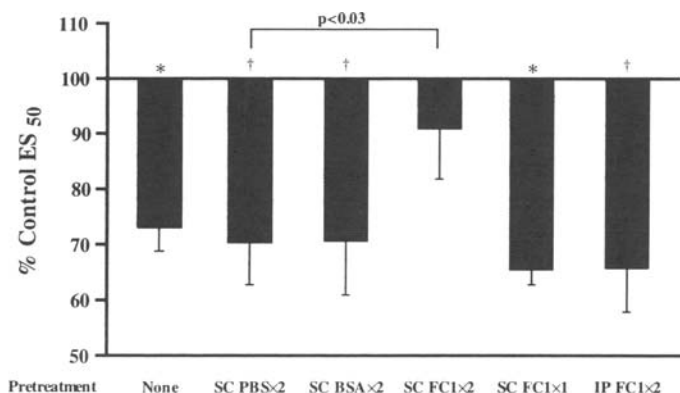


Figure 1. Airway hyperresponsiveness in sensitized animals can be prevented by subcutaneous injection of peptide. AR was analyzed 2 d after completion of sensitization to nebulized antigen by electrical field stimulation of tracheal smooth muscle preparations. AR was expressed as a percentage of control ES_{50} . The mean ($\pm$ SEM) ES_{50} for nonsensitized (PBS-exposed) control animals ($n = 22$) was 4.1 ± 0.2 Hz and this value was taken as 100%. AR was analyzed in several groups of mice sensitized to Fel d I chain 1 (FC1) via the airways: mice receiving no pretreatment (none, $n = 12$), mice receiving 2 subcutaneous injections of PBS (Days -10 and -5) (SC PBS × 2, $n = 16$), mice receiving 2 subcutaneous injections of BSA (SC BSA × 2, $n = 7$), mice receiving 2 subcutaneous injections of FC1 (SC FC1 × 2, $n = 13$), mice receiving a single subcutaneous injection of FC1 (Day -5) (SC FC1 × 1, $n = 7$), or mice receiving 2 intraperitoneal injections of FC1 (IP FC1 × 2, $n = 8$). Presented are the means $\pm$ SD for each group from independent experiments using 3 to 4 mice in each group. There were significant differences (* $p < 0.02$ and † $p < 0.05$) compared with nonsensitized control mice.

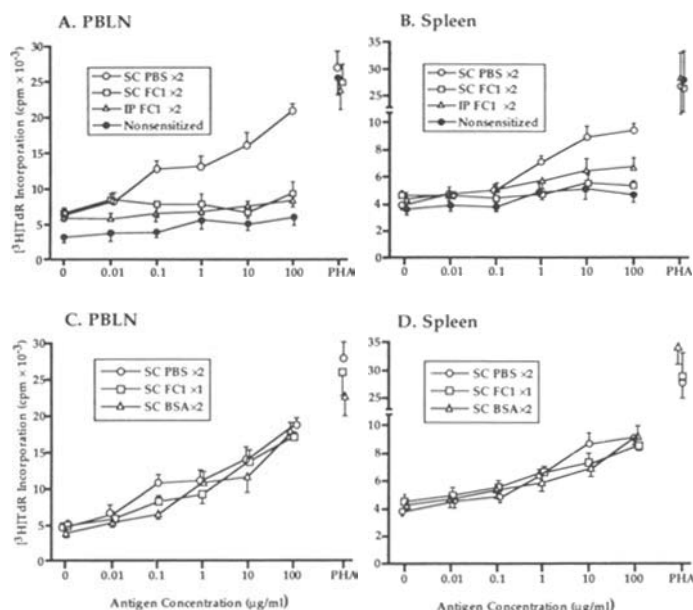


Figure 2. Peptide injection suppresses the antigen-specific lymphoproliferative response to subsequent peptide sensitization. Pooled PBLN and spleens were obtained from the different treatment groups as described in the legend to Figure 1. MNC from the PBLN (A and C) and spleens (B and D) were prepared and incubated with the indicated concentrations of Fel d I chain 1 or $1 \mu\text{g/ml}$ of PHA. The proliferative response was measured by addition of $[^3\text{H}]\text{-thymidine}$ (TdR) for the last 6 h of 3-d cultures and incorporation assayed. The results are expressed as means (cpm) $\pm$ the SD of triplicate determinations.

the concentrations of Fel d I-specific IgE and IgG1 antibodies (Figure 3A, B). In contrast, specific IgG titers in these two groups were significantly increased (Figure 3C). Pretreatment alone did not elicit any specific IgE (IgG1) or IgG responses. A single pretreatment with Fel d I chain 1 or BSA had no significant effect on the antibody responses. Further, the effects of the treatments on Ig production were confined to antigen-specific antibody responses, since no significant changes were observed in total IgE and IgG concentrations among the different treatment groups (Figure 4).

