

Topical thrombin-induced IgE-mediated anaphylaxis: RAST analysis and skin test studies

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Bovine topical thrombin (BTT) is a heterologous plasma thrombin concentrate that has been frequently used for the hemostasis since the 1940s. Recently, three patients in Japan went into shock after the topical application of BTT at lesion sites, and two of these patients had received BTT repeatedly. The clinical symptoms and the increased anti-BTT percent RAST counts suggest that these reactions were shock mediated by anti-BTT IgE antibodies. The RAST-inhibition analysis suggested that the antigenic substance(s) were bovine-specific moiety(ies) mainly involved in the contaminant rather than bovine thrombin itself. The skin tests were studied to predict such allergic reactions. The intracutaneous test provoked nonspecific reactions even at the low concentrations of BTT. The prospective study on the predictive value of the prick test with 1000 U/ml (1 mg/ml) of BTT in 192 patients suggested that it is useful to detect highly sensitive patients. In addition, the increased levels of anti-BTT IgE antibodies in patients 1 month after the single administration of BTT suggested the immunogenicity of the topical application of BTT. (J ALLERGY CLIN IMMUNOL 1991;88:620-9.)

Key words: Topical thrombin, shock, anaphylaxis, IgE antibody, RAST, bovine, skin test, intravascular coagulation, immunogenicity, prediction

BTT is a heterologous plasma thrombin concentrate usually used for hemostasis of hemorrhage that is difficult to control by ordinary means, such as compression and ligation.¹⁻⁴ In spite of its popular use since the 1940s, only a small number of studies have described its adverse reactions: a potential danger of intravascular coagulation caused by erroneous injection of BTT into the blood vessels^{5,6} and the delayed-type hypersensitivity.⁷ Recently, however, a case of anaphylactic shock after the topical application of BTT was reported.⁸ Confronted with this study,

Abbreviations used

BTT: Bovine topical thrombin
SDS: Sodium dodecyl sulfate
PAGE: Polyacrylamide gel electrophoresis
Ag: Antigen
Ab: Antibody

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Supported in part by the Manabe Medical Foundation, Tokyo, Japan.

Received for publication May 30, 1990.

Revised May 16, 1991.

Accepted for publication May 22, 1991.

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1/1/31236

multicenter monitoring of BTT-induced adverse reactions was conducted in Japan from 1986 to 1989. The clinical study and the RAST analysis of the sera from patients with BTT-induced adverse reactions revealed that three cases of BTT-induced shock and two cases of BTT-induced occupational rhinitis and dyspnea were anaphylaxis mediated by anti-BTT IgE Ab. This finding resulted in our establishing a useful and simple skin test to delineate the state of sensitization and to predict the likelihood of the occurrence of such life-threatening anaphylactic reactions.

We describe in this article the clinical features of the cases with BTT-induced anaphylaxis and the re-

sults of RAST analyses. The prospective study on the skin test and the immunogenic potential of topical application of BTT are also described.

MATERIAL AND METHODS

Substance

Bovine thrombin preparation, thrombin topical, was donated by Warner-Lambert K.K., Tokyo. Human thrombin preparation was purchased from Midori-Juji Co., Tokyo. One thrombin unit is defined as the activity to convert standard fibrinogen into fibrin in 15 seconds at 37° C, pH 7.4. Semipurified thrombin and thrombin-free contaminant were kindly prepared by Dr. Michio Matsuda in Jichi Medical College using affinity-column chromatography.

PAGE

The materials were dissolved in 62.5 mmol/L of Tris HCl buffer, pH 6.8, containing 10% glycerol, 2.3% SDS, and 50 mmol/L of dithiothreitol, and loaded on a 12.5% SDS-polyacrylamide gel. After the electrophoresis, the proteins were fixed and stained with 0.1% Coomassie blue.

Sera

Sera from patients with BTT-induced adverse reactions were obtained within 2 weeks after the adverse reactions and stored at -20° C until assay.

