

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

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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-lipoxygenase 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* ■■■■;■■■:■■■-■■■.)

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 Ca²⁺ 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,

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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

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 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 $37.5^{\circ}\text{C} \leq T \leq 40^{\circ}\text{C}$), *mild reaction* (score ≤ 2 and $34.5^{\circ}\text{C} \leq T \leq 37.5^{\circ}\text{C}$), or *severe or life-threatening reaction* (score ≥ 3 and $T \leq 34.5^{\circ}\text{C}$).

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 IgG₁ and IgE

Peripheral blood was collected 1 week after the last sensitization, and levels of serum peanut-specific IgG₁ 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 $\mu\text{g}/\text{mL}$ 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 (phycoerythrin conjugated), 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 Fc ϵ RI (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⁺ Fc ϵ RI⁺ cells (Fig 1, B and C) along with significant upregulation of Fc ϵ RI 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 Fc ϵ RI 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.

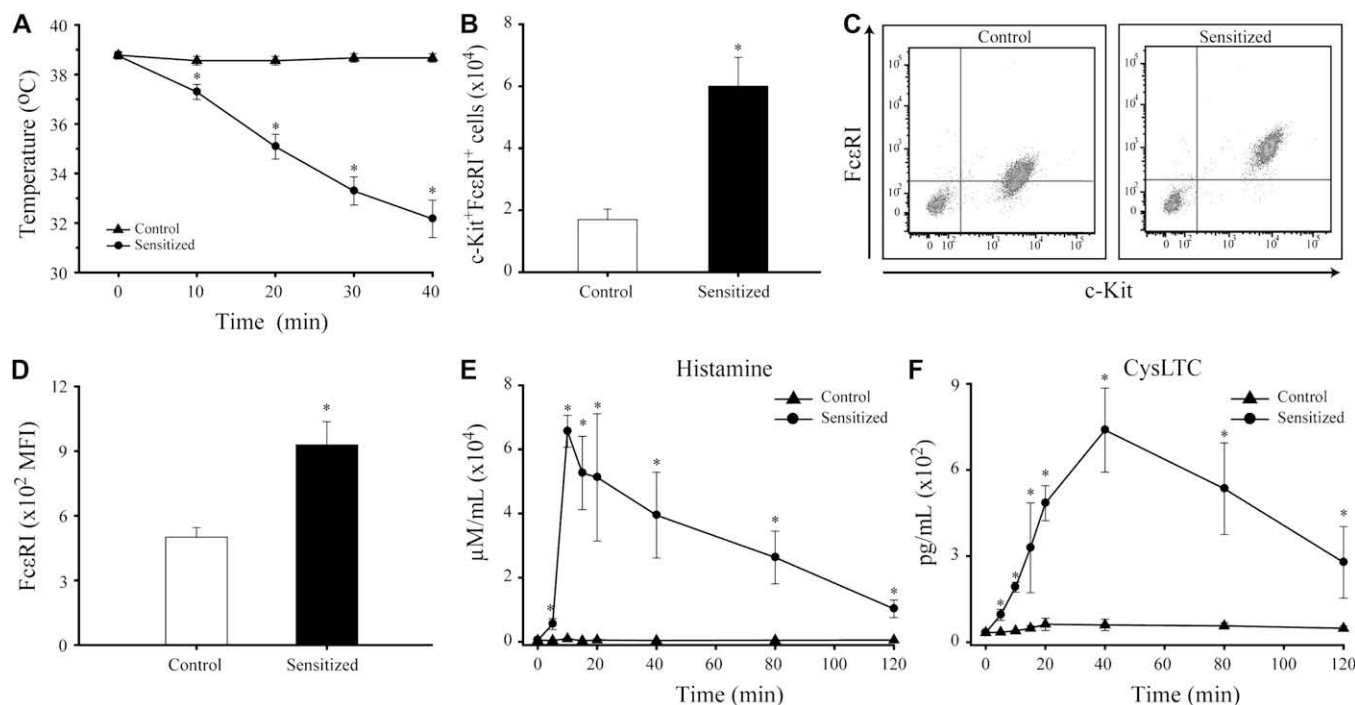


FIG 1. Peanut-induced anaphylaxis is preceded by upregulation of FcεRI on c-kit⁺ cells and accompanied by release of mediators. **A**, Rectal temperature of control (▲) and sensitized (●) mice after challenge. **B**, FcεRI⁺ and c-kit⁺ peritoneal cells. **C**, Representative fluorescence-activated cell sorting plot. **D**, Mean fluorescence intensity (MFI) of FcεRI on c-kit⁺ cells. **E**, Histamine levels. **F**, Leukotriene levels. Means ± SEMs from 3 independent experiments; n = 8 mice/group. *P < .05 relative to control.

As shown in Fig 2, A and B, blockade of this pathway did not affect the extent and/or kinetics of the anaphylactic reactions. Moreover, we observed comparable levels of histamine in plasma from untreated versus treated mice (Fig 2, C). Conversely, cysLTs were nearly undetected in plasma from treated, but not untreated, mice, indicating that pharmacologic inhibition of the 5-LO pathway was successful (Fig 2, D). In agreement with these findings, mice with a targeted disruption in the 5-LO gene manifested the same degree of anaphylaxis as their wild-type counterparts after challenge (data not shown).

