

Decreased Nasal Tolerance to Allergic Asthma in Mice Fed an Amino Acid–Based Protein-Free Diet

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ABSTRACT: Intranasal (i.n.) administration of soluble proteins induces a state of specific unresponsiveness to subsequent immunization, known as nasal tolerance. It is thought that newborns are less susceptible to nasal tolerance induction. Recently, we have shown that feeding adult animals with a protein-free diet (Aa) resulted in their arrest at an immature immunological profile. Here, we examined the effects of the Aa diet on the development of nasal tolerance to ovalbumin (OVA) in a murine model of allergic asthma. Nasal OVA administration suppressed almost totally the OVA-induced asthma-like responses (airway eosinophilia, type 2 cytokine production, and OVA-specific IgE antibodies) in chow- or casein-fed BALB/c mice. In contrast, in Aa-fed animals the suppression of asthma-like responses by nasal OVA was partial, being effective in suppressing airway eosinophilia, but not airway type 2 cytokine or OVA-specific IgE response. We conclude that animals fed the Aa diet are more resistant to the induction of nasal tolerance. Our animal model may mimic the features of the immune system of human infants.

KEYWORDS: diet; tolerance; asthma; eosinophils; IgE

INTRODUCTION

Allergic asthma results from an intrapulmonary allergen-driven Th2 response and is characterized by intermittent airway obstruction, airway hyperreactivity, and airway inflammation. Intranasal (i.n.) administration of soluble proteins (allergens) before immunization induces a state of specific unresponsiveness known as nasal tolerance.¹ However, in human infants airborne allergens may cause long-term allergic sensitization instead of inducing tolerance.² Indeed, mucosal tolerance to allergens is relatively slow to develop in newborns.^{3,4}

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We have previously shown that mice fed a diet in which intact dietary proteins were replaced by equivalent amounts of amino acids from weaning (day 21 of life), presented an immunological profile similar to neonates.⁵ Adult animals reared in this balanced amino acid-based protein-free diet (Aa-fed mice) grew and looked like normal mice but showed local and systemic abnormalities in their immune system. Aa-fed mice had poorly developed gut-associated lymphoid tissue (GALT), and presented a predominant Th2 profile, resembling preweaned mice.⁵ Most of the reports on the impairment in Th1 responses in neonates refers to the neonatal period as the early postnatal time in suckling animals.^{4,6} In the present work, we examined whether animals fed an Aa diet are susceptible to the induction of nasal tolerance. We used ovalbumin (OVA) as allergen in an established murine model of allergic asthma.⁷

