

IgE and mast cell responses on intestinal allergen exposure: A murine model to study the onset of food allergy

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Objective: Allergic reactions to food are characterized by enhanced allergen-specific IgE serum levels and the activation of intestinal mast cells. Here we describe a murine model for the onset of food allergy and the role of cytokines in the regulation of food-induced IgE responses.

Methods: Mice were primed systemically with low doses of alum-precipitated ovalbumin. Subsequent intragastric challenge led to enhanced sensitization.

Results: Compared with baseline ovalbumin-specific IgE levels before challenge (0.23 ± 0.06 optical density [OD] units), ovalbumin-challenged mice showed significantly elevated IgE levels (0.86 ± 0.23 OD units) after intragastric challenge, which were not observed in control animals (0.29 ± 0.06 OD units). IgE levels mirrored intestinal mast cell activation, measured by decreased histamine levels in duodenal specimens, in ovalbumin-challenged mice (92.6 ± 7.9 ng/0.1 gm tissue weight) but not in saline-challenged mice (135.4 ± 18.3 ng/0.1 gm tissue weight), compared with baseline levels (141.1 ± 4.1 ng/0.1 gm tissue weight). Changes in IgE and histamine levels after intragastric challenge could be blocked by treating the animals with neutralizing antibodies against IL-4 or IL-10. Although it is generally accepted that ingestion of food allergens leads to a state of immunologic unresponsiveness (i.e., oral tolerance), it is shown here that low-dose systemic priming followed by intragastric challenge leads to sensitization instead of unresponsiveness.

Conclusions: Our murine model shows an important correlation between T_{H2} cytokines, IgE production, and histamine release. Hence, this *in vivo* model provides a useful tool with which the complex mechanism underlying sensitization to food allergens can be studied. (*J Allergy Clin Immunol* 1997;99:94-9.)

Key words: IgE, histamine, food allergy

In genetically predisposed or atopic patients, food allergy is generally caused by an overproduction of IgE directed to common dietary proteins.¹ Recently, atopy has been described as an inherent bias in the CD4⁺ T-cell-mediated response to specific antigens presented at mucosal surfaces, which results in a dominance of T cells of the T

Abbreviations used

IFN- γ : Interferon- γ
PBS: Phosphate-buffered saline
OD units: Optical density units

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helper 2 (T_{H2}) phenotype.² This specific T-cell subset produces predominantly IL-4, IL-10, and little or no interferon- γ (IFN- γ), a situation that favors IgE production.³ Moreover, both IL-4 and IL-10 inhibit the production of T_{H1} cytokines, such as IFN- γ and IL-2, indicating that T_{H1} and T_{H2} cytokines act reciprocally on the induction and persistence of IgE responses *in vivo*.⁴

The full significance of local IgE in food allergy has not been established yet. Increased numbers of

IgE-bearing cells, presumably mast cells, have been described in mucosal specimens from patients allergic to food.⁵ Binding of allergen-IgE complexes to the high-affinity FcεRI receptor on mast cells is known to induce mast cell activation and subsequent degranulation.¹ The release of stored and newly formed mediators leads to vasodilation and increased vascular permeability.⁶ Other factors released by mast cells, such as tumor necrosis factor-α and IL-4, can modulate the expression of adhesion molecules (e.g., E-selectin) on the endothelium of smaller blood vessels, in which proximity mast cells are often localized.⁷ This altered expression might stimulate the recruitment of new effector cells into the small bowel. In addition, studies in rodents have demonstrated that alterations in intestinal salt and water transport after a local challenge were associated with mucosal histamine depletion.⁸ These studies indicate that mast cells are important effector cells in IgE-mediated allergic reactions to food.

Recently, mast cells were suggested to be directly involved in regulating IgE production, independently of T cells.⁹ On stimulation, mast cells express CD40 ligand⁹ and produce IL-4,¹⁰ the minimal signals required to induce B-cell isotype switching to IgE producing plasma cells. Furthermore, human cord blood-derived T cells were found to lack CD40 ligand membrane expression, possibly because of transcriptional downregulation.¹¹ Taken together, mast cells are likely to play an important role in both the induction and maintenance of allergic reactions in early childhood.