RAST analysis

Two milligrams per milliliter of BTT was conjugated to paper disks activated by cyanogen bromide; 50 µl of each serum sample was applied to a Phadebas RAST kit (Pharmacia Diagnostics, Uppsala, Sweden) to determine anti-BTT IgE Abs. The percent RAST count was determined with the following equation:

$$\% \text{ RAST count} = \frac{\text{Patient's serum (cpm)} - \text{background (cpm)}}{\text{Total } ^{125}\text{I-labeled anti-IgE (cpm)} - \text{background (cpm)}} \times 100$$

In some experiments, Phadezym RAST kits (Pharmacia Diagnostics) were used instead of Phadebas kits. Experiments were performed in duplicate.

In the RAST-inhibition assay, sera were preincubated with various concentrations of thrombins (each from 0.1 to 1000 U/ml, i.e., BTT from 0.1 to 1000 µg/ml, semipurified bovine thrombin from 0.017 to 170 µg/ml, human topical thrombin from 0.05 to 500 µg/ml, and thrombin-free contaminant from 0.031 to 310 µg/ml) or saline (negative control) for 1 hour in preparation for assay. Percent inhibition was determined by the following equation:

$$\% \text{ Inhibition} = \left(1 - \frac{\text{Inhibitor } (\% \text{ RAST count})}{\text{Saline } (\% \text{ RAST count})} \right) \times 100$$

Skin testing

In the prick test, one drop of saline (negative control), 1 mg/ml of histamine (positive control), and BTT solution

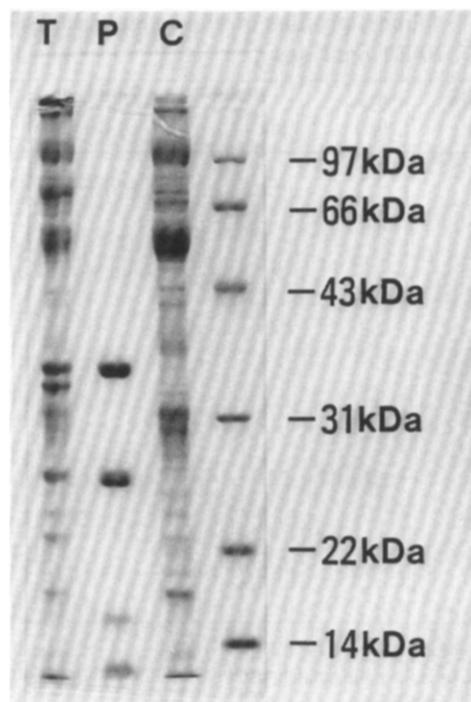


FIG. 1. SDS-PAGE of BTT and the fractions. Crude BTT (T), semipurified thrombin (P), and thrombin-free contaminant (C) were loaded on a 12.5% SDS-polyacrylamide.

were placed on the volar side of the forearm approximately 3 cm apart from each other. The skin was pricked by a sterile needle through each drop to prevent bleeding. The diameters (maximum diameter times minimum diameter) of wheal and flare and other local reactions were recorded 15 minutes after the prick. When the mean diameter of wheal was <4 mm and less than twice that of the negative control (saline), and the diameter of flare was <15 mm, the reactions were defined as negative. Otherwise, the reactions were defined as positive.⁹ An intracutaneous test was conducted by injecting 20 µl of saline, 10 µg/ml of histamine, and BTT solution. The diameters (maximum diameter times minimum diameter) of wheal and flare were recorded 15 minutes after the injection. When the mean diameter of wheal was not <9 mm or the mean diameter of flare was not <20 mm 15 minutes after the injection, the reactions were defined as positive. When the mean diameter of wheal was <6 mm and that of flare was <10 mm, the reaction was defined as negative. Intermediate reactions were defined as doubtful.⁹

Selection of nonallergic healthy volunteers

Nonallergic healthy volunteers who matched all the following seven criteria were selected: (1) 20- to 50-year-old men, (2) no past history of allergic diseases, (3) no serious abnormal results in physical as well as routine laboratory tests, including urine, blood count, chest x-ray, and blood chemistry, (4) normal serum IgE level, (5) no Ag eliciting marked positive reactions with pseudopods and/or areola

TABLE I. Clinical profiles of the patients with BTT-induced anaphylactic reactions and the results of anti-BTT RAST analyses

Patient No	Age (yr)	Sex	Disease	Previous use	Administration route
11	55	M	Urinary bladder carcinoma	19 v/4 yr†	Urinary bladder irrigation
12	32	F	Lacerated wound of pubis	(-)	Sprinkling beneath the skin implant
13	53	M	Burns	20 v/2 yr§	Sprinkling beneath the skin implant
21	38	M	Angioneurotic edema	5 yr	Inhalation
22	33	F	None	3 yr	Inhalation

V, Vial; ND, not done.

