

Experimental gastrointestinal allergy enhances pulmonary responses to specific and unrelated allergens

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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 neo-aeroallergen 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

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

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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; $\text{ALK}[\text{SO}_4]_2 \cdot 12\text{H}_2\text{O}$; 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 ($\times 40$ 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 $\pm$ 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 $\pm$ 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.

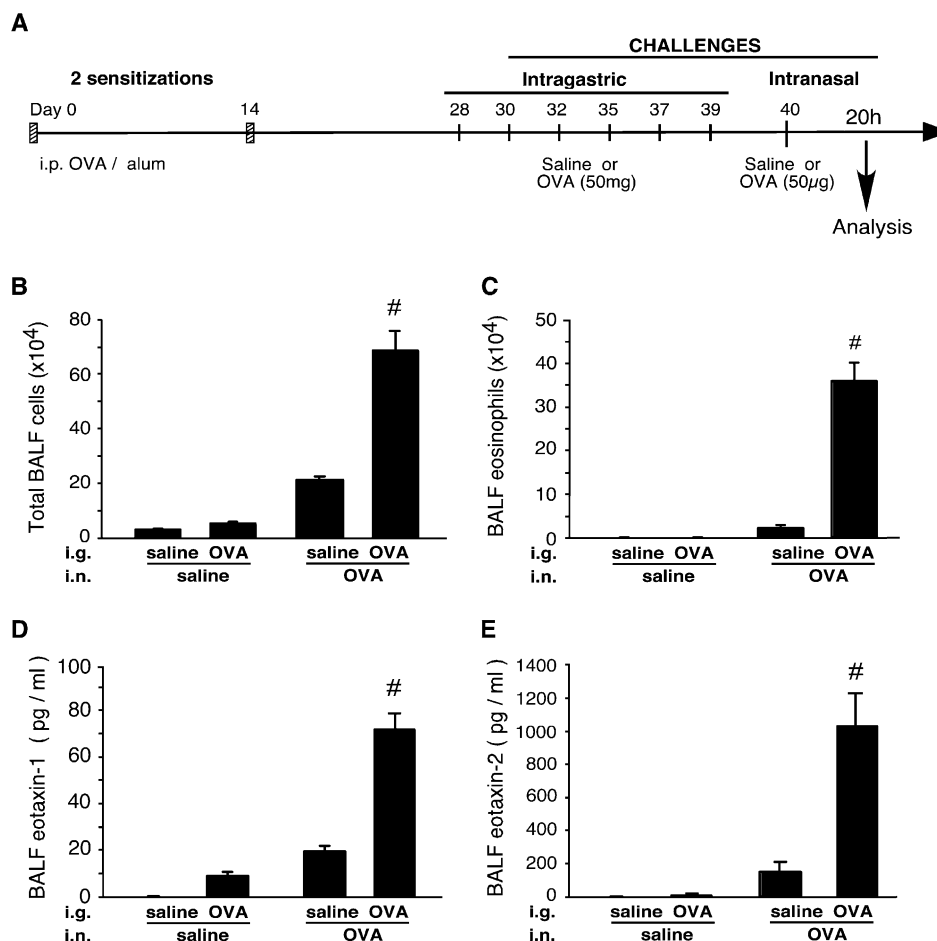


FIG 1. OVA-induced gastrointestinal allergy increases BALF inflammation after a single intranasal allergen challenge. **A**, After OVA/alum sensitization and 6 intragastric saline or OVA challenges, mice underwent a single intranasal challenge with saline or 50 μ g of OVA as depicted. Total number of cells recruited into the BALF (**B**), as well as BALF eosinophils (**C**), eotaxin-1 levels (**D**), and eotaxin-2 levels (**E**) were assessed 20 hours after the intranasal challenge and expressed as mean $\pm$ SEM of 3 separate experiments ($n = 8$ -19 mice per group). Statistical significance between mice with and without gastrointestinal allergy was assessed by using the Mann-Whitney U test ($\#P < .001$). *i.p.*, Intraperitoneal; *i.g.*, intragastric; *i.n.*, intranasal.

Multiple comparisons were done with 1-way ANOVA, followed by the appropriate post-hoc test.

