Peanuts can contribute to anaphylactic shock by activating complement

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Background: Peanut allergy is the most common food-related cause of lethal anaphylaxis and, unlike other food allergies, typically persists into adulthood. Resistance to digestion and dendritic cell activation by the major peanut allergen Ara h 1 are reported to contribute to its allergenicity.

Objective: We sought to evaluate whether peanut molecules might also promote anaphylaxis through an innate immune mechanism.

Methods: Naive mice were treated with a β-adrenergic receptor antagonist and long-acting IL-4 to increase sensitivity to vasoactive mediators and injected with peanut extract (PE). Shock was detected and quantified by means of rectal thermometry. Gene-deficient mice and specific antagonists were used to determine the roles of specific cell types, complement, Fc receptors, and vasoactive mediators in shock pathogenesis.

Results: PE induces dose-dependent shock. PE activates complement in vivo and in vitro in mice and human subjects. C3a and, to a lesser extent, stimulatory immunoglobulin receptors contribute to PE-induced shock. PE-induced shock depends more on macrophages and basophils than on mast cells. Platelet-activating factor and, to a lesser extent, histamine contribute to PE-induced shock. PE induces shock in the absence of the adaptive immune system. LPS contamination is not responsible for PE-induced shock. PE and IgE-mediated mast cell degranulation synergistically induce shock. Tree nuts have similar effects to PE, and skim milk and egg white do not.

Conclusion: Peanuts can contribute to shock by causing production of C3a, which stimulates macrophages, basophils, and mast cells to produce platelet-activating factor and histamine. (J Allergy Clin Immunol 2009;123:342-51.)

Key words: Peanut, C3a, complement, anaphylaxis, shock, macrophages, mast cells, basophils, platelet-activating factor, histamine

Peanut allergy affects approximately 1% of Americans and is the leading cause of food allergy–related death in the United States. Unlike most food allergies, which appear in children but resolve with age, childhood peanut allergy usually persists into adulthood and can reappear in individuals who have become peanut tolerant.

Previous studies suggest that more than one peanut characteristic contributes to allergenicity. Roasting, which is used to process most peanuts consumed in the United States, increases peanut allergenicity. Peanuts contain relatively large quantities of at least 8 proteins that express strong B- and T-cell epitopes and elicit IgE antibody responses. Resistance of peanut allergens to digestion increases the likelihood that sufficient allergen will be absorbed systemically to induce an IgE antibody response and trigger IgE-mediated anaphylaxis. Ara h 1, a strong peanut allergen, activates dendritic cell signaling pathways that induce these cells to present antigens in a way that promotes a proallergic Th2 response by binding to intercellular adhesion molecule 1–grabbing nonintegrin molecule (CD209).

The ability of peanuts to induce an IgE antibody response does not depend entirely on the 8 major peanut allergens, however, because purified major peanut allergens are considerably less immunogenic than a crude peanut extract (PE). This suggests that other “matrix” components of peanuts might have adjuvant properties that promote a Th2 immune response. These considerations suggest that PE has proinflammatory effects that mediate its adjuvant activity and raise the possibility that such effects might even contribute directly to peanut-induced shock. Studies performed to investigate this possibility demonstrate that PE indeed contributes to shock induction by means of immunoglobulin lin-independent activation of the complement system with production of the anaphylatoxin C3a.

METHODS Allergen extracts

Commercial roasted peanuts were ground in a blender (Scovill Manufacturing Company, Racine, Wis) in 0.1 mol/L ammonium bicarbonate (pH 9.0). Insoluble material was removed by means of centrifugation after 4 hours of incubation at room temperature. The supernatant was dialyzed overnight at 4°C against 0.15 mol/L NaCl and then fractionated by means of ammonium sulfate precipitation, with retention of the fraction that was soluble in 25% saturated ammonium sulfate and insoluble in 80% saturated ammonium sulfate. This fraction was resuspended in water and dialyzed 4 times against 0.15 mol/L NaCl. Refrigerated PE solutions were brought to room temperature to dissolve cryoprecipitates before injection into mice.