Transfer of Cells from Peptide-treated Mice into Sensitized Recipients

In view of the response to subcutaneous injection of the peptide on these immediate hypersensitivity responses, we examined the capacity of cells from animals primed by subcutaneous peptide injection to prevent these responses in recipient mice exposed to aerosolized peptide. Spleen MNCs from mice pretreated with two subcutaneous injections of PBS or Fel d I chain 1 were used in transfer experiments. In mice receiving cells from PBS-pretreated mice, subsequent nebulization with Fel d I chain 1 resulted in immediate cutaneous responses, increased AR, and increased concentrations of anti-Fel d I IgE and IgG1 antibodies (Figures 5 and 6; Table 2) to the same degree as observed in primary, sensitized control mice (Figures 1 and 3; Table 1). In recipients of cells from mice pretreated subcutaneously with peptide on Days -10 and -5 , a reduction in the size of the wheal responses (Table 2) and no alteration of AR (Figure 5) were observed following sensitization. The degree of inhibition of the responses was comparable to that observed in primary, subcutaneously peptide-treated mice. The transfer of cells from subcutaneously peptide-treated mice also prevented the increases in serum anti-Fel d I

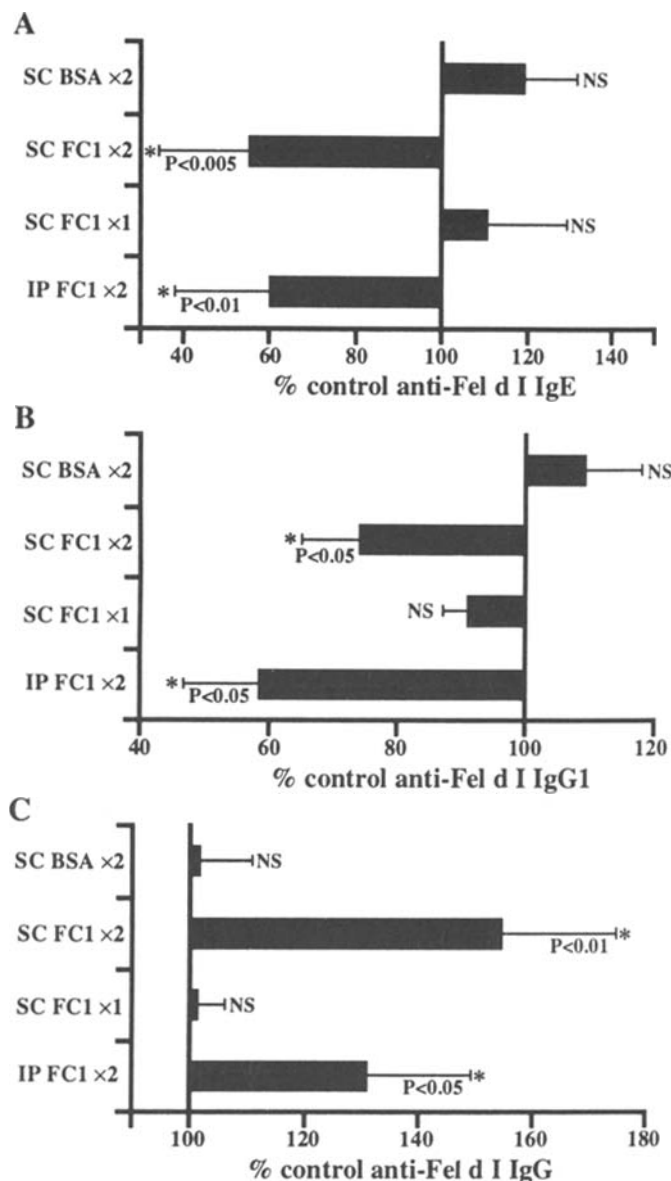


Figure 3. Peptide injection decreases specific IgE and IgG1 antibody production and increases specific IgG in the serum. Serum was obtained from the different treatment groups described in Figure 1, 1 d after completion of nebulization. Antibody concentrations were determined by ELISA and expressed as a percentage of control. The average anti-Fel d antibody titers (ELISA Units/ml) ($\pm$ SD) for sensitized control animals (pretreated with PBS) were IgE, 11.0 ± 2.6 ; IgG1, 7.7 ± 2.5 ; and IgG, 78.9 ± 13.1 EU/ml, respectively, and these values were taken as 100%. Shown are the mean $\pm$ SD for anti-Fel d I IgE (A), anti-Fel d I IgG1 (B), and anti-Fel d I IgG (C) from three separate experiments in each group ($n = 12$ mice). *Significant differences to sensitized control are shown. NS = not significant.

