

Hierarchical suppression of asthma-like responses by mucosal tolerance

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Background: Mucosal tolerance can be induced by oral or nasal administration of soluble proteins and results in the suppression of cellular and/or humoral immune responses to the specific antigen.

Objective: To compare the effect of oral or nasal ovalbumin administration before, during or after immunization on the development of cellular and humoral immune responses by using a murine asthma model.

Methods: To induce lung allergic inflammation, animals were immunized twice with ovalbumin/aluminum hydroxide gel and challenged twice with ovalbumin. To induce tolerance, BALB/c mice received ovalbumin by the oral or nasal routes for 3 consecutive days. The ovalbumin administration was initiated before (day -7), during (day 0), or after immunization (day 7). **Results:** Airway eosinophilia, airway hyperreactivity, mucus hypersecretion, and cytokine production were suppressed when oral or nasal ovalbumin administration was initiated before immunization. Oral but not nasal ovalbumin exposure suppressed ovalbumin-specific nonanaphylactic IgG₁ antibodies, whereas both routes suppressed the production of anaphylactic IgG₁ and IgE antibodies. Mucosal ovalbumin administration at day 0 inhibited all T_H2-mediated allergic parameters but not nonanaphylactic IgG₁ antibodies. Finally, ovalbumin exposure 7 days after immunization was still effective in suppressing lung allergy but not ovalbumin-specific anaphylactic IgG₁ and IgE antibodies. **Conclusion:** We show that the effectiveness of mucosal tolerance depends on route and time and presents a hierarchical pattern of suppression in the following order: lung allergic responses > anaphylactic antibodies > ovalbumin-specific IgG₁. (*J Allergy Clin Immunol* 2006;117:283-90.)

Key words: Tolerance, asthma, anaphylactic antibodies, AHR

Allergic sensitizations are commonly induced by environmental antigens such as house dust mites, grass pollen, and animal proteins, leading to diseases that include asthma, rhinitis, and atopic dermatitis. These disorders affect 10% to 15% of Western populations, and their prevalence has doubled in the last decade.¹

Allergic asthma is a chronic respiratory disease characterized by allergen-induced early and late bronchial obstructive reactions, which are associated respectively with anaphylactic antibodies that induce activation of mast cells on allergen interaction; and with T_H2 cells, eosinophils, and neutrophils that release various inflammatory mediators. These inflammatory mediators induce mucus hypersecretion and the development of airway hyperresponsiveness (AHR) to a variety of stimuli, including allergens, and pharmacological agents such as histamine and methacholine.^{2,3}

Usually, mucosal exposure to nonpathogenic antigens results in a state of hyporesponsiveness, known as *mucosal tolerance*, which is revealed on immunogenic contact with the same antigen.⁴ We have shown that oral ovalbumin exposure prevented the development of asthma-like responses, such as airway eosinophilia, bronchial hyperreactivity, and IgE production, in different mouse strains, even in T/B monoclonal mice that produce high levels of IgE and lack naturally occurring regulatory T cells.⁵⁻⁷ Several studies presented evidence that ovalbumin delivered by the nasal route prevents the production of allergen-specific IgE antibodies.^{8,9} Other authors have demonstrated that aerosolized ovalbumin can suppress key features of experimental allergic asthma.¹⁰ However, no systematic study comparing the effect of oral versus nasal tolerance on the development of asthma-like responses has been reported. Also, there is no information on whether nasal tolerance could be induced after immunization. Thus, we compared the effectiveness of oral or nasal ovalbumin exposure before, during, or after immunization in inhibiting key phenotypes of experimental asthma, such as airway allergic inflammation, AHR, and the production of ovalbumin-specific IgE or IgG₁ anaphylactic antibodies.¹¹

Herein, we show that mucosal tolerance can present a hierarchical pattern of inhibition of immune responses that is dependent on the route and timing of ovalbumin exposure. Hierarchy in tolerance induction was evidenced by the facts that (1) cellular lung allergic responses are more susceptible to suppression than the ovalbumin-specific IgG₁ and IgE anaphylactic antibody production,

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Abbreviations used

AHR: Airway hyperreactivity
BAL: Bronchoalveolar lavage
DC: Dendritic cell
PAS: Periodic acid-Schiff
PCA: Passive cutaneous anaphylaxis
Penh: Enhanced pause

and (2) only oral tolerance could prevent the production of ovalbumin-specific nonanaphylactic IgG₁ antibodies.

METHODS

Mice

BALB/c mice bred and housed under specific pathogen-free conditions were obtained from our own Animal Breeding Unit (Biotério de Camundongos Isogênicos ICB-USP, São Paulo, Brazil). Male mice 4 to 8 weeks old were used in all experiments, with 5 mice per group. Mice were treated according to Animal Welfare guidelines of the Biomedical Science Institute (USP, Brazil).