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Extracts were prepared through a similar process from commercial roasted almonds, cashews, and walnuts. Egg white was aspirated directly from a fresh chicken egg. Fresh commercial skim milk was centrifuged at 10,000 rpm to remove residual fat and particulate matter and filtered through a 0.45-μm filter before use.

**LPS removal**

LPS was removed from PE with an Endotrap red purification system (Profos AG, Regensburg, Germany), according to the manufacturer’s protocol. More than 99% of the LPS in PE preparations was eliminated by using this treatment, as determined by a Limulus Amebocyte Lysate assay with the Limulus Amebocyte Lysate QCL-1000 kit from Cambrex Biosciences (East Rutherford, NJ). Purified LPS was a gift of Stephanie Vogel.

**Mice**

BALB/c wild-type and FcRγ-deficient mice11 were purchased from Taconic (Petersburgh, NY); C57BL/6 μMT,12 mixed background C3-deficient,13 BALB/c C5aR-deficient,14 BALB/c C3aR-deficient,15 and BALB/c Rag1-deficient16 mice and BALB/c mice with severe combined immunodeficiency17 were purchased from the Jackson Laboratory (Bar Harbor, Me). C57BL/6 Toll-like receptor (TLR) 4−,18 TLR2−,19 and MyD88-deficient20 mice were a gift of Christopher Karp (Cincinnati Children’s Hospital Medical Center [CCHMC]). BALB/c FABP-IL-9 transgenic mice21 were bred in the animal facility at CCHMC. Mixed background FcRγ/C3 double-deficient mice were generated by breeding C3-deficient mice with FcRγ-deficient mice. The PCR primers 5′-ACC CTA CTC TAC TGT CGA CTC AAG, 5′-CTC GTG CTT TAC GGT ATC GCC, and 5′-CTC ACG GCT GGC TAT AGC TGC CTT were used to detect homozygous FcRγ-deficient mice. A genotyping protocol recommended by the Jackson Laboratory’s online site was used to detect homozygous C3-deficient mice. Four 8- to 12-week-old mice were used per group, except where noted otherwise. All experiments were performed with prior approval by the CCHMC Institutional Animal Care and Use Committee.

**ELISAs**

Mouse mast cell protease 1 (MMCP-1) levels were measured in serum obtained from blood drawn 2 hours after challenge (unless other specified) with an ELISA kit purchased from Moredun Scientific (Midlothian, Scotland). Plasma histamine levels were determined with an ELISA kit purchased from IBL Hamburg (Hamburg, Germany). The amount of C3a protein in mouse and human plasma was determined by means of ELISA. For the mouse C3a assay, microtiter plate wells were coated with purified rat anti-mouse C3a antibody (catalog no. 558251, BD PharMingen), horseradish peroxidase (HRP)–streptavidin, and SuperSignal ELISA substrate (Pierce Biotechnology, Cheshire, United Kingdom). Purified native mouse C3a (catalog no. 558618, BD PharMingen) was used as a standard. For the human C3a assay, microtiter plate wells were coated with purified mouse anti-human C3a mAb (catalog no. GAU 013-16-02, Affinity BioReagents, Golden, Colo), followed by biotin-labeled mouse anti-human C3a mAb (catalog no. GAU 017-01-02B, Affinity BioReagents), HRP-streptavidin, and SuperSignal ELISA substrate (Pierce Biotechnology). Purified native human C3a (Binding Site, Birmingham, United Kingdom) was used as a standard.
minutes before challenge or with 30 mg/kg of the C3aR antagonist SB29015726 (EMD Chemicals, Inc, Gibbstown, NJ) 3 hours before challenge. Mice were depleted of mast cells by injecting 1 mg of anti–c-kit antibody (ACK2) intravenously and then intraperitoneally every other day for 14 days. Control mice received equal doses of an isotype-matched control antibody (J1.2).

Complement activation

Mouse or human plasma was prepared by centrifuging blood that had been collected in EDTA-coated tubes and was immediately used or frozen until use. Plasma (50 μL) was mixed with 2 μL of 1 mol/L CaCl2, 2 μL of 1 mol/L MgCl2, and 5 μL of PE and then incubated for 10 minutes at 37°C to test for activation of complement. The reaction was stopped by adding 11 μL of 0.7 mol/L EDTA (pH 8.0) and cooling to 0°C.