To examine the role of histamine, peanut-sensitized mice were administered H1 and H2 receptor antagonists 30 minutes before challenge. Our data indicate that blocking H1 and H2 receptors did not change the severity or the course of the anaphylactic reaction (Fig 2, E and F). Moreover, we observed comparable levels of histamine and cysLT (Fig 2, G and H) in plasma between treated and untreated mice after challenge, suggesting that the extent of MC degranulation was equivalent in both groups. Thus, antihistamines, at least when given alone, do not ameliorate the clinical manifestations of PIA.

Impact of PAF-R antagonism on the severity and duration of PIA responses

Peanut-sensitized mice were treated with a PAF-R antagonist once before challenge and monitored for 40 minutes. Targeting PAF activity considerably lessened the severity of the reactions (Fig 3, A, B, and E). In fact, ~50% of treated mice developed either no or mild anaphylactic reactions. Notably, there was a considerable difference in the number of mice whose core

temperature dropped by more than 4°C (87% in untreated vs 48% in treated), suggesting that PAF antagonism considerably prevented life-threatening reactions. Moreover, although 15% of untreated mice developed convulsions, no seizures were observed in the treated group. Of note, the levels of peanut-specific IgE and IgG1 were similar among groups, indicating equivalent sensitization (data not shown). Together, our data demonstrate that the blockade of PAF-R substantially prevents severe anaphylactic reactions to peanuts.

To investigate whether PAF-R antagonism had an impact on the time of recovery from an anaphylactic reaction, we monitored clinical responses for 120 minutes after peanut challenge. We observed a clear difference in the number of mice that reached recovery temperature (≥37°C) within 120 minutes after challenge (83% in treated vs 43% in untreated; Fig 3, C and D). It should be noted that mice experiencing prolonged severe reactions showed a slower rate of recovery and, in some cases, did not show any clinical improvement during the observation period (Fig 3, F). Importantly, ~30% of untreated, but only 9% of treated, mice experienced severe hypothermia 120 minutes after challenge, thus decreasing their likelihood of recovering fully and, correspondingly, increasing the probability of death. In fact, ~20% of untreated, but none of the treated, mice died within 48 hours after challenge. Collectively, these data provide evidence that PAF-R antagonism attenuates the magnitude and duration of PIA reactions.