Immunization and induction of allergic airway response

Animals were immunized with 4 µg ovalbumin (grade V; Sigma-Aldrich, St Louis, Mo) adsorbed to 1.6 mg aluminum hydroxide gel in 0.4 mL PBS injected subcutaneously on days 0 and 7. On days 14 and 21, the animals were challenged intranasally with 10 µg ovalbumin in 50 µL PBS as previously described.¹² The control group consisted of untreated mice. All determinations were performed 24 hours after the last ovalbumin challenge.

Mucosal tolerance

For induction of oral tolerance, BALB/c mice received 1% ovalbumin solution dissolved in the drinking water on 5 consecutive days. The average consumption of ovalbumin solution is 3 to 5 mL/day/mouse. Thus, each animal ingested approximately 30 to 50 mg ovalbumin per day. For induction of nasal tolerance, animals were slightly anesthetized, and 100 µg ovalbumin/50 µL sterile PBS was dripped in the nostrils with a micropipette on 3 consecutive days. Oral or nasal ovalbumin administration was initiated 7 days before immunization or at day 0 or day 7 of immunization (see protocol, Fig 1).

Determination of AHR

Airway responsiveness to increasing doses of methacholine was assessed 24 hours after the last ovalbumin challenge in conscious mice by using a single-chamber, whole-body plethysmograph (Buxco Electronics Inc, Wilmington, NC), as previously described.¹² The enhanced pause (Penh), a dimensionless value, was used to determine airway responsiveness. To establish baseline Penh values, unrestrained mice were exposed for 2.5 minutes to nebulized PBS (Aeroneb Lab micropump nebulizer; Aerogen, Mountain View, CA) and were subsequently exposed to increasing concentrations (3, 6, 12, and 25 mg/mL) of nebulized methacholine (Sigma-Aldrich) in PBS. After each nebulization, recordings were taken for 5 minutes. The Penh values measured during each 5-minute sequence were averaged and expressed for each methacholine concentration.

Bronchoalveolar lavage fluid

Mice were deeply anesthetized by intraperitoneal injection of an anesthetic solution containing ketamine (Ketamina Agener; União

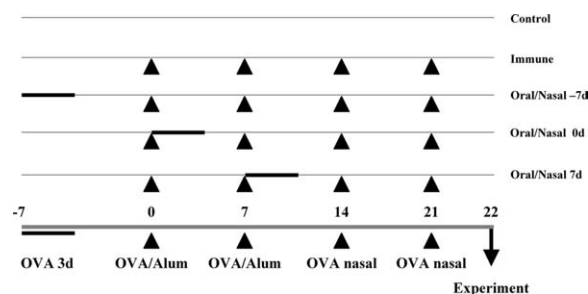


FIG 1. Experimental protocols. BALB/c mice received 1% ovalbumin (OVA) in drinking water or 100 µg ovalbumin intranasally on 3 consecutive days before immunization (day -7) or after immunization (day 0 or day 7). Animals were ovalbumin immunized and challenged as described in Methods.

Química Farmacêutica Nacional S/A, SP, Brazil) and chloral hydrate (Labsynth, SP, Brazil). The tracheae were cannulated and lungs were lavaged 3 times with 0.5 mL PBS. Total and differential cell counts of bronchoalveolar lavage fluid were determined by hemocytometer and cytopsin preparation stained with Instant-Prov (Newprov, Pinhais, Brazil).

IL-10 and TGF-β production by spleen cells

Animals received 30 mg ovalbumin by oral gavage for 5 consecutive days or 100 µg nasal ovalbumin for 3 consecutive days to induce mucosal tolerance. Two days later, the animals were immunized once with ovalbumin/alum, and 7 days later, spleen cells were collected. The cells were incubated with 100 µg ovalbumin for 40 hours and 72 hours, respectively, for IL-10 and TGF-β determinations, as described below.

Cytokines determination

The levels of IL-4, IL-5, IL-10, IL-13, and TGF-β in the bronchoalveolar lavage fluid or in supernatants of spleen or lymph nodes cells were assessed by a sandwich kit ELISA according to the manufacturer's recommendations, as previously described.⁶ Values are expressed as pg/mL deduced from standards run in parallel with recombinant cytokines. The limit of detection was 2 pg/mL, 10 pg/mL, and 31 pg/mL for IL-4, IL-5, and IL-13, respectively. Purified and biotinylated antibodies and standard purified cytokines were purchased from R&D Systems (Minneapolis, Minn).

Fluorescence-activated cell sorting analysis

Single cell suspensions in staining buffer (PBS containing 2% FCS and 0.1% NaN₃) were incubated for 45 minutes at 4°C with the antibodies combination. Samples were analyzed in a FACSCalibur instrument (BD). CD4 fluorescein isothiocyanate-labeled antibodies were purchased from BD Biosciences-Pharmingen (Franklin Lakes, NJ).