IgE and IgG1 antibody concentrations and the decrease in specific IgG antibodies (Figure 6). No significant changes were observed in total IgE and IgG concentrations between the groups (data not shown).

DISCUSSION

One of the purposes of this study was to develop a model of allergic (airways) sensitization to immunogenic peptides that could be used to elucidate the pathogenesis of allergic responses.

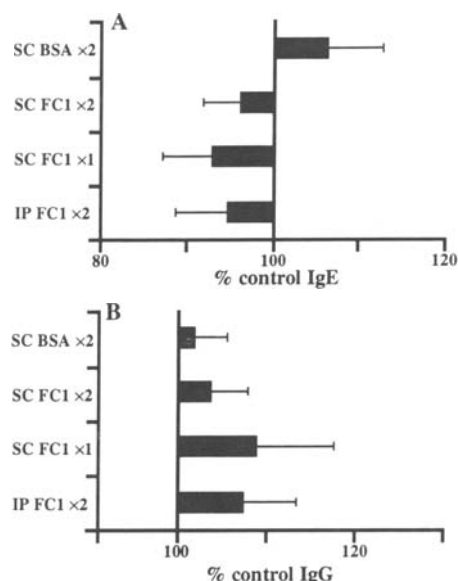


Figure 4. Effect of pretreatment on total IgE and IgG serum concentrations. Total IgE and IgG in the same serum samples described in Figure 3 were determined by ELISA. The average total IgE and IgG serum levels ($\pm$ SD) for sensitized control mice (pretreated with PBS) were 2.5 ± 0.7 ng/ml and 315 ± 45 μ g/ml, respectively, and these values were taken as 100%. Presented are the means $\pm$ SD for total IgE (A) and IgG (B). None of the differences were statistically significant.

In this approach, we were able to monitor altered airway responsiveness and to analyze the induction of tolerance or prevention of allergic sensitization by determining the effect of subcutaneous injections of the peptide on immediate hypersensitivity and AR. BALB/c mice are known to be a high IgE-responding strain (21). Previous studies demonstrated that in BALB/c mice, repeated exposure via the airways to antigenic proteins such as rag-

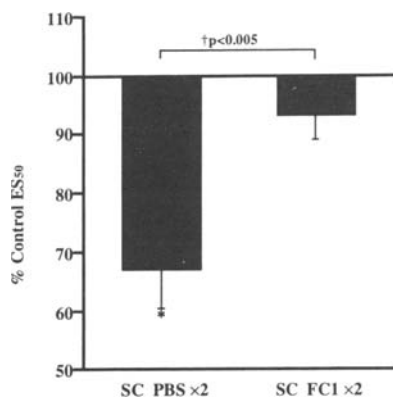


Figure 5. Spleen cells from mice pretreated with subcutaneous peptide transfer the ability to prevent altered AR. Splenic MNC from mice receiving PBS subcutaneously (SC PBS $\times$ 2) or Fel d I chain 1 subcutaneously (SC FC1 $\times$ 2), 10 and 5 d earlier were injected intravenously to recipients, and AR was determined 2 d after completion of the sensitization protocol. The mean ES₅₀ for the same nonsensitized control mice described in Figure 1 was taken as 100% and the percentage of control ES₅₀ for each recipient group was calculated. Shown are the mean $\pm$ SD from two separate experiments with eight mice in each group. *p < 0.01 compared with nonsensitized control mice. †There is a significant (p < 0.005) difference between the groups.