*Both the prick test and scratch test were conducted with 1000 U/ml of BTT. The results are expressed by diameters of wheal per diameters of flare (judgement).

†Mean anti-BTT percent RAST count was $1.35\% \pm 0.15\%$ in normal volunteers ($N = 35$).

‡Total vials (1 vial equals 5000 U) per years; BTT was administered with a certain intervals during the years of treatment.

§The counts for the total ^{125}I -labeled anti-IgE and for the background were 33470 ± 252.5 and 61.3 ± 4.9 cpm, respectively.

and no more than five Ags eliciting positive reactions among the 10 common Ags in the prick test panel, (6) negative reaction to cow's milk and BTT vehicle (1.6 mg/ml of calcium chloride dihydrate, 9.06 mg/ml of aminoacetic acid, and 1.746 mg/ml of sodium chloride) in the prick test, and (7) normal reactivity to 10 $\mu\text{g}/\text{ml}$ of histamine and 40 $\mu\text{g}/\text{ml}$ of compound 48/80 in the intracutaneous test.

Evaluation of maximal concentrations of BTT in skin reactions

Skin reactions of 22 nonallergic healthy volunteers to various concentrations of BTT were studied, starting with the lowest concentration (1.0 U/ml for the prick test and 0.001 U/ml for the intracutaneous test) of BTT, vehicle, and saline. When the immediate reaction was negative, the concentration of BTT was increased stepwise 10 times to 1000 U/ml for the prick test and to 100 U/ml for the intracutaneous test.

Prospective study on the predictive value of the prick test with 1000 U/ml BTT

The predictive value of the prick test with 1000 U/ml of BTT was studied in 196 patients who needed BTT because of hemorrhage in 28 institutions in Japan. The subject group consisted of 105 male and 91 female subjects from 5 to 83 years old. Fourteen patients had a history of allergy, but none had an overt history of allergy to bovine materials. Tests were done after informed consent was obtained.

The prick test was conducted with 1000 U/ml of BTT, 1 mg/ml of histamine, and saline the day before the administration of BTT. The venous blood was also aspirated for laboratory tests. BTT was not administered because of the reluctance of the patients to receive BTT after a positive reaction. Blood samples were obtained for the RAST analysis instead. When the reaction was negative, the intracutaneous test with 0.1 U/ml of BTT and saline (negative control) was conducted. When the immediate intracutaneous test was positive, BTT was not administered, and blood samples were obtained. This intracutaneous test was conducted to confirm that highly sensitive patients were not missed. When the test was negative, BTT was administered. Adverse reactions were monitored after the administration, and blood samples were collected at the onset of any adverse reaction. When no adverse reaction was observed, the prick test and blood collection were conducted 1 month after the treatment to determine whether the patients were sensitized.

RESULTS

Clinical profiles of patients with BTT-induced hypersensitivity reactions

Clinical profiles of five cases with BTT-induced, immediate-type hypersensitivity reactions and the results of anti-BTT IgE RAST are listed in Table I. Three patients (Nos. 11, 12, and 13) experienced shock reaction with or without other symptoms, such as urticaria, flush, fever, vomiting, and dyspnea, appearing within 15 minutes after the topical application