To assess the anaphylactic response more comprehensively, we developed parameters to grade severity as *global*, *moderate*, and *life-threatening*. To this end, we constructed a mathematical model describing the overall behavior of the anaphylactic

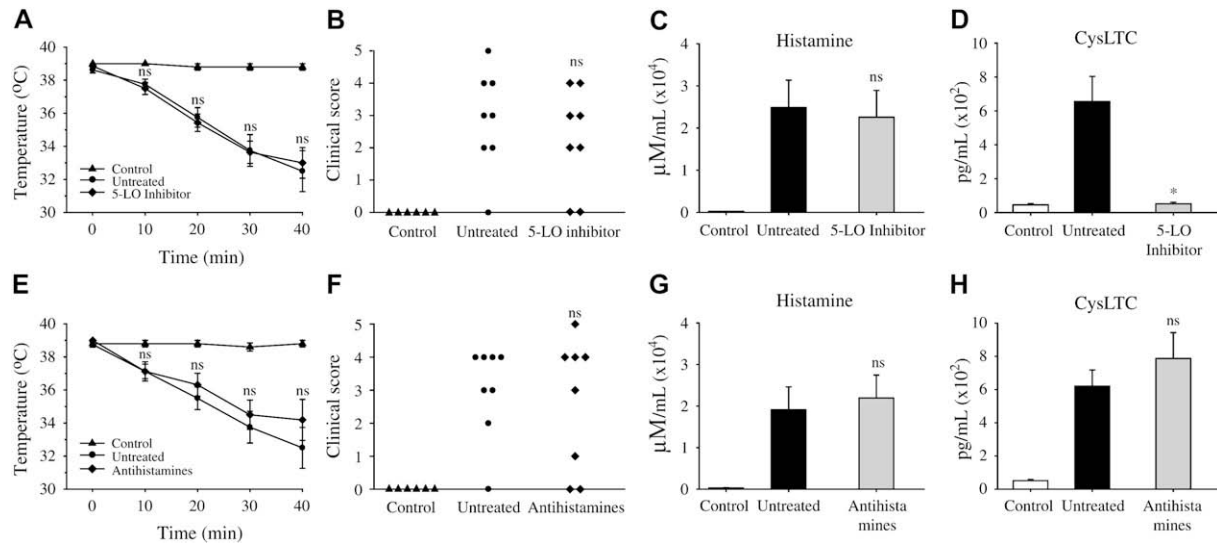


FIG 2. Histamine and leukotrienes are nonessential mediators but are biomarkers of PIA. Mice were treated with (A-D) a 5-LO inhibitor or (E-H) histamine receptor antagonists. (A and E) rectal temperature and (B and F) clinical symptoms from control (▲), untreated (●), and treated (◆) groups. (C and G) Histamine and (D and H) leukotriene levels after challenge. Representative data from 3 independent experiments; n = 8-12 mice/group. **P* < .05 relative to control. ns, Not significant.

response for each experimental group (see this article's Supplementary Methods in the Online Repository at www.jacionline.org). By using mean experimental data for untreated mice, we derived an equation that represents the pattern of change in core temperature as long as 120 minutes after challenge. The same equation was then fit to the other experimental group (mice treated with PAF-R antagonist) to derive the numerical coefficients that accurately depict the response. These equations were subsequently used to generate modeled data and validated (Fig 3, G). As shown in Fig 3, H, we defined *global severity* as the area of the response under 39°C (AU_{39°C}), whereas *life-threatening severity* was depicted as the area of the response under 35°C (AU_{35°C}). *Moderate severity* was deemed as the difference between global and life-threatening severity (AU_{39°C} - AU_{35°C}). Given that these areas were calculated using mean group temperatures at each time point, they describe the overall trend of the response. Hence, the measure of AU_{35°C} does not accurately differentiate life-threatening conditions from the overall response. In other words, the AU_{35°C} is not representative of those mice that experienced life-threatening anaphylaxis exclusively but rather encompasses the response of the entire population. Therefore, we introduced an additional parameter, "events under 35°C", to provide a more exclusive assessment of life-threatening severity. Finally, we evaluated the duration of life-threatening conditions by calculating the time each response was below 35°C.