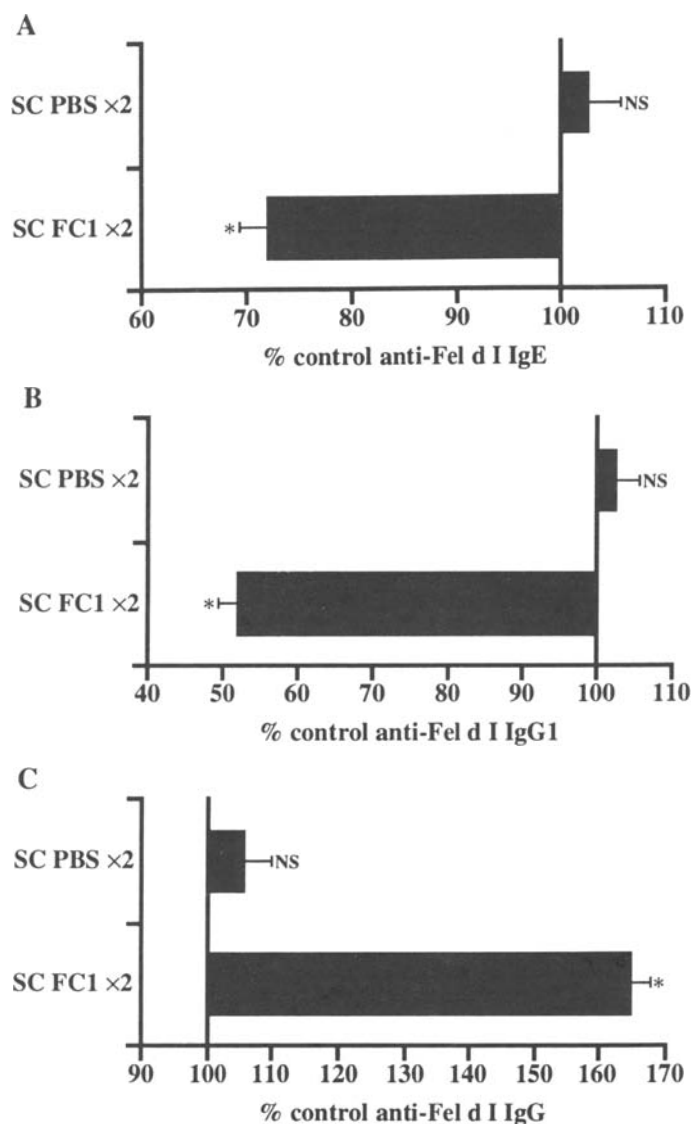


Figure 6. Transfer of cells from pretreated animals inhibits specific IgE and IgG1 antibody production. Serum was obtained from the same recipients as described in the legend to Figure 5, 1 d after completion of sensitization protocol and following cell transfer. The average anti-Fel d I antibody titers (ELISA Units/ml) for the sensitized control mice described in Figure 3 were taken as 100% and the percentages of control antibody concentrations were determined. Shown are the mean $\pm$ SD for anti-Fel d I IgE (A), anti-Fel d I IgG1 (B), and anti-Fel d I IgG (C). *p < 0.05 compared with sensitized control mice.

weed extract or ovalbumin induced a systemic, antigen-specific IgE response (14, 15). The IgE response was associated with the acquisition of immediate cutaneous hypersensitivity (16) and the development of increased AR (19). In this study, we sensitized mice by airway exposure to another immunogenic peptide which is derived from the major cat allergen, Fel d I. This peptide was used to sensitize mice as well as to pretreat them. Fel d I contains two peptide chains, which have been shown to be the major human T-cell epitopes in the protein (18). Intraperitoneal injection of Fel d I chain 1 in complete Freund's adjuvant was shown to induce T cell-dependent immune responses and subcutaneous administration of the peptide tolerated these responses (9). We first established that exposure to this peptide via the airways was capable of inducing immediate cutaneous reactivity and altered

TABLE 2
EFFECT OF CELL TRANSFER FROM SUBCUTANEOUSLY
PRETREATED DONORS ON IMMEDIATE
CUTANEOUS HYPERSENSITIVITY*

Pretreatment of Donor	Nebulization	Wheal Diameter† (mm)	Positive Responders (no.)
SC PBS ×2	FC1	13.5 ± 1.7	8/8
SC FC1 ×2	FC1	6.0 ± 0.8‡	8/8

* Cutaneous reactivity to Fel d I chain 1 (FC1) was determined in the same recipients as described in Figure 5, 1 d after completion of the nebulization protocol following cell transfer. The size of wheal responses was assessed 15 min after intradermal injection of FC1. A positive skin test response was defined by the development of a wheal ≥ 3 mm in diameter.

