

Mechanisms preventing allergen-induced airways hyperreactivity: Role of tolerance and immune deviation

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Background: Aeroallergens continuously enter the respiratory tract of atopic individuals and provoke the development of asthma characterized by airway hyperreactivity (AHR) and inflammation. By contrast, nonatopic individuals are exposed to the same aeroallergens, but airway inflammation does not develop. However, the mechanisms that prevent allergen-induced respiratory diseases in nonatopic subjects are poorly characterized. **Objective:** In this study we compared the role of allergen-specific T-cell tolerance and immune deviation in conferring protection against the development of allergen-induced AHR. **Methods:** We exposed mice to intranasal ovalbumin (OVA) to induce T-cell tolerance and examined its effects on the subsequent development of AHR and inflammation. **Results:** We demonstrated that exposure of mice to intranasal OVA resulted in peripheral CD4⁺ T-cell unresponsiveness that very efficiently prevented not only the development of AHR but also greatly inhibited airway inflammation and OVA-specific IgE production. The induction of peripheral T-cell tolerance and protection against AHR were not dependent on the presence of IFN- γ or IL-4. The development of AHR was also prevented by an OVA-specific T_H1-biased immune response induced by inhalation of OVA in the presence of IL-12. However, the OVA-specific T_H1 response was associated with a significant degree of pulmonary inflammation. **Conclusion:** These results indicate that both allergen-specific T-cell tolerance and T_H1-biased immune deviation prevent the development of AHR, but T_H1 responses are associated with significantly greater inflammation in the lung than is associated with T-cell unresponsiveness. Therefore CD4⁺ T-cell unresponsiveness critically regulates immune responses to aeroallergens and protects against the development of allergic disease and asthma. (*J Allergy Clin Immunol* 2000;106:239-46.)

Key words: Allergy, airway hyperreactivity, T lymphocytes, tolerance, immune deviation

Abbreviations used

AHR: Airway hyperreactivity
BAL: Bronchoalveolar lavage
H&E: Hematoxylin and eosin stain
OVA: Ovalbumin
Penh: Enhanced pause

The effector cells and secreted factors that cause allergic disease and asthma are well characterized, but the specific immunologic mechanisms that protect against the development of these problems are poorly understood. Thus although CD4⁺ T_H2 cells secreting high levels of IL-4, IL-5, IL-9, and IL-13 play critical roles in the pathogenesis of asthma and allergy,¹⁻⁴ T_H1 cells have been assumed to counterbalance the inflammatory response orchestrated by T_H2 cells. However, there is accumulating evidence indicating that T_H1 cells may not fully protect against allergic disease and asthma and that additional more complex mechanisms are involved in regulating the development of these diseases.

The idea that T_H1 cells downmodulate T_H2 cells in allergic disease and asthma was extrapolated from models of parasite infection (eg, with *Leishmania* species), where there is good experimental evidence demonstrating that T_H1 inhibits the development of T_H2 cells.⁵ In the context of allergy, the idea that T_H1 cells protect against allergic inflammation is supported by studies demonstrating that allergen-specific T cells cloned from healthy nonallergic individuals display a T_H1 cytokine profile.⁶⁻⁸ Furthermore, epidemiologic studies showed that the incidence of allergy and asthma is reduced in patients with diseases caused by overexpression of T_H1 cytokines, such as tuberculosis and multiple sclerosis.^{9,10} In addition, the beneficial role of T_H1 cells in allergic disease is supported by studies demonstrating that allergen-specific CD8⁺ T cells secreting enhanced amounts of IFN- γ maintain homeostasis after allergen inhalation.^{11,12} Thus many investigators have concluded that T_H1 cells protect against asthma and allergy and can reverse the effects of T_H2-driven inflammation.

However, although the production of IFN- γ may be associated with regulation of allergen-specific immune responses, other observations suggest that allergic disease and asthma are not exclusively regulated by the selective

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expansion of allergen-specific T_H1 cells. First, allergen-specific T cells in the peripheral blood and the lungs of patients with asthma also produce IFN- γ , which appears to actually contribute to the severity of the disease.^{1,13} Moreover, we and others have demonstrated that classical T_H1 cells, when adoptively transferred into mice, do not counterbalance effector T_H2 cells but rather codominantly generate inflammatory responses in the lungs.¹⁴ Furthermore, in contrast to the significant inflammation found in the lungs of patients with asthma, the airways of nonallergic subjects are characterized by the absence of inflammatory changes, including the absence of T_H1 cells. This suggests that other regulatory mechanisms, distinct from the T_H1 -biased immune responses, are involved in suppressing inflammation and protecting against allergic disease and asthma. In this study we examined the effect of T-cell tolerance induced by respiratory exposure to ovalbumin (OVA) on the subsequent development of airway hyperactivity (AHR) and inflammation.