BTT applied (vial/form)	Symptoms	Skin test*	Anti-BTT % RAST count†
2 v/solution	Shock Flush Drowsiness	Prick 7 × 10/47 × 45 (+)	21.5§
1 v/powder	Shock Urticaria	ND	13.8
1 v/powder	Shock Dyspnea Fever Vomiting Urticaria	Scratch 11 × 12/21 × 23 (+)	29.9
Unknown/powder	Rhinitis Asthma Conjunctivitis	Prick 39 × 9/67 × 30 (+)	32.7
Unknown/powder	Rhinitis Dyspnea	ND	21.8

of BTT. All these patients received BTT locally, one patient in solution for urinary bladder irrigation and the other two patients received BTT in powder form on skin lesions. All patients recovered from shock within a few hours with treatment. Patients had no history of allergy. Two of the three patients had been repeatedly exposed to topical application of BTT with some intervals. Sera from these three patients revealed a high anti-BTT RAST count ($>13\%$) in contrast to results from 35 normal volunteers ($1.35\% \pm 0.15\%$) who had never been exposed to BTT. The reaction results suggested that they were anaphylactic shock mediated by anti-BTT IgE Abs. Positive reactions for 1000 U/ml of BTT both in the prick test (patient No. 11) and in the scratch test (patient No. 13) also matched this mechanism. The diagnostic value of the skin testing will be discussed later.

Two pharmacists (Nos. 21 and 22) experienced rhinitis, conjunctivitis, and dyspnea in preparing the application powder of BTT after a few years of occupational exposure. The anti-BTT percent RAST counts of these two pharmacists were as high as counts of patients with anaphylactic shock. One pharmacist (No. 21) had positive prick test reactions for 1000 U/ml BTT. It was suggested that these reactions were also mediated by anti-BTT IgE Abs.

Antigenic substance(s) for IgE Abs in the sera from patients with topical thrombin-induced anaphylaxis

To study an antigenic substance(s) for the IgE Abs, a RAST-inhibition assay was performed with crude

BTT, semipurified bovine thrombin, thrombin-free BTT contaminant, and human topical thrombin. Crude BTT demonstrated >10 protein bands in SDS-PAGE. Semipurified thrombin contained α - (37.7 kd) and β -thrombin (28.0 kd), whereas the contaminant demonstrated no visible bands corresponding to the thrombins (Fig. 1). As illustrated in Fig. 2, crude BTT and thrombin-free BTT contaminant demonstrated parallel and dose-dependent inhibition, whereas semipurified thrombin demonstrated little inhibition. In addition, the human topical thrombin preparation demonstrated no inhibition. Furthermore, the prick test in one patient (No. 11) was positive with pseudopod for crude BTT and thrombin-free BTT contaminants, positive for semipurified bovine thrombin and negative for human topical thrombin. These results suggested that relevant antigenic substance(s) for the IgE Abs were bovine-specific moiety(ies) mainly involved in the contaminant rather than the bovine thrombin itself.

Skin testing studies

Evaluation in normal volunteers. To establish a simple and useful skin test for BTT-induced allergic reactions, the maximum concentrations of BTT that were not associated with nonspecific reactions in the prick test and the intracutaneous test were studied. From 45 volunteers, 22 healthy nonallergic volunteers were selected as described in METHODS. The percent RAST count of these 22 patients was $1.39\% \pm 0.13\%$ (mean $\pm$ SD). As presented in Table II, no positive reactions were observed by the prick test,