Antibody treatment

IgE-mediated anaphylaxis was induced by injecting mice intravenously with 80 μg of EM-95 (rat IgG2a anti-mouse IgE mAb).38

Characterization of shock

Severity of shock was assessed by means of rectal thermometry.22,29

IL-4/anti–IL-4 mAb complexes

IL-4/anti–IL-4 mAb complexes (IL-4C) were prepared by mixing IL-4 and propranolol and then incubated for activation of complement. The reaction was stopped by adding 11 μL of 0.7 mol/L EDTA (pH 8.0) and cooling to 0°C.

complement, bind more avidly than free IgG to FcyRs, or interact simultaneously with FcyRs and cytokine receptors because they contain a single IgG antibody molecule, and the anti–IL-4 mAb used, BVD4-1D11, blocks IL-4 binding to the IL-4 receptor α chain.30

Induction of shock with PE

Mice were pretreated for 24 hours with IL-4C (1 μg of IL-4 plus 5 μg of BVD4-1D11 rat IgG2b anti-mouse IL-4 mAb per mouse) and for 20 minutes with propranolol (35 μg per mouse). Both propranolol and IL-4C were injected intravenously. Mice were challenged intravenously with PE (250 μg per mouse unless other specified).

Oral inoculation

Oral inoculation was performed with intragastric feeding needles (catalog no. 01–290-2B; Thermo Fisher Scientific, Rochester, NY). Mice were deprived of food for 3 to 4 hours before each intragastric challenge.

Basophil depletion

Mice were injected intravenously with 35 μg of Ba103, a nonactivating depleting mAb to the basophil-specific antigen CD200R3,31,32 Preliminary experiments demonstrated approximately 80% depletion of splenic and bone marrow basophils, which were identified as FcRI+‘c-kit+’ cells.31

Worm inoculation

BALB/c mice were inoculated subcutaneously with 500 Nippostrongylus brasiliensis third-stage infectious larvae (L3).34

FIG 2. PE-induced shock is MyD88, TLR2, TLR4, and LPS independent. A, C57BL/6 wild-type and MyD88-deficient mice (left panel) or wild-type and TLR2- and TLR4-deficient mice (right panel) were pretreated with IL-4C and propranolol and challenged with 250 μg of PE and then followed with rectal thermometry for development of shock. B, BALB/c mice pretreated with IL-4C and propranolol were injected intravenously with 10 or 50 μg of Salmonella typhimurium LPS or 300 μg of PE and followed for development of shock.
which can exacerbate anaphylactic shock. Mice pretreated in the absence of B and T cells, age-, sex-, and genetic background–matched BALB/c wild-type and Rag1-deficient mice (upper panel) or C57BL/6 wild-type and μMT mice (lower panel) were pretreated with IL-4C and propranolol and injected intravenously with 250 μg of PE and followed with rectal thermometry for development of hypothermia.

Statistics

Data were analyzed for statistical significance with ANOVA and Fisher protected least significant difference tests by using Statview software. P values of less than .05 were considered statistically significant.

RESULTS

PE induces shock in IL-4C/propranolol-pretreated mice

Because peanuts are responsible for such a large percentage of severe anaphylaxis in most developed countries, we hypothesized that they might induce shock through an innate immune mechanism in addition to the classical IgE/mast cell/vasoactive mediator pathway. To investigate this possibility, we evaluated whether injecting nonimmune mice with water-soluble PE would cause shock (detected as hypothermia22). When PE-treated mice did not have hypothermia (data not shown), we increased the sensitivity of our model by pretreating mice with a long-acting form of IL-4–adrenergic antagonist propranolol, which protected least significant difference tests by using Statview software. PE induces shock in otherwise healthy mice, even after sensitization with IL-4C and propranolol. Because this suggested that PE must be absorbed systemically to induce shock, we evaluated whether shock is induced by PE ingestion in mice in which increased intestinal permeability has been induced by infection with the intestinal worm parasite *N. brasiliensis.* Significant hypothermia was induced in these mice by means of oral inoculation of PE (Fig 1, B) but not by means of oral saline inoculation (data not shown).

