

Pathogenic role of thromboxane A2 in immediate food hypersensitivity reactions in children

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Background: Food hypersensitivity, from the standpoint of pathogenesis as well as clinical management, remains controversial. During the food allergen-induced immediate hypersensitivity reaction, various chemical mediators are released.

Objective: The purpose of our study was to determine whether thromboxane A2 participates in food antigen-induced responses in children with food hypersensitivity.

Methods: Nine open food challenges were performed in nine patients with suspected food hypersensitivity. Plasma thromboxane B2 and histamine levels were measured during a 24-hour period following the challenge.

Results: All the patients demonstrated immediate reactions after food challenge. The mean plasma thromboxane B2 level (a marker of thromboxane A2 activity) rose significantly at two hours and three hours after the challenge. Simultaneously, the mean plasma histamine level rose significantly at two hours and three hours after the challenge.

Conclusions: The results suggest that thromboxane A2 may play a pathogenic role in part in the immediate reaction after food challenge and that thromboxane A2 is probably released from a common cellular source (eg, mast cell) with histamine and/or by a common mechanism (eg, IgE-dependent platelet activation).

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INTRODUCTION

The pathogenesis and clinical management of food hypersensitivity remain controversial. Although a variety of immunologic and nonimmunologic mechanisms have been implicated, there is no clear consensus as to whether a single mechanism (eg, IgE immune response) or a spectrum of mechanisms is involved. During the food allergen-induced immediate hypersensitivity reaction, various chemical mediators are released.

Thromboxane A2 is one of the chemical mediators. It is a potent inducer of platelet aggregation¹ and also acts as a vasoconstrictor and a bronchoconstrictor.² Further, it has been reported that thromboxane A2 induces bronchial hyperresponsiveness in asthma³⁻⁵ and is

also involved in the allergen-induced late cutaneous responses.⁶⁻⁸

The purpose of the present study was to determine whether thromboxane A2 participates in food antigen-induced responses in children with food hypersensitivity. Thromboxane A2 has a half-life of about 30 seconds and is rapidly hydrolyzed to a stable, biologically inactive metabolite, thromboxane B2.¹ The short half-life of thromboxane A2 precludes its measurement at physiologic concentrations. We therefore determined the time course of plasma thromboxane B2 (the stable metabolite of thromboxane A2) and histamine (a major chemical mediator in immediate hypersensitivity) levels following food challenges.

MATERIALS AND METHODS

Subjects

Nine patients with suspected food hypersensitivity were included in this study (Table 1). The parents granted

informed consent for this study. Patients ranged in age from 10 months to 6 years (mean, 3.3 years). Six of the nine patients had episodes of food-induced immediate symptoms. All nine patients had improvement in their symptoms after an elimination diet. Offending foods, however, were not confirmed by double-blind placebo-controlled food challenge. In addition to the history, these patients had both positive antigen-specific IgE and positive skin prick tests for the offending food. All the patients had been instructed to eliminate the offending food.

Food Challenge

Patients were instructed to discontinue antihistamines for at least seven days and beta-agonists, theophylline, cromolyn, and topical corticosteroids for at least 12 hours before the study. No patients were receiving oral corticosteroids.

Nine open food challenges with egg (seven) and milk (two) were performed in nine patients early in the morning.

The amount of food antigen administered as a test dose was governed by the degree of suspected hypersensitivity based on the history of clinical reactivity. Foods administered for challenges were hen egg (¼ to 1 boiled egg) and cow milk (50 to 100 mL).

Plasma Thromboxane B2 and Histamine Determinations

An intravenous catheter with a heparin-lock was placed in the brachial vein of each subject, and heparinized-saline was kept in the catheter to keep the system anticoagulated. Blood samples were obtained prior to challenge and at 0.5, 1, 2, 3, 4, 6, 8, and 24 hours after challenge.