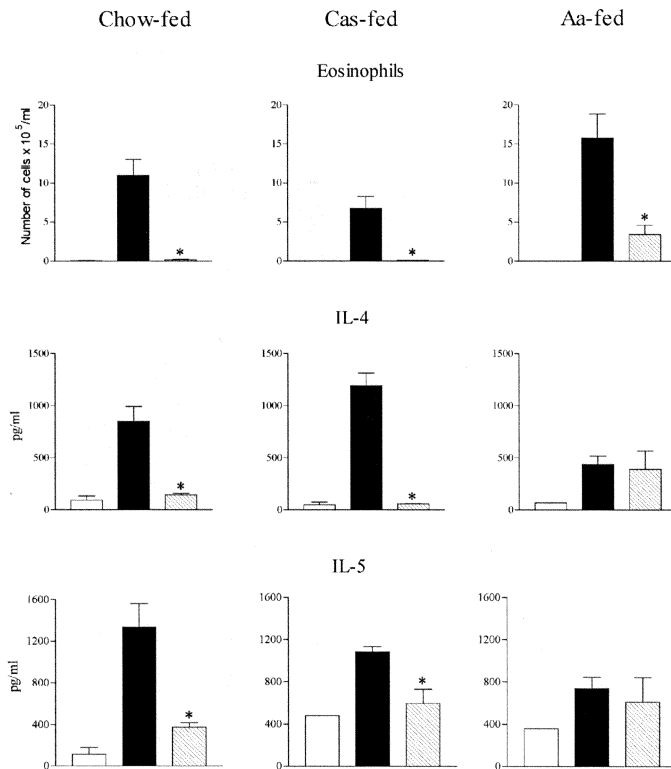


FIGURE 1. The effect of nasal OVA administration on antigen-induced airway eosinophilic inflammation and BAL fluid IL-4 and IL-5 levels in Chow-fed (*left*), Cas-fed (*middle*), or Aa-fed (*right*) BALB/c mice. Groups of five mice received OVA 100 μ g i.n. (nasal) or PBS i.n. (immune) for three consecutive days, before OVA-alum immunization and challenge. The control group received only OVA intranasally. Experiments were performed 24 h after the last OVA challenge. Results are expressed as means $\pm$ standard error of the mean (SEM). *Significant difference ($P < .05$) between values of Immune versus Nasal groups. *Open bars*, control; *black bars*, immune; *striped bars*, nasal.

MATERIALS AND METHODS

BALB/c mice were fed normal chow (Chow), casein (Cas)-, or an amino acid-based protein-free diet (Aa) after weaning (21 days of life), as previously described.⁵ At 7 weeks of age, groups of five animals received OVA 100 μ g/50 μ L PBS i.n. delivered into the nostrils for 3 consecutive days to induce nasal tolerance. Control animals received only 50 μ L PBS. Four days after, the animals were immunized, and boosted at weekly intervals, by a subcutaneous injection of OVA (Sigma grade V) 4 μ g/1.6 mg alum, followed by two intranasal OVA challenges with 10 μ g OVA/50 μ L PBS. Twenty-four hours after the last intranasal challenge, lungs were lavaged (BAL). Total and differential cell counts and the levels of IL-4 and IL-5 in the BAL fluid were assessed by hemocytometer and cytospin preparation, and by ELISA, respectively, and OVA-specific IgE levels of serum samples were related to an internal standard that was set at 10,000 arbitrary units (A.U.) as previously described.⁷

RESULTS

Both normal Chow- and Cas-fed BALB/c mice that received OVA intranasally before OVA immunization and challenge showed almost complete inhibition (>99%) of airway eosinophilia when compared with OVA-immunized and challenged animals that received i.n. PBS (FIG. 1). The inhibitory effect of nasal OVA administration was less pronounced (~75% of inhibition) in Aa-fed animals (FIG. 1). Similar results were obtained when the total number of BAL cells was determined (data not shown).

Nasal tolerance in Chow- or Cas-fed animals also suppressed airway type 2 cytokine production (IL-4 and IL-5) (FIG. 1). In addition, intranasal OVA administration resulted in a significant suppression of OVA-specific IgE antibody production

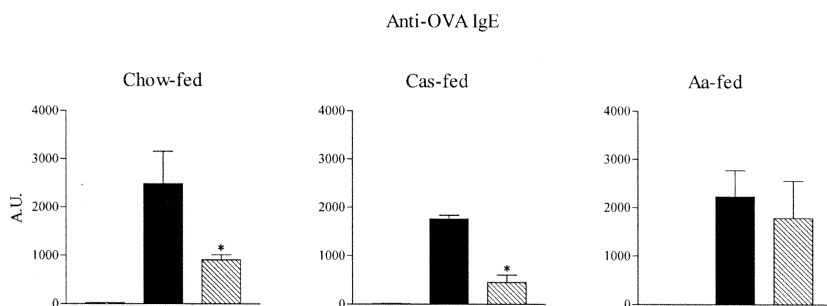


FIGURE 2. The effect of nasal OVA administration on antigen-induced anti-OVA IgE antibody production in Chow-fed (*left*), Cas-fed (*middle*), or Aa-fed (*right*) BALB/c mice. Groups of five mice received OVA 100 μ g i.n. (nasal) or PBS i.n. (immune) for three consecutive days before OVA-alum immunization and challenge. The control group received only OVA intranasally. Experiments were performed 24 h after last OVA challenge. Results are expressed as arbitrary units of means $\pm$ standard error of the mean (SEM). *Significant difference ($P < .05$) between values of immune versus nasal groups. *Open bars*, control; *black bars*, immune; *striped bars*, nasal.

in Chow- or Cas-fed animals (FIG. 2). In contrast, nasal OVA administration did not suppress type 2 cytokine production or OVA-specific IgE response in Aa-fed animals (FIGS. 1 and 2).

