

A murine model of peanut anaphylaxis: T- and B-cell responses to a major peanut allergen mimic human responses

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Background: Peanut allergy affects 0.6% of the US population. At the present time, allergen avoidance is the only therapeutic option. Animal models of food-induced anaphylaxis would facilitate attempts to design novel immunotherapeutic strategies for the treatment of peanut allergy.

Objective: The purpose of this study was to develop a murine model of IgE-mediated peanut hypersensitivity that closely mimics human peanut allergy.

Methods: C3H/HeJ mice sensitized orally with freshly ground whole peanut and cholera toxin as adjuvant were challenged orally 3 and 5 weeks later with crude peanut extract. Anaphylactic reactions were determined. T- and B-cell responses to Ara h 1 and Ara h 2, the major peanut allergens, were characterized by evaluating splenocyte proliferative responses and IgE antibody concentrations. Furthermore, IgE antibodies in the sera of patients with peanut allergy and mice were compared for antibody binding to Ara h 2 isoforms and allergenic epitopes.

Results: Peanut-specific IgE was induced by oral peanut sensitization, and hypersensitivity reactions were provoked by feeding peanut to sensitized mice. The symptoms were similar to those seen in human subjects. Ara h 1- and Ara h 2-specific antibodies were present in the sera of mice with peanut allergy. Furthermore, these Ara h 2-specific IgE antibodies bound the same Ara h 2 isoforms and major allergenic epitopes as antibodies in the sera of human subjects with peanut allergy. Splenocytes from mice with peanut allergy exhibited proliferative responses to Ara h 1 and Ara h 2.

Conclusion: This murine model of peanut allergy mimics the clinical and immunologic characteristics of peanut allergy in human subjects and should be a useful tool for developing immunotherapeutic approaches for the treatment of peanut allergy. (*J Allergy Clin Immunol* 2000;106:150-8.)

Key words: Peanut anaphylaxis, animal model, B- and T-cell responses

Peanuts are highly allergenic and may cause severe allergic reactions in sensitized children and adults.^{1,2} The clinical features of peanut allergy (PNA) are frequently expressed as acute IgE-mediated reactions after ingestion of peanuts.³⁻⁸ Peanuts and tree nuts together account for the majority of fatal and near-fatal, food-induced, anaphylactic reactions in the United States.³ The prevalence of PNA has increased in the past decades⁸ and now affects about 1.5 million Americans. Unlike other childhood food allergies, such as cow's milk and egg allergy, PNA is rarely outgrown.^{1,3,9-11} Given the severity, prevalence, and frequently lifelong persistence of PNA and the lack of preventive or curative therapy for PNA, researchers have been investigating the allergenicity of peanuts and the immunopathogenic mechanisms of PNA to develop new immunotherapies. However, the probability of a life-threatening reaction after exposure to even minute amounts of peanut limits human testing and reinforces the need for a suitable animal model.

Two major allergenic peanut proteins have been identified, Ara h 1 and Ara h 2, which are recognized by more than 95% of patients with PNA.¹²⁻¹⁴ A third protein, Ara h 3, is recognized by about 45% of the patients with PNA.¹⁵ The characterization and cloning of these proteins may make it possible to develop new forms of immunotherapies, such as immunization with peanut allergen plasmid-DNA, peptide fragments, or engineered recombinant proteins. However, there has been no completely suitable animal model of PNA to test the efficacy and safety of immunologic therapies. We previously used a model with intraperitoneal sensitization and challenge to investigate the efficacy of immunization with pAra h 2 DNA, a plasmid DNA encoding the major peanut allergen Ara h 2.^{16,17} Roy et al¹⁸ recently used our Ara h 2 plasmid vector in a similar mouse model to investigate the preventive effect of orally administered, DNA-embedded, chitosin particles. However, models using intraperitoneal food sensitization and challenge are not satisfactory models of human food allergy, which is provoked by allergen ingestion. Furthermore, it is unlikely that peanut-specific immune responses are the same as those seen in human PNA because the route of antigen delivery has been shown to affect the nature of an immune response.¹⁹

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Abbreviations used

CMH:	Cow's milk hypersensitivity
Con A:	Concanavalin A
CPE:	Crude peanut extract
PBST:	PBS containing 0.05% Tween
PCA:	Passive cutaneous anaphylaxis
PNA:	Peanut allergy
RT:	Room temperature
TBST:	TRIS-buffered saline containing 1% Tween

The most significant obstacle to the development of food allergy models is the strong innate tendency of animals to have immunologic tolerance to ingested antigens. Various studies in mice have shown that oral tolerance is influenced by strain,^{20,21} age at first feeding,²²⁻²⁴ and the dose and nature of antigen.^{25,26} Cholera toxin, a potent mucosal adjuvant for secretory IgA production in some mouse strains,^{27,28} has been shown to activate T_{H2} cells and promote production of IL-4 and IgE antibodies.^{29,30} We recently used a combination of experimental conditions to generate the first murine model of IgE-mediated cow's milk hypersensitivity (CMH), which closely resembles the clinical features of immediate cow's milk allergy in human subjects.³¹ However, T- and B-cell responses to cow's milk proteins (α -casein, β -casein, α -lactalbumin, and β -lactoglobulin) in that model have not yet been fully characterized.