Histologic analyses

After BAL collection, lungs were perfused via the right ventricle with 10 mL PBS to remove residual blood and immersed in 10% phosphate-buffered formalin for 24 hours and then in 70% ethanol until embedded in paraffin. Tissues were sliced, and 5-µm sections were stained with periodic acid-Schiff (PAS)/hematoxylin for light microscopy evaluation of mucus production. Ten to 15 bronchi of serial nonconsecutive lung sections per animal were scored for mucus index. Small or median bronchi were captured under light microscopy by using a digital camera, and a quantitative digital morphometric analysis was performed by using the application program

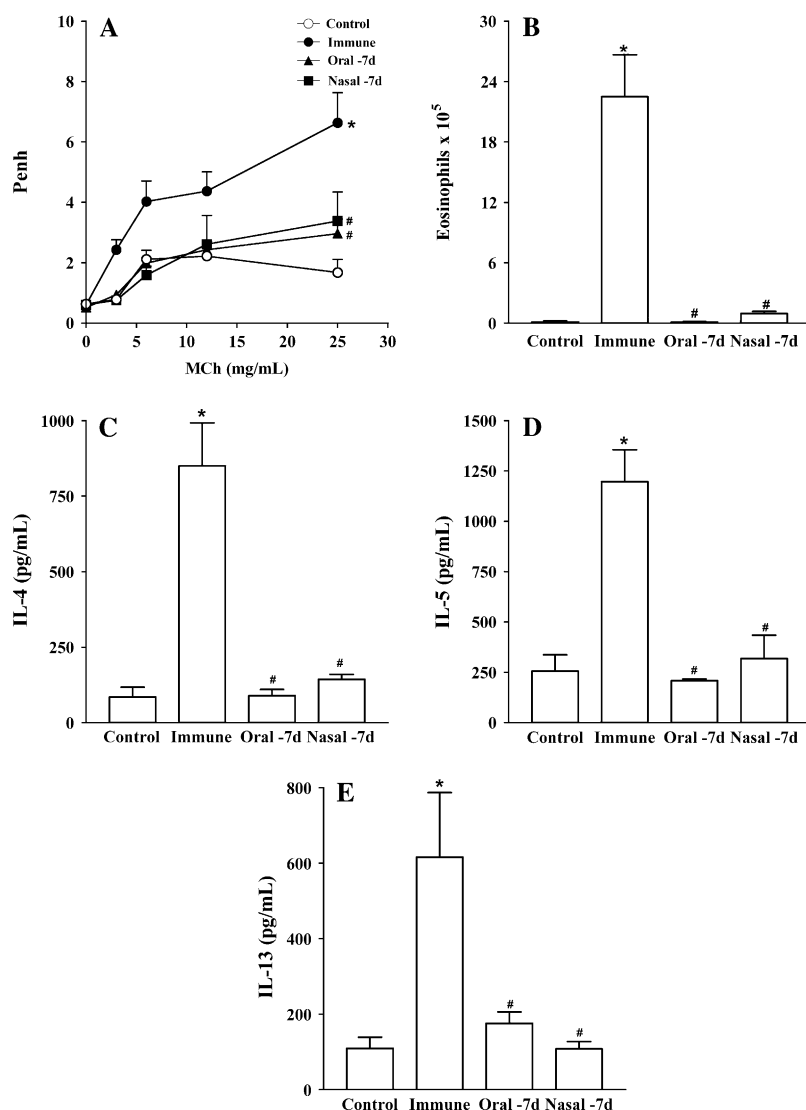


FIG 2. Asthma-like responses in animals exposed to ovalbumin before immunization. Airway hyperreactivity (A), airway eosinophilia (B), and IL-4 (C), IL-5 (D), and IL-13 (E) in the BAL. Values represent the means $\pm$ SEMs, $n = 5$, representative of 2 experiments. *Significant difference ($P < .05$) versus control or #versus immune. MCh, Methacholine.

Metamorph 6.0 (Universal Images Corp, West Chester, Pa). The circumference area of bronchia and the PAS-stained area were electronically measured, and the mucus index was determined by the following formula: (PAS stained area/bronchial circumference area) $\times$ 100.

Determination of serum ovalbumin-specific IgG₁ and IgG_{2a}

Serum was obtained on day 21 for measurement of ovalbumin-specific IgG₁ and IgG_{2a} antibodies by sandwich ELISA as previously described.⁶ Briefly, Nunc-Immuno Plates Maxi-Sorb (Nalge Nunc International, Rochester, NY) were coated with ovalbumin in carbonate buffer, pH 9.6, and incubated overnight at 4°C. After washing with PBS-Tween 0.05%, and blocked with 0.25% casein in PBS, the serum samples were added and incubated for 1 hour. The bounded antibodies were revealed by the addition of goat antimouse IgG₁ or

IgG_{2a} for 1 hour followed by washings and the addition of peroxidase-conjugated rabbit antigoat IgG (heavy and light chains) antibodies (Southern Biotechnology, Birmingham, Ala) for 1 hour. The reaction was developed by the addition of 100 μ L/well of substrate (ortho-phenylenediamine) and absorbance of the samples determined at 490 nm. The concentrations of IgG₁ and IgG_{2a} antibodies were estimated by comparison with IgG₁ and IgG_{2a} standards run in parallel (Southern Biotechnology).