Blood samples were collected in siliconized glass tubes containing EDTA + indomethacin for thromboxane B2 assay and plastic tubes contain-

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Table 1. Background of Patients with Suspected Food Hypersensitivity in the Study

Patient No	Age, Sex	Primary Diagnosis	CAP RAST* class, UA/mL	IgE, IU/mL
1	1y,M	Urticaria	Egg white 3(3.52) †Der p 0(0.35>)	64
2	2y,M	Atopic dermatitis	Egg white 4(19.8)	110
3	4y,M	Urticaria	Der p 3(4.87)	
4	3y,M	Atopic dermatitis	Cow milk 6(100<)	6100
5	3y,M	Bronchial asthma	Der p 5(95.7)	
6	5y,M	Bronchial asthma	Egg white 4(19.0)	480
7	10m,M	Angioedema	Der p 4(23.5)	
8	2y,F	Bronchial asthma	Cow milk 2(1.94)	610
9	6y,M	Urticaria	Der p 6(100<)	
		Angioedema	Egg white 4(40.4)	910
		Bronchial asthma	Der p 5(97.1)	
		Angioedema	Egg white 3(13.3)	80
		Angioedema	Der p3(12.8)	
		Atopic dermatitis	Egg white 3(8.28)	3791
		Bronchial asthma	Der p 6(100<)	
		Atopic dermatitis	Egg white 4(23.3)	588
		Bronchial asthma	Der p 0(0.35>)	

* CAP-RAST: CAP-radioallergosorbent test.
† Der p: *Dermatophagoides pteronyssinus*.

ing EDTA for histamine assay and these tubes were immediately placed on ice. After collection the samples were centrifuged at 1000 *g* for ten minutes at 4°C, and the plasma was pipetted into polypropylene tubes. Care was taken not to disturb the buffy coat. Samples were stored at –40°C until assayed for thromboxane B2 and histamine.

Plasma thromboxane B2 levels were measured by EIA with a kit, sensitive to 0.2 pg per well supplied by Amersham (Buckinghamshire, England) after extraction using modifications of the procedures reported by Skrinska

and Lucas.⁹ Bond elute C18 and Si extraction columns supplied by Analytichem International and Varian SPP (CA, USA) were used.

Microtiter plates were set up with sufficient wells to enable the running of all blanks, standards, and samples as required. A 100-μL aliquot of assay buffer was placed into the nonspecific binding wells, 50 μL assay buffer into the zero standard wells, and 50 μL of each standard or sample into the appropriate wells. Next, 50 μL of anti-serum was added to each well except the blank and nonspecific binding

wells, and 50 μL of thromboxane B2 peroxidase conjugate was added to all wells except the blank. The plates were covered with the lids provided and incubated at 15 to 30°C for one hour. All the wells were aspirated and washed four times with 400 μL of wash buffer. A 150-μL aliquot of enzyme substrate was dispensed into each well, and mixed for 15 minutes at 15 to 30°C. One hundred microliters of 1M sulphuric acid was placed into each well, and the optical density was determined in a plate reader at 450 nm within 30 minutes. Resulting optical density measurements using from 0.5 to 64 pg per well of thromboxane B2 standard (provided with the kit) were used for construction of the standard curves. Concentrations of unknown samples were determined by extrapolation.

Plasma histamine concentrations were measured by RIA with a kit supplied by Immunotech (Marseille, France)¹⁰ which is sensitive to 0.5 nM (nmol/L). Samples were thawed and 100 μL of plasma or the standard solution was placed into designated “acylation tubes.” Next, 50 μL of acylation buffer was added to each tube which was followed by vortex mixing