In the present study we used a similar approach to generate a murine model of peanut-induced anaphylaxis.³¹ We demonstrated that peanut-specific IgE and immediate hypersensitivity reactions to peanuts can be induced by intragastric administration of peanut antigen. To validate the immunologic relevance of this model to human PNA, T- and B-cell responses to major peanut proteins were evaluated by comparing splenic T cell-proliferative responses and B-cell anti-Ara h 1 and anti-Ara h 2 IgE antibody production. Furthermore, IgE antibody binding to Ara h 2 isoforms and allergenic epitopes were compared. We showed that both T- and B-cell responses to the major peanut allergens in this model are similar to those found in patients with PNA.

METHODS

Mice and reagents

Female C3H/HeJ mice 5 weeks of age were purchased from the Jackson Laboratory (Bar Harbor, Me) and maintained on peanut-free chow under specific pathogen-free conditions. Standard guidelines³² for the care and use of animals were followed.

Freshly ground whole peanut was used as the antigen. Crude peanut extracts (CPEs), Ara h 1 and Ara h 2, were prepared as described previously.^{12,13} Cholera toxin was purchased from List Biological Laboratories, Inc (Campbell, Calif). Concanavalin A (Con A) and dinitrophenyl-albumin were purchased from Sigma (St Louis, Mo). Antibodies for ELISAs were purchased from the Binding Site, Inc, or PharMingen (San Diego, Calif).

Intragastric sensitization and challenge

Mice were sensitized by means of intragastric gavage with 5 mg (equivalent to 1 mg of peanut protein; low dose) or 25 mg (equivalent to 5 mg of peanut protein; high dose) per mouse of ground

whole peanut together with 10 μ g per mouse of cholera toxin on day 0 and again on day 7. Three weeks after the initial sensitization, mice were fasted overnight and challenged with intragastric gavage with CPE of 10 mg per mouse divided into 2 doses at 30- to 40-minute intervals. Mice surviving the first challenge were rechallenged at week 5. Cholera toxin sham-sensitized mice and naive mice were challenged in the same manner.

Measurement of peanut-specific IgE

To monitor serum IgE antibody responses, tail vein blood was obtained at weekly intervals after initial sensitization. Sera were collected and stored at -80°C . Levels of peanut-specific IgE were measured by using ELISA. Briefly, Immulon II 96-well plates (Dynatech Laboratories, Inc, Chantilly, Va) were coated with 2 μ g/mL CPE in coating buffer, pH 9.6 (Sigma, St Louis, Mo). All subsequent steps followed the protocol described previously.¹⁶ IgE antibodies specific for Ara h 1 and Ara h 2 were monitored in pooled sera from peanut-sensitized mice. Plates were coated with Ara h 1 or Ara h 2 (2 μ g/mL), and subsequent steps were performed as described previously.¹⁶ All analyses were performed in duplicate, and coefficients of variation of greater than 10% were repeated to ensure a high degree of precision.

Assessment of hypersensitivity reactions

Anaphylactic symptoms were evaluated for 30 to 40 minutes after the second challenge dose by using a scoring system that was modified slightly from previous reports^{31,33,34}: 0, no symptoms; 1, scratching and rubbing around the nose and head; 2, puffiness around the eyes and mouth, diarrhea, pilar erecti, reduced activity, and/or decreased activity with increased respiratory rate; 3, wheezing, labored respiration, and cyanosis around the mouth and the tail; 4, no activity after prodding or tremor and convulsion; 5, death.

Measurement of plasma histamine levels

To determine plasma histamine levels, blood was collected 30 minutes after the second intragastric gavage challenge. Plasma was prepared as previously described^{16,31} and stored at -80°C until analyzed. Histamine levels were determined by using an enzyme immunoassay kit (ImmunoTECH Inc, Marseille, France), as described by the manufacturer.

Histology

Mast cell degranulation during systemic anaphylaxis was assessed by examination of ear samples collected immediately after anaphylactic death or 40 minutes after challenge from surviving mice as previously described.^{16,30} Tissues were fixed in 10% neutral-buffered formalin, and 5- μ m paraffin sections were stained with toluidine blue or Giemsa stain. Sections from 3 sites of each mouse ear were examined by an observer unaware of their identities using light microscopy at 400 $\times$. A degranulated mast cell was defined as a toluidine blue- or Giemsa-positive cell with 5 or more distinctly stained granules completely outside of the cell. Four hundred mast cells in each ear sample were classified.