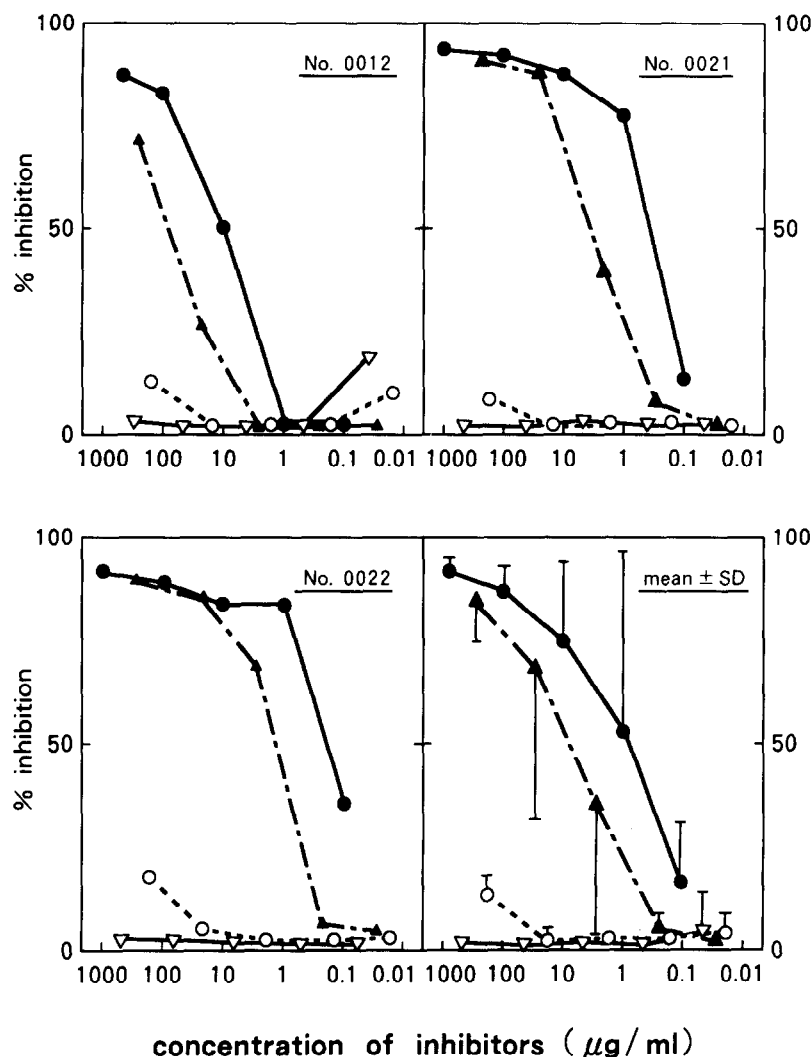


FIG. 2. RAST-inhibition test. Sera from patients with IgE-induced anaphylaxis were preincubated with serial concentration of crude BTT (●), semipurified bovine thrombin (○), thrombin-free BTT contaminant (▲), human topical thrombin (▽), and control saline in preparation for the anti-BTT IgE RAST assay. One microgram of the materials demonstrated thrombin activity of 0.95, 5.88, <0.001, and 2.04 U, respectively. The mean counts per minute of the total ^{125} I-labeled anti-IgE and the background were 12,600 and 46, respectively. Data are expressed as means of duplicate experiments. The means $\pm$ SD of three sera are also illustrated in the lower right panel.

even at the highest concentration of 1000 U/ml (1.05 mg/ml). However, wheal-and-flare reactions were recorded in the intracutaneous tests with 0.01 U/ml or higher concentrations of BTT. The mean diameters of the wheal and flare after correcting for the saline control increased almost in a dose-dependent fashion, although the increase was significant only at 10 and 100 μ g/ml for the flare and at 100 μ g/ml for the wheal compared to the control (Fig. 3).

The results revealed that the maximum concentration causing no nonspecific reactions for the prick test was 1000 U/ml or more, whereas the concentration for the intracutaneous test was as low as 0.001 U/ml.

The prick test with 1000 U/ml BTT was chosen for additional study in patients.

Prospective study on the predictive value of the prick test with 1000 U/ml of BTT. Predictive value of the prick test with 1000 U/ml of BTT was studied in 196 patients who needed BTT in 28 institutions.

In the pretreatment skin tests, four subjects were positive for the prick test and one patient was positive for the intracutaneous test with 0.01 U/ml of BTT, although the patient was negative for the prick test (Table III).