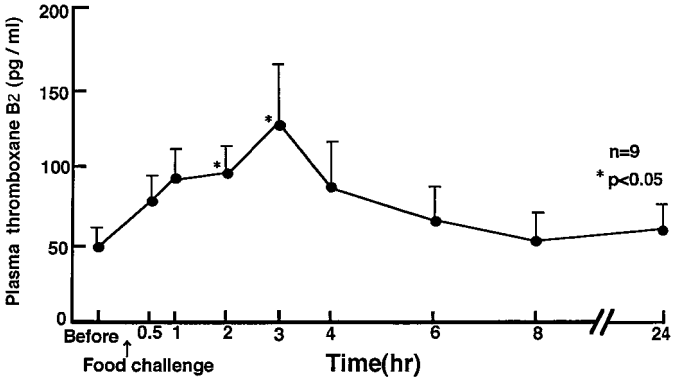


Figure 1. Changes in plasma thromboxane B2 levels in all nine patients with immediate reactions after food challenges.

until a powdered acylation reagent was completely solubilized. Next, 1 mL of iodinated [^{125}I] acylated histamine was added, following which the tubes were mixed by vortex. After transferring a 0.5-mL aliquot each of these test solutions to monoclonal antibody-coated tubes, incubation at 4°C was allowed to proceed for 16 to 20 hours. Solutions were aspirated and discarded and the antibody-coated tubes counted in a gamma counter. Resulting counts per minute from 0.5 to 150 nM of histamine standard solutions (provided with the kit) were used for construction of the standard curves. Concentrations of unknown samples were determined by extrapolation.

Statistical Analysis

Statistical evaluations were performed using Wilcoxon test and Spearman's correlation coefficient; $P < .05$ was considered significant. Data are reported as mean $\pm$ SEM.

RESULTS

Symptoms Induced by Food Challenge

Nine food challenges were performed in nine patients with suspected food hypersensitivity, and all the nine challenges were interpreted as being positive. Nine positive challenges elicited symptoms that were cutaneous consisting of pruritus, erythema, urticaria, and angioedema (patients 1, 7, and 8); both cutaneous and gastrointestinal consisting of pruritus, erythema, urticaria, angioedema, nausea, vomiting, and abdominal pain (patients 2 and no. 5); and both cutaneous and gastrointestinal plus respiratory consisting of pruritus, erythema, urticaria, angioedema, nausea, abdominal pain, coughing, and wheezing (patients 3, no. 4, no. 6 and no. 9). All of the symptoms occurred within two hours after challenge. Although none of the patients had been diagnosed as having gastrointestinal manifestations, six of the nine patients demonstrated gastrointestinal symptoms after challenge.

Plasma Thromboxane B2 and Histamine Levels

Six of the nine patients with immediate reactions showed a large rise in their plasma thromboxane B2 and histamine levels after food challenges.

In nine positive challenges, the mean plasma thromboxane B2 level rose from the prechallenge levels of 49.6 ± 9.7 pg/mL to 92.7 ± 20.4 (two hours after the challenge) and 128.8 ± 34.3 (three hours after the challenge), ($P < .05$) (Fig 1).

The mean plasma histamine level was significantly increased from the prechallenge levels of 2.89 ± 0.38 nM to 21.47 ± 8.86 (two hours after the challenge) and 28.22 ± 11.99 (three hours after the challenge), ($P < .05$) (Fig 2).

The maximum increase of plasma thromboxane B2 above the prechallenge levels after food challenge was significantly correlated with the maximum increase of plasma histamine in each subject (Fig 3).

In the nine patients with immediate reactions, the time of peak of symptoms nearly matched the time of peak levels of plasma thromboxane B2 and histamine. There was an apparent correlation of the severity of symptoms with plasma thromboxane B2 and histamine levels.

The platelet count was measured with plasma thromboxane B2 and histamine levels following food challenges in only two patients (8 and 9). In these patients, individual changes in plasma thrombox-

ane B2 and histamine levels followed food challenges (Fig 4).

Patient 8 demonstrated a mild immediate reaction after challenge to egg. Symptoms occurred at 15 minutes after the challenge. Pruritus and erythema localized to the face occurred at 15 minutes after the challenge. All symptoms cleared within two hours of the challenge. Plasma thromboxane B2 and histamine levels were not elevated after the challenge. The platelet count did not increase after the challenge either.