Determination of ovalbumin-specific IgE

For ovalbumin-specific serum IgE determinations, plates were coated overnight at 4°C with 2 mg/mL goat-antimouse IgE antibodies (Southern Biotechnology). The serum samples were added, and subsequently, biotin-labeled ovalbumin was added. The bound ovalbumin-biotin was revealed by extrAvidin Peroxidase conjugate (Sigma-Aldrich) as previously described.⁶ Hyperimmune serum from

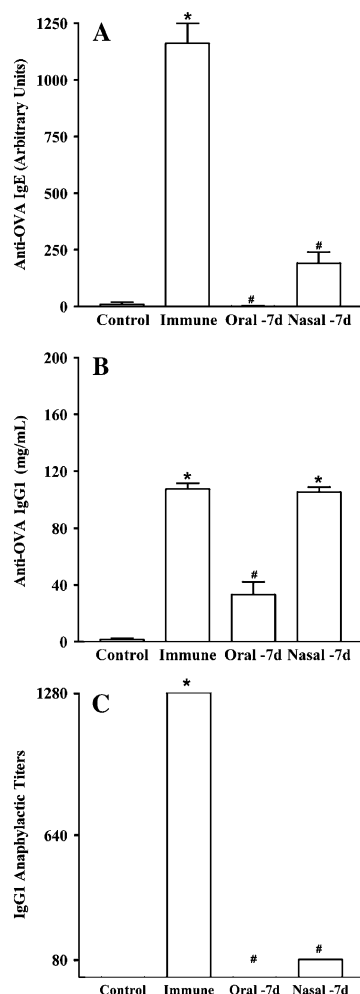


FIG 3. Ovalbumin (OVA)-specific antibody production in animals exposed to OVA before immunization. OVA-specific IgE (**A**), IgG₁ (**B**), and anaphylactic IgG₁ (**C**) production was determined in sera of control, immune, oral, or nasal groups. Values represent the means $\pm$ SEMs, $n = 5$, representative of 2 experiments. *Significant difference ($P < .05$) versus control or #versus immune.

ovalbumin/alum-immunized BALB/c mice was used for IgE standard and arbitrarily assigned at 10,000 U/mL.

Determination of anaphylactic IgG₁

Titration of anaphylactic IgG₁ antibodies was performed by passive cutaneous anaphylaxis (PCA) reactions as described by Ovary.¹³ BALB/c mice were shaved on the back, and 3 intradermal injections (0.05 mL) of serial sera dilutions were made in each side of the dorsal skin. Because IgE antibodies are heat-labile, serum aliquots were incubated at 56°C for 1 hour to inactivate IgE anaphylactic activity. Two hours later, mice were challenged intravenously with 0.5 mL 0.25% Evans blue solution containing 250 μ g ovalbumin. All tests were made in triplicate, and PCA titers were expressed as the reciprocal of the highest dilution that gave a lesion of >5 mm in diameter.

Statistical analysis

ANOVA was used to determine the levels of difference between all groups. Comparisons of all pairs were performed by Tukey-

Kramer honest significant difference test. Values for all measurements are expressed as means $\pm$ SEMs, and P values for significance were set to .05.

RESULTS

Effects of oral versus nasal ovalbumin exposure before immunization on the development of asthma-like responses

We first examined the effects of ovalbumin exposure by the oral or nasal routes before immunization on the development of airway allergic responses. We found that ovalbumin immunized and challenged animals developed AHR as revealed by Penh to increasing doses of methacholine compared with control animals (Fig 2, A). Also, animals immunized and challenged with ovalbumin presented an intense airway eosinophilic inflammation (Fig 2, B) and high levels of IL-4 (Fig 2, C), IL-5 (Fig 2, D), and IL-13 (Fig 2, E) in the BAL. In contrast, oral or nasal ovalbumin administration suppressed AHR (Fig 2, A), airway eosinophilia (Fig 2, B), and the production of type 2 cytokines (Fig 2, C-E). These data document that oral or nasal ovalbumin administration before immunization suppressed equally all pulmonary asthma-like responses.

Effect of oral versus nasal ovalbumin administration before immunization on ovalbumin-specific IgG₁ and IgE production

We next compared the effect of oral versus nasal ovalbumin administration on ovalbumin-specific IgE and IgG₁ production. We found that oral as well as nasal administration of ovalbumin efficiently suppressed IgE production compared with the immune group (Fig 3, A). These data are in accordance with previous reports.^{6,8} Regarding IgG₁ production, we found that oral but not nasal ovalbumin exposure inhibited ovalbumin-specific IgG₁ antibodies (Fig 3, B). Because murine IgG₁ antibodies can be classified according to their anaphylactic activity,¹⁴ experiments were undertaken to determine whether the ovalbumin-specific IgG₁ antibodies detected in animals exposed to ovalbumin by nasal route present anaphylactic activity. We found that anaphylactic IgG₁ was totally inhibited in animals that received ovalbumin by the nasal route (Fig 3, C). Taken together, these data indicate that oral or nasal tolerance suppresses ovalbumin-specific IgE and IgG₁ anaphylactic antibodies. However, only oral tolerance blocks the production of nonanaphylactic IgG₁ antibodies.