The model shows that treatment with a PAF-R antagonist resulted in ~50% decrease in global severity. In addition, the number of "events under 35°C" was 41 in treated mice versus 133 in untreated mice, indicating that untreated mice approached life-threatening conditions to a much greater extent (Fig 3, H). This is relevant because mice experiencing a severe drop in core temperature are less likely to recover and thus succumb to challenge. Moreover, treated mice were under life-threatening conditions for ~75% less time compared with untreated mice (Fig 3, H).

Systemic anaphylactic reactions are often associated with excessive vascular permeability and fluid extravasation.^{2,16,17} In

accordance with this, we observed that the blockade of PAF-R substantially reduced vascular leakage, as indicated by a 57% decrease in the content of albumin in the PL fluid of treated mice compared with untreated mice after challenge. The treatment was also associated with a significant decrease in the levels of histamine and cysLTs in plasma after challenge (see this article's Table E1 in the Online Repository at www.jacionline.org).

Impact of concurrent blockade of histamine and PAF receptors in peanut-induced anaphylaxis

The complexity of the interactions among vasoactive mediators involved in allergic disease has led to the suggestion that control of clinical manifestations may require the blockade of various mediators.^{18,19} Hence, we treated peanut-sensitized mice with a PAF-R antagonist along with H1 and H2 receptor antagonists (Fig 4, A-E). As shown in Fig 4, D all but 1 mouse developed mild, if any, anaphylactic reactions, indicating that concurrent blockade of PAF and histamine has the greatest beneficial effect in the prevention of PIA.

To compare the efficacy of the various interventions, we used the aforementioned mathematical model (see this article's Supplementary Methods in the Online Repository at www.jacionline.org). The model showed that the global response to 120 minutes (AU_{39°C}) in the group treated with the combination therapy never fell below 35°C (Fig 4 F). Similarly, the measure of moderate severity reflects the success of the combination therapy in the prevention of severe drops in core temperature. In fact, although PAF antagonism had a partial effect on moderate severity, histamine blockade did not. Importantly, the number of "events under 35°C" was 75, 33, 16, and 0 for the untreated, antihistamines, PAF-R antagonism, and combined treatment groups, respectively, indicating that combination therapy was the most successful intervention to prevent drastic reductions in temperature and, hence, development of life-threatening reactions (Fig 4 F). Similarly, the proportion of mice reaching partial or full recovery at 120 minutes was greatest when treated with