Passive cutaneous hypersensitivity testing

Sera were obtained from 6 mice sensitized with a low dose of peanut and pooled. Passive cutaneous anaphylaxis (PCA) tests were performed as previously described,^{31,35} with a slight modification. Briefly, the abdomens of naive mice were shaved 1 day before intradermal injection of 50 μ L of heated (56°C for 3 hours) or unheated sera (1:5 dilution). Control mice received an equal amount of diluted naive serum. Twenty-four hours later, mice were injected intravenously with 100 μ L of 0.5% Evan's blue dye, immediately followed by an intradermal injection of 50 μ L of CPE (4 mg/mL).

Thirty minutes after the dye-antigen administration, the mice were killed, the skin of the belly was inverted, and reactions were examined for visible blue color. A reaction was scored as positive if the bluing of the skin at the injection sites was greater than 3 mm in diameter in any direction.

Proliferation assays

Spleens were removed from peanut-sensitized and naive mice after rechallenge at week 5. Spleen cells were isolated and suspended in complete culture medium (RPMI-1640 plus 10% FBS, 1% penicillin-streptomycin, and 1% glutamine). Spleen cells (1×10^6 /well in 0.2 mL) were incubated in triplicate cultures in microwell plates in the presence or absence of CPE, Ara h 1, or Ara h 2 (10 or 50 μ g/mL). Cells stimulated with Con A (2 μ g/mL) were used as positive controls. Six days later, the cultures were pulsed for 18 hours with 1 μ Ci per well of tritiated thymidine. The cells were harvested, and the incorporated radioactivity was counted in a β -scintillation counter. The results were expressed as counts per minute.

Two-dimensional gel electrophoresis and immunoblotting

Two-dimensional gel electrophoresis was used to separate peanut proteins by using previously described methods with slight modifications.^{13,36,37} The first dimension consisted of an isoelectric focusing gel in glass tubing. After making the gel mixture with a pH gradient of 3.5 to 10 (BioRad Laboratories), 200- μ g samples were loaded and focused with a BioRad Protean II xi 2-D cell at 200 V for 2 hours, 500 V for 2 hours, and 800 V overnight. The second-dimension gel, SDS-PAGE, used an 18% polyacrylamide separating and a 4% stacking gel, as previously described.^{13,38} Electrophoresis was performed for 18 hours at 25 mA per 14 cm by 12 cm gel with a set limit of 150 V in a Hoefer Apparatus (Pharmacia Biotech).

Proteins were transferred from the separating gel to a 0.22- μ m nitrocellulose membrane in a TRIS-glycine buffer containing 20% methanol. The procedure was performed in a Hoefer transfer unit for 14 hours at 100 mA. To assure proper protein separation and quality of transfer, one nitrocellulose membrane from each pair was stained with Amido-black stain, whereas both polyacrylamide gels were stained with Coomassie brilliant blue.

After removal from the transblot apparatus, the nitrocellulose membranes were placed in blocking solution (PBS containing 0.5% gelatin, 0.05% Tween, and 0.001% thimerosal) overnight at room temperature (RT) on a rocking platform. The nitrocellulose blot was then washed 3 times with PBS containing 0.05% Tween (PBST) and incubated with pooled sera from highly sensitive patients with peanut allergy (1:10 dilution in a blocking solution) for 2 hours at RT. After rinsing and washing 4 times with PBST, alkaline phosphatase-conjugated goat anti-human IgE (KPL, 0.5 μ g/mL) was added and incubated at RT for 2 hours. After rinsing and washing with PBST 4 times, the blot was developed with the BCIP/NBT Phosphatase Substrate System (KPL) for 5 minutes. The reaction was stopped by washing the nitrocellulose membrane with distilled water, and the blot was air-dried.

For characterization of mouse IgE antibody binding to allergenic peanut proteins, the nitrocellulose blot was prepared as above. The blot was incubated with pooled sera from peanut-sensitive mice (1:10 dilution) overnight at RT, followed by extensive washes with PBST and another overnight incubation in 0.75 μ g/mL sheep anti-mouse IgE (The Binding Site). The blot was then washed 4 times, and 0.3 μ g/mL horseradish peroxidase-conjugated donkey anti-sheep IgG (The Binding Site) was added. After 2 hours incubation at RT, the blot was washed and developed with TMB Membrane Substrate Three Component System (KPL) for 15 minutes, washed with distilled water, and air-dried.