METHODS

Animals

Five- to 6-week-old BALB/c or BALB/c IL-4^{-/-} mice were purchased from Jackson Laboratories (Bar Harbor, Me) and were housed in pathogen-free conditions at the laboratory animal facilities of Stanford University in accordance with the guidelines of the National Institutes of Health.

Immunization protocol

Mice were exposed intranasally to 100 μ g of OVA (grade V; Sigma Chemical Co, St Louis, Mo) in 30 μ L of normal saline solution in the absence or presence of 1 μ g of recombinant IL-12 (generously provided by Stanley Wolf, Genetic Institute) or recombinant IL-4 (PeproTech, Rocky Hill, NJ) on 3 consecutive days. Control mice received an equivalent volume of intranasal PBS. Ten days after the last administration of intranasal OVA, the mice were immunized intraperitoneally with 50 μ g of OVA in 2 mg of aluminum hydroxide (alum). Ten days later, the mice were rechallenged intranasally with OVA (50 μ g) for 3 days. Some mice received a 2-mg neutralizing anti-IFN- γ mAb intraperitoneally (XMG1.2; a generous gift of Dr R. Coffman, DNAX) before and during all immunizations.

Measurement of airways responsiveness

Airway responsiveness was assessed by methacholine-induced airflow obstruction from conscious mice placed in a whole-body plethysmograph, as previously described.¹⁵ Measurements of methacholine responsiveness were obtained by exposing mice for 2 minutes to nebulized NaCl 0.9%, followed by incremental doses of nebulized methacholine and monitoring enhanced pause (Penh). Results were expressed for each methacholine concentration as the percentage of baseline Penh values after NaCl 0.9% exposure.

Analysis of airways inflammation

The trachea was cannulated, the lungs were lavaged with 0.4 mL of PBS 3 times, and the fluid was pooled. Cells in the lavage fluid were counted, and bronchoalveolar lavage (BAL) cell differentials were determined on slide preparations stained with Hansel Stain (Lide Laboratories, Florissant, Mo). At least 200 cells were differentiated by light microscopy on the basis of conventional morphologic criteria. In some animals the lungs were removed, fixed in 10% formalin, routinely processed, and embedded in paraffin wax.

Five-micrometer sections were prepared and stained with hematoxylin and eosin stain (H&E).

In vitro proliferation and cytokine assays

Spleen or bronchial lymph node cells were harvested, passed through a nylon mesh, and cultured (5×10^5 cells/well) with or without OVA in 0.2 mL of Dulbecco's Modified Eagle's medium (Sigma) supplemented with 10% FCS (Gemini Bioproducts, Calabasas, Calif), 2 mmol/L glutamine, 20 μ g/mL gentamicin, and 5×10^{-5} mol/L 2-mercaptoethanol, as previously described.¹⁶ The levels of IL-4, IL-5, IL-10, IL-13, and IFN- γ in the supernatants were assayed by ELISA, as previously described.¹⁶

Measurement of OVA-specific immunoglobulins

Mice were bled at the time of death, and OVA-specific antibodies were measured by using modified OVA-specific IgG1, IgG2a, and IgE ELISAs, as previously described.^{16,17} The standard for the OVA-specific IgE ELISA was a monoclonal anti-OVA IgE.¹⁸

Statistical analysis

Results are expressed as means $\pm$ SD, except for the results for airway hyperactivity, in which SEs are provided. Pairs of groups were compared by using the unpaired two-tailed Student *t* test.

RESULTS

We examined the effects of intranasal exposure to OVA on the subsequent response to intraperitoneal immunization with OVA in alum. Five days after the intraperitoneal injection, splenic T cells were isolated and cultured in vitro with OVA. Fig 1, A, shows that these T cells proliferated poorly compared with T cells from control mice, which received intranasal PBS before immunization with OVA in alum. The inhibition of T-cell proliferation was OVA specific and did not affect the ability of the mice to respond to immunization with other nonrelevant antigens (data not shown).

To determine whether intranasal exposure to OVA influenced the development of allergen-induced AHR, BALB/c mice were pre-exposed to intranasal OVA or control PBS before treatment with a protocol to induce AHR and eosinophilic inflammation. This protocol included intraperitoneal immunization with OVA in alum and subsequent repeated respiratory challenge with OVA. Twenty-four hours after the last dose of intranasal OVA, AHR in response to inhaled methacholine was assessed. As expected, mice that had received intranasal PBS before systemic and respiratory immunization had significant AHR (Fig 1, B). On the contrary, mice pre-exposed to intranasal OVA had normal airway reactivity similar to airway reactivity observed in naive mice (Fig 1, B).

