Experimental gastrointestinal allergy enhances pulmonary responses to specific and unrelated allergens

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Mechanisms of asthma and allergic inflammation

Background: Gastrointestinal allergy often precedes or coexists with respiratory allergy.

Objective: We hypothesized that established experimental gastrointestinal allergy would prime for the development of allergic respiratory responses.

Methods: BALB/c mice were sensitized with ovalbumin (OVA) in the presence of aluminum potassium sulfate and then subjected to intragastric saline or OVA challenges. After the development of allergen-induced gastrointestinal allergy, mice were intranasally exposed to either saline, OVA, or a neoaeroallergen house dust mite (HDM) extract. Airway inflammation (eg, bronchoalveolar lavage fluid cellularity, cytokine levels, and OVA-specific antibody levels) and airway responsiveness to methacholine exposure were assessed after intranasal allergen exposure.

Results: A single intranasal exposure to OVA induced significantly more airway inflammation in intragastric OVA-challenged mice compared with that seen in intragastric saline-treated mice. Kinetic analysis revealed that the observed amplification of lung inflammation was sustained for up to 12 days after the last intragastric OVA challenge after resolution of blood eosinophilia. When mice with gastrointestinal allergy were repeatedly challenged with HDM in the respiratory tract, they experienced enhanced airway inflammation, including bronchoalveolar lavage fluid eosinophilia and increased IL-13 levels.

Conclusion: Taken together, our results demonstrate that OVA-induced gastrointestinal allergy enhances not only allergic airway responses to OVA but also to HDM, an unrelated aeroallergen.

Clinical implications: Experimental gastrointestinal allergy primes for responses to allergens in the respiratory tract, enhancing antigen-specific antibody and T_H2 cytokine production, airway inflammation, and airway hyper-responsiveness. (J Allergy Clin Immunol 2006;118:420-7.)

Key words: *Allergy, lung, intestine, eosinophil, eotaxin, IL-13, house dust mite, ovalbumin, mice*

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The most common clinical manifestations of allergic responses to food involve the gastrointestinal tract, but the skin and respiratory tract are also frequently involved.[1,2] Gastrointestinal allergy to ingested foods often precedes or coexists with upper and lower respiratory tract symptoms.[1,2] A wide variety of respiratory symptoms have been attributed to food allergy, including nasal congestion, rhinorrhea, sneezing, coughing, and wheezing. Although food-induced respiratory symptoms are less frequent, their presence generally indicates a more severe disease manifestation.[3] The incidence of respiratory reactions induced by food is estimated to be between 2% and 8% in children and adults with asthma.[2] Although oral ingestion is the primary route of exposure to food that results in respiratory symptoms, asthmatic responses can also occur from direct inhalation of aerosolized food particles.[4,5] Food-induced asthma is generally observed during IgE-mediated systemic allergic reactions to ingested proteins.[6] Notably, food allergy in early childhood is associated with an increased risk of allergic airway disease.[7-9]

Allergen sensitization is an important risk factor for asthma development because approximately two thirds of asthmatic patients are sensitized to at least one inhalant allergen.[10] Indeed, sensitization to multiple allergens increases with age, suggesting that polysensitization is the natural history of allergy in atopic individuals.[10] Although numerous studies have focused on the cause for the increase in the incidence of allergic disease over the last few decades,[11,12] it remains unclear how an atopic individual progresses from nonsensitization to polysensitization. Furthermore, the role of an established allergen-specific T_H2-mediated inflammatory response in the sensitization to new antigens is unclear.

We have recently developed a mouse model of oral allergen–induced gastrointestinal allergy accompanied by strong T_H2-associated humoral and cellular responses, including intestinal eosinophilia and mastocytosis and the occurrence of diarrhea.[13,14] We hypothesized that an established inflammation in the gastrointestinal tract predisposes to allergic airway responses. Accordingly, we

Abbreviations used

Alum: Aluminum potassium sulfate
BALF: Bronchoalveolar lavage fluid
HDM: House dust mite
OVA: Ovalbumin
PAS: Periodic acid–Schiff
Penh: Enhanced pause

subjected mice to an intranasal challenge with the same food allergen (ovalbumin [OVA]) and examined the consequences of pre-existing gastrointestinal allergy on the development of respiratory immune responses. Furthermore, we examined collateral priming to an unrelated aeroallergen (house dust mite [HDM]) in mice with gastrointestinal allergy.

METHODS

Animals

BALB/c mice were obtained from the National Cancer Institute (Bethesda, Md). Experiments were performed on sex- and age-matched mice (5-7 weeks). All mice were housed according to institutional guidelines in a pathogen-free environment. Water was available continuously through automatic ports, and a commercial mouse diet was provided *ad libitum*.