† Results are expressed as mean ± SD.

‡ $p < 0.05$ for wheal diameter compared with sensitized, nonpretreated mice.

AR. In association with these changes, sensitization to the peptide triggered production of antigen-specific IgE and IgG1 antibody responses and cell proliferative responses, similar to what we observed following sensitization to ovalbumin (20). These findings indicate that Fel d I chain 1 peptide contains a T- and B-cell epitope which is important in the development of immediate hypersensitivity and airway hyperresponsiveness in BALB/c mice.

Pretreatment of the mice by subcutaneous (or intraperitoneal) injection of the same peptide used to sensitize the animals had significant effects on the subsequent development of antigen-specific IgE and IgG1 antibody responses and immediate cutaneous reactivity. Further, this is the first demonstration that subcutaneous injections of the immunogenic peptide not only reduce IgE-mediated hypersensitivity responses but can also prevent airway hyperresponsiveness in mice sensitized by airway exposure to the peptide. Thus, subcutaneous or intraperitoneal administration of immunogenic peptide identifies immunoregulatory effects at several different levels: suppression of antigen-specific IgE production associated with reciprocally increased concentrations of specific IgG antibodies; tolerization of cell proliferative responses; and prevention of immediate cutaneous hypersensitivity and airway hyperresponsiveness.

It is well documented that IgE production is under the regulatory control of distinct subsets of T cells. In mice, IgE production by B cells is stimulated by activated helper T cells, which are classified as Th2 and capable of secreting IL-4; they are essential for the induction of IgE isotype class switch (22–25). The results of this study demonstrate that peptide administered subcutaneously or intraperitoneally prevented specific IgE production in response to sensitization with the same antigenic peptide. Suppression of antigen-specific antibody production has been shown in previous investigations using proteins conjugated to synthetic chemicals. Tolerance induced in this way was also selective for IgE, but the mechanism of tolerance induction was not defined (11, 26). Secondly, we show that subcutaneous or intraperitoneal administration of the peptide resulted in significantly diminished proliferative responses of PBLN and spleen cells to the peptide. One of the possible explanations for this diminished response was that subcutaneously administered peptide induces clonal anergy of a specific T-cell population. In previous reports, *in vivo* T-cell tolerance to antigen challenge has been shown using soluble antigen, modified antigen, or antigenic epitopes (9, 27, 28). In *in vitro* models of tolerization, Th2-type T-cell clones can be tolerized as well as Th1 cells (29, 30).

In order to begin to define the mechanisms underlying suppression of the allergic reactions by peptide pretreatment, we examined the ability of cell components transferred from mice primed by subcutaneous peptide injections to prevent the development of immediate hypersensitivity and airway hyperrespon-

siveness in sensitized recipient mice. Spleen cells from mice primed with subcutaneously administered peptide could transfer the ability to suppress antigen-specific IgE and IgG1 antibody responses, to reduce cutaneous reactivity, and to prevent altered AR. These data suggest that subcutaneous peptide administration results in the activation of specific cell populations which negatively regulate IgE production and AR rather than the induction of functional anergy or deletion of T cells specific to the peptide. We are currently characterizing the subset(s) of splenic T cells capable of transferring suppression. Cytokine profiles of cells from mice sensitized with or without peptide pretreatment and cells from mice primed by subcutaneous injection alone remain to be investigated because there is a possibility that peptide pretreatment might exert an effect on antigen-specific T-cell populations and the lymphokines they produce. For example, interferon- γ , the major cytokine associated with inhibition of IgE production, could be one of the candidates involved in the negative regulatory circuit (31).