The downregulation of the OVA-specific proliferative response was associated with failure of the T cells to secrete T_H1 and T_H2 cytokines. Thus very low levels of IFN- γ , IL-4, IL-5, IL-10, and IL-13 were present in the culture supernatants of spleen cells from mice pre-exposed to intranasal OVA (Fig 2). In contrast, enhanced amounts of these cytokines were observed in cultures of cells from control mice pretreated with intranasal PBS (Fig 2). Tumor growth factor β was not detected in any of the cultures performed (data not shown). In addition, the circulating levels

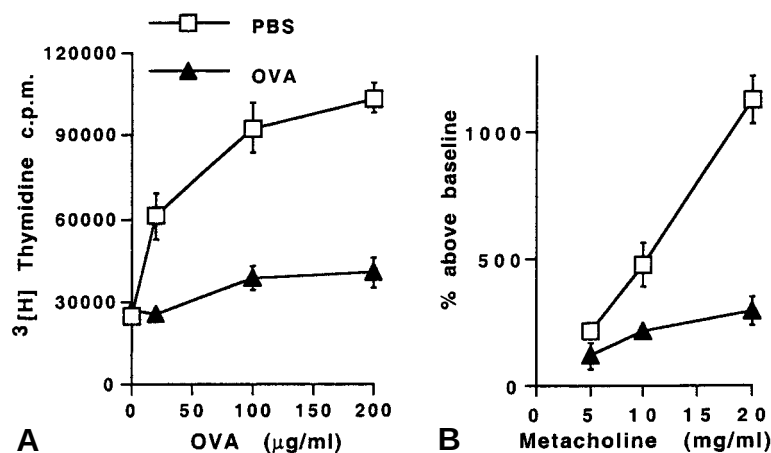


FIG 1. A, Exposure to intranasal OVA induces antigen-specific T-cell unresponsiveness. BALB/c mice were exposed to intranasal PBS or OVA (100 μg) on 3 consecutive days. Ten days later, the mice were immunized intraperitoneally with OVA (50 μg) in alum, and after 5 days, spleen cells were harvested and cultured in vitro with or without increasing concentrations of OVA. The proliferation was determined by measuring tritiated thymidine incorporation. Representative results from 6 experiments with 5 to 6 mice per group are shown. The results are expressed as means ± SD counts per minute of triplicate cultures. **B**, Induction of intranasal tolerance prevents the development of AHR and inflammation on subsequent allergen exposure. BALB/c mice pretreated with intranasal PBS or OVA (100 μg × 3) were immunized intraperitoneally with OVA (50 μg) in alum and rechallenged with intranasal OVA (50 μg × 3) 10 days later. Twenty-four hours after the last administration of intranasal OVA, airway hyperreactivity in response to inhaled methacholine was measured by using a whole-body plethysmograph. The results are expressed as means ± SE of the percentage above baseline Penh values and are representative of 6 experiments with 5 to 6 mice per group. The *P* value at 20 mg/mL methacholine comparing the two groups was .0001.