Mice pretreated with IL-4C and propranolol were challenged with fresh egg white or skim milk or water-soluble extracts of cashews, walnuts, and almonds to determine whether other allergens might share the effects of PE. Shock was induced by 250 μg of PE and, to a lesser extent, by 250 μg of cashew, walnut, and almond extracts (Fig 1, C, and data not shown). In contrast, 2000 μg of egg white protein failed to induce shock, and 2000 μg of skim milk protein had only a trivial effect (Fig 1, C).

PE-induced shock is TLR independent

Because our preparations of PE contain LPS, we were concerned that contamination with LPS or other TLR ligands might be responsible for PE-induced shock. Three different approaches were used to investigate this possibility, and all make it unlikely. First, PE injection induced severe hypothermia in MyD88−, TLR2−, and TLR4-deficient mice, despite the failure of LPS, bacterial lipoprotein, or both to induce shock in these mice (Fig 2, A). Second, removal of more than 99% of LPS from PE did not alter its ability to induce hypothermia (Fig 2, B). Third, shock induced by injecting mice with large quantities of LPS developed much more slowly than PE-induced shock (Fig 2, C). Thus shock induction by PE is not a result of LPS contamination.

PE induces shock in the absence of antibody

PE might induce shock by reacting with natural IgG or IgE antibodies. PE was injected into Rag1−deficient mice, which lack B and T cells, or μMT mice, which lack B cells, to investigate this possibility. Results demonstrate that PE induces shock in both antibody-deficient mouse strains (Fig 3). Shock develops in mice with severe combined immunodeficiency in a similar manner (data not shown). Thus PE induces shock in the absence of the adaptive immune system.

PE-induced shock is more macrophage and basophil dependent than mast cell dependent

Because shock can be mediated by macrophages, basophils, and mast cells in antibody-dependent anaphylaxis,22,32 we evaluated whether these cell types contribute to PE-induced shock. Elimination or inactivation of most macrophages with either gadolinium23 or clodronate-containing liposomes24 considerably reduced the severity of PE-induced shock (Fig 4, A). Elimination of most basophils with a specific nonactivating antibody to CD200R3 (Ba103) also partially suppressed PE-induced shock, and the combination of Ba103 and gadolinium had a slightly greater suppressive effect than gadolinium alone (Fig 4, B). In contrast, although PE induces some mast cell degranulation, as shown by slightly increased serum levels of MMCP-1 (Fig 4, A), mast cell depletion with anti–c-kit mAb did not detectably inhibit shock severity (Fig 4, C). Mast cell depletion in this
experiment was sufficient to decrease the MMCP-1 and histamine responses to anti-IgE mAb by approximately 95% and 80%, respectively (Fig 4, C), and almost totally blocked the histamine response to PE (Fig 4, D). Inhibition of mast cell degranulation by treating mice with 30 mg/kg cromolyn every 12 hours for 60 hours21 also had no effect on PE-induced shock (not shown). Thus macrophages and basophils appear to be more important than mast cells in PE-induced shock.

PE-induced shock is predominantly PAF dependent but partially histamine dependent

Mast cells, basophils, and macrophages predominantly contribute to the pathogenesis of anaphylaxis through their secretion of histamine and PAF.22,32 The involvement of basophils and macrophages in PE-induced shock led us to evaluate whether the same mediators are also involved in this process. An experiment performed with a specific PAF receptor antagonist demonstrated that PE-induced shock is substantially ameliorated by a PAF receptor antagonist (Fig 5). Surprisingly, in view of our studies with anti–c-kit mAb (Fig 4), antihistamine treatment also inhibited PE-induced shock, and the combination of PAF and histamine antagonists suppressed shock more completely than the PAF antagonist alone (Fig 5). Thus PAF is more important than histamine in PAF-induced shock pathogenesis, which is consistent with the greater role of macrophages and basophils than mast cells, but both mediators appear to be involved.

