Epicutaneous sensitization results in IgE-dependent intestinal mast cell expansion and food-induced anaphylaxis

Lisa M. Bartnikas, MD,[a]* Michael F. Gurish, PhD,[b]* Oliver T. Burton, PhD,[a] Sabine Leisten, MS,[a] Erin Janssen, MD, PhD,[a] Hans C. Oettgen, MD, PhD,[a] Jacqueline Beaupré, BA,[a] Christopher N. Lewis, BA,[a] K. Frank Austen, MD,[b] Stephanie Schulte, MD,[c] Jason L. Hornick, MD, PhD,[c] Raif S. Geha, MD,[a]* and Michiko K. Oyoshi, PhD[a]* *Boston, Mass*

Background: Sensitization to food antigen can occur through cutaneous exposure.
Objective: We sought to test the hypothesis that epicutaneous sensitization with food antigen predisposes to IgE-mediated anaphylaxis on oral allergen challenge.
Methods: BALB/c mice were epicutaneously sensitized by repeated application of ovalbumin (OVA) to tape-stripped skin over 7 weeks or orally immunized with OVA and cholera toxin (CT) weekly for 8 weeks and then orally challenged with OVA. Body temperature was monitored, and serum mouse mast cell protease 1 levels were determined after challenge. Tissue mast cell (MC) counts were examined by using chloroacetate esterase staining. Levels of serum OVA-specific IgE and IgG_1 antibodies and cytokines in supernatants of OVA-stimulated splenocytes were measured by means of ELISA. Serum IL-4 levels were measured by using an *in vivo* cytokine capture assay.
Results: Epicutaneously sensitized mice exhibited expansion of connective tissue MCs in the jejunum, increased serum IL-4 levels, and systemic anaphylaxis after oral challenge, as evidenced by decreased body temperature and increased serum mouse mast cell protease 1 levels. Intestinal MC expansion and anaphylaxis were IgE dependent because they did not occur in epicutaneously sensitized $IgE^{-/-}$ mice. Mice orally immunized with OVA plus CT did not have increased serum IL-4 levels, expanded intestinal MCs, or anaphylaxis after oral challenge, despite OVA-specific IgE levels and splenocyte cytokine production in response to OVA stimulation, which were comparable with those of epicutaneously sensitized mice.
Conclusion: Epicutaneously sensitized mice, but not mice orally immunized with antigen plus CT, have expansion of intestinal MCs and IgE-mediated anaphylaxis after single oral antigen challenge. IgE is necessary but not sufficient for food anaphylaxis, and MC expansion in the gut can play an important role in the development of anaphylaxis. (J Allergy Clin Immunol 2013;131:451-60.)

Key words: *Food allergy, epicutaneous sensitization, IgE, mast cells, anaphylaxis*

Anaphylaxis to food results from IgE-mediated sensitivity to a food allergen. However, IgE antibodies to foods can exist in subjects who can ingest the foods without experiencing anaphylaxis,[1] suggesting that factors other than IgE might be required. In many cases allergic reactions to foods occur on the first known ingestion, suggesting that routes other than the oral one might be important in sensitization. Epidemiologic data suggest that sensitization to peanut protein can occur in children through the application of peanut oil to inflamed skin,[2] which is consistent with the skin being an important route of allergen sensitization.

Altered skin barrier function in patients with atopic dermatitis (AD) is thought to promote cutaneous sensitization to environmental antigens, including food proteins, potentially leading to the development of food allergies. Little is known about how to prevent the development of food allergy in atopic patients, and presently, there is no cure for it. Current therapy relies on allergen avoidance and treatment of severe reactions with epinephrine. We have used a mouse model of allergic skin inflammation with many features of AD[3,4] to demonstrate that epicutaneous sensitization, but not oral immunization, with the food antigen ovalbumin (OVA) results in IgE-dependent expansion of intestinal mast cells (MCs) and IgE-mediated anaphylaxis after oral challenge.

METHODS

Mice

BALB/c mice were purchased from Charles River Laboratories (Wilmington, Mass). $IgE^{-/-}$ mice on a BALB/c background were previously reported.[5] All mice were housed in a specific pathogen-free environment and fed an OVA-free diet. All procedures were performed in accordance with the Animal Care and Use Committee of Boston Children's Hospital.