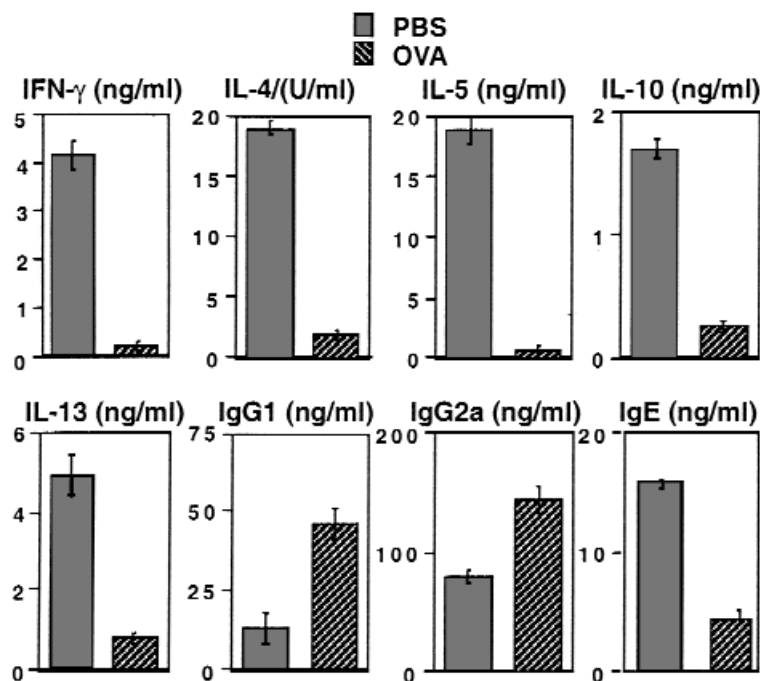


FIG 2. Induction of intranasal tolerance is associated with downregulation of T_H1 and T_H2 cytokines and IgE production. BALB/c mice were treated as described in Fig 1, B. Spleen cells harvested 1 day after the last intranasal immunization with OVA were restimulated in vitro with OVA (100 μg/mL). One representative experiment of 5 is shown. Similar results were obtained with bronchial lymph node cells. Cells cultured in vitro without OVA failed to produce cytokines. Results are expressed as means ± SD for duplicate or triplicate cultures. Similarly treated mice were bled 2 weeks after the intraperitoneal immunization, and the serum levels of OVA-specific IgG1, IgG2a, and IgE were quantitated by means of isotype-specific ELISA. Results are expressed as means ± SD of triplicate determinations.

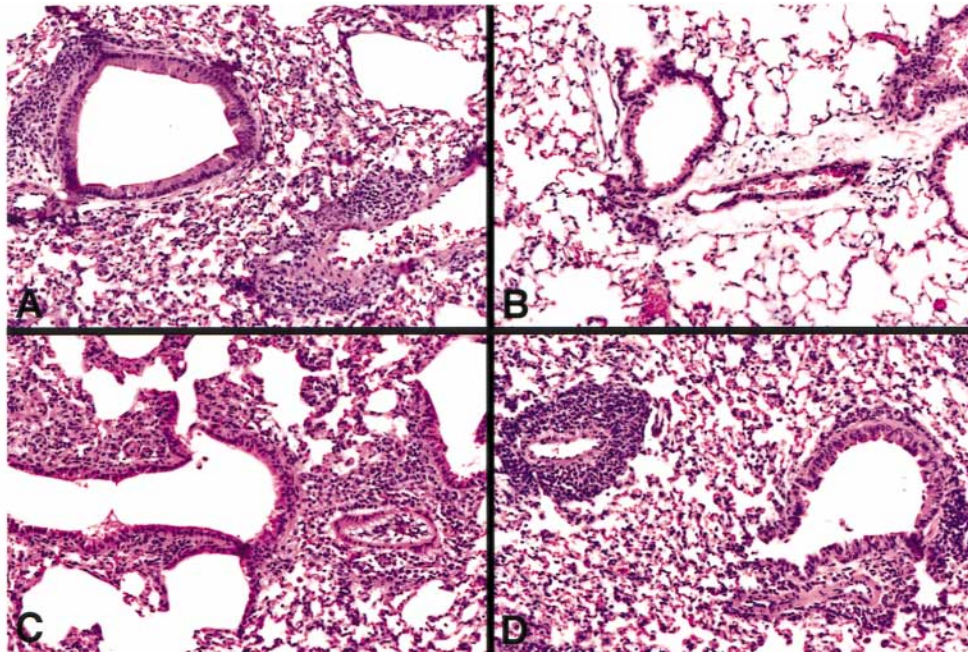


FIG 3. Lung histology after allergen sensitization of mice pre-exposed to intranasal OVA with or without IL-12 or IL-4. BALB/c mice pre-exposed to intranasal PBS or OVA with and without IL-12 or IL-4 on 3 consecutive days were immunized intraperitoneally with OVA in alum on day 13 and rechallenged with intranasal OVA 10 days later. One day after the last dose of intranasal OVA, the lungs were removed, embedded in paraffin, sectioned, and stained with H&E. Shown are examples (H&E 200 $\times$) of mice pretreated with the following. **A**, Pretreatment with intranasal PBS shows that dense mononuclear cell infiltrates are present in peribronchial and perivascular areas, consisting primarily of eosinophils and lymphocytes. **B**, Pretreatment with intranasal OVA alone shows that normal lung histology is present, with a notable absence of inflammation. **C**, Pretreatment with intranasal OVA plus IL-12 shows that perivascular inflammatory infiltrates of slightly reduced intensity compared with the PBS group are present. **D**, Pretreatment with intranasal OVA plus IL-4 shows that severe inflammatory infiltrates are present around the airways and vessels, similar to those observed in the PBS group.

of OVA-specific IgE were substantially reduced in mice pre-exposed to intranasal OVA compared with the high amounts detected in control mice pretreated with PBS (Fig 2). However, the serum levels of OVA-specific IgG1 and IgG2a were elevated in both groups of mice.