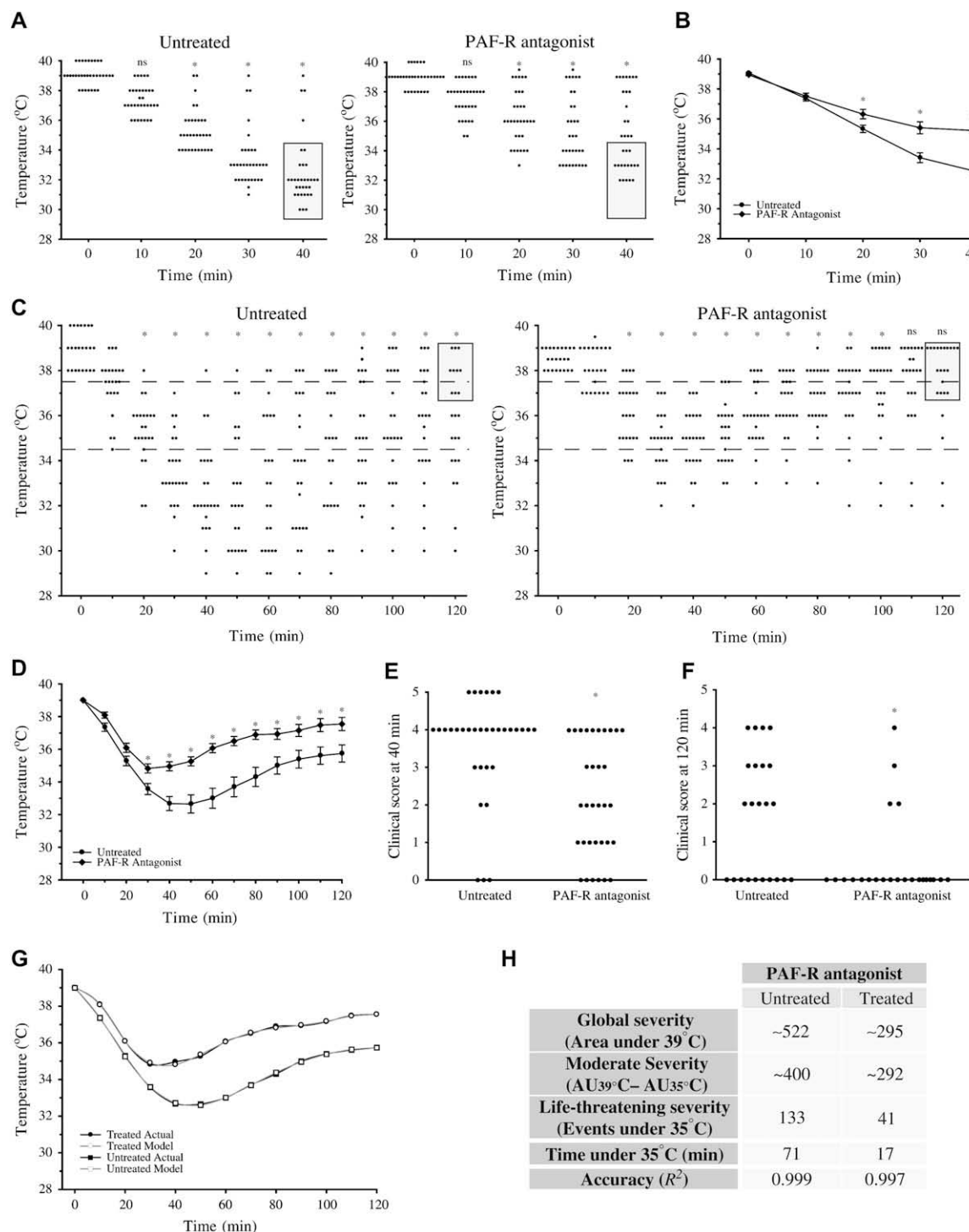


FIG 3. Treatment with a PAF-R antagonist diminishes the severity of peanut-induced anaphylaxis and accelerates recovery. (**A and B**) Rectal temperature and (**E**) anaphylactic symptoms as long as 40 minutes after challenge. (**C and D**) Rectal temperature and (**F**) anaphylactic symptoms as long as 120 minutes after challenge. The severity of the reaction was graded using a mathematical model. **G**, Comparison between actual (■, untreated actual and ●, treated actual) and modeled (□, untreated model and ⊖, treated model) data. **H**, Assessment of the anaphylactic response after treatment. Pooled data from 3 independent experiments; * $P < .05$ relative to time 0. ns, Not significant.

combination therapy. Of note, all of these mice achieved some level of recovery, with 66% of them exhibiting full recovery.

Last, we observed that combined treatment resulted in significantly less vascular permeability, as indicated by an 82%

decrease in the content of albumin in the PL fluid of treated mice compared with untreated mice after challenge. Likewise, combination therapy was associated with a considerable decrease in the levels of histamine and CysLTs in plasma (see Table E1).