In this study high IgE responder BALB/c mice were primed with a low dose of alum-precipitated ovalbumin, a well-known food allergen derived from chicken egg white. To study food-induced allergic sensitization, the effects of an intragastric ovalbumin challenge on serum IgE levels and histamine release in the small bowel and the influence of concomitant cytokine neutralization on the induction of IgE responses were studied.

METHODS

Animals

Female BALB/c mice were obtained from Harlan CPB (Zeist, The Netherlands). All mice were 8 to 12 weeks old at the start of the experiments. The mice were bred and kept on an ovalbumin-free diet and had access to water and food ad libitum. All experiments were approved by the Animal Experiments Committee of the Vrije Universiteit Amsterdam.

Sensitization protocol

Mice were sensitized intraperitoneally on day zero with 0.5 ml of a freshly prepared solution of 4 μg/ml of ovalbumin type VII (Sigma, St. Louis, Mo.) in 0.15 mol/L phosphate-buffered saline (PBS), adsorbed on 1 mg of dried aluminum hydroxide gel (F2200; Reheis Chemical Company, Berkeley Heights, N.J.), a kind gift of Dr. E. Svensjö (Astra Draco, Lund, Sweden). The mice received an intraperitoneal boost of 1 μg of ovalbumin in 0.5 ml of PBS without adjuvant on day 14. On day 18, the mice were challenged intragastrically with 1 mg of ovalbumin dissolved in 0.2 ml of PBS. Control mice were sensitized as described previously but received PBS intragastrically.

Anti-cytokine treatment

Animals were injected intraperitoneally at the time of challenge with rat monoclonal IgG₁ antibody specific for either murine IL-4 (11B11, 2 mg/mouse¹²) or murine IL-10 (JES5-2A5, 1 mg/mouse¹³). Control animals received an isotype-matched control monoclonal antibody, directed to *Escherichia coli* β-galactosidase (GL113¹³). All antibodies were purified as described elsewhere.¹⁴

Determination of antibody levels in serum

Both total and ovalbumin-specific IgE antibodies were determined by using a modified capture ELISA. Plates were coated overnight at 4°C with monoclonal rat anti-mouse IgE, EM-95,¹⁵ cultured, and purified as described elsewhere.¹⁴ EM-95 was used at a concentration of 1 μg/ml in PBS. As detecting antibody for total IgE, biotinylated rat-anti-mouse IgE was used (Pharmingen, San Diego, Calif.) at a concentration of 4 μg/ml. Ovalbumin-specific IgE was detected with appropriately diluted digoxigenin-coupled ovalbumin, prepared with digoxigenin-3-O-methylcarbonyl-ε-aminocaproic acid N-hydroxy-succinimide ester (Boehringer, Mannheim, Germany). Subsequently, the plates were either incubated with peroxidase-conjugated streptavidin (DAKO, Glostrup, Denmark) at a concentration of 1.6 μg/ml or with peroxidase-coupled sheep anti-DIG Fab fragments (Boehringer). Finally, 2 mg/ml O-phenylenediamine-dihydrochloride (Sigma) and 0.015% hydrogen peroxide in 0.1 mol/L phosphate-citrate buffer were used for color development. The absorbance was determined at 490 nm and expressed as optical density (OD) units.

Histamine levels in whole gut tissue homogenates

To study histamine content in the small intestines after intragastric allergen provocation, the first 5 cm of the duodenum, distal from the ligament of Treitz, was collected. Specimens were flushed with 5 ml of cold 0.9% NaCl solution, and the weight was determined. Tissue was then homogenized with 1 ml of 0.9% NaCl. After thorough mixing, 200 μl of 12% perchloric acid was added, and samples were centrifuged for 10 minutes at 10,000 g. Subsequently, 1 ml of supernatant was