PE-induced shock is predominantly C3 dependent

Taken together, our observations indicate that PE can induce shock by stimulating macrophage, basophil, and mast cell production of PAF and histamine through an immunoglobulin-independent mechanism. Because macrophages and mast cells can be activated to secrete vasoactive mediators by C3a and C5a,39-41 which are produced by complement activation,42 this suggested that PE might activate complement through the alternative or lectin pathway.43 To test the possibility that PE induces shock through a complement-dependent mechanism, we first compared the ability of PE to induce shock in wild-type mice

FIG 4. PE-induced shock depends more on macrophages and basophils than on mast cells but is accompanied by mast cell activation. A, BALB/c mice were treated with saline (no macrophage depletion) or with gadolinium or clodronate-containing liposomes to deplete macrophages and then pretreated with IL-4C and propranolol, injected intravenously with 250 μg of PE, and followed with rectal thermometry. Sera from mice injected with saline or PE were analyzed for MMCP-1 content by means of ELISA. B, BALB/c mice were treated with gadolinium to deplete macrophages, 35 μg of Ba103 anti-basophil mAb to deplete basophils, both gadolinium and Ba103, or a control mAb isotype matched to Ba103. Mice were then pretreated with IL-4C and propranolol, injected intravenously with 250 μg of PE, and followed with rectal thermometry. C, BALB/c mice were treated for 15 days with 0.5 mg per mouse of anti-c-kit mAb by means of intraperitoneal injection every other day to deplete mast cells or an isotype-matched control mAb and then pretreated with IL-4C and propranolol and challenged intravenously with 250 μg of PE. Sera obtained from mice after injection of an activating anti-IgE mAb were assayed for MMCP-1 content or histamine content by means of ELISA. D, BALB/c mice treated with saline or anti-c-kit mAb as in Fig 4, B, were challenged with saline or PE and bled 5 minutes later. Sera were assayed for histamine content by means of ELISA. *P < .05 compared with saline in Fig 4, A and C, and control mAb in Fig 4, B. **P < .05 for anti-c-kit mAb compared with saline in Fig 4, C.
and C3-deficient mice, which lack the complement component that is central to all 3 complement activation pathways. To evaluate an alternative possibility, that macrophages, basophils and mast cells are activated by PE through an FcR-dependent pathway, we compared shock induction by PE in wild-type versus FcRy-deficient mice, which lack the polypeptide essential for all known mouse activating Fc receptors. Results (Fig 6) demonstrate that PE induces shock normally in FcRy-deficient mice, to only a slight extent in C3-deficient mice, and not at all in mice deficient in both C3 and FcRy. C3aR- and C5aR-deficient mice and antagonists to these 2 receptors were used to evaluate the roles of each complement-generated anaphylatoxin in PE-induced shock. Results of these experiments (Fig 6, B and C) demonstrate an important role for C3a but not for C5a. Analysis of serum from mice injected with saline or PE confirmed that PE rapidly activates complement with production of C3a (Fig 6, D).

To determine whether these observations with a mouse model might be relevant to human subjects, we also evaluated the ability of PE, cashew extract, egg white, and skim milk to activate mouse and human complement in vitro using C3a-specific ELISAs as a readout. PE and cashew extract increased C3a levels in both mouse and human plasma (Fig 6, E) in a dose-dependent manner (Fig 6, F). In contrast, skim milk and egg white, which fail to induce shock in IL-4C/propranolol–pretreated mice, had little effect. The mouse assay was considerably more sensitive than the human assay. This might reflect assay properties rather than a difference in the relative ability of PE to activate mouse versus human complement because a similar difference was observed when fixed gram-negative bacteria were used to activate complement (data not shown).