RESULTS

Gastrointestinal allergy increases BALF inflammation after a single intranasal allergen challenge

We have previously shown that intraperitoneal sensitization with OVA/aluminum potassium sulfate (alum) primes for gastrointestinal allergy after intragastric OVA challenges.¹⁴ In addition to gastrointestinal mastocytosis and eosinophilia, the mice also display systemic T_H2 responses, including blood eosinophilia and increased OVA-specific IgG1 and IgE levels. We hypothesized that distant mucosal sites, such as the lung, might also be primed by this T_H2 response. We were thus interested in assessing the importance of gastrointestinal

inflammation on responses to respiratory OVA exposure. After intragastric saline or OVA administration, mice were exposed to a single intranasal saline or OVA challenge (Fig 1, A). To evaluate the effect of OVA/alum sensitization alone on the intranasal challenge, we intragastrically challenged mice with saline. In the absence of OVA-induced gastrointestinal allergy, a significant but limited increase in total BALF cells was observed ($21.0 \pm 1.5 \times 10^4$ vs $3.0 \pm 0.4 \times 10^4$, $P < .0001$) after a single intranasal OVA or saline challenge, respectively. In mice with established gastrointestinal allergy, intranasal OVA challenge resulted in significant BALF inflammation compared with that seen in mice intranasally challenged with saline ($68.4 \pm 7.3 \times 10^4$ vs $5.2 \pm 0.6 \times 10^4$, $P < .0001$). More importantly, when compared with mice intragastrically challenged with saline, a 3-fold increase was observed after airway OVA exposure (Fig 1, B). The majority of infiltrating BALF cells in