Effect of oral versus nasal ovalbumin administration on IL-10 and TGF- β production by spleen cells

We have recently shown that depletion of TGF- β during ovalbumin feeding reverts the suppression of IgG₁ antibodies.⁷ Thus, we sought to determine whether oral but not nasal ovalbumin administration results in the production of TGF- β in lymphoid organs. Because nasal

tolerance has been associated with IL-10 production,¹⁵ we also determined the levels of IL-10 in these experiments. Animals received oral ovalbumin by gavage or nasal ovalbumin by instillation, and 2 days later, they were immunized with ovalbumin/alum. Seven days after immunization, spleen cells were collected and incubated with ovalbumin as described in Methods. As shown in Table I, spleen cells from animals that received ovalbumin by the oral route showed an enhanced production of TGF- β , whereas animals that received nasal ovalbumin produced higher levels of IL-10 than the oral group. These results indicate that oral exposure results in preferential production of TGF- β .

Effects of oral versus nasal ovalbumin administration initiated at day 0 or day 7 of immunization on the development of asthma-like responses

Next we compared the effects of oral versus nasal ovalbumin administration on asthma-like responses when ovalbumin exposure is initiated at day 0 or day 7 of immunization (see protocol, Fig 1, A). We found that oral or nasal ovalbumin administration initiated at day 0 or day 7 inhibited AHR when compared with immune group (Fig 4, A). However, the suppression of AHR (Penh values) of animals that received ovalbumin at day 7 was less pronounced than that obtained at day 0 (Fig 4, A). Analyses of bronchoalveolar lavage fluid revealed that oral or nasal ovalbumin administration was equally effective in suppressing airway eosinophilia (Fig 4, B) and IL-5 and IL-13 production (Fig 4, C and D). Because eosinophil migration into airways appears to be mediated by CD4⁺ T_H2 cells,² we performed a fluorescence-activated cell sorting analysis to quantify the number of CD4⁺ T cells in BAL when ovalbumin exposure is initiated at day 7 of immunization. Similar to what we found with eosinophil influx, a significant influx of CD4⁺ T cells was detected in immune animals but not in mice that received oral or nasal ovalbumin (Fig 4, E).

Finally, we found that mucus formation was also suppressed by oral or nasal tolerance, although the suppressive effect at day 7 was less pronounced than that obtained at day 0 (Fig 5). In summary, our data demonstrate that mucosal tolerance suppresses all pulmonary allergic responses.

Effects of oral versus nasal ovalbumin administration initiated at day 0 or day 7 of immunization on ovalbumin-specific IgG₁ and IgE production

We were interested in determining the effect of oral versus nasal ovalbumin administration at day 0 or day 7 of immunization on ovalbumin-specific IgG₁ and IgE production. As shown in Fig 6, A, neither oral nor nasal ovalbumin administration inhibited IgG₁ production compared with the immune group. Although ovalbumin-specific IgG₁ production was not inhibited, these antibodies failed to induce an exuberant PCA reaction, revealing the non-anaphylactic nature of these antibodies (Fig 6, B and C).

TABLE I. Production of IL-10 and TGF- β by spleen cells after induction of oral or nasal tolerance (pg/mL)

	Control	Oral	Nasal
IL-10	ND	1489 $\pm$ 203*	2053 $\pm$ 224*
TGF- β	25 $\pm$ 13	723 $\pm$ 55*	147 $\pm$ 11

* $P < .05$ versus control.

However, when mucosal ovalbumin administration was initiated 7 days after immunization, the production of IgG₁ and IgE anaphylactic antibodies was not inhibited. As shown in Fig 6, B and C, the levels of antibodies obtained in the oral 7 or nasal 7 groups were not statistically different from those found in the immune group. Taken together, these results indicate that mucosal tolerance downregulates more efficiently anaphylactic IgG₁ and IgE than nonanaphylactic IgG₁ antibodies.