**Shock is synergistically induced by PE and IgE-stimulated mast cell degranulation**

The requirement for presensitization with propranolol and IL-4C makes it unlikely that peanut ingestion can induce shock in healthy individuals (or even those with increased intestinal permeability) solely through direct activation of complement. In contrast, it seemed possible that limited complement activation by PE might contribute to shock induction by acting synergistically with IgE-induced mast cell degranulation in individuals who have IgE antibodies to peanut antigens. To test this possibility, wild-type, C3-deficient, and FcRy-deficient mice pretreated only with a low dose of propranolol were challenged with anti-IgE mAb, PE, or both and evaluated for development of shock. PE failed to induce detectable hypothermia in wild-type mice pretreated with low-dose propranolol (not shown) but considerably exacerbated shock when these mice were also challenged with anti-IgE mAb (Fig 7, left panel). This synergism was C3-dependent because PE did not exacerbate anti-IgE mAb-induced hypothermia in C3-deficient mice (Fig 7, middle panel). Furthermore, PE had little effect on shock development in this system when anti-IgE mAb–induced mast cell degranulation was blocked by the absence of FcRy (Fig 7, right panel). Synergistic induction of shock by PE and anti-IgE mAb was also observed in wild-type mice in the absence of any sensitization with propranolol or IL-4C (Fig 7, B) and when PE was administered orally rather than intravenously (Fig 7, D). Oral administration of a large dose of PE caused a rapid increase in plasma C3a concentrations that was small but statistically significant (Fig 7, C). This increase was considerably greater in mice that expressed an IL-9 transgene in their intestinal epithelium, which causes an increase in intestinal permeability, than in normal mice (Fig 7, C). Oral administration of PE also slightly but significantly enhanced the severity of shock induced by intravenous administration of anti-IgE mAb (Fig 7, D).

**DISCUSSION**

Our observations, when combined with those published by other investigators, suggest that the high incidence, persistence, and severity of peanut allergy might result from a combination of properties that make it a perfect allergen: high content of several poorly digestible proteins that have strong B- and T-cell epitopes, the ability of at least 1 of these proteins to directly activate antigen-presenting cells, and the ability of soluble peanut molecules to rapidly activate complement with production of large amounts of the anaphylatoxin C3a. The rapid production of C3a through an antibody-independent pathway stimulates macrophages, basophils, and, to a lesser extent, mast cells to secrete PAF and histamine, which contribute to the induction of shock by increasing vascular permeability. C5a, another complement-derived anaphylatoxin, does not appear to be important in this process, even though it strongly promotes leukocyte activation and migration in immune complex disease, and both C3a and C5a can contribute to the pathogenesis of asthma and sepsis.

Although our studies consistently demonstrate that PAF contributes more than histamine to PE-induced shock, the results of our experiments that evaluate the importance of histamine appear inconsistent. A role for histamine is supported by evidence that PE induces a histamine response and the ability of the H1 antagonist triprolidine to decrease the severity of PE-induced shock. The suppressive effect of triprolidine was most obvious in experiments that compared the effect of a PAF antagonist with the combined effect of this antagonist plus triprolidine (Fig 5). In contrast, treatment of mice with anti-c-kit mAb, which kills mast cells...
FIG 6. PE-induced shock is mediated predominantly by C3a. A, Wild-type, FcRγ-deficient, C3-deficient, and FcRγ/C3 double-deficient mice on the same genetic background were pretreated with IL-4C and propranolol, injected intravenously with 250 μg of PE, and followed for 2 hours with rectal thermometry. B, C3aR-deficient, C5aR-deficient, and wild-type mice on the same BALB/c genetic background were pretreated with IL-4 and propranolol as described above, challenged intravenously with 250 μg of PE, and followed for the next 2 hours with rectal thermometry. C, BALB/c mice were pretreated with propranolol and IL-4C. Mice were also injected intraperitoneally with 0.6 mg of the C3aR antagonist SB290157 3 hours before challenge with PE, intravenously with 20 μg of the C5aR antagonist A8Δ71-73 20 minutes before challenge with PE, or both. Mice were followed with rectal thermometry for 115 minutes after intravenous challenge with 400 μg of PE. D, Sera from mice injected intravenously with saline or with 300 μg of PE were analyzed for C3a concentration by means of ELISA. *P < .05 compared with saline. 1P < .05 compared with 5 minutes. E, The abilities of saline, egg white, skim milk, cashew extract, and PE to activate mouse and complement with production of C3a were determined. F, Mouse and human plasma were treated with the amounts of PE indicated, and C3a levels were determined by means of ELISA.
by eliminating an essential growth factor, had no effect on PE-induced shock, even though it almost completely blocked histamine production (Fig 4). This suggests that triprolidine inhibits PE-induced shock by blocking the effects of a mediator other than histamine (eg, bradykinin) or that treatment of mice with anti–c-kit mAb enhances mast cell–independent pathways of inflammation (eg, c-kit can promote mast cell production of PAF acetylhydrolase, which catabolizes PAF).