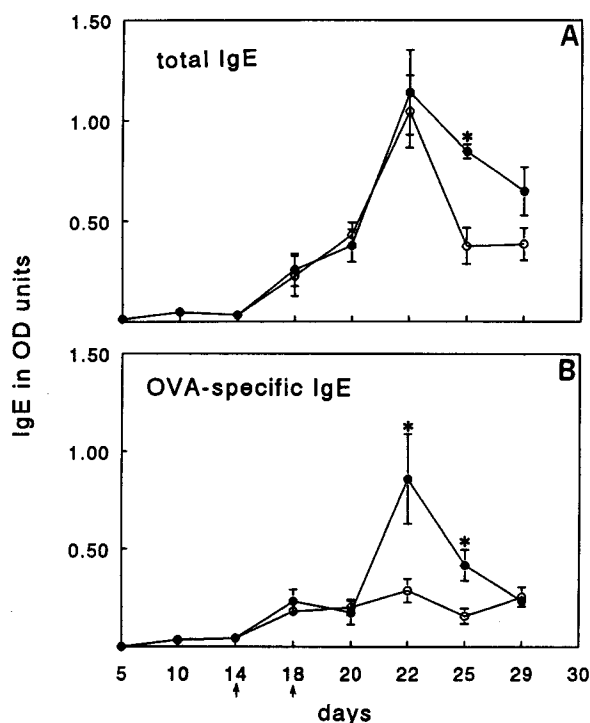


FIG. 1. Total (A) and ovalbumin-specific (B) IgE serum levels after systemic sensitization and single intragastric ovalbumin challenge. Mice were primed and boosted with 1 μ g of ovalbumin on days 0 and 14, respectively, and challenged intragastrically on day 18 with 1 mg of ovalbumin (filled circles). Control mice were primed and boosted but received PBS intragastrically (open circles). Mean serum levels of four mice per group are expressed as mean OD units $\pm$ SEM. * $p < 0.05$. Arrows indicate systemic boost and intragastric challenge given on days 14 and 18, respectively. OVA, Ovalbumin.

collected and again mixed with 200 μ l of 12% perchloric acid. After centrifugation, supernatant was collected and stored at -20°C until further analysis. Histamine was measured by fluorometric analysis as described by Siraganian.¹⁶

Statistical analysis

Differences between groups were analyzed by using the Student's *t* test. Probability (*p*) values less than 0.05 were considered to be significant.

RESULTS

Serum IgE levels after intragastric ovalbumin provocation

Systemic sensitization with 1 μ g of alum-precipitated ovalbumin leads to a significant increase of total IgE in the serum, which peaks on day 22, 8 days after the intraperitoneal boost (Fig. 1, A). When these animals were additionally challenged on day 18 with an intragastric dose of soluble ovalbumin, no enhancement of total serum IgE

was seen, but the peak response was found to decline more slowly in ovalbumin-challenged mice, compared with PBS-challenged control mice. Interestingly, when ovalbumin-specific serum IgE levels were measured, the intragastric challenge led to a rapid increase of allergen-specific IgE, which peaked 4 days after the intragastric ovalbumin provocation (Fig. 1, B). However, ovalbumin-specific IgG₁ serum levels were only slightly increased in ovalbumin-challenged mice compared with control PBS-challenged mice (data not shown). Moreover, when a high dose of alum-precipitated ovalbumin (100 μ g) was used for priming, only slightly elevated ovalbumin-specific IgE levels were found, whereas ovalbumin-specific IgG₁ levels were significantly increased (data not shown). These results indicate that ingestion of allergen after previous systemic sensitization with a low dose of allergen results in significantly increased allergen-specific serum IgE levels.

Effect of neutralization of IL-4 or IL-10 on serum IgE responses induced after intragastric ovalbumin provocation

To study the role of IL-4 and IL-10 in IgE responses induced by intragastric ovalbumin provocation, mice were injected intraperitoneally with neutralizing doses of either 2 mg of anti-IL-4 or 1 mg of anti-IL-10 at the time of challenge. Control mice received isotype-matched rat antibodies (GL113). The mice were bled several days after the intragastric challenge, and total and ovalbumin-specific IgE levels were determined by ELISA. Neutralization of either IL-4 or IL-10 resulted in a significant reduction of the total IgE response at 2 and 4 days after the intragastric challenge as compared with the GL113-treated control mice (Fig. 2, A). Similar results were obtained for ovalbumin-specific IgE levels (Fig. 2, B). These results suggest that both IL-4 and IL-10 are required for optimum IgE production on intragastric challenge.