The reduction in AHR by prior exposure to intranasal OVA correlated with a reduction in airway inflammation. Control mice that received intranasal PBS before treatment with the protocol to induce AHR had dense peribronchiolar, perivascular, and interstitial inflammation, consisting primarily of eosinophils and lymphocytes (Fig 3, A). In contrast, the lungs of mice tolerized by pre-exposure to intranasal OVA were almost normal, with only rare sites of inflammatory infiltrates (Fig 3, B). Mice immunized intraperitoneally with OVA in alum but not rechallenged with intranasal OVA had normal lung histology (data not shown), indicating that the respiratory challenge with OVA was responsible for the inflammatory changes observed in the lungs. The severity of airway inflammation was reflected in BAL fluid eosinophilia. Thus control mice pre-exposed to intranasal PBS had a marked influx of eosinophils in the airway, whereas the number of eosinophils present in BAL fluid from mice tolerized after exposure to intranasal OVA was significantly reduced (data not shown).

The presence of IFN- γ and IL-4 is of critical importance in determining the phenotype of CD4⁺ T-cell responses and the potential development of inflammatory reactions.¹⁹ To determine whether the induction of intranasal tolerance and protection from subsequent development of AHR and inflammation were influenced by the presence of these two cytokines, BALB/c IL-4^{-/-} mice, as well as BALB/c mice that received neutralizing anti-IFN- γ mAb before and during all immunizations, were treated with our sensitization protocol. Cells from PBS-pretreated mice lacking IL-4 or IFN- γ after intraperitoneal immunization with OVA proliferated vigorously, as did cells from similarly treated wild-type control mice (data not shown). On the contrary, cells from mice pre-exposed to intranasal OVA failed to proliferate, regardless of the presence or absence of active IL-4 or IFN- γ , indicating that the induction of CD4⁺ T-cell unresponsiveness was independent of IL-4 and IFN- γ . Moreover, the protective effect of pre-exposure to intranasal OVA on the development of AHR was not dependent on the presence of these two cytokines. Thus exposure of IL-4^{-/-} mice or mice lacking IFN- γ to intranasal OVA still prevented the induction of AHR, as was observed in wild-type mice (data not shown). Control IL-4^{-/-} or IFN- γ -deficient mice pretreated with intranasal PBS had sig-

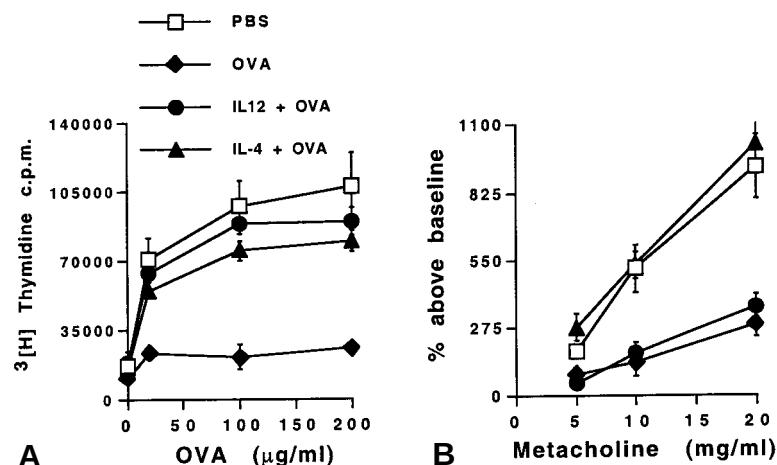


FIG 4. **A**, The presence of IL-12 or IL-4 at the time of primary exposure to intranasal OVA prevents the induction of tolerance. BALB/c mice pre-exposed to intranasal PBS or OVA with and without IL-12 or IL-4 on 3 consecutive days were immunized intraperitoneally with OVA in alum 10 days later. After 5 days, the proliferation of spleen cells was assessed in response to *in vitro* restimulation with increasing concentrations of OVA. The proliferation was determined by measuring tritiated thymidine incorporation. Results, expressed as mean $\pm$ SD counts per minute of triplicate cultures, from a representative experiment with 5 to 6 mice per group are shown. **B**, Induction of allergen-specific $\text{T}_\text{H}1$ responses prevents the development of AHR but not inflammation. BALB/c mice pre-exposed to intranasal PBS or OVA with and without IL-12 or IL-4 on 3 consecutive days were subsequently treated as described in Fig 1, *B*. Twenty-four hours after the last administration of intranasal OVA, AHR in response to inhaled methacholine was measured. Representative results, with 5 to 6 mice per group, are shown expressed as mean $\pm$ SE of the percentage above baseline Penh values. The *P* values at 20 mg/mL methacholine comparing the PBS group with the OVA group, and those for the IL-12 plus OVA group were less than .001 and less than .002, respectively.