Epicutaneous sensitization and oral immunization

Epicutaneous sensitization of mice was performed, as previously described.[3] Each mouse had a total of three 1-week exposures to OVA (grade V; Sigma, St Louis, Mo) applied as a patch to tape-stripped skin and separated by 2-week rest intervals.

From [a]the Division of Immunology, Boston Children's Hospital, and the Department of Pediatrics, Harvard Medical School; [b]the Division of Rheumatology, Immunology, and Allergy, Brigham and Women's Hospital, and the Department of Medicine, Harvard Medical School; and [c]the Department of Pathology, Brigham and Women's Hospital and Harvard Medical School.

*These authors contributed equally to this work.

Supported by National Institutes of Health (NIH) grants AR-047417 and R01-AI083516. L.M.B. was supported by training grant T32-AI-007512 and the 2010 American Academy of Allergy, Asthma & Immunology/Food Allergy Initiative Howard Gittis Memorial Third/Fourth Year Fellowship/Junior Faculty Research Award. M.K.O. was supported by the Harvard Digestive Disease Center, NIH grant P30 DK34845, and the Boston Children's Hospital Faculty Career Development Fellowship/Eleanor and Miles Shore Program for Scholars in Medicine at Harvard Medical School. M.F.G. was supported by National Institute of Allergy and Infectious Diseases (NIAID) grant RO1-AI083516. H.C.O. was supported by NIH/NIAID grant R56 AI100889-01 and US Environmental Protection Agency grant 83482501.

Disclosure of potential conflict of interest: S. Leisten has received research support from the German Academic Exchange Service (DAAD). R. S. Geha has received research support from the National Institutes of Health. The rest of the authors declare that they have no relevant conflicts of interest.

Received for publication December 23, 2011; revised November 2, 2012; accepted for publication November 8, 2012.

Corresponding author: Michiko K. Oyoshi, PhD or Raif S. Geha, MD, Karp 10, Division of Immunology, Boston Children's Hospital, 300 Longwood Ave, Boston, MA 02115. E-mail: Michiko.Oyoshi@childrens.harvard.edu Or: Raif.Geha@childrens.harvard.edu.

0091-6749/$36.00

© 2013 American Academy of Allergy, Asthma & Immunology

http://dx.doi.org/10.1016/j.jaci.2012.11.032

Abbreviations used
AD: Atopic dermatitis
CAE: Chloroacetate esterase
CT: Cholera toxin
CTMC: Connective tissue mast cell
IL-4R: IL-4 receptor
MC: Mast cell
MLN: Mesenteric lymph node
mMCP-1: Mouse mast cell protease 1
OVA: Ovalbumin
Treg: Regulatory T
WT: Wild-type

Oral immunization of mice was performed, as previously described.[6] Briefly, 4- to 6-week-old mice were enterally (subsequently referred to as orally) immunized by means of gavage once a week for 7 weeks with 5 mg of OVA and 10 μg of cholera toxin (CT; azide free; List Biological Laboratories, Campbell, Calif) in 150 μL of normal saline or placebo (10 μg of CT alone in 150 μL of normal saline) by using a ball-ended mouse feeding needle.

Induction and measurement of systemic anaphylaxis

At week 7 (epicutaneous sensitization model) or week 8 (oral immunization model), mice received a bolus oral challenge with 100 mg of OVA or intravenous challenge with 100 μg of OVA. Temperature changes were measured by using the DAS-6006 Smart Probe and transponders (Biomedic Data Systems, Seaford, Del) injected subcutaneously. Mice were killed at 60 minutes after challenge to collect serum and harvest tissues.

Serum antibody measurement

OVA-specific IgG_1 and IgE levels were determined by means of ELISA, as previously described.[3]

In vitro cytokine production and proliferation assay

Spleen single-cell suspensions were cultured at 2×10^6/mL in the presence of OVA (200 μg/mL) for 96 hours, as described previously.[7] Cytokine secretion in supernatants was measured by means of ELISA per the manufacturer's instructions (IL-4 and IFN-γ, eBioscience, San Diego, Calif; IL-13, R&D Systems, Minneapolis, Minn). Splenocyte proliferation was measured by using tritiated thymidine incorporation after 72 hours of culture.