The involvement of ovalbumin-specific IgE in local histamine release in the small bowel

Mast cells are thought to play a crucial role in the induction of allergic reactions to food. By measuring the total amount of histamine in the small bowel specimens on the day of challenge and several days after the challenge, the effects of an intragastric ovalbumin challenge on the local mast cell population were studied. Control PBS-challenged mice showed neither an increase in ovalbumin-specific IgE levels nor a significant difference

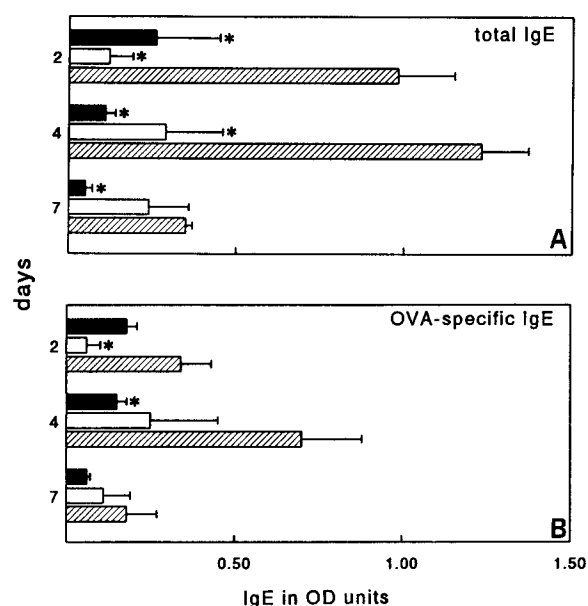


FIG. 2. Effect of anti-IL-4 (black bars) and anti-IL-10 (open bars) administration on the day of challenge on total (A) and ovalbumin-specific (B) serum IgE responses. Mice were primed and boosted with 1 μ g of ovalbumin on days 0 and 14, respectively, and challenged with 1 mg of ovalbumin on day 18. At 2, 4, and 7 days after challenge, sera were collected and analyzed with an ELISA. Control mice (hatched bars) received an isotype-matched control antibody (GL113). Mean serum levels of four mice per group are expressed as mean OD units $\pm$ SEM. * p < 0.05. OVA, Ovalbumin.

in histamine levels compared with baseline histamine levels on the day of challenge (Fig. 3, A). On the contrary, ovalbumin-provoked mice showed a significant reduction in histamine levels at 4 days after challenge, whereas a significant rise in ovalbumin-specific IgE serum levels was observed at the same time point (Fig. 3, B). No significant changes in histamine levels of ovalbumin-challenged mice were observed at 6, 12, or 24 hours after intragastric challenge (data not shown). Reduction of histamine levels was found to be transient, because histamine levels recovered rapidly over the next 3 days.

Neutralization of either IL-4 or IL-10 blocks mast cell reactivity in the small bowel

To further determine the role of IgE in the activation of mast cells, as suggested by the concomitant rise in ovalbumin-specific IgE and decrease in local histamine levels, the ovalbumin-specific IgE production after an intragastric challenge was abolished by injection of neutralizing antibodies against IL-4 or IL-10. When hista-