Patient 9 had a moderate immediate reaction after challenge with egg. Symptoms occurred at 20 minutes after the challenge. Abdominal pain and pruritus occurred at 20 minutes; rhinorrhea, sneezing, coughing and mild wheezing at one and one-half hours; and erythema and urticaria at two and one-half hours. All symptoms cleared within four hours of the challenge. Plasma thromboxane B2 levels increased progressively from the prechallenge level of 21 pg/mL to 127 at three hours, and then declined to the baseline within six hours. Plasma histamine levels rose from the prechallenge level of 1.6 nM to 49.0 at three hours, then declined to the baseline within six hours. The platelet count increased progressively from the prechallenge count of $33.2 \times 10^4/\mu\text{L}$ to 46.4 at three hours, and then declined to baseline within six hours.

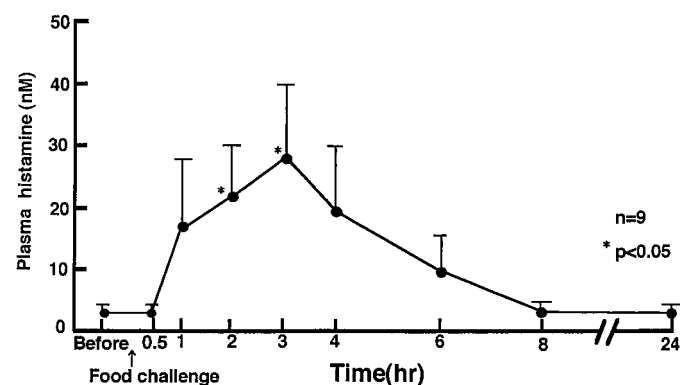


Figure 2. Changes in plasma histamine levels in all nine patients with immediate reactions after food challenges.

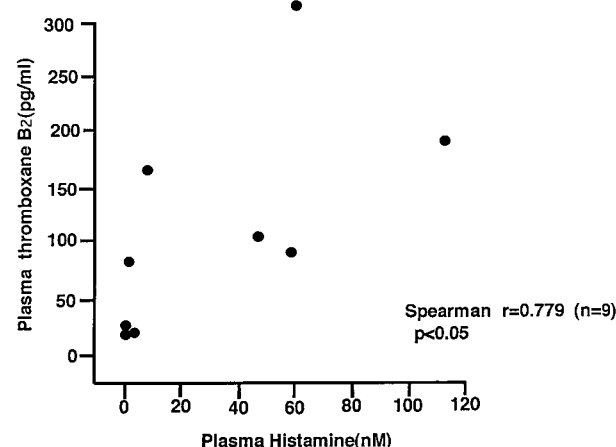


Figure 3. Correlation between the maximum increase of plasma thromboxane B2 and histamine above prechallenge levels after food challenges in all nine patients.

DISCUSSION

Thromboxane A2 is a potent inducer of platelet aggregation¹ and also acts as a vasoconstrictor and a bronchoconstrictor.² Recently, it has been reported that thromboxane A2 may correlate with airway microvascular leakage.¹¹ It has a half-life of about 30 seconds and is rapidly hydrolyzed into a stable, biologically inactive metabolite, thromboxane B2.¹ The short half-life of thromboxane A2 precludes its measurement at physiologic concentrations. Consequently, the measurement of thromboxane B2, which

has a half-life of 20 to 30 minutes, is accepted as an indicator of thromboxane A2 production.^{12,13}

Elevated thromboxane B2 levels in complex biologic fluids have been observed in a variety of allergic conditions. Elevated thromboxane B2 levels in plasma^{14,15} and bronchoalveolar lavage fluid¹⁶ have been recorded in asthmatic patients following allergen inhalation challenge, and elevated thromboxane B2 levels in skin blister fluid in patients suffering from IgE-mediated allergic diseases following

allergen skin testing.¹⁷ Furthermore, it has been reported that thromboxane A2 induces bronchial hyperresponsiveness in asthma³⁻⁵ and is also involved in allergen-induced late cutaneous responses.⁶⁻⁸ There have been, however, few reports of plasma thromboxane B2 levels following food challenge in patients with food hypersensitivity.¹⁸ Further, there have been no reports on the prolonged time course of plasma thromboxane B2 after food challenge.