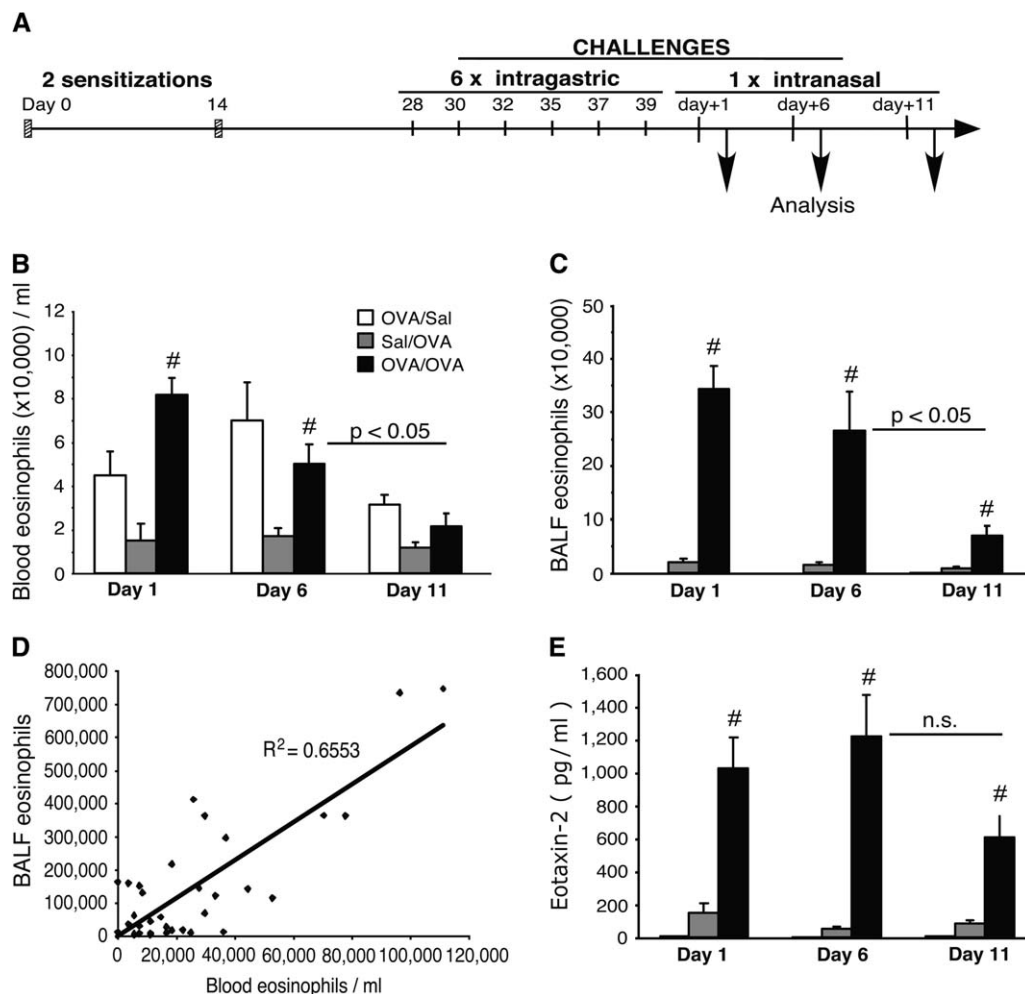


FIG 2. Eosinophilia after delayed intranasal OVA challenge. **A**, All mice received a single intranasal challenge with OVA or saline either 1, 6, or 11 days after the last intragastric challenge. **B**, Peripheral blood eosinophil levels were assessed by means of Discombe staining and expressed as the mean $\pm$ SEM of 2 to 4 separate experiments. **C**, Total number of BALF eosinophils and BALF eotaxin-2 levels (**E**) were assessed 20 hours after the intranasal challenge. All BALF results are expressed as the mean $\pm$ SEM of 4 separate experiments ($n = 8-16$ mice per group). Statistical significance between mice with or without allergic diarrhea was assessed by using the Mann-Whitney U test (# $P < .005$). Comparisons between day 1, day 6, and day 11 were done with 1-way ANOVA with the Dunn post-hoc test. **D**, Correlation between total BALF eosinophil numbers and blood eosinophil levels was assessed in all mice challenged intranasally with OVA. The results of the Spearman non-parametric correlation test were significant ($P < .005$). Sal, Saline; n.s., not significant.

mice with gastrointestinal allergy were eosinophils ($52.1\% \pm 2.7\%$ vs $9.0\% \pm 2.2\%$), resulting in a 15-fold increase in total BALF eosinophils (Fig 1, C).

Because eotaxin-1 and eotaxin-2 are eosinophil-selective chemokines,¹⁸ we measured protein levels in the BALF. Importantly, when mice with and without gastrointestinal allergy were compared, BALF eotaxin-1 (Fig 1, D) and eotaxin-2 (Fig 1, E) levels were significantly increased 3.5-fold and 7-fold, respectively ($P < .0001$). Taken together with a recent report demonstrating that BALF eotaxin-2 (and not eotaxin-1) represents the main chemokine responsible for eosinophil accumulation into the BALF,¹⁹ our results suggest that gastrointestinal allergy promotes the secretion of increased amounts of eotaxin-2 in the BALF, thus inducing a profound BALF eosinophilia.

To examine the kinetics of this experimental system, we administered intranasal OVA on various days after the last gastrointestinal OVA challenge (specifically 1, 6, or 11 days after intragastric OVA challenge). We then examined blood and BALF eosinophilia 20 hours after intranasal OVA challenge (Fig 2, A). The importance of allergen-induced intestinal inflammation on eosinophil blood levels is highlighted by the absence of any blood eosinophilia after the intranasal OVA challenge in mice without allergen-induced diarrhea (Fig 2, B, saline/OVA group). After allergic diarrhea and in the absence of any new allergen challenge to the intestine or the lungs, blood eosinophilia returned to baseline levels around day 12 (Fig 2, B). Gastrointestinal eosinophil levels, which were still increased 48 hours after the last OVA challenge