Induction of intestinal anaphylaxis

Mice were sensitized and intragastrically challenged with chicken OVA (grade V; A-5503; Sigma-Aldrich, St Louis, Mo), as previously described.[14] Briefly, the mice were intraperitoneally sensitized twice with 50 μg of OVA in the presence of 1 mg of the aluminum potassium sulfate adjuvant (alum; $ALK[SO_4]_2$-$12H_2O$; A-7210; Sigma-Aldrich, Milwaukee, Wis). Two weeks later, the mice were fed either 50 mg of OVA suspended in 250 μL or 0.9% saline as a control through an intragastric feeding needle (22-gauge, 1.5-inch, 1.25-mm ball; 01-290-2B; Fisher Scientific, Pittsburg, Pa). The challenges were performed 3 times per week (every other day) for a total of 6 challenges. The mice were observed for up to 1 hour after each challenge to observe the induction of intestinal anaphylaxis, as evidenced by the onset of profuse liquid diarrhea.

OVA-induced airway inflammation

One day after the completion of the intragastric challenges, the mice were subjected to a single intranasal administration with 50 μL of OVA (1 mg/mL) or sterile saline. A protocol of delayed asthma induction was performed with a single intranasal challenge at either 6 or 11 days after the last intragastric challenge to isolate the effect of the systemic response after intestinal allergy.

Induction of cross-sensitization to HDM

Sensitization and intragastric challenges were performed with OVA, as described above. The mice were subjected to a series of 4 intranasal challenges with 50 μL of saline or HDM 1 day after the third, fifth, and sixth intragastric challenges, respectively. HDM extract (Greer laboratories, Lenoir, NC) was resuspended in saline at a concentration of 1.3 mg/mL (50 μL represents 65 μg of total protein and 10 μg of Der p 1 according to the manufacturer).

Airway function measurements

Increases in airway resistance to aerosolized methacholine (3.125-50 mg/mL) were determined as enhanced pause (Penh), a dimensionless value, after 5 minutes of aerosol exposure by using whole-body plethysmography, as previously reported.[15] The data are expressed as the percentage increase in Penh over baseline (PBS).

Bronchoalveolar lavage fluid

Mice were killed by means of carbon dioxide inhalation 20 hours after the last intranasal challenge, and bronchoalveolar lavage fluid (BALF) was obtained as previously reported.[16] Slides were stained with a modified Diff-Quick protocol (HEMA3 from Fisher Scientific, Kalamazoo, Mich). All samples were evaluated in a blind manner.

Lung histopathology

The lungs were fixed in formalin, embedded in paraffin, and stained with either hematoxylin and eosin or periodic acid–Schiff (PAS). PAS-stained airway goblet cells were enumerated by means of light microscopic examination (×40 objective), as previously reported.[16] The number of PAS-positive and PAS-negative epithelial cells in individual bronchioles was counted to quantitate the level of mucus expression in the airway. At least 3 medium-sized bronchioles (defined by having approximately 75-150 luminal airway epithelial cells) were counted in each slide. Results are expressed as the percentage of PAS-positive cells per bronchiole, which is calculated from the number of PAS-positive epithelial cells per bronchiole divided by the total number of epithelial cells of each bronchiole.

Quantification of blood eosinophils

Blood was collected by means of retro-orbital bleeding, as previously described,[17] and diluted (1:10) in Discombe's solution, which was prepared by mixing 1 volume of 1% aqueous eosin Y (Fischer Scientific, Fair Lawn, NJ) with 1 volume of acetone and finally diluting 1:10 in water. For each sample, eosinophils per cubic millimeter of the hemocytometer chamber (Hausser Scientific Partnership, Horsham, Pa) were counted, and the final result was expressed as the number of eosinophils per milliliter of blood.

ELISA measurements

BALF and plasma were collected 20 hours after the last intranasal challenge. IL-13 BALF levels and total IgE BALF and plasma levels were measured by using an ELISA kit, according to the manufacturer's instructions (R&D Systems, Minneapolis, Minn, and BD Biosciences-Pharmingen, San Diego, Calif, respectively). Allergen-specific IgG1 and IgA levels were measured after coating 96-well Immulon plates with 100 μL of OVA (100 μg/mL) in carbonate buffer and overnight incubation. Blocking was done with 10% FCS in PBS for sera samples and 3% BSA for BALF samples, and all washes were performed with 0.1% Tween-20 in PBS. Plasma samples were diluted 1:1000 for IgG1 and 1:10 for IgA, whereas BALF samples were undiluted for IgA and diluted 1:20 for IgG1 before serial dilutions of 1:4. After 2 hours of incubation, plates were washed, and horseradish peroxidase–conjugated rabbit anti-mouse IgG1 (1:1000; X56; BD Pharmingen) was added. The plates were developed with tetramethylbenzidine (TMB Substrate Reagent set; BD Pharmingen) and quenched with 2 M HCL. The OD was read within 5 minutes at 450 nm on an Ultra Microplate Reader (Bio-tek Instruments, Winooski, Vt). Data represent the mean ± SEM of the plasma dilution to obtain a specific OD within the linear portion of the curve for all samples assayed.

Statistical analysis

Data are expressed as means ± SEM. Statistical significance comparing different sets of mice was determined by using the Student unpaired t test or the Mann-Whitney U test when appropriate.

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