Mapping of mouse IgE-binding epitopes

The 157 amino acids comprising Ara h 2 were synthesized as 73 overlapping peptides. Each peptide was 13 amino acids long and offset from the adjacent peptide by 2 amino acids. Individual peptides were synthesized on a derivatized cellulose membrane by Genosys Biotechnologies (Houston, Tex). The cellulose membrane containing the synthesized peptides was washed with TRIS-buffered saline containing 1% Tween (TBST) and then incubated with blocking solution of TBST containing 1% BSA overnight at 4°C. After blocking, the membrane was incubated for 15 hours at 4°C with pooled sera from peanut-sensitized mice (3600 ng/mL IgE) that had been diluted 1:10 in a solution containing TBST and 1% BSA. Primary antibody was detected with iodine 125-labeled anti-IgE antibody. The secondary antibody is a rat anti-mouse IgE mAb (Southern Biotechnology Associates, Birmingham, Ala) iodinated by DiaMed, Inc (Windham, Me; iodine 125 label, 18.6 μ Ci/ μ g specific activity). The membrane was exposed to X-ray film, and densitometric scans were made of the autoradiographs to determine the relative amounts of IgE bound to each peptide.

RESULTS

Increased peanut-specific IgE after intragastric peanut sensitization and challenge

To determine peanut-specific IgE responses, sera from each group of mice were obtained weekly after intragastric gavage sensitization and 1 day before challenges. Peanut-specific IgE concentrations increased significantly from week 1 through week 5 in mice sensitized with low-dose peanut (5 mg per mouse) and from week 2 through week 5 in mice sensitized with high-dose peanut (25 mg per mouse; Fig 1). Peanut-specific IgE levels were significantly higher in the low-dose group than in the high-dose group at both week 3 and week 5. In a preliminary study we found that sensitizing doses of 0.1 mg of peanut per mouse and 1.0 mg of peanut per mouse failed to induce peanut-specific IgE response at any time point between week 1 and week 5 after sensitization (data not shown). In this study we found that although both 5-mg and 25-mg sensitization doses could induce peanut-specific IgE, 5 mg was a more effective dose.

Systemic anaphylactic reactions after intragastric challenge

Three weeks after the initial sensitization, mice were fed with CPE by means of intragastric gavage at 30- to 40-minute intervals. Systemic anaphylactic symptoms were evident within 10 to 15 minutes after the first dose, and the severity of the anaphylaxis was evaluated at 30 to 40 minutes after the second dose. The initial reactions consisted primarily of cutaneous reactions with puffiness around the eyes and mouth, diarrhea, or both followed by respiratory reactions, such as wheezing and labored respiration. The most severe reactions provoked loss of consciousness and death (Fig 2, A). Mice sensitized with the low dose (5 mg per mouse plus cholera toxin) of whole peanut exhibited more severe reactions than those sensitized with the high dose (25 mg per mouse plus cholera

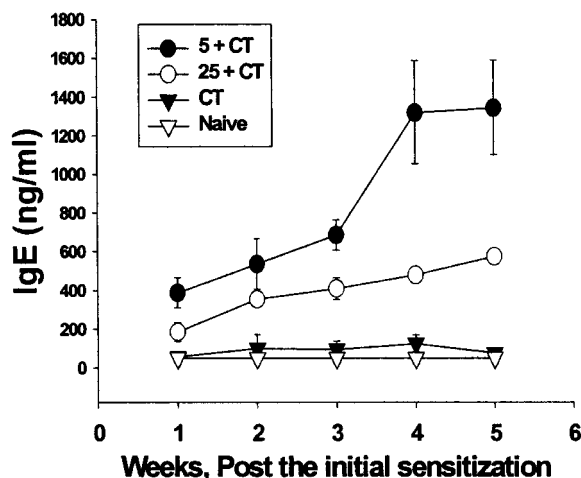


FIG 1. Levels of peanut-specific IgE. Sera from different groups of mice ($n = 8-16$) were obtained weekly after initial peanut sensitization. Peanut-specific IgE levels were determined by using ELISA. Data are given as means $\pm$ SEM of 3 to 4 experiments. CT, Cholera toxin.

