Concurrent blockade of platelet-activating factor and histamine prevents life-threatening peanut-induced anaphylactic reactions

Katherine Arias, BHSc,[a] Moiz Baig, BSc,[a] Marc Colangelo, BHSc,[a] Derek Chu, BHSc,[a] Tina Walker, BSc,[a] Susanna Goncharova, MD, PhD,[a] Anthony Coyle, PhD,[c] Peter Vadas, MD, PhD,[d] Susan Waserman, MD, MSc,[b] and Manel Jordana, MD, PhD[a] *Hamilton and Toronto, Ontario, Canada, and Gaithersburg, Md*

Background: Food anaphylaxis is an acute and life-threatening systemic allergic reaction. Fatality registries place peanut as the most common culprit of fatal and near-fatal reactions in North America. Because prophylaxis and treatment have advanced little in recent years, it is imperative to evaluate novel therapies.
Objective: To investigate the impact of blocking mast cell mediators in a mouse model of peanut-induced anaphylaxis.
Methods: Mice were sensitized with peanut protein and cholera toxin via oral gavage weekly for 4 weeks. One week after the last sensitization, separate groups of mice were treated with either a (1) 5-lypoxygenase inhibitor, (2) a platelet-activating factor (PAF) receptor antagonist, (3) histamine receptor antagonists, or (4) a PAF receptor antagonist along with histamine receptor antagonists before peanut challenge.
Results: Treatment targeting either leukotrienes or histamine alone had no beneficial effects. In contrast, PAF antagonism significantly attenuated the magnitude and duration of the anaphylactic reactions. Particularly, it prevented severe reactions. Moreover, 83% of PAF-treated versus 43% of untreated mice reached recovery within 120 minutes after peanut challenge. Notably, combined blockade of PAF and histamine had a clearly greater beneficial effect. In fact, all but 1 mouse developed mild, if any, anaphylactic reactions. In addition, combination therapy was associated with a significant decrease in vascular leakage and release of vasoactive mediators after peanut challenge.
Conclusion: Combination therapy blocking both PAF and histamine markedly reduces the severity of peanut-induced anaphylaxis, and thus it may be a potential life-saving therapeutic approach in peanut and, likely, other food-induced anaphylaxis. (J Allergy Clin Immunol 2009;124:307-14.)

Key words: *Peanut allergy, anaphylaxis, mast cells, histamine, PAF, leukotrienes, mathematical modeling*

Immediate hypersensitivity reactions to foods account for one third to one half of anaphylaxis cases worldwide.[1,2] Anaphylaxis is an acute and life-threatening systemic allergic reaction, and food anaphylaxis fatality registries in the United States implicate peanuts as the major culprit of fatal reactions.[3] Recent studies in North America and the United Kingdom have reported that prevalence rates of peanut allergy (PA) among schoolchildren are currently higher than 1%.[4,5] Although other food allergies often resolve during the first years of life, peanut hypersensitivity usually persists.[6] Prophylaxis and treatment for PA have advanced little in recent years, and intervention remains limited to strict avoidance. However, accidental ingestion is common, with a reported annual incidence rate of 14.3% among schoolchildren in Montreal, Quebec, Canada.[7]

Mechanistically, anaphylaxis is a hypersensitivity reaction involving the release of mediators from mast cells (MCs) and basophils after allergen interaction with cell-bound IgE. Mediators include vasoactive amines (eg, histamine), proteases, and lipid-derived mediators such as leukotrienes, prostaglandins, and platelet-activating factor (PAF). It is thought that the extent of mediator release closely correlates with the severity and persistence of the anaphylactic reaction. However, the relative contribution of these mediators in the physiopathology of food-induced anaphylaxis is unknown.