Among the four subjects with positive prick test,

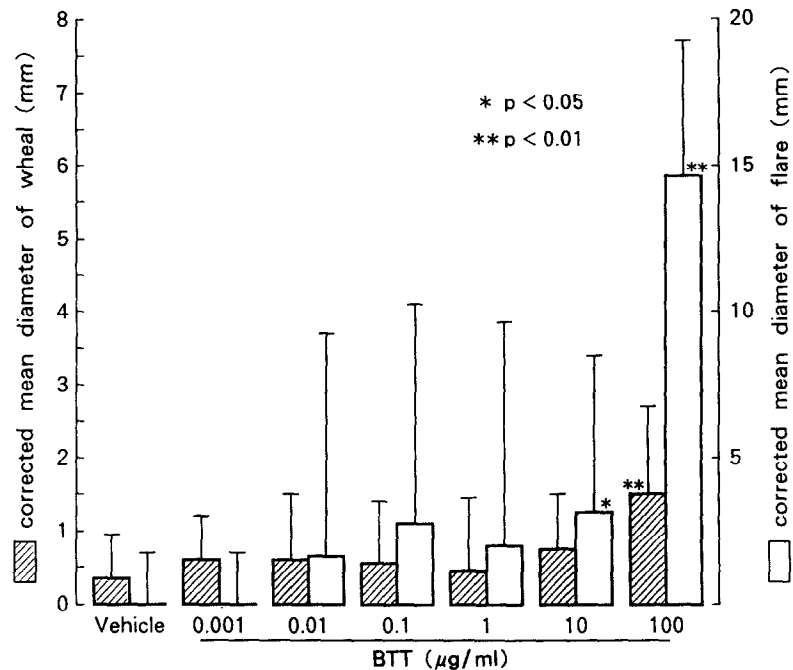


FIG. 3. The wheal-and-flare reactions provoked by the various concentrations of BTT in nonallergic patients. The mean diameters of wheal-and-flare reactions provoked by sequential concentration of BTT both in the prick test and in the intracutaneous test in 22 normal, nonallergic volunteers are illustrated. The values were corrected for the saline control; * $p < 0.05$; ** $p < 0.01$.

TABLE II. Skin test with BTT in nonallergic healthy volunteers

	Prick test					Intracutaneous test						
	BTT (U/ml)					BTT (U/ml)						
	Vehicle	1	10	100	1000	Vehicle	0.001	0.01	0.1	1	10	100
No. of reactions												
Negative	22	22	22	22	22	22	22	20	19	19	12	1
Doubtful*						0	0	1	2	1	7	6
Positive	0	0	0	0	0	0	0	1	0	1	1	8
No. of patients not tested†	0	0	0	0	0	0	0	0	1	1	2	7‡

*Only for the intracutaneous test; criteria for judgment are described in METHODS.
†The tests were not done because of the positive reactions at the previous lower concentration.
‡The tests were not done for four patients with doubtful reactions at 10 U/ml because they complained of pain at injection sites.

two (Nos. 2101 and 9105) demonstrated significantly high percent RAST counts comparable to counts of patients with anaphylactic shock. One patient had a history of urticaria and the other patient had atopic dermatitis. The other two patients with positive prick tests (Nos. 9401 and No. 9701) demonstrated normal percent RAST count; one patient, No. 9701, was administered BTT because of the urgent need for hemostasis and did not experience any adverse reaction. The percent RAST count of the patient (No. 6101) with a positive intracutaneous test and a negative prick

test was also within the normal limits. No allergic reaction was observed after the treatment in 191 patients who were negative for the pretreatment prick test.

The prick test and the RAST were conducted 1 month after the BTT administration in 182 patients who were initially negative for the prick test. Then posttreatment prick test was positive in one patient. The mean $\pm$ SD of the percent RAST counts (2.14 ± 2.58) was significantly higher ($p < 0.01$) than that of 35 normal subjects (1.35 ± 0.15) who

TABLE III. Findings obtained in patients who demonstrated positive skin reactions before BTT administration

Pt. No.	Prick test	Intracutaneous test	Treatment	Systemic reactions	% RAST counts*	History of allergy
2101	0 × 0/20 × 25 (+)†	8 × 11/33 × 47 (+)	No	—	8.3	Urticaria
6101	0 × 1/2 × 2 (—)	9 × 10/24 × 21 (+)	No	—	1.1	No
9105	6 × 8/10 × 18 (+)	not done	No	—	10.5	Atopic dermatitis
9401	3 × 3/10 × 10 (+)	4 × 4/12 × 12 (—)	No	—	1.6	No
9701	6 × 5/21 × 10 (+)	not done	Yes	No	2.1	No

*The percent RAST counts (mean ± SD) of 35 sera from normal healthy volunteers was 1.35% ± 0.15%. The net counts for the total ¹²⁵I-labeled anti-IgE and for the background were 33470.3 ± 252.5 and 61.3 ± 4.9 cpm, respectively.