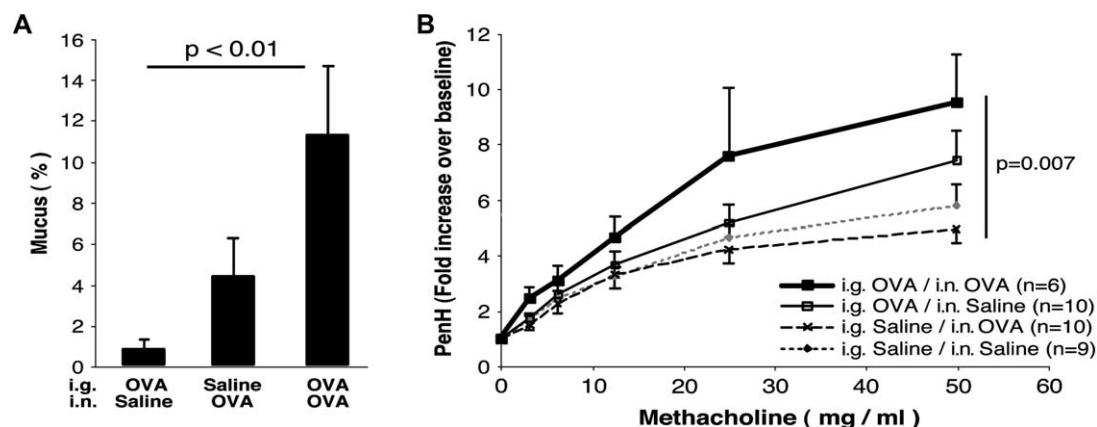


FIG 3. Gastrointestinal allergy followed by a single intranasal allergen challenge increases allergic airway responses. **A**, The percentage of mucus-producing epithelial cells was assessed in bronchioles 20 hours after the intranasal OVA challenge ($n = 10$ –15 mice per group). **B**, Methacholine sensitivity was determined 20 hours after the single intranasal saline or OVA challenge by means of whole-body plethysmography (Buxco). Results are expressed as mean $\pm$ SEM of the fold increase over baseline (6–10 mice per group), and statistical significance for multiple comparisons was assessed by using 1-way ANOVA with the Tukey-Kramer post-hoc test. *i.g.*, Intragastric; *i.n.*, intranasal.

(497 ± 95 and 515 ± 159 eosinophils/mm² after intranasal saline or OVA challenge on day 1, respectively) returned to baseline within 1 week (256 ± 48 and 249 ± 32 eosinophils/mm², respectively).

Importantly, BALF eosinophil levels were still significantly higher on day 11 in mice with allergic diarrhea when compared with those seen in mice challenged intragastrically with saline (Fig 2, C). Although a correlation exists between blood eosinophilia and increased eosinophil recruitment into the BALF (Fig 2, D; $P < .005$), in the absence of an allergen challenge to the airways, no BALF eosinophilia was observed (Fig 2, C). In accordance with its role in eosinophil recruitment into the BALF,¹⁹ eotaxin-2 BALF levels were still increased on day 11 (Fig 2, E) and correlated well with sustained eosinophil BALF levels ($P < .0001$).

Gastrointestinal allergy followed by a single intranasal allergen challenge increases allergic airway responses

In addition to airway eosinophilia, other hallmarks of allergic lung inflammation include IL-13 production, mucus secretion, and airway hyperresponsiveness.²⁰ Although IL-13 BALF levels were barely detectable (data not shown), an increased number of mucus-producing epithelial cells was observed after the single intranasal OVA challenge (Fig 3, A). When subjected to increasing doses of aerosolized methacholine, mice with gastrointestinal allergy had increased Penh values after intranasal OVA exposure compared with values seen in mice with gastrointestinal allergy but exposed to intranasal saline (Fig 3, B; $P < .007$). Of note, gastrointestinal allergy by itself failed to promote increased Penh values (data not shown). This was further confirmed when comparing mice intranasally challenged with saline (Fig 3, B). Taken together, these results show that allergen-induced inflammatory events taking place in the intestinal mucosa

have a profound effect on pulmonary responses to the same antigen.

OVA-induced gastrointestinal allergy increases BALF eosinophilia after exposure to an unrelated aeroallergen (HDM) challenge