nificant AHR. With regard to airway inflammation, exposure of IL-4^{-/-} or IFN- γ -deficient mice to intranasal OVA also reduced the number of inflammatory cells in BAL fluid, irrespective of the presence or absence of active IL-4 and IFN- γ (data not shown). Control IL-4^{-/-} or IFN- γ -deficient mice pretreated with intranasal PBS had greatly increased numbers of cells in BAL fluid, as did PBS-pretreated wild-type control mice, although the overall number of cells in BAL fluid of all IL-4^{-/-} mice was reduced. These results together indicate that the protective effect of intranasal tolerance induction against the development of allergic respiratory disease did not require the presence of IL-4 or IFN- γ .

We next determined whether the induction of an OVA-specific $\text{T}_\text{H}1$ response in the lungs of mice would protect against the development of AHR. For these experiments, BALB/c mice received intranasal OVA and IL-12 and were subsequently treated with the standard protocol to induce AHR. After the intraperitoneal immunization, splenic T cells proliferated vigorously *in vitro* when stimulated with OVA (Fig 4, *A*), indicating that treatment with OVA plus IL-12 abrogated the induction of T-cell unresponsiveness to OVA. However, Fig 4, *B*, shows that pretreatment of mice either with intranasal OVA (induction of T-cell unresponsiveness) or with intranasal OVA plus IL-12 (induction of $\text{T}_\text{H}1$ responsiveness) prevented the development of AHR. The OVA-specific $\text{T}_\text{H}1$ response reduced AHR to levels observed in naive unimmunized mice, which was similar to that observed in

mice rendered unresponsive to OVA by exposure to intranasal OVA. In contrast, control mice pretreated with intranasal OVA plus IL-4 had T cells that proliferated vigorously when stimulated *in vitro* with OVA (Fig 4, *A*) and had increased AHR (Fig 4, *B*).

Although mice that received OVA plus IL-12 had normal airway reactivity, this treatment resulted in the development of a significant inflammatory response in the airways. Thus the histology of the lungs of mice pretreated with OVA plus IL-12 was very abnormal, with significant peribronchiolar and perivascular infiltrates, consisting mainly of lymphocytes, but very few eosinophils (Fig 3, *C*). BAL fluid from mice pretreated with OVA plus IL-12 had more than twice as many inflammatory cells than did BAL fluid from mice tolerized with intranasal OVA alone (data not shown). On the contrary, the lungs of mice rendered unresponsive to OVA by exposure to intranasal OVA were almost normal (Fig 3, *B*). Control mice pretreated with intranasal OVA plus IL-4 had marked inflammatory changes in the airways, consisting mainly of lymphocytes and eosinophils (Fig 3, *D*), and significant airway eosinophilia was noted in BAL fluid (data not shown).

As expected, the cytokine profile of T cells from mice pre-exposed to intranasal OVA plus IL-12 was characterized by high levels of IFN- γ (Fig 5), whereas mice pretreated with tolerizing doses of intranasal OVA alone produced low levels of IFN- γ , IL-4, IL-5, IL-10, and IL-13. Mice pretreated with intranasal OVA plus IL-12 also had high circulating levels of OVA-specific IgG2a and