The data from these investigations of *in vivo* responses provide additional insight into the relationship between IgE responses and the induction of allergic reactions. The reduction of antigen-specific immediate cutaneous reactivity in the peptide-pretreated groups was paralleled by decreases in serum-specific IgE and IgG1 antibodies. In addition to IgE, murine IgG1 antibodies are capable of sensitizing mast cells (32, 33). However, it is difficult to simply conclude that the reduction in concentrations of IgE or IgG1 was responsible for the reduced skin test reactions. One possibility is that the increase in antigen-specific IgG could have blocked IgE and IgG1-mediated reactions. To further clarify this issue, it will be necessary to carry out experiments in which antigen-specific IgE is passively transferred to animals in combination with peptide pretreatment. The induction of IgE responses has also been associated with the development of increased AR (14, 31, 34). It is generally understood that the presence of IgE antibodies is a necessary trigger of immediate responses but alone is not sufficient for the development of increased AR. Several lines of evidence indicate that reactive T cells may have a more direct influence on AR by releasing inflammatory factors that interfere with physiologic regulatory systems in the control of airway function (35–37). The present study highlights a discordance in the effect of peptide pretreatment on IgE responses and AR. Although subcutaneous administration of peptide resulted in almost complete normalization of AR, the reduction of specific IgE was not complete when compared with the absence of specific IgE antibodies in nonsensitized animals. Moreover, although Fel d I chain 1 injected intraperitoneally could reduce antigen-specific IgE responses and induce antigen-specific IgG, pretreatment by the intraperitoneal route failed to modulate AR. These findings indicate that alteration in airway status may not directly parallel changes in serum concentrations of IgE antibodies. Both subcutaneous and intraperitoneal administration of peptide reduced serum-specific IgE levels to roughly the same extent but were nevertheless incomplete. A certain concentration of specific IgE may be sufficient to trigger the allergic response in the airways, and in the case of subcutaneous peptide pretreatment, different components of the response other than IgE could have been affected, resulting in the prevention of altered AR. One possible effect of subcutaneous peptide pretreatment is that only the subcutaneous route could modulate effector cell functions that play a role in the development of increased AR in association with IgE-mediated immediate reactions.

This study identifies several important variables which need to be considered for successful intervention or prevention of allergic sensitization. The route of peptide or protein administration as shown in this study may have implication for the responses that can be modified. This is similar to our findings when inter-

feron- γ was used to modify similar responses. Whereas intraperitoneally administered interferon- γ was effective in reducing total serum IgE, only nebulized interferon- γ was effective in modulating antigen-specific responses following sensitization via the airways (31). A critical component distinguishing routes of sensitization and desensitization may reside in the differences in the capacity of antigen-presenting cells (APC) at different sites to process antigens and recruit specific T cells with different functions.

This highlights a second variable, namely the nature of the protein or peptide used in sensitization and pretreatment. In this study, the same peptide was used for both phases. The peptide Fel d I chain 1 is only one of the T-cell epitopes recognized in native Fel d I protein. Further studies on the characterization and bioactivity of the different peptides are necessary. The structural features of these peptides are likely to differ in terms of their ability to sensitize or tolerize a particular response, in large part related to their interactions with APC and ultimately specific T-cell subsets.

A third variable is related to the schedule and amounts administered for pretreatment. We demonstrated that at least two injections of 150 μ g of Fel d I chain 1 were required to affect *in vivo* and *in vitro* responses. The concentration of peptide injected could be important for differentiation of Ig isotypes, as it is documented that large doses or repeated exposure to antigen leads to IgG responses and inhibition of IgE production (38). The timing of the injections at Days -10 and -5 was based on previous studies examining the tolerization of T-cell responses to the peptide (9) and preliminary studies evaluating the effects of this schedule on inhibiting the development of immediate hypersensitivity responses. Ongoing studies are attempting to define the best regimens for preventing the development of IgE/IgG1 antibody production and preventing airway hyperresponsiveness, recognizing that they may not coincide.

A number of approaches to use peptides for induction of tolerance or immunologic unresponsiveness to allergen are currently under study in many laboratories. This mouse model of allergic sensitization via the airways and the ability of peptide pretreatment to interfere not only with immediate hypersensitivity responses but also the response of airways altered by sensitization, provides an important advance toward guiding the development of such new approaches to modulate ongoing allergic responses.

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