To determine whether gastrointestinal allergy would prime for allergic respiratory responses to an unrelated antigen, we exposed sensitized mice to intragastric saline or OVA and to 3 or 4 intranasal doses of HDM, as described in Fig 4, A. Although a significant increase in total BALF eosinophil numbers was observed after only 3 intranasal challenges ($14 \pm 8 \times 10^3$ vs $73 \pm 21 \times 10^3$, $P < .05$), we opted for a fourth HDM administration 1 week later to maximize the adaptive immune response to HDM. Indeed, only 1 week after the first administration of HDM, HDM-specific IgG1 levels were barely detectable after the third intranasal challenge (titers ranged between undetectable and 1:100). However, after the fourth intranasal HDM challenge, significant levels of HDM-specific IgG1 were observed in the plasma and BALF of HDM-challenged mice. Importantly, no significant increase in HDM-specific IgG1 blood levels was observed in HDM-exposed mice with gastrointestinal allergy compared with levels seen in mice only exposed to HDM (titers were $1:1216 \pm 255$ vs $1:1103 \pm 217$, respectively). Similarly, BALF levels of HDM-specific IgG1 and IgA were also not induced (data not shown). Furthermore, spleen and lymph node cells were isolated and stimulated *in vitro* with either OVA or HDM. Although all spleen cells proliferated to OVA (because all mice were intraperitoneally sensitized to OVA), only the mice challenged with HDM proliferated to HDM, albeit weakly and inconsistently (data not shown). No significant increase in HDM-induced proliferation could therefore be observed in either draining lymph node or spleen cells of mice with OVA-induced gastrointestinal allergy.