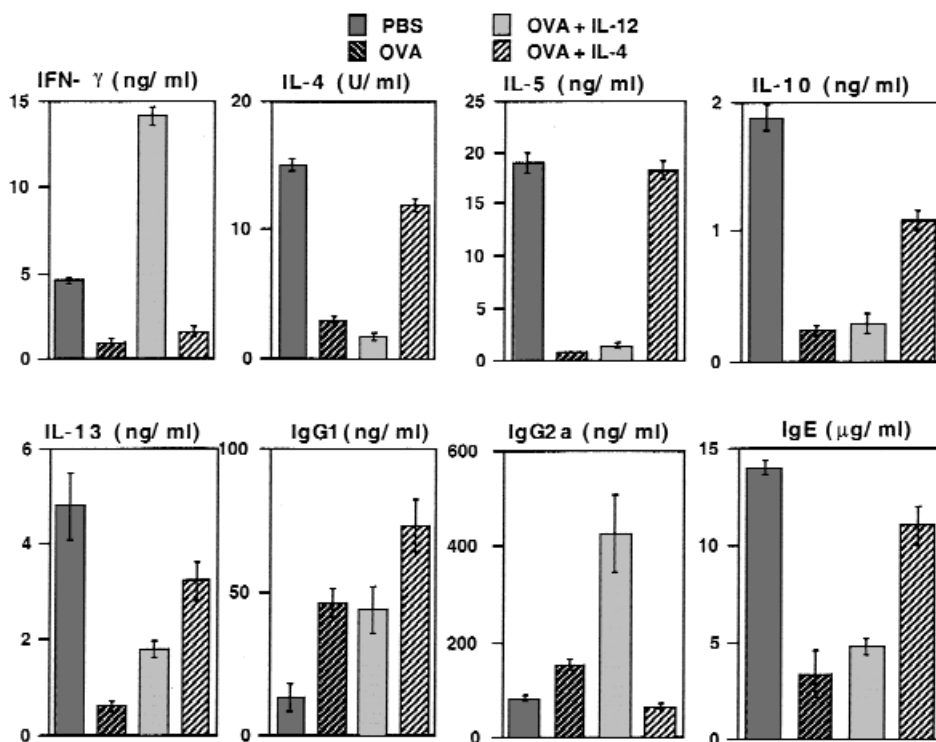


FIG 5. Disruption of intranasal tolerance is associated with immune deviation and functional T_H cell responses. BALB/c mice were treated as described in Fig 4, B. Spleen cells harvested 1 day after the last intranasal immunization with OVA were restimulated in vitro with OVA (100 μ g/mL). One representative experiment of 5 is shown. Similar results were obtained with bronchial lymph node cells. Cells cultured in vitro without OVA failed to produce cytokines. Results are expressed as means $\pm$ SD for duplicate or triplicate cultures. Similarly treated mice were bled 2 weeks after the intraperitoneal immunization, and the serum levels of OVA-specific IgG1, IgG2a, and IgE were quantitated by using isotype-specific ELISA. Results are expressed as means $\pm$ SD of triplicate determinations.

reduced amounts of IgE. As expected, in control mice pretreated with intranasal OVA plus IL-4 before intraperitoneal immunization, production of IL-4, IL-5, IL-10, and IL-13 was increased, as were serum levels of OVA-specific IgE (Fig 5).

DISCUSSION

The respiratory mucosa of both atopic and nonatopic individuals is constantly exposed to a wide variety of environmental aeroallergens, but the clinical responses in these populations differ considerably, with T_H2 -biased allergic inflammation and AHR developing in the former and protective immunity developing in the latter. However, the specific mechanisms that downregulate the development of inflammation and AHR in normal nonatopic individuals have been poorly characterized. In this study we showed that the induction of $CD4^+$ T-cell unresponsiveness that was generated by exposure to OVA through the intranasal route effectively in the absence of inflammatory signals prevented the development of OVA-induced AHR and inflammation. Other mechanisms, such as immune deviation toward the T_H1 phenotype, also protected against the development of AHR, but this

measure was not as effective in preventing pulmonary inflammation. These results indicate that functional T-cell unresponsiveness downregulates aberrant T_H2 reactivity in the lungs in a manner that is more effective than T_H1 -biased immunity. Moreover, these results suggest that functional T-cell unresponsiveness may be an important mechanism responsible for protection of normal nonatopic individuals from the development of AHR and detrimental allergic inflammatory responses against aeroallergens.

The pathogenesis of allergic disorders and asthma has been attributed to excessive production of T_H2 cytokines. In addition, because T_H2 cells are pathogenic for asthma, the opposite cell type (ie, the T_H1 cell) has been assumed to protect against asthma.^{6,7,20} However, if T_H1 cells counterbalance T_H2 cells in the airway and protect against asthma, then one would predict that T_H1 cells would be prominent in the lungs of normal nonasthmatic individuals. On the contrary, very few lymphocytes are found in the lungs of normal individuals, and in fact, T_H1 cells are often present in chronic severe allergic inflammation and appear to exacerbate asthma.^{1,13} Thus although T_H1 responses may prevent the development of AHR, T_H1 responses have the capacity to cause tissue-

damaging inflammation in respiratory mucosa, which suggests that other immunologic mechanisms may be important in downregulating asthmatic symptoms and airway inflammation in normal individuals.