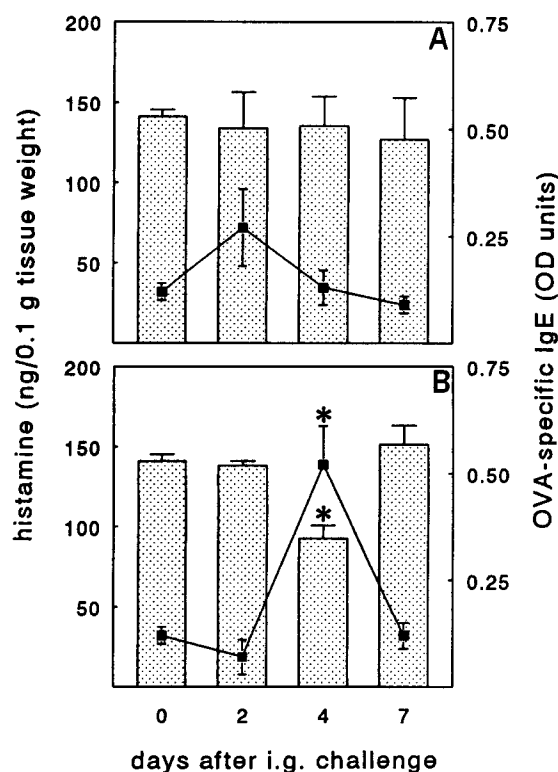


FIG. 3. Effect of intragastric ovalbumin challenge on local histamine levels measured in small bowel tissue homogenates several days after challenge (bars). Corresponding sera were collected and analyzed with an ELISA for ovalbumin-specific IgE levels (continuous line). Mean histamine levels in nanograms per 0.1 gm of tissue weight and ovalbumin-specific IgE levels in OD units $\pm$ SEM of four mice per group of both control PBS (A) and ovalbumin-challenged mice (B) are shown. * p < 0.05. OVA, Ovalbumin; i.g., intragastric.

mine levels in these animals were determined (Fig. 4), the histamine reduction 4 days after ovalbumin challenge (bar B) was halted by either anti-IL-4 (bar C) or anti-IL-10 injection (bar D) at the time of challenge. These results indicate an important direct role for both cytokines in the induction of allergen-specific IgE and an indirect role in mast cell activation in the small intestines.

DISCUSSION

Egg allergy is a common cause of allergic reactions to food in children. All four predominant egg white proteins (ovalbumin, ovotransferrin, ovomucoid, and lysozyme) have been found to cause IgE production.¹⁷ Although ovalbumin is a suitable allergen, used in many experimental models, current evidence points to ovomucoid as the major egg allergen for human beings.¹⁸

The murine model described in this article dem-

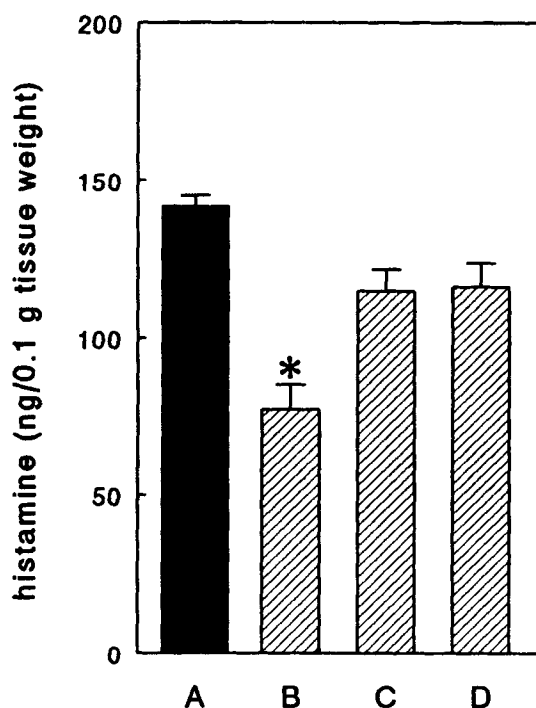


FIG. 4. Effect of anti-IL-4 and anti-IL-10 on histamine levels in the small bowel. Mice were primed and boosted intraperitoneally with 1 μ g of ovalbumin on days 0 and 14 and challenged intragastrically with 1 mg of ovalbumin on day 18. At the time of challenge, mice received either PBS (bar B), anti-IL-4 (bar C), or anti-IL-10 (bar D) intraperitoneally. Four days after the challenge, histamine levels were measured in small bowel homogenates and compared with baseline histamine levels on the day of challenge (bar A). Mean histamine levels of five mice per group $\pm$ SEM are shown. * $p < 0.05$.