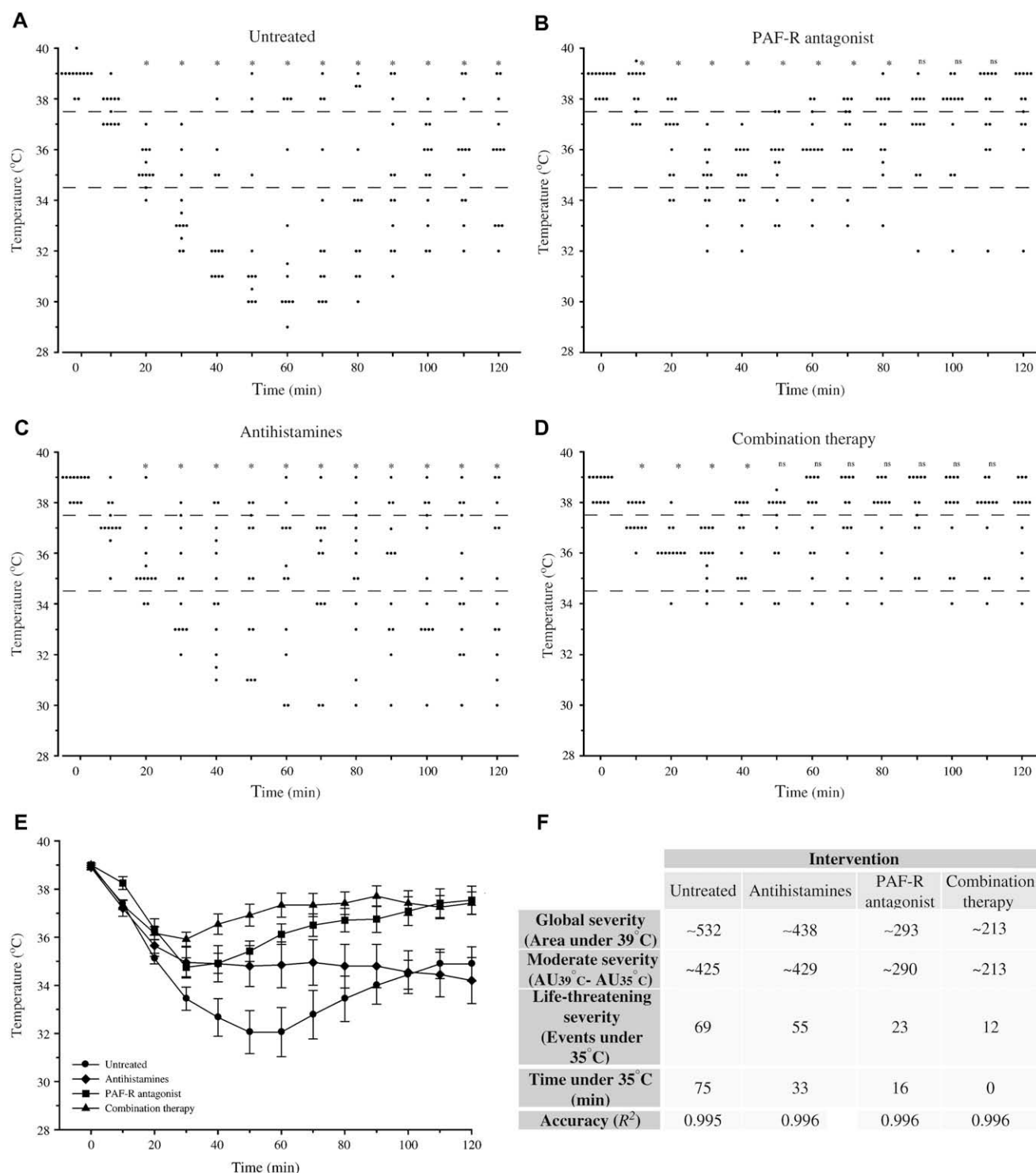


FIG 4. Concomitant treatment with PAF-R antagonist and antihistamines prevents life-threatening and prolonged PIA. Mice were treated as follows: **(A)** drug vehicle, **(B)** PAF-R antagonist, **(C)** histamine receptor antagonist. **D**, Treatments **B** and **C** administered concurrently. **E**, Mean rectal temperatures. **F**, Mathematical parameters to compare the efficacy of the interventions. * $P < .05$ relative to time 0. ns, Not significant.