DISCUSSION

Allergic diseases are caused by an aberrant T_H2 immunity to nonpathogenic proteins. Therefore, the control of harmful T-cell responses is of central relevance to allergy therapy. Previous studies using the ovalbumin model of allergic airway inflammation showed that antigen administration at mucosal surfaces (oral or nasal) resulted in peripheral tolerance, prevention of T-cell priming, and consequently, inhibition of lung allergic pathology.^{6,10} However, studies comparing the effectiveness of oral versus nasal routes in the suppression of different allergic responses have not been performed. We found that suppression of T_H2-mediated responses by mucosal ovalbumin administration were dependent on dose, route, and timing of ovalbumin exposure. Nasal tolerance could be induced by the administration of 3 intranasal doses of 100 μ g ovalbumin, whereas the induction of oral tolerance required roughly 500-fold higher dose of ovalbumin. Lower doses of ovalbumin (10 μ g for nasal or 0.1% for oral tolerance) were less effective in suppressing lung allergy or ovalbumin-specific antibodies (data not shown). Although different doses of ovalbumin were used for oral and nasal ovalbumin administration, both routes inhibited the development of key phenotypes of asthma such as airway eosinophilic inflammation, AHR, mucus hyperproduction, and ovalbumin-specific IgE antibodies. However, only oral ovalbumin administration inhibited IgG₁ antiovalbumin antibodies.

It has been proposed that the mucosal milieu creates tolerogenic dendritic cells (DCs) that might induce different regulatory T cells.^{15,16} It was shown that pulmonary DCs isolated after nasal ovalbumin administration produce IL-10 and induce T_H1 regulatory cells, whereas DCs from gut produce TGF- β and induce T_H3 regulatory cells.¹⁵ Thus, it is possible that inhibition of IgG₁ antibodies by oral but not nasal tolerance might depend on TGF- β production by mesenteric DCs. Indeed, we have recently shown that IgG₁ production is restored when