Platelet-activating factor is a phospholipid secreted by MCs, monocytes, and fixed tissue macrophages.[8] By binding to a G-protein–coupled transmembrane receptor, PAF mediates cellular responses, including Ca^{2+} mobilization, platelet aggregation, and vasodilatation.[9,10] In addition to its role as a physiological mediator, PAF has been associated with the pathogenesis of anaphylactic shock. Indeed, administration of PAF to mice can lead to bronchoconstriction, hypotension, and increased vascular permeability, causing pulmonary edema and impaired cardiac and renal function. Importantly, PAF-R deficiency and administration of PAF-R antagonists can prevent PAF-induced lethal anaphylaxis in animal models.[10-12] However, the contribution of PAF to food-induced anaphylaxis remains to be elucidated. Of interest, a human study showed that the severity of peanut-induced anaphylaxis (PIA) correlates with the levels of PAF in serum.[13] Here, we examined the impact of pharmacologic interventions targeting either metabolic pathways or mediator receptors in an experimental mouse model of PIA. We observed that blockade

From [a]the Department of Pathology and Molecular Medicine, Centre for Gene Therapeutics, and [b]the Department of Medicine, McMaster University, Hamilton; [c]the Department of Inflammation and Autoimmunity, MedImmune Inc, Gaithersburg; and [d]the Departments of Medicine, Division of Allergy and Clinical Immunology, and Emergency Medicine, St Michael's Hospital, Toronto.

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Reprint requests: Manel Jordana, MD, PhD, Department of Pathology and Molecular Medicine, Centre for Gene Therapeutics, McMaster University, MDCL Room 4013, 1200 Main Street West, Hamilton, Ontario, Canada L8N 3Z5. E-mail: jordanam@mcmaster.ca.

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Abbreviations used
5-LO: 5-Lipoxygenase
AU: Area under response
cysLT: Cysteinyl leukotriene
MC: Mast cell
PA: Peanut allergy
PAF: Platelet-activating factor
PAF-R: Platelet-activating factor receptor
PIA: Peanut-induced anaphylaxis
PL: Peritoneal lavage

of PAF activity significantly prevented prolonged and life-threatening PIA reactions. Interestingly, combination therapy targeting PAF and histamine receptors had a synergistic effect in the prevention of PIA.

METHODS

Animals

Female C57BL/6 mice (6-8 weeks old) were purchased from Charles River Laboratories. All experiments described in this study were approved by the Animal Research Ethics Board of McMaster University.

Grading of the severity of PIA responses

Mice were sensitized and challenged as previously described.[14] Mice were carefully monitored for either 40 or 120 minutes immediately after peanut challenge, and anaphylactic responses were evaluated as previously described.[14] To grade the severity of the anaphylactic response, we used a 3-point grading scheme. Accordingly, anaphylactic reactions were classified as *no reaction* (score 0 and 37.5°C $\leq$ T $\leq$ 40°C), *mild reaction* (score $\leq$2 and 34.5°C $\leq$ T $\leq$ 37.5°C), or *severe or life-threatening reaction* (score $\geq$3 and T $\leq$ 34.5°C).

Determination of vasoactive mediators

Histamine and leukotriene levels were determined in plasma by using enzyme immunoassay kits (Beckman Coulter Canada, Inc, Mississauga, and Cedarlane Laboratories Ltd, Hornby, Canada, respectively) according to the manufacturers' specifications.

Analysis of vascular leakage

Vascular permeability was determined by measuring albumin levels in the peritoneal lavage (PL) fluid as previously described.[14] Albumin content was quantified in the supernatants by using an enzyme immunoassay kit (Immunology Consultants Laboratory, Inc, Newberg, Ore) following the manufacturer's instructions.

Measurement of peanut-specific IgG_1 and IgE

Peripheral blood was collected 1 week after the last sensitization, and levels of serum peanut-specific IgG_1 and IgE were measured by using a previously described sandwich ELISA.[14]

Pharmacologic interventions

Inhibition of leukotrienes. Peanut-sensitized mice were administered a 5-lipoxygenase (5-LO) inhibitor, Zileuton (50 mg/kg; Cayman, Ann Arbor, Mich), twice, 24 hours and 1 hour before challenge orally. The control group received 0.5% hydroxyethyl cellulose (vehicle) in 100 μL saline at equivalent time points.