In the present study, plasma thromboxane B2 and histamine levels were determined in patients with suspected food hypersensitivity during a 24-hour period following food challenge. All the patients demonstrated immediate reactions after food challenge. The mean plasma thromboxane B2 level rose significantly at two hours and three hours after the challenge. Simultaneously, the mean plasma histamine level rose significantly at two hours and three hours after the challenge. The maximum increase of plasma thromboxane B2 above the prechallenge levels after food challenge was significantly correlated with the maximum increase of plasma histamine, and there was an apparent correlation of the severity of symptoms with plasma thromboxane B2 and histamine levels after food challenge. Furthermore, the time of peak symptoms nearly matched the time of peak levels of plasma thromboxane B2 and histamine individually.

As in our previous study of plasma histamine and tryptase in patients with immediate reactions following food challenge,¹⁹ the elevation of plasma histamine indicates mast cell activation by the IgE-dependent mechanism. Thromboxane A2 is known to be generated from several cell types such as platelets,¹ macrophages,²⁰ mast cells,²¹⁻²⁴ neutrophils, or eosinophils.²⁰ Thromboxane A2, however, is thought to arise predominantly from platelets and macrophages, although thromboxane A2 is probably generated in smaller quantities by mast cells.²⁴ Mast cells and basophils with high affinity IgE receptors, and platelets, macrophages, eosinophils, and lymphocytes with low affinity IgE receptors, are capable of responding to allergen stimu-

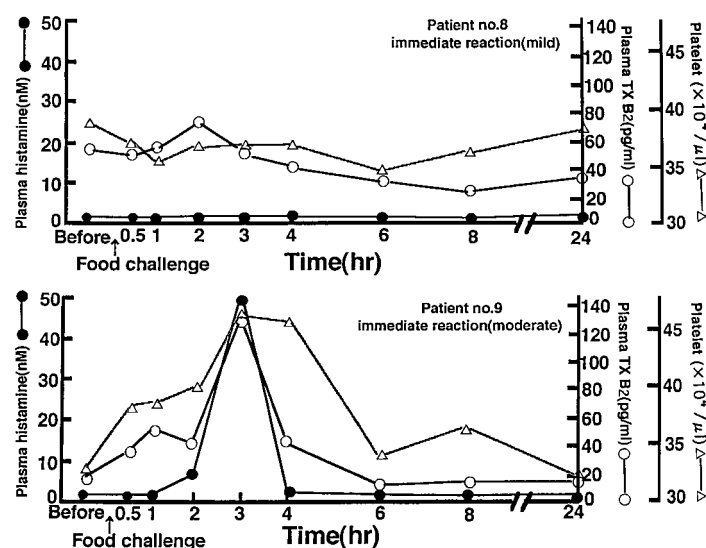


Figure 4. Time course of plasma thromboxane B2, histamine levels, and the platelet count after food challenges in patients 8 and 9.

lation with release of preformed mediators and generation of various prostanoids and leukotrienes.^{25,26} In fact, human platelets can bind IgE, and cross-linking with anti-IgE or antigen leads to platelet activation and secretion.^{27,28}

In conclusion, the results suggest that thromboxane A2 may play a pathogenic role in part in an immediate reaction after food challenge and is probably released from a common cellular source with histamine (eg, mast cell) and/or by a common mechanism (eg, IgE-dependent platelet activation).

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