DISCUSSION

The results presented here show that nasal tolerance is fully effective in suppressing asthma-like responses in Chow- or Cas-fed animals, but not in Aa-fed animals. The resistance of Aa-fed animals to nasal tolerance was clearly documented by the elevated production of IL-4 and IL-5 cytokines, as well as the high levels of OVA-specific IgE antibodies in the nasally treated group. Interestingly, airway eosinophilia was significantly suppressed (~75%) in these mice despite the high levels of IL-5 attained in their BAL fluid. It is known that IL-5 and eotaxin cooperate locally in pulmonary tissues to selectively and synergistically promote eosinophilia.⁸ Thus, the inhibition of airway eosinophilia observed in Aa-fed animals could be attributed in part to inhibition of chemokines such as eotaxin, or RANTES and MIP-1 α that are also involved in eosinophilic inflammation.^{9,10} Conversely, the high production of IgE antibodies in these Aa-fed mice is consistent with the lack of suppression of IL-4 by intranasal OVA administration, inasmuch as IL-4 and IL-13 are key cytokines for IgE production.^{11,12}

Our results are in consonance with a previous study showing that the nasal tolerance for IgE response does not function during the early postnatal period,³ and we extend this finding to type 2 cytokine production and airway eosinophilia. The mechanism(s) responsible for the partial resistance to nasal tolerance in Aa-fed animals remains to be determined.

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REFERENCES

1. HOLT, P.G. 1997. Development of sensitization versus tolerance to inhalant allergens during early life. *Pediatr. Pulmonol. Suppl.* **16**: 6–7.
2. HOLT, P.G. 1994. Immunoprophylaxis of atopy: light at the end of the tunnel? *Immunol. Today* **15**: 484–489.
3. HOLT, P.G., J. VINES & D. BRITTEN. 1988. Suppression of IgE responses by antigen inhalation: failure of tolerance mechanism(s) in newborn rats. *Immunology* **63**: 591–593.
4. NELSON, D.J. & P.G. HOLT. 1995. Defective regional immunity in the respiratory tract of neonates is attributable to hyporesponsiveness of local dendritic cells to activation signals. *J. Immunol.* **155**: 3517–3524.
5. MENEZES, J.S. *et al.* 2003. Stimulation by food proteins plays a critical role in the maturation of the immune system. *Int. Immunol.* **15**: 447–455.
6. HUSBAND, A.J. & M. GLEESON. 1996. Ontogeny of mucosal immunity. Environmental and behavioral influences. *Brain Behav. Immun.* **10**: 188–204.

7. RUSSO, M. *et al.* 2001. Suppression of asthma-like responses in different mouse strains by oral tolerance. *Am. J. Respir. Cell Mol. Biol.* **24**: 518–526.
8. FOSTER, P.S. *et al.* 2001. Elemental signals regulating eosinophil accumulation in the lung. *Immunol. Rev.* **179**: 173–181.
9. JIA, G.Q. *et al.* 1999. Selective eosinophil transendothelial migration triggered by eotaxin via modulation of Mac-1/ICAM-1 and VLA-4/VCAM-1 interactions. *Int. Immunol.* **11**: 1–10.
10. GONZALO, J.A. *et al.* 1996. Eosinophil recruitment to the lung in a murine model of allergic inflammation. The role of T cells, chemokines, and adhesion receptors. *J. Clin. Invest.* **98**: 2332–2345.
11. FINKELMAN, F.D. *et al.* 1988. IL-4 is required to generate and sustain in vivo IgE responses. *J. Immunol.* **141**: 2335–2341.
12. WILLS-KARP, M. *et al.* 1998. Interleukin-13: central mediator of allergic asthma. *Science* **282**: 2258–2261.