Blockade of PAF and histamine receptors. Peanut-sensitized mice were treated with a PAF receptor antagonist (50 mg/kg), ABT491 (Sigma, St Louis, Mo), in 100 μL PBS orally 1 hour before challenge. A separate group of sensitized mice were injected histamine receptor antagonists (mepyramine [3 mg/kg], an H1 receptor antagonist; and cimetidine [10 mg/kg], an H2 receptor antagonist; Cedarlane, Mississauga, Ontario, Canada) in 200 μL PBS intravenously 30 minutes before challenge. In some cases, treatments were administered concurrently.

Flow cytometry of peritoneal MCs

Peritoneal cells were harvested as previously described.[14] For cytometric analysis, the cells were preincubated with B3B4 and 2.4G2 mAbs for 10 minutes at 4°C.[15] The cells were then incubated with 10 μg/mL IgE mAb (Sigma, Oakville, Ontario, Canada) for 20 minutes at 4°C and subsequently washed and stained with anti-IgE (fluorescein isothiocyanate conjugated), anti–c-kit (phycoerythrinconjugated), and anti-CD11b (phycoerythrin-Cy7 conjugated) mAbs at 4°C for 30 minutes. All antibodies were purchased from BD PharMingen, Mississauga, Ontario, Canada. Data were collected by using a LSRII (BD Biosciences, San Jose, Calif) flow cytometer and analyzed by using FlowJo 6.4.2 software (Tree Star Inc, Ashland, Ore). Peritoneal MCs were identified as c-kit$^+$IgE$^+$ cells.

Statistical analysis

Statistical analysis was performed by using SIGMAStat (Systat Software Inc, San Jose, Calif). When applicable, results were analyzed using 1-way or 2-way ANOVA with repeated measures followed by the Tukey *post hoc* test. An unpaired Student *t* test (2-tailed) was used when only 2 sets of continuous data were compared. A P value <.05 was considered statistically significant.

RESULTS

PIA is characterized by the acute and release of vasoactive mediators

Oral sensitization with peanut protein and cholera toxin followed by an intraperitoneal challenge with crude peanut extract led to an acute and robust anaphylactic response as indicated by severe clinical symptoms and a significant drop in core body temperature (Fig 1, *A*). We previously demonstrated that the elicitation of PIA is mediated, to a large extent, by MCs, IgE, and FcεRI (IgE high-affinity receptor).[14] Here, we show that sensitization with peanut resulted in a 3-fold increase in the number of peritoneal c-kit$^+$FcεRI$^+$ cells (Fig 1, *B and C*) along with significant upregulation of FcεRI expression (Fig 1, *D*). Moreover, we detected significantly greater levels of histamine (Fig 1, *E*) and cysteinyl leukotrienes (cysLTs; Fig 1, *F*) in the plasma of peanut-sensitized mice after challenge compared with control mice. Interestingly, the kinetics of mediator production are distinct. Although histamine peaked early after challenge (10 minutes), remained substantially elevated for 1 hour, and slowly decreased over time, cysLT progressively increased on challenge, reaching maximum levels at 40 minutes and gradually declining within 2 to 3 hours after challenge (Fig 1, *E and F*). Together, these data suggest that sensitization with peanut results in IgE-mediated upregulation of MC FcεRI expression, ultimately decreasing the threshold for degranulation and, consequently, amplifying the release of vasoactive mediators.

Impact of leukotrienes and histamine blockade in PIA

To assess the role of leukotrienes in PIA, peanut-sensitized mice were administered a 5-LO inhibitor twice before challenge. As shown in Fig 2, *A and B*, blockade of this pathway did not