†Diameters of wheal per diameters of flare.

TABLE IV. Percent of patients whose percent RAST counts were >3% and the mean percent RAST count in various ways of BTT-administration

Administration route	No. of patients with allergy	No. of patients with % RAST counts of 3% or more (%)	% RAST count (mean ± SD)
Local application onto the skin injury or operation wounds	12/144	14/144 (9.7)	2.10 ± 2.72
Urinary bladder irrigation with local sprinkling	0/4	0/4 (0)	1.75 ± 0.60
Urinary bladder irrigation	0/12	3/12 (25.0)	2.66 ± 2.18
Oral	1/22	3/22 (13.4)	2.27 ± 2.30
Total	13/182	20/182 (11.0)	2.14 ± 2.59*
Normal control subjects	0/35	0/35 (0)	1.35 ± 0.15

*Significantly different from that of the normal control subjects ($p < 0.01$).

had never received BTT. As illustrated in Fig. 4, 20 patients (11% of the 182 patients), including the patient with positive posttreatment prick test reactions, demonstrated percent RAST count of 3% or more and formed a population separable from the normal subjects. Only two of the 20 patients had a history of allergic diseases.

Both the percentages of the cases with percent RAST counts of 3% or more and the mean percent RAST counts were not significantly different across the different routes of administration, although the percentage of positive RAST was high in the group with urinary irrigation (Table IV).

The relation between the results of the prick test and the percent RAST counts of sera obtained at the same time from five patients with positive skin tests before the treatment and 182 patients 1 month after the treatment is presented in Table V. When the percent RAST count of 3% or more was defined as positive, as described in Fig. 4, the specificity, sensitivity, and efficiency of the prick test was 98.8%, 13.6%, and 88.8%, respectively.

DISCUSSION

BTT is a concentrate of bovine plasma thrombin and contains various bovine plasma proteins. It is not surprising that such heterologous proteins administered onto broken skin or mucous membranes sensitize some people and evoke anaphylactic reactions in highly sensitized patients.

However, no studies described BTT-induced anaphylactic reactions until Usuda et al.⁸ reported a case of BTT-induced anaphylactic shock in 1986. During multicenter monitoring in Japan from 1986 to 1989, three cases of BTT-induced anaphylactic shock were referred, along with two pharmacists with BTT-induced occupational rhinitis and dyspnea. These patients with BTT-induced anaphylactic shock had other symptoms of anaphylaxis, such as urticaria, vomiting, dyspnea, and flush. Symptoms started within 15 minutes after the topical application of BTT. The RAST analysis of sera from these patients revealed significantly high anti-BTT IgE percent RAST counts. These results suggested that these three cases of BTT-induced shock were mediated by anti-BTT IgE Abs.

TABLE V. The relation between the results of prick test and the percent RAST count

	% RAST count	
	Positive (3% or more)	Negative (<3%)
Prick test		
Positive	3	2
Negative	19	163

The three patients with shock received BTT topically to the lesions in the skin or via urinary bladder irrigation. They had no overt history of food allergy to beef or cow's milk, suggesting that the absence of a history of food allergy to bovine materials can not rule out the occurrence of BTT-induced anaphylaxis when it is applied topically to nonalimentary tract lesions. Absorption of the intact BTT through the wide wounds appeared to have some relation to the occurrence of anaphylaxis in the highly sensitized patients.

Two of the three patients with shock received repeated applications of BTT with certain intervals. No adverse reactions were noticed on the previous occasion. It was likely that the patients were sensitized and/or the reactions were accelerated during the repeat administration. Therefore, caution should be used in patients who have received BTT repeatedly, even if they had not demonstrated allergic reactions previously.

The RAST-inhibition test demonstrated that the antigenic substance(s) in BTT were mainly involved in the contaminant rather than in the bovine thrombin itself. BTT contained >10 protein bands on SDS-PAGE analysis. It remains to be investigated which substance(s) is(are) the relevant antigen(s).