Serum mouse mast cell protease 1 levels

Mouse mast cell protease 1 (mMCP-1) concentrations were measured in sera collected 1 day before and 60 minutes after oral challenge by means of ELISA per the manufacturer's instructions (eBioscience).

Histologic analysis of MCs

Tissue specimens were fixed in 4% paraformaldehyde and embedded in glycolmethacrylate, and sections were stained with chloroacetate esterase (CAE) for quantification of MCs, as previously described.[8] Tissue sections were examined by investigators who were blind to the identities of the samples. MCs were counted in 10 high-power fields at a magnification of ×400.

In vivo cytokine capture assay for IL-4

The *in vivo* cytokine capture assay for IL-4 was performed, as previously described.[9] Briefly, mice were intravenously injected with 10 μg of biotin anti–IL-4 mAb (BVD6-24G2, eBioscience) and bled 16 hours later. Serum IL-4 levels were determined by using ELISA.

Il4 mRNA expression in mesenteric lymph nodes

Total RNA was extracted from homogenized mesenteric lymph nodes (MLNs) with the RNAqueous extraction kit (Ambion, Austin, Tex). cDNA was generated with the iScript cDNA synthesis kit (Bio-Rad Laboratories, Hercules, Calif). Quantitative real-time PCR was done with the TaqMan Gene Expression Assay, universal PCR master mix, and ABI Prism 7300 sequence detection system (Applied Biosystems, Foster City, Calif). Fold induction of IL-4 gene expression was calculated by using the comparative method for relative quantitation with normalization to the internal control β_2-microglobulin, as described previously.[6]

Statistical analysis

Statistical significance was determined by using GraphPad Prism, version 4.0a (GraphPad Software, La Jolla, Calif). Statistical differences were calculated by using the Student *t* test (between 2 groups) and 2-way ANOVA (between curves). A *P* value of less than .05 was considered statistically significant.

RESULTS

Epicutaneous sensitization with OVA results in anaphylaxis after oral antigen challenge

Wild-type (WT) BALB/c mice were subjected to 3 cycles of epicutaneous sensitization with OVA or saline as a control and then orally challenged with OVA the day after the last cycle of sensitization (Fig 1, *A*). Epicutaneous sensitization with OVA resulted in the generation of OVA-specific IgE and IgG_1 antibodies (Fig 1, *B*), as previously published.[3,10] After oral challenge, mice epicutaneously sensitized with OVA exhibited a significant decrease in core body temperature (Fig 1, *C*) and significantly increased β-chymase mMCP-1 levels expressed in mucosal MCs[8,11,12] compared with values seen in control animals epicutaneously sensitized with saline (Fig 1, *D*). Oral challenge of epicutaneously sensitized mice with saline caused no detectable changes in core body temperature or mMCP-1 levels (Fig 1, *C* and *D*). WT BALB/c mice epicutaneously sensitized with 100 μg of peanut extract over 7 weeks and then orally challenged with 100 mg of peanut flour also exhibited a significant decrease in temperature and an increase in serum mMCP-1 levels (see Fig E1 in this article's Online Repository at www.jacionline.org). These results indicate that cutaneous introduction of antigen promotes anaphylaxis and MC degranulation after oral antigen challenge.

Anaphylaxis in epicutaneously sensitized mice is IgE dependent

Both IgE and IgG_1 antibodies can mediate anaphylaxis in mice.[12] We used $IgE^{-/-}$ mice to examine the role of IgE in our model. After epicutaneous sensitization with OVA, $IgE^{-/-}$ mice exhibited comparable levels of OVA-specific IgG_1 compared with WT mice (Fig 2, *A*) but no detectable OVA-specific IgE (data not shown). $IgE^{-/-}$ mice epicutaneously sensitized with OVA did not experience decreased body temperature (Fig 2, *B*) or increased serum mMCP-1 levels (Fig 2, *C*) after oral OVA challenge. These results suggest that IgE is necessary for the development of anaphylaxis in our model.

Download English Version:

https://daneshyari.com/en/article/3198246

Download Persian Version:

https://daneshyari.com/article/3198246

Daneshyari.com