Although PE contains some LPS, which stimulates C3a production through the alternative complement activation pathway, as well as the classical pathway, PE-induced shock was not caused by LPS contamination. This was demonstrated by 3 sets of studies: (1) TLR2, TLR4, and MyD88 deficiency did not protect mice from PE-induced shock; (2) removal of more than 99% of LPS from PE also failed to diminish PE-induced shock; and (3) LPS induction of hypothermia was shown to be less rapid and more prolonged than that induced by PE.

Detection of PE-induced shock required development of a system that allows a normally inapparent insult to become obvious. Systemic anaphylaxis in mice is mediated predominantly by vascular leak, which causes hypotension that is reflected by hypothermia. Development of hypotension is normally limited by the magnitude of vasoactive mediator effects on vascular endothelial cells that increase their permeability and by β-adrenergic–dependent increases in vascular tone, heart rate, and myocardial contractility that compensate for decreased intravascular volume. With these observations in mind, we made mice more sensitive to PE-induced shock by pretreating them with IL-4, which increases sensitivity to vasoactive mediators, and with propranolol, which blocks β-adrenergic compensatory mechanisms and can increase the severity of human anaphylaxis. Pretreatment with IL-4C and propranolol does not induce shock by itself but increases the ability of ingested allergens to induce IgE-dependent anaphylaxis (unpublished data).

The requirement for presensitization of mice with IL-4 and propranolol to allow PE to induce shock makes it unlikely that peanut-mediated complement activation induces shock by itself in the absence of other insults. This is consistent with the ability of normal rodents and most human subjects to eat large quantities of peanuts without untoward effects and the observation that a negative RAST test result is highly predictive of peanut tolerance in human subjects. In contrast, PE-induced C3a production probably acts synergistically with IgE/FcεRI-dependent mast cell degranulation to exacerbate anaphylaxis. This hypothesis is supported by our observation that the severity of IgE-mediated anaphylaxis is enhanced by PE, even in the absence of both IL-4 and propranolol and even when PE is administered orally.

Although we do not know whether our mouse model results are directly relevant to peanut-induced anaphylaxis in human subjects, it is notable that PE can activate complement in both mouse and human plasma in vitro (Fig 6). It is also notable that complement is activated by hymenoptera venom, the most common cause of severe, non–food-related human anaphylaxis, and by metabolites of penicillin, the most common cause of severe
drug-induced human anaphylaxis. Thus exacerbation of anaphylaxis by complement activation might not be restricted to mice or to our PE model; rather complement activation products might have a general role in the pathogenesis of severe IgE-mediated anaphylaxis.

In contrast to peanuts and tree nuts, which activate complement and are associated with severe anaphylaxis, milk and egg white have little ability to activate complement in vivo or in vitro and typically cause relatively mild allergic reactions. It is tempting to speculate that this association between complement activation and induction of severe anaphylaxis is not coincidental but reflects complement exacerbation of the effector phase of anaphylaxis.

Because activated complement is a potent adjuvant, peanut activation of complement might contribute to induction of the IgE response to peanut allergens in addition to the effector phase of peanut-induced shock. This possibility is consistent with the considerably poorer immunogenicity of purified major peanut allergens than unfraccionated PE and with our initial observations that 2 purified major peanut allergens, Ara h 1 and Ara h 2, have little or no ability to induce shock (data not shown). Instead, we find that a low-molecular-weight (<5 kD) fraction of PE, which has a partial amino acid sequence that does not match that of any of the 8 major peanut allergens, is particularly effective at inducing shock when injected into mice intravenously (unpublished data). Studies are underway to identify this molecule and to determine whether it can act as an adjuvant that promotes Th2 responses to major peanut allergens. Identification of this putative adjuvant might provide a strategy for engineering peanuts that have reduced allergenicity.

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Clinical implications: Peanut-induced C3a might act synergistically with IgE-dependent mast cell activation to cause shock in patients with peanut allergy and might contribute to peanut induction of an IgE response.

REFERENCES