DISCUSSION

In North America, food allergies are the most common cause of severe anaphylactic reactions, with peanuts and tree nuts accounting for ~80% of the life-threatening episodes.³ Unfortunately,

pharmacologic interventions for food allergies have advanced little in the past decades. Several lines of evidence indicate that the release of vasoactive mediators is associated with the clinical manifestations of anaphylaxis.¹⁷ Nevertheless, the relative

contribution, if any, of these mediators in the pathogenesis of PIA is not well understood. Hence, the objective of this study was to investigate the impact of the pharmacologic blockade of MC-associated mediators, particularly leukotrienes, histamine, and PAF in a mouse model of PIA.

The protocol used evokes the rapid onset of severe anaphylaxis as indicated by severe clinical symptoms, a significant drop in core temperature, marked release of vasoactive mediators, and pronounced vascular leakage. As MC-deficient (kitW[−]/kitW[−]) mice do not experience any measurable anaphylactic reactions in this model, we speculate that the mediators responsible for the elicitation of anaphylactic symptoms are primarily produced by MCs. Our data demonstrate that sensitization with peanut increased the number of peritoneal MCs and FcεRI surface expression. We have previously reported that the number of oral sensitizations progressively enhances the level of circulating peanut-specific IgE.¹⁴ Because IgE is a major regulator of FcεRI expression,¹⁵ it is likely that increased levels of peanut-specific IgE resulted in greater FcεRI density and occupancy. This is relevant to our findings because previous studies have shown that IgE-dependent upregulation of FcεRI expression significantly enhances the ability of MCs to release proinflammatory mediators in response to challenge with IgE and specific antigen.¹⁵

Recent studies by Khodoun et al²⁰ in which peanut was delivered intravenously to IL-4 + propranolol-pretreated mice suggest that innate mechanisms such as complement (ie, C3a) act synergistically with IgE/FcεRI-dependent MC degranulation to exacerbate anaphylaxis. In this model, peanuts might contribute to shock by causing production of C3a, which stimulates macrophages, basophils, and MCs to produce mediators including histamine and PAF.

We investigated the relative contribution of some vasoactive mediators in the elicitation of PIA. Our data indicate that pharmacological inhibition of cysLT did not have a significant effect on the extent of PIA. These findings do not negate the potential value of leukotriene levels in the management of anaphylaxis. Indeed, laboratory tests to support the clinical suspicion of anaphylaxis such as serum levels of histamine and tryptase have been rather disappointing to date.²¹ Because leukotriene levels gradually increase in plasma after peanut challenge and remain elevated for a considerable period of time, they may represent a useful biomarker of anaphylaxis. In this regard, the presence of leukotrienes and their metabolites has been previously reported in urine during and after anaphylaxis.²²

Presently, antihistamines are often given as adjuvant therapies. However, the benefit of antihistamines in systemic anaphylaxis has not been conclusively assessed.²³ Antihistamines have a slow onset of action and cannot block events that occur subsequent to histamine binding to its receptors. Because plasma histamine peaks early in anaphylaxis and rapidly returns to baseline, it is thought that the timing of administration of antihistamines is the main factor limiting their effectiveness. We treated peanut-sensitized mice with H1 and H2 receptor antagonists. This treatment did not have an impact on the severity or the course of the anaphylactic reaction. Antihistamines were administered before challenge; thus, the lack of effect cannot be attributed to inadequate timing. There is accumulating evidence linking PAF to the pathology of anaphylactic shock in animals.^{10,11,19} Importantly, Vadas et al¹³ recently showed that circulating PAF levels correlate with the severity of anaphylactic reactions in human beings. However, causality between PAF levels and severity of

anaphylaxis could not, understandably, be ascertained in this study. Our data demonstrate that treatment with a PAF-R antagonist substantially reduces the severity and length of PIA reactions. Moreover, we observed that blockade of PAF-R was associated with decreased vascular leakage and a reduction in histamine and cysLT levels in plasma. *In vitro* studies have shown that PAF can directly induce histamine release and *de novo* synthesis of leukotriene C4 from various cell types.^{24,25} It is, therefore, plausible that counteracting PAF may have resulted in a concurrent decrease in other unidentified mediators involved in the anaphylactic response. Last, we evaluated whether administration of H1 and H2 receptor antagonists had a further effect in the prevention of PIA. Our data demonstrate a remarkable benefit when PAF and histamine receptors are blocked concurrently.