onstrates two important characteristics of food-induced allergic reactions, elevated serum allergen-specific IgE levels and concomitant histamine release in the small bowel, probably as a result of mast cell or basophil activation. Although the production of food allergen-specific IgE antibodies is assumed to be crucial in mast cell-induced food allergic reactions, a direct relationship between serum IgE and local histamine release, as seen in this model, has not been described in animal models for food hypersensitivity reactions. Our data confirm IgE-induced proportional local physiologic changes as previously described in a rat model.¹⁹ In this model changes in water and electrolyte absorption in the small intestines were seen within 2 hours after an intraluminal allergen provocation, performed 2 weeks after priming. In our model, however, a marked delay in histamine release was observed. Interestingly, histamine release was found to be related to the amount of

ovalbumin-specific IgE in serum, resulting in histamine release 4 days after intragastric challenge when IgE levels were peaking, as shown in Fig. 3. This delay could result from binding of ovalbumin-anti-ovalbumin IgE complexes to mast cells, rather than cross-linking by antigen of cell-bound IgE. A role of such complexes in the activation of rodent mast cells has been described.²⁰

Although *in vitro* studies have demonstrated that mast cells in small bowel tissue from sensitized animals are responsible for abnormalities in ion transport and changes in permeability that precede allergic reactions to food,²¹ intragastric provocation in our model was not sufficient to induce clinical symptoms such as diarrhea. This may be species-specific, although when IL-9 transgenic mice, which have abundant numbers of chymase-positive mucosal mast cells in their gastrointestinal tract compared with BALB/c mice, were used, systemic sensitization followed by an intragastric challenge resulted in very high levels of ovalbumin-specific IgE and concomitant local edema in the small bowel (manuscript in preparation). These data suggest that clinical gastrointestinal symptoms only occur *in vivo* when increased numbers of mast cells are present and activated. Therefore the model described in this article does not reflect the clinical situation as seen in patients with food allergy.

Primary allergic sensitization to food allergens occurs in early life and is characterized by transient high levels of IgE antibodies. Interestingly, initial IgE production in response to common dietary antigens has been demonstrated in both atopic and healthy children. However, atopic children usually display higher allergen-specific IgE serum levels than healthy children, a situation that often precedes clinical symptoms.²² It has been shown that substantial mast cell activation can be induced when mast cells are sensitized with low-affinity IgE antibodies cross-linked with high-valency antigens, such as aggregated ovalbumin.²⁰ This mechanism for mast cell activation could account for an exquisite sensitivity of mast cells for allergen-IgE complexes. Moreover, patients with allergy might respond to a higher number of allergen epitopes than nonallergic subjects, resulting in clinical symptoms even when low percentages of allergen-specific IgE are bound to mast cells. Our data confirm these *in vitro* results, because we were able to show the release of histamine early after the induction of an IgE response, when affinity maturation and memory B-cell formation has not yet occurred.

In our model IgE production was found to be highly IL-4-dependent, as shown in Fig. 2. These

results are characteristic for primary IgE responses, because memory IgE responses in vivo were found to be less IL-4-dependent and not susceptible to IFN- γ , indicating that memory IgE responses are not tightly controlled by T cells.²³ It remains speculative whether rapid induction of IFN- γ -producing, allergen-specific CD8-positive suppressor T cells or induction of CD4-positive T_{H1} cells is responsible for the transient character of the induced IgE response. Neutralization of IL-10 was found to decrease serum IgE levels as well, probably by indirect stimulation of endogenous IFN- γ production, because it is known that IL-10 produced by T_{H2} cells inhibits IFN- γ production by T_{H1} cells.²⁴ These results point to an important role for CD4-positive T_{H1} cells in controlling intragastrically induced IgE responses. Taken together, these results demonstrate that both IL-4 and IL-10 are crucial in the induction of allergen-specific IgE through the gastrointestinal tract. Although IgE is necessary for primary mast cell activation, these cytokines are indirectly involved in mast cell activation, because neutralization of either IL-4 or IL-10 was found to inhibit histamine release significantly.

In conclusion, our murine model represents the early phase of sensitization to food-related allergens. Further research will focus on the role of distinct T-cell subsets and their cytokines in the regulation of intragastrically induced IgE responses and the role of mast cells in this complex process.

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