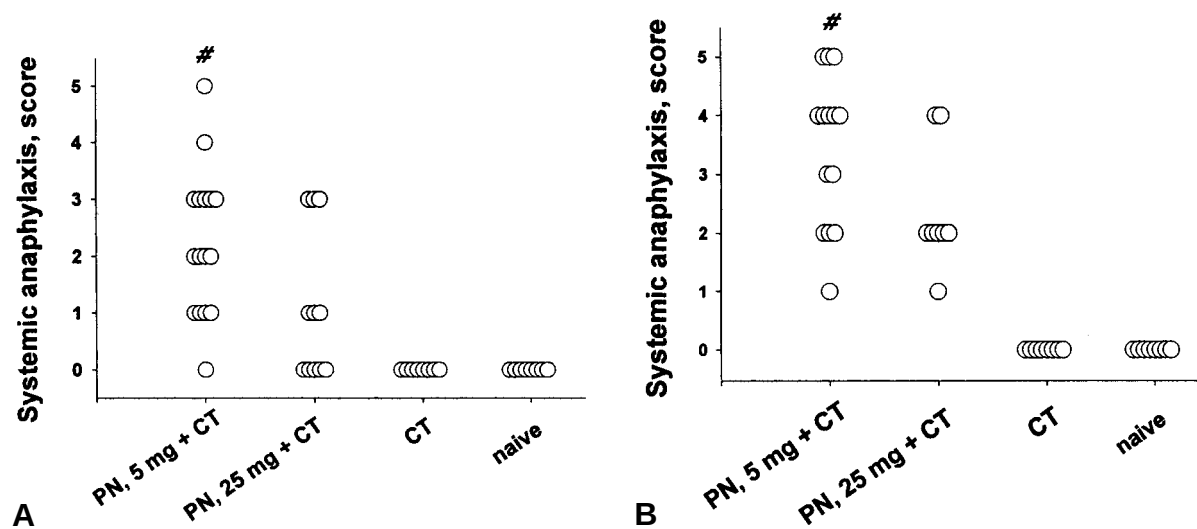


FIG 2. Peanut (PN) antigen-induced systemic anaphylaxis. Mice ($n = 8-16$) were sensitized by means of intragastric gavage with 5 or 25 mg of ground whole peanut plus cholera toxin (CT). At week 3, mice were challenged by means of intragastric gavage with 10 mg of CPE per mouse. Symptoms of anaphylaxis were scored as described in the "Methods" section (A). Mice surviving the first challenge at week 3 were rechallenged at week 5, and the symptoms were scored as above (B). Open circles indicate individual mice. Data are the combined results of 3 to 4 individual experiments. # $P < .05$ versus the high-dose group.

toxin). Fatal or near-fatal anaphylaxis occurred in 12.5% of low dose-sensitized mice but in none of the high dose-sensitized mice. Sham-sensitized and naïve mice did not show any symptoms of anaphylaxis.

Two weeks after the first challenge, the surviving mice were rechallenged. Systemic anaphylactic reactions were again provoked and were more severe than those induced by the first challenge at week 3 with both 5 mg (symptom score week 5 vs week 3, $P = .009$) and 25 mg of peanut (symptom score week 5 vs week 3, $P = .03$; (Fig

2, B). In a preliminary study we saw no significant anaphylactic reactions if sensitized mice were challenged only at week 5 after the initial sensitization (data not shown). Thus the challenge at week 3 appears to have served as an additional boosting dose. As seen at the week 3 challenge, symptom scores at week 5 (rechallenge) were also significantly higher in the group sensitized with 5 mg of peanut than in the group sensitized with 25 mg ($P < .05$). Because sensitization with 5 mg per mouse was optimal for the induction of peanut-spe-

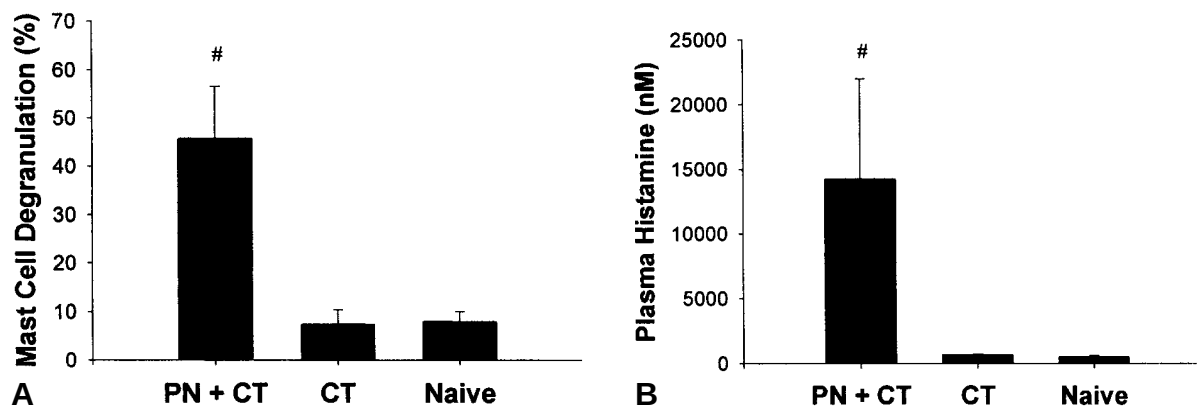


FIG 3. A, Degranulation of mast cells. Ear samples were collected immediately after anaphylaxis-related death or 40 minutes after challenge of surviving mice and fixed. Five micrometers of toluidine blue- or Giemsa-stained paraffin sections were examined by means of light microscopy at 400 $\times$. Four hundred mast cells were classified for each ear sample. Values are expressed as means $\pm$ SEM of 3 to 4 mice per group. $\#P < .001$ versus controls. **B,** Plasma histamine levels. Thirty minutes after peanut (PN) challenge, blood from each group of mice ($n = 4$) was collected, and histamine levels were determined by using a commercial enzyme immunoassay kit. $\#P < .05$ versus controls. CT, Cholera toxin.