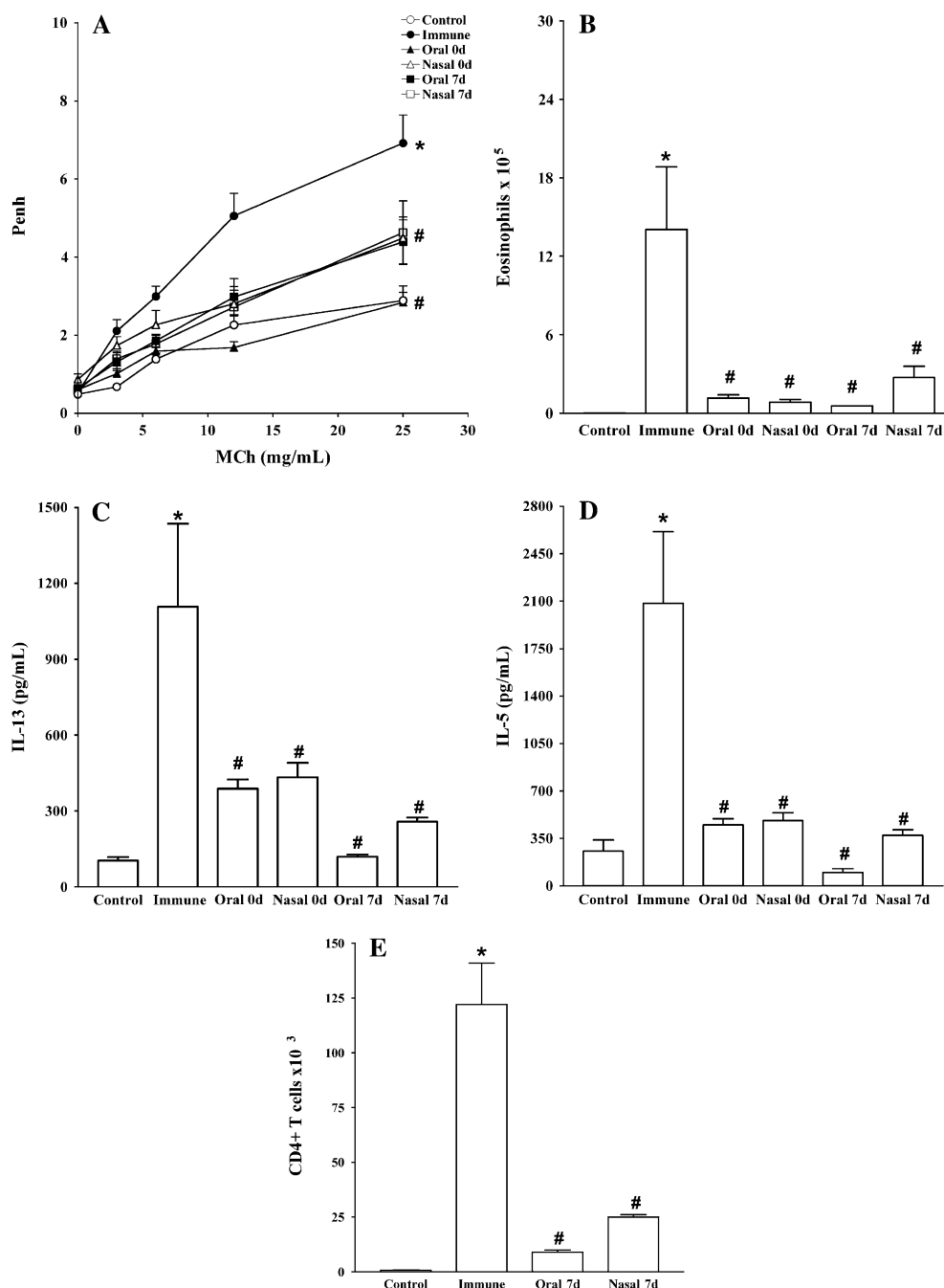


FIG 4. Asthma-like responses in animals exposed to ovalbumin after immunization. Airway hyperreactivity (A), airway eosinophilia (B), IL-5 (C), and IL-13 (D) production and CD4⁺ T cells (E) in the BAL. Values represent the means $\pm$ SEMs, $n = 5$, representative of 2 experiments. *Significant difference ($P < .05$) versus control or #versus immune. MCh, Methacholine.

animals are treated with anti-TGF- β antibodies during ovalbumin feeding.⁷ Here we showed that spleen cells from orally tolerized animals produced higher levels of TGF- β than those from nasal group. Conversely, the nasal group presented an enhanced IL-10 production. Another interesting finding that emerged from our study is that ovalbumin-specific IgG₁ antibodies detected in animals that were nasally tolerized could not induce passive

cutaneous anaphylaxis. Faquim-Mauro et al¹⁴ presented evidence that IgG₁ antibodies that induce mast cell degranulation are IL-4-dependent, whereas the production of nonanaphylactic IgG₁ antibodies is independent of IL-4. Using rats, McMennamin et al¹⁷ reported that nasal tolerance inhibits the production of IgE and IgG_{2b} anaphylactic antibodies but not total IgG. We found that during nasal tolerance, the production of anaphylactic

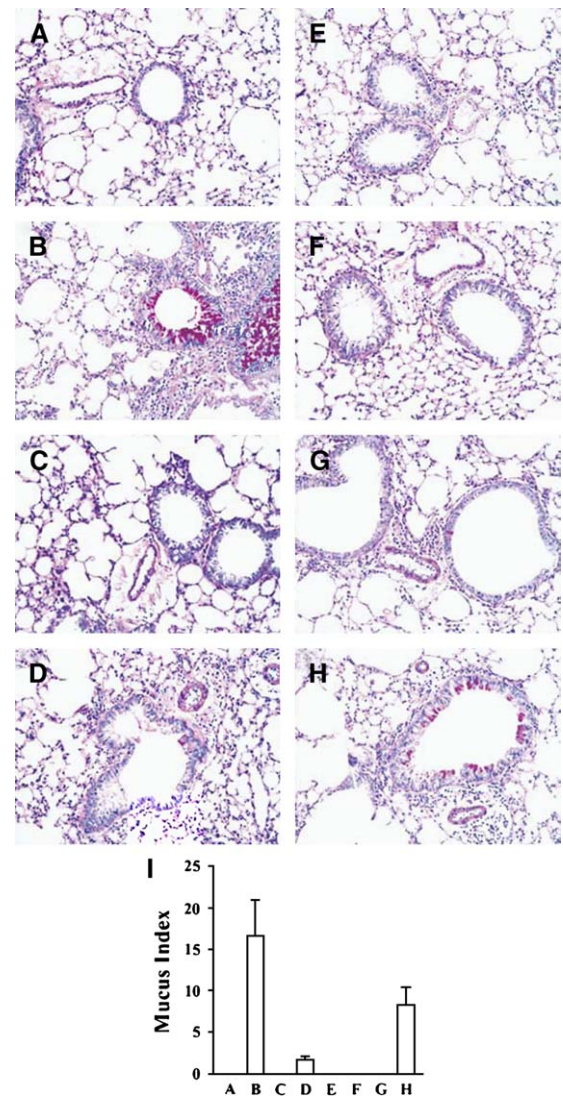


FIG 5. Histology and mucus index. PAS staining (A–H); mucus index (I). Control (A), immune (B), oral 7d (C), nasal 7d (D), oral 0d (E), nasal 0d (F), oral –7d (G), nasal –7d (H). Values represent the means $\pm$ SEMs, $n = 5$, representative of 2 experiments. *Significant difference ($P < .05$) versus control or #versus immune.

antibodies is impaired; however, ovalbumin-specific non-anaphylactic IgG₁ production, which is IL-4-independent, is preserved. In conclusion, our results indicate that irrespective of the route, mucosal tolerance initiated before immunization was very effective in preventing all cellular and humoral T_H2-mediated allergic responses.