To examine these mechanisms, we established a system in which naive mice were exposed to OVA through the respiratory mucosa to induce antigen-specific T-cell unresponsiveness before treatment with a protocol known to induce AHR and eosinophilic inflammation. Exposure of mice to intranasal OVA in the absence of inflammatory signals resulted in the development of peripheral, OVA-specific, CD4⁺ T-cell tolerance. Tolerance was characterized by a transient activation and expansion of OVA-specific CD4⁺ T cells, followed by a significant degree of T-cell deletion and failure of remaining T cells to produce high levels of T_H1 or T_H2 cytokines (functional inactivation).^{21,22} This T-cell unresponsiveness was associated with a reduced production of OVA-specific IgE but with a capacity to produce IgG1 and IgG2a. The presence of immunosuppressive cytokines, such as IL-10, tumor growth factor β ,²² or IFN- γ and IL-4, was not required for the induction of CD4⁺ T-cell tolerance. Nevertheless, the induction of T-cell tolerance was highly effective in preventing subsequent development of AHR and in preventing airway inflammation when the mice were rechallenged in the lung with OVA. These results suggest that the absence of functional OVA-specific T_H effector cells in the mice and tolerization of such cells by exposure to intranasal OVA was responsible for the reduction in airway reactivity.

Intranasal exposure to antigens, such as myelin basic protein, is known to induce peripheral CD4 T-cell tolerance and to protect against the development of T_H1-mediated autoimmune diseases, such as experimental allergic encephalomyelitis.²³ However, intranasal tolerance has not been previously studied in the context of preventing the most important feature of asthma, AHR. Previous studies have indicated that inhalation of antigen induces CD8⁺ or CD4⁺ regulatory cells that inhibit IgE production and tracheal eosinophilia.^{11,12,24-26} However, because AHR is not dependent on the presence of eosinophils^{27,28} or IgE,¹⁴ our data, showing that peripheral T-cell tolerance indeed protects against AHR, demonstrates for the first time that peripheral T-cell tolerance may in fact be extremely important in preventing the clinical symptoms of asthma.

We compared the protective effects on AHR of peripheral tolerance induction with that of a T_H1-biased immune response, which is believed to protect against allergic inflammation. An OVA-specific T_H1 response was generated by intranasal administration of IL-12 with OVA because the presence of IL-12 strongly influences T-cell differentiation and preferentially promotes the acquisition of the T_H1 effector phenotype¹⁹ and because tracheal administration of IL-12 has been shown to prevent airway eosinophilia and AHR.²⁹ Consistent with previous reports, exposure of the mice to intranasal OVA plus IL-12 resulted in T_H1-biased immunity characterized by increased production of IFN- γ and IgG2a, which prevent-

ed AHR on subsequent rechallenge with intranasal OVA. Although the absence of elevated airway reactivity correlated with reduced accumulation of eosinophils in the lungs, the generation of active OVA-specific T_H1 immunity was accompanied by a significant pulmonary inflammatory response, consisting mainly of monocytes and lymphocytes, which can be associated with pulmonary tissue damage and dysfunction.^{14,30-32} Although it is possible that a less vigorous OVA-specific T_H1 response might produce less airway inflammation, it is becoming clear that classical T_H1 responses may be harmful at mucosal sites.¹⁴ Because the induction of antigen-specific T-cell tolerance was equally effective in preventing AHR but was much more effective in reducing airway inflammation, we conclude that the induction of peripheral tolerance is a safer and more efficacious mechanism in the prevention of allergic disease and asthma.

Taken together, our results suggest that exposure to aeroallergens under normal circumstances in nonallergic individuals results in allergen-specific unresponsiveness characterized by a lack of functional allergen-specific CD4⁺ T_H cells, reduced IgE production, and protection against the development of AHR and inflammation on further allergen exposure. In addition, these results suggest that therapeutic interventions for asthma and allergy should focus on inducing allergen-specific T-cell tolerance, rather than on promotion of T_H1 responses with agents such as IL-12 or naked DNA plasmids or CpG motifs, which might induce detrimental T_H1-biased inflammation in respiratory mucosa.^{16,17,33} For example, oral-sublingual allergen immunotherapy may be effective in therapy for allergic disease because it induces antigen-specific tolerance.³⁴ In addition, conventional allergen immunotherapy may be clinically effective because it induces tolerance,^{35,36} anergy, and/or deletion of the allergen-specific CD4⁺ T cells.^{37,38} Further investigations are required to elucidate the tolerance mechanisms that protect against allergic disease and to develop novel approaches for the induction of allergen-specific T-cell tolerance.

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