TABLE I. PCA reactions after injection of heated or nonheated immune sera

Donor (immunization, ig)	Heat inactivation (56°C for 3 hours)	Diameter, mm (mean $\pm$ SE)	Positive reaction	
			n/total	%
Peanut + cholera toxin	–	8.28 $\pm$ 1.20*	7/7	100
Peanut + cholera toxin	+	1.16 $\pm$ 0.40	0/6	0
Naive	–	1.20 $\pm$ 0.37	0/5	0

Naive mice ($n = 5-7$) were injected with heated or nonheated immune sera followed by intravenous antigen-Evan's blue dye injection. PCA reactions were assessed as described in the "Methods" section.

ig, Intragastric gavage.

* $P < .001$ versus heated immune sera.

cific IgE, as well as anaphylactic reactions after challenge, this dose was used for all subsequent studies.

Increased mast cell degranulation and histamine release after intragastric challenge

The percentage of degranulated mast cells in ear tissues were significantly greater in peanut-sensitized mice than in control mice after peanut challenge (Fig 3, A). Consistent with this finding, plasma histamine levels also were significantly increased in peanut-sensitized mice compared with cholera toxin sham-sensitized and naive mice (Fig 3, B). These results suggest that histamine (and probably other mediators) released from mast cells contributed to the symptoms of peanut-induced anaphylaxis.

PCA reactions

To confirm that IgE was responsible for the induction of peanut hypersensitivity and to rule out IgG1-mediated anaphylaxis, PCA testing was performed. Injection of sera from mice with peanut allergy, but not normal mice, induced PCA reactions. These reactions were eliminated by heat (56°C for 3 hours) inactivation of immune sera (Table I), indicating that IgE is the reagenic antibody in this model.

T cell-proliferative responses to whole peanut and the major peanut allergens Ara h 1 and Ara h 2 resemble those of human PNA

To characterize T-cell responses to whole peanut or major allergens in this model, spleen cells from peanut-allergic mice or naive mice were cultured with crude extract, Ara h 1, or Ara h 2. Although cells from both mice with peanut allergy and naive mice showed significant proliferative responses to Con A stimulation, cells from mice with peanut allergy, but not from naive mice, exhibited significant proliferative responses to crude peanut, Ara h 1, and Ara h 2 stimulation (Fig 4). These results demonstrated that the T-cell responses and the major allergens were similar to those observed in allergic patients.³⁹

B-cell IgE responses to the major allergens Ara h 1 and Ara h 2 resemble those of human PNA

To determine B-cell responses to the major allergens, IgE antibodies against Ara h 1 and Ara h 2 were measured in pooled sera of peanut-sensitized mice and naive

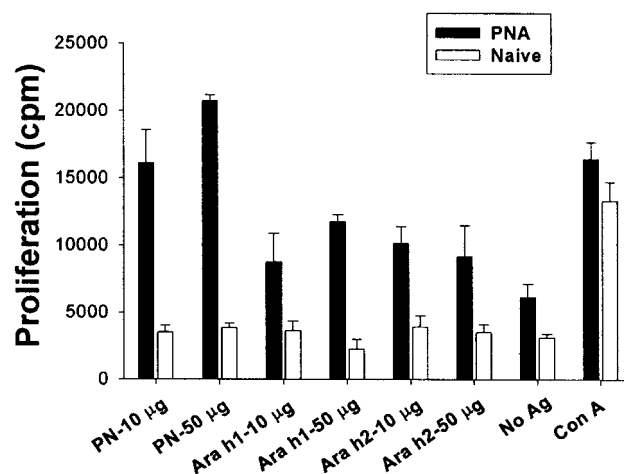


FIG 4. Splenocyte proliferative responses to peanut (PN), Ara h 1, and Ara h 2 stimulation. Triplicate cultures of pooled spleen cells from allergic mice ($n = 2$) and naive mice ($n = 2$), respectively, were stimulated with 10 and 50 $\mu\text{g/mL}$ crude extract, Ara h 1, or Ara h 2. Cells cultured in medium alone or with Con A served as controls. Six days later, the cultures received an 18-hour pulse of 1 μCi per well of tritiated thymidine. The cells were harvested, and the incorporated radioactivity was counted. The results are expressed as counts per minute.