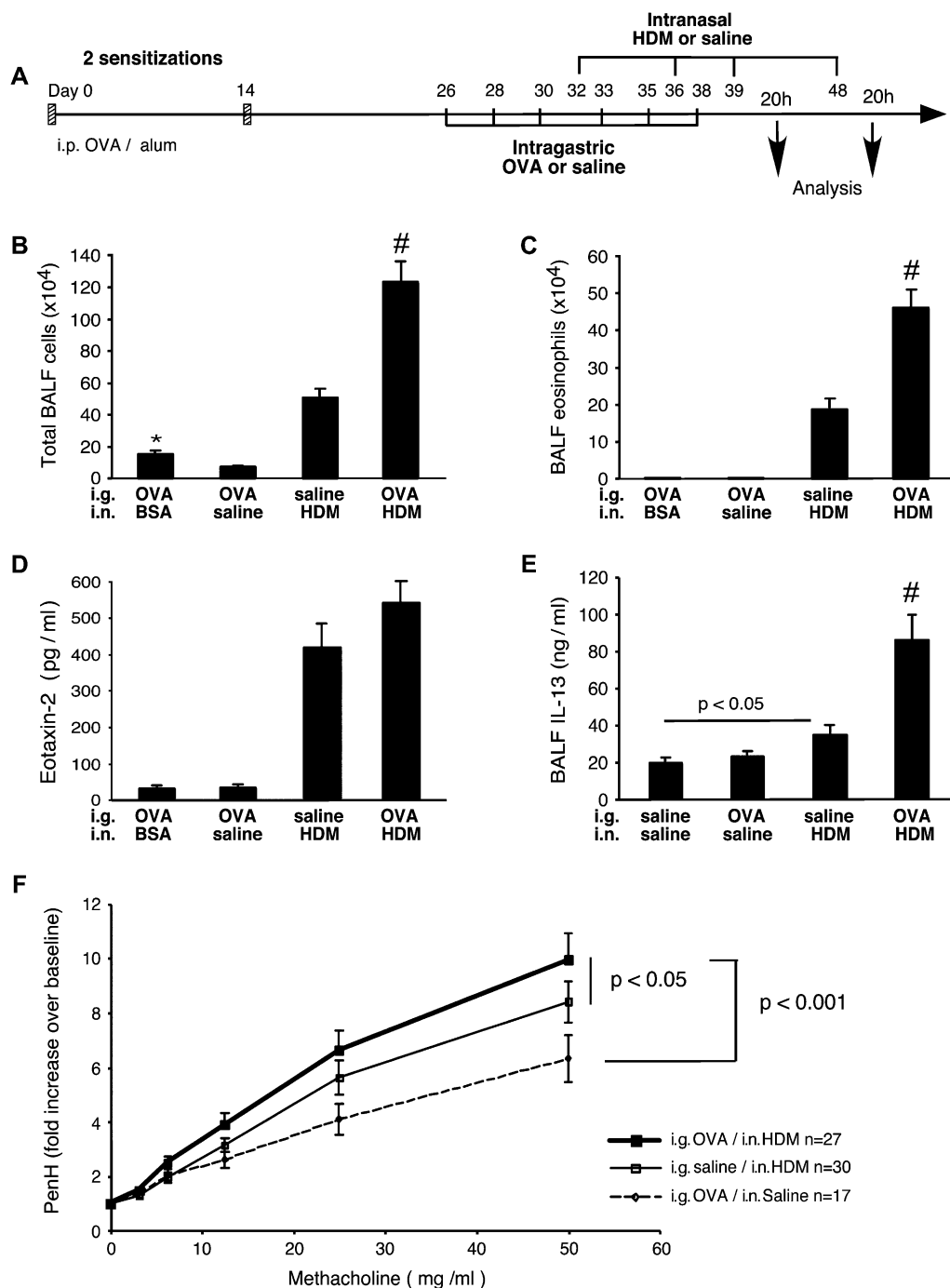


FIG 4. BALF eosinophilia after intranasal challenge with an unrelated allergen, HDM. **A**, Timeline representation with intragastric OVA challenges and intranasal HDM administration. Total number of BALF cells (**B**) and BALF eosinophilia (**C**) were assessed 20 hours after the fourth intranasal saline or HDM challenge. All results are expressed as mean $\pm$ SEM of 3 separate experiments for 4 $\times$ HDM ($n = 17$ -19 mice per group). Eotaxin-2 (**D**) and IL-13 (**E**) BALF levels were assessed 20 hours after 4 intranasal HDM challenges ($n = 17$ -20 mice per group). Statistical significance between mice with and without gastrointestinal allergy was assessed by using the Mann-Whitney U test or t test when appropriate (* $P < .05$, # $P < .01$). **F**, Methacholine sensitivity was determined 20 hours after the single intranasal saline or HDM challenge by means of whole-body plethysmography (Buxco). Results are expressed as mean $\pm$ SEM of the fold increase over baseline (17-30 mice per group), and statistical significance for multiple comparisons was assessed by using 1-way ANOVA with the Tukey-Kramer post-hoc test. *i.p.*, Intraperitoneal; *i.g.*, intragastric; *i.n.*, intranasal.

Nevertheless, a significant increase in total BALF cells was observed in mice with allergic diarrhea when compared with the BALF inflammation observed in mice only challenged with HDM (Fig 4, B; $P < .001$). Importantly, exposure to HDM led to significantly higher BALF eosinophilia in mice with acute OVA-induced diarrhea (Fig 4, C; $P < .05$). Of note, neutrophils and lymphocytes were also significantly increased after HDM challenges in mice with OVA-induced gastrointestinal allergy (data not shown), albeit more modestly than BALF eosinophils.

To determine whether the inflammatory response in the lung was allergen specific, we intranasally challenged mice with gastrointestinal allergy with BSA instead of HDM. Although a mild but significant increase in total BALF cells was observed (Fig 4, B), BALF eosinophilia was not significantly different from that seen after a saline challenge ($2.0 \pm 0.9 \times 10^3$ vs $1.2 \pm 0.5 \times 10^3$, $P > .05$) and was not associated with increased BALF eotaxin-2 levels (Fig 4, D).

IL-13 is an essential cytokine for allergic airway responses,²⁰ and as such, we hypothesized that gastrointestinal allergy would prime for respiratory allergen-induced IL-13 production. Notably, in mice with OVA-induced allergic diarrhea, respiratory HDM exposure was associated with increased IL-13 BALF levels (Fig 4, E). Next we assessed airway responses by means of whole-body plethysmography. When subjected to increasing doses of aerosolized methacholine, mice with intranasal HDM exposure had a significant increase in Penh values (Fig 4, F; $P < .001$). Notably, a modest increase in Penh was observed in mice with acute diarrhea when compared with that seen in their respective control animals (Fig 4, F; $P < .05$).

DISCUSSION

The present study demonstrates that allergic reactions at one mucosal site can prime for similar responses in distant mucosal sites. Although several studies have suggested that skin sensitization can lead to an increase in allergic airway responses,^{15,21-24} the present study demonstrates that gastrointestinal allergic reactions increase the severity of the pulmonary response after an acute allergen exposure in the lung. As such, our experimental model offers new insight into the cross-talk between gastrointestinal allergy and asthma.