We also presented evidence that the effectiveness of mucosal ovalbumin administration in suppressing T-cell immunity declines when mucosal antigen delivery starts after immunization. First, the nonanaphylactic subtype of IgG₁ was inhibited by neither oral nor nasal antigen exposure of ovalbumin at day 0. Also, the production of anaphylactic IgG₁ and IgE antibodies was not inhibited when mucosal ovalbumin exposure occurred 7 days after immunization. In sharp contrast and surprisingly, lung

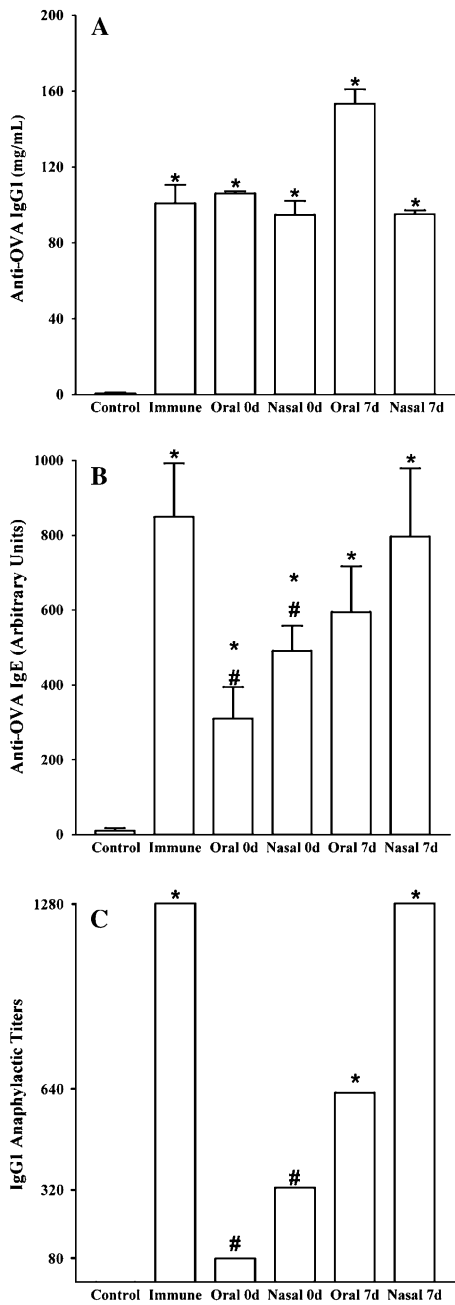


FIG 6. Ovalbumin (OVA)-specific antibody production in animals exposed to OVA after immunization. OVA-specific IgE (A), IgG₁ (B), and anaphylactic IgG₁ (C) production was determined in control, immune, oral, or nasal groups. Values represent the means $\pm$ SEMs, $n = 5$, representative of 2 experiments. *Significant difference ($P < .05$) versus control or #versus immune.

allergic responses (eosinophilia, AHR, and so forth) were all suppressed. Although airway allergic responses were suppressed by mucosal tolerance, the effects of oral tolerization were always more pronounced than those obtained by nasal tolerance. This might be related to the dose of ovalbumin or to the way that ovalbumin is administered (continuous versus 1 dose), because

previous work had shown that continuous ovalbumin feeding is more effective than a single gavage in inducing tolerance.¹⁸

Our study raises an intriguing question regarding T-cell tolerance: why T-cell unresponsiveness achieved by mucosal Ag administration initiated during or after immunization is selective for T_H2-mediated pulmonary allergic responses but not for T-cell-mediated antibody production. Although different subtypes of regulatory T cells or suppressor cytokines and mechanisms such as anergy, deletion, and immune deviation have increasingly been defined as important in mediating T-cell unresponsiveness, we do not know what mechanisms govern this differential and selective T-cell unresponsiveness.

It is hard to envisage how anergy/deletion, regulatory T cells, or suppressive cytokines could operate in a system that presents suppressed airway allergic responses but not anaphylactic antibody production. An alternative explanation for the selective T-cell unresponsiveness might be related to the ability of T cells to traffic in the body and exert their activities. In other words, airway allergic responses are not triggered because T cells from animals exposed to ovalbumin might be unable to migrate from lymphoid organs to the lung. However, they might be able to activate B cells in lymphoid organs. This notion is in line with the findings of Schaerli et al¹⁹ and Schaerli and Moser²⁰ showing that primed nonpolarized CD4⁺ T cells, expressing the chemokine receptor CXCR5 can provide help to B cells, but that these cells do not have the ability to move to sites of inflammation. Actually, to migrate from lymphoid organs to sites of allergic reactions, CD4⁺ T cells, upon activation, downregulate lymphoid homing receptor molecules such as CCR7 and CD62L and upregulate molecules involved in lymphocyte trafficking to inflamed tissue such as CCR4, CCR8, LFA-1, and VLA-4.²¹ Thus, the differential expression of chemokine receptors and adhesion molecules on T cells might explain the hierarchical pattern of T-cell responses observed in our study. Nevertheless, more studies are required to ascertain whether this hypothesis is correct.

Finally, we speculate that during exposure to airborne or foodborne allergens, similar to what we found in mice, certain individuals might develop T cells that could activate B cells for IgE production, but these T cells might be unable to migrate to airways and provoke allergic lung inflammation. This type of immune response might explain why individuals with allergy and high levels of IgE do not necessarily develop asthma.

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