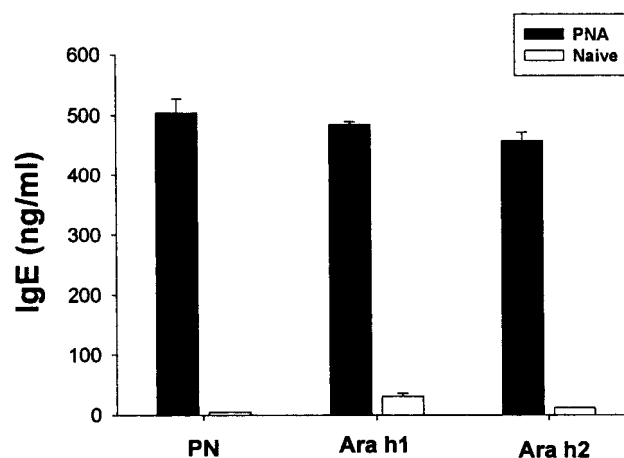


FIG 5. Concentration of peanut (PN)-, Ara h 1-, and Ara h 2-specific IgE. Pooled sera from mice with peanut allergy or naive mice ($n = 6$) were prepared. The levels of peanut-, Ara h 1-, and Ara h 2-specific IgE were determined by using ELISA.

mice. Both Ara h 1- and Ara h 2-specific IgE were present in the sera of mice with peanut allergy (Fig 5). These results demonstrated that B-cell IgE responses to allergens in this model resemble those in human PNA.

Comparison of IgE antibody binding of mice and human subjects with peanut allergy to the major allergen Ara h 2

After the detection of anti-Ara h 1- and anti-Ara h 2-specific IgE antibodies in pooled sera of mice with peanut allergy, we compared IgE antibody binding of mice and human subjects with peanut allergy to the

major allergen Ara h 2 fractions by using two-dimensional gel electrophoresis and immunoblotting. Fig 6, A, shows that human IgE recognizes 8 Ara h 2 isoforms that have been previously characterized (Sampson et al, manuscript in preparation). Fig 6, B, shows that IgE from peanut-sensitized mice recognized the same Ara h 2 isoforms as human IgE.

Ara h 2 IgE-binding epitopes are similar in mice and human subjects

The finding that mouse IgE recognized the same Ara h 2 isoforms as human IgE suggested that mouse IgE and human IgE might bind to similar Ara h 2 epitopes. To

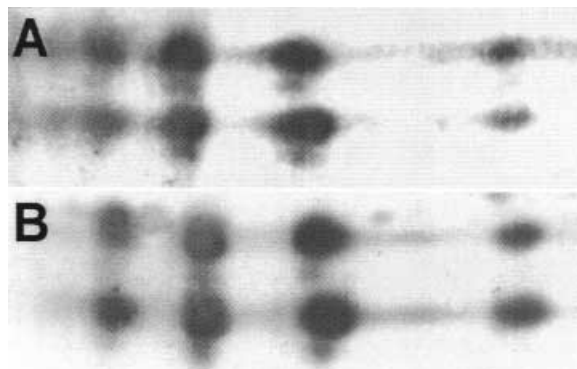


FIG 6. Comparison of mouse and patient IgE antibody binding to Ara h 2 isoforms. Crude protein extract (200 μ g) resolved by two-dimensional SDS-PAGE on nitrocellulose membranes was probed with pooled sera from patients with peanut allergy (**A**) or allergic mice (**B**) as described in the "Methods" section.

TABLE II. Comparison of mouse and patient IgE antibody binding to Ara h 2 epitopes

Mouse IgE-binding epitope	Human IgE-binding epitope	Ara h 2 position	Percentage of IgE binding (mouse)
LFLAAH (1)	HASARQQWEL (1)	M9-15, H15-24	10.8
RQQWELQGDRR (2)	QWELQGDR (2)	M19-29, H21-28	10.9
RCQSQLERA (3)	DRRCQSQLER (3)	M29-37, H27-36	4.4
	LRPCEQHLMQ (4)	H39-48	
DEDSYERDP (4)	KIQRDEDS (5)	M53-61, H49-56	35.0
YERDPYSPS (5)	RDYPSP (6)	M57-65, H59-64	14.3
YSPSPYD (6)	SQDPYSPS (7)	M69-75, H65-72	7.0
QQEQQFK (7)	LQGRQQ (8)	M121-127, H117-122	4.0
KRELRNLPQ (8)	KRELRN (9)	M127-135, H127-132	4.1
RNLPQQCGL (9)		M131-139	3.1
CGLRAPQ (10)		M137-143	3.1
QRCDLDV (11)	QRCDLDVE (10)	M143-149, H143-152	3.0

Numbers in parentheses indicate epitope numbers.

M, Mouse; H, human.