We first demonstrated that OVA-induced gastrointestinal allergy significantly increased airway inflammation compared with sensitization alone. Although neutrophils normally represent the major infiltrating granulocyte during the first 24 hours after a single intranasal allergen challenge, the increased recruitment of eosinophils compared with neutrophils is likely the combined result of increased eotaxin-2 BALF levels and increased eosinophil blood levels resulting from gastrointestinal eosinophilia. Although blood eosinophilia correlated with BALF eosinophilia after intranasal challenge, this is not the case in the absence of intranasal allergen exposure, in which neither lung eotaxin-2 mRNA levels (data not shown) nor BALF eotaxin-2 protein levels were significantly increased. Only

eotaxin-2 BALF levels strongly correlated with BALF eosinophil levels in all groups. Indeed, in the context of an IL-13-driven allergic airway response, eotaxin-2 has been shown to have a central role in eosinophil recruitment from the lung tissue into the airway lumen.^{19,25}

Although airway neutrophilia does not generally affect development of airway hyperresponsiveness,²⁶ several recent studies have convincingly demonstrated that eosinophil recruitment to the lungs has a critical role in several hallmarks of experimental asthma, including hyperresponsiveness to methacholine challenge.²⁷⁻²⁹ Indeed, we demonstrate increased airway hyperresponsiveness after a single intranasal OVA challenge in mice with gastrointestinal allergy compared with that seen in control mice not subjected to gastrointestinal allergen challenge. Interestingly, it has recently been demonstrated that overexpressing eotaxin-1, specifically in the small intestine, results in intestinal eosinophilia associated with bronchial hyperresponsiveness.³⁰ However, gastrointestinal allergy by itself failed to induce airway hyperresponsiveness (data not shown and Fig 3). Although the 2 models differ quite significantly, it remains possible that the modest increase observed in the Penh ratio could become significant with a larger number of mice.

More relevant to the natural course of the disease, we were able to demonstrate that gastrointestinal allergy primes for allergic responses to unrelated allergens (HDM). Although a single dose of HDM administered 1 day after the last intragastric OVA challenge did not induce any BALF inflammation over that seen after a saline control (data not shown), a significant eosinophil infiltrate occurred after 3 challenges. After a fourth challenge, 2 weeks after the first airway exposure to HDM, a significant BALF inflammation was associated with HDM-specific T- and B-cell responses. A recent study using a regimen of 5 HDM doses per week for up to 7 weeks demonstrated that after a week of exposure to HDM, BALF inflammation was not significantly different from that seen in naive mice; continuous exposure over a 3-week period was needed to generate an HDM-specific T_H2 humoral response,³¹ which was likely mediated by GM-CSF.³² The proteolytic activity of the major mite allergen Der p 1 is believed to have a role in disrupting the airway epithelium³³ and promoting a local T_H2 response.³⁴

To better understand why gastrointestinal allergy to ingested foods often precedes or coexists with upper and lower respiratory tract symptoms,^{1,2} our study focused on the role that gastrointestinal allergic reactions have on the development of allergic airway responses to an unrelated aeroallergen. It has been suggested that pre-exposure to HDM a few hours before airway exposure to OVA could facilitate the penetration of the bystander allergen and overcome tolerance normally observed after multiple intranasal OVA doses, probably because of the proteolytic activity of Der p 1.^{35,36} Rather than investigating the additive effects of OVA and HDM administration to a single compartment, we decided to examine 2 distinct mucosal tissues relevant to the food allergen OVA and the aeroallergen HDM. Notably, OVA-induced gastrointestinal allergy was associated with increased HDM-induced

airway inflammation. Furthermore, although IL-13 BALF levels were significantly higher after acute allergic diarrhea, gastrointestinal allergy had no significant effect on HDM-specific immune responses under our experimental conditions. Perhaps additional HDM challenges might yield a significant increase in HDM-specific immune responses in mice with gastrointestinal allergy because at least 10 doses of HDM have been shown to be required for HDM-specific lymphocyte immunity.^{32,35} Taken together, our results demonstrate cross talk between the development of gastrointestinal and respiratory allergy and establish that gastrointestinal allergy primes for allergic respiratory responses. Furthermore, these results provide an experimental framework to dissect critical mechanisms that might be operational during induction of natural allergic disease.

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