The beneficial effect of concomitant blockade of vasoactive mediators has been previously demonstrated. Brandt et al¹⁸ showed that complete inhibition of ovalbumin-induced allergic diarrhea required the targeting of 2 mediators, PAF and serotonin. Of note, neutralization of either mediator alone did not have any significant effect. Interestingly, we observed that the absence of serotonin in the periphery does not ameliorate PIA reactions. In fact, mice with a targeted disruption in the tryptophan hydroxylase 1 gene (tph1^{−/−} mice) manifested the same degree of anaphylaxis as their wild-type counterparts after peanut challenge (data not shown). Nevertheless, we cannot rule out the potential benefit of concurrent inhibition of PAF and serotonin in our model. It is, however, possible that serotonin has a rather distinctive role in the elicitation of local allergic intestinal permeability and, thus, differentially contributes to the etiology of allergic diarrhea.

In summary, our data demonstrate that treatment with a PAF-R antagonist significantly diminishes the severity of PIA and accelerates the recovery from anaphylactic reactions. In addition, the combination of a PAF-R antagonist and antihistamines resulted in dramatic beneficial results. We should note that these findings were made in an experimental model that elicits robust anaphylactic reactions. Expectedly, then, a greater benefit may be anticipated in less severe reactions. We suggest that these findings have implications beyond their inherent, direct, therapeutic value. Indeed, the evaluation of promising strategies such as conventional immunotherapy or anti-IgE treatment is substantially limited by the potential of serious adverse effects. In this context, the pharmacologic strategy that we have investigated may also be viewed as a risk-reducing therapy—in other words, an ancillary therapy that may facilitate the implementation of other therapeutic strategies for PA and, most likely, other food allergies.

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Clinical implications: Concomitant blockade of PAF and histamine may represent a life-saving therapy for food-induced anaphylaxis and a risk-reducing approach that facilitates the implementation of other therapies (ie, conventional immunotherapy or anti-IgE).

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SUPPLEMENTARY METHODS

Mathematical Model

Mathematical equations were generated from the mean experimental data from curve-fitting techniques by using FindGraph 1.821 (UNIPHIZ Lab, Vancouver, British Columbia, Canada). For each experimental group, temperature (T) is expressed as a function of time (t):

$$T=39+x_a t+x_b t^2+x_c t^3+x_d t^4+x_e t^5+x_f t^6+x_g t^7+x_h t^8$$

where x_n represents numerical coefficients unique to individual group. The areas above each curve were derived by using Mathematica 6.0.2.1 (Wolfram Research Inc, Champaign, Ill). Accuracy of the model was measured through linear regression, comparing actual and simulated data for each group.

TABLE E1. Mice were treated as follows: histamine receptor antagonists (antihistamines), PAF-R antagonist, or both (combination therapy). Histamine and CysLT levels were analyzed in plasma and albumin was quantified in peritoneal lavage. Means $\pm$ SEMs from 3 independent experiments; n = 8-12 mice/group

	Intervention			
	Untreated	PAF-R antagonist	Antihistamines	Combination therapy
Albumin ($\times 10^5$ ng/mL)	3.41 $\pm$ 1.16	1.45 $\pm$ 0.52	3.78 $\pm$ 1.39	0.60 $\pm$ 0.45*
Histamine ($\times 10^4$ μ M/mL)	1.88 $\pm$ 0.55	1.03 $\pm$ 0.32	1.65 $\pm$ 0.50	0.51 $\pm$ 0.17*
CysLT ($\times 10^2$ pg/mL)	5.70 $\pm$ 0.97	1.87 $\pm$ 0.39*	7.36 $\pm$ 1.56	0.70 $\pm$ 0.22*†

* $P < .05$ to untreated mice.

† $P < .05$ to PAF-R antagonist.