Human IgE-binding epitopes are from Stanley et al, *Arch Biochem Biophys* 1997;342:244-53.

confirm this possibility, we mapped mouse Ara h 2 IgE-binding epitopes. Seventy-three overlapping peptides representing the amino acid sequence of the Ara h 2 protein were synthesized, as indicated above, to determine which regions were recognized by serum IgE. These peptides were probed with pooled sera from peanut-sensitized mice. Table II depicts the 11 IgE-binding epitopes identified. These epitopes were distributed throughout the length of the Ara h 2 protein, with 9 of 11 in essentially the same regions as those previously identified as human IgE-binding epitopes.⁴⁰

Because the mouse Ara h 2 IgE-binding epitopes were similar to the human epitopes, we wondered whether the same epitopes were also immunodominant. In human subjects Ara h 2 amino acid residues 57-74 are considered to be immunodominant because they are recognized by IgE from all peanut-sensitive patients. In addition, serum IgE antibodies that recognize this region represent the majority of Ara h 2-specific IgE found in patients with PNA.⁴⁰ To determine which, if any, of the 11 mouse epitopes was immunodominant, the intensity of IgE binding to each peptide was determined as a function of the pool's total IgE binding to all epitopes. The region represented by amino acid residues 53-75 bound 56.3%

of the Ara h 2-specific mouse IgE, indicating that, as has been observed in human subjects, this region is also immunodominant in peanut-sensitized mice.

DISCUSSION

In the present study we generated a murine model of anaphylaxis in which peanut-specific IgE was induced by oral sensitization, and systemic anaphylactic symptoms were provoked by ingestion. These symptoms involved multiple target organs, including the skin, gastrointestinal tract, and respiratory system. The most severe reactions were fatal. The anaphylactic reactions in this model mimic the clinical characteristics of anaphylactic reactions in human subjects. Although the precise mechanisms involved in the pathogenesis of systemic anaphylaxis after ingestion of peanut are largely unknown, the findings of markedly increased cutaneous mast cell degranulation and elevated plasma histamine in this model suggest that histamine released from activated mast cells contributed to the symptoms of systemic anaphylaxis.

Previous studies, including ours, have shown that IgG1 induces anaphylaxis in some strains of mice. However, in those models serum antigen-specific IgE was not pres-

ent.^{16,41-43} In this study we found that peanut-specific IgE significantly increased after oral sensitization and that PCA reactions were abrogated by heat inactivation of immune sera, thereby strongly implicating IgE as the reagenic antibody in this model. We also found that lower doses of peanut induced higher IgE levels and more severe reactions than higher sensitizing doses. Different responses to high and low antigen doses in this model are in accordance with our findings in the model of CMH, as well as other studies showing that high antigen doses induce tolerance.^{25,26} This phenomenon (ie, high-dose tolerance/low-dose sensitization) may be due to low-dose induction of regulatory cell activation and high-dose induction of clonal deletion or anergy, as described by Chen and Weiner.⁴⁴ However, low and high doses are relative, and very low doses also fail to induce an IgE response.

The hypersensitivity reactions in our model of CMH,³¹ as well as in this model of PNA are both IgE mediated, and the anaphylactic symptoms after oral challenge are essentially the same. However, compared with CMH, induction of peanut-specific IgE required a lower sensitization dose (5 mg per mouse of whole peanut, equivalent to 1 mg of peanut protein vs 10 mg of cow's milk protein per mouse), fewer sensitizing doses (one boosting dose of peanut vs 5 boosting doses of cow's milk), and a shorter sensitization period (3-week sensitization to peanut vs 6-week sensitization to cow's milk). More importantly, we found that peanut hypersensitivity could be induced in mice of 5 to 6 weeks of age (adult), as well as 3-week-old mice (immediately after weaning), whereas CMH could be induced only by sensitization of 3-week-old mice and not adult mice. These results suggest that peanut protein is more allergenic than cow's milk protein and that peanuts can induce hypersensitivity at a later age. The differences between these two models reflect findings in human CMH and PNA.^{45,46}

To further assess the suitability of this model for testing novel approaches to immunotherapy, we assessed T- and B-cell responses to the major peanut allergen proteins Ara h 1 and Ara h 2. We found that spleen cells from mice with PNA exhibited significant proliferative responses to Ara h 1 and Ara h 2, as well as to whole peanut protein stimulation. We also found that both Ara h 1- and Ara h 2-specific IgE were present in the sera of mice with PNA. Moreover, we demonstrated that IgE from peanut-sensitized mice recognized the same Ara h 2 isoforms as IgE from patients with PNA. Of particular significance is the finding that mouse IgE and human IgE bind to similar (9/11) Ara h 2 epitopes and share the same immunodominant region (H57-74/M53-75). This immunologic similarity to human PNA is an important feature of this model because it will allow us to explore clinically relevant immunotherapeutic strategies using this model before launching similar studies in human subjects.

In conclusion, we have established a mouse model of peanut-induced anaphylaxis, using intragastric sensitization and challenge, that closely reflects the clinical characteristics of PNA in human subjects. Peanut-induced

anaphylaxis in this model is IgE mediated, and mast cell degranulation and histamine release are associated with the anaphylactic symptoms. We further demonstrated that B- and T-cell responses of mice with PNA were similar to those observed in human subjects with PNA. This model closely mimics physiologic and immunologic phenomena seen in human subjects with PNA. Studies are underway in our laboratory to test the efficacy of pAra h 2 DNA immunization and Ara h2 peptide desensitization with this newly generated mouse model of peanut hypersensitivity.

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