Human topical thrombin preparations have also been used frequently in Japan. The homologous material should cause little, if any, allergic reactions. However, BTT will continue to be used until a safer and more effective agent is introduced, since human topical thrombin preparations are relatively expensive and may not be completely free from human viral infectious hazards.

Therefore, we have studied the skin tests for a useful and simple way to recognize the state of sensitization and to predict the likelihood of anaphylaxis.

It is generally believed that prick testing has advantages over intracutaneous testing in the ease of interpreting results, safety, simplicity, speed, less discomfort to the patients, and stability of the allergens. In addition, there is better correlation with clinical diseases.¹⁰

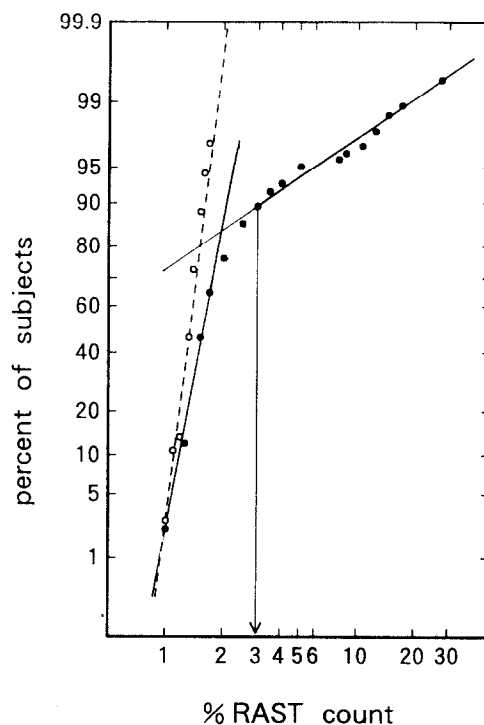


FIG. 4. Cumulative frequency distribution of the percent RAST count in patients 1 month after the BTT treatment. Cumulative percent frequency of 182 patients (●) who received BTT 1 month before and 35 normal subjects (○) are plotted by percent RAST count on a normal probability paper.¹³ The distribution of the normal subjects fits a straight line (---) illustrating that they had the normal distribution. The plot of the patients is described by two composite lines (—), the lower almost parallel to that of the normal subjects and the upper at higher percent RAST counts, illustrating that they can be separated into two populations. The cutoff value, 3%, was determined by outlier test according to the method of Grubbs et al.¹⁴ at the level of 5% from the percent RAST counts of the normal subjects and is illustrated by an arrow.

Our study on nonallergic healthy volunteers revealed that the intracutaneous test with 0.01 U/ml (10 ng/ml) or higher concentrations of BTT caused positive immediate reactions. The mean diameters of wheal and flare corrected for the saline control increased almost in a dose-dependent fashion, although the increase was significant only at 10 U/ml (10 µg/ml) or more. In contrast, a prick test with 1 to 1000 U/ml (1.04 mg/ml) of BTT caused no reactions. Belin and Nelson¹¹ reported a similar observation that false positive reactions occurred at protein concentrations of 10 µg/ml or more by intracutaneous test and 10 mg or more by prick test. The concentration of Ag required to elicit a positive intracutaneous reaction is 1000 to 10,000 times lower than that necessary for a positive prick test reaction.^{10, 11} The prick test at 1000 U/ml is 100 to 1000 times more sensitive in detecting

sensitized patients than the intracutaneous test at 0.001 U/ml.

Therefore, the diagnostic and predictive value of the prick test at 1000 U/ml was further evaluated in 196 patients who needed BTT. One patient demonstrated a negative prick test but positive intracutaneous test reactions for 0.1 U/ml of BTT (Table IV). The percent RAST value of this patient (No. 6101) was within the normal limits. Since the above study has demonstrated that nonspecific reactions may be produced by the intracutaneous test with 0.01 U/ml or higher concentrations of BTT, it is likely that the reactions were also nonspecific.

Four patients demonstrated positive prick test reactions. In this group of patients, no BTT treatment was performed, and the blood was obtained instead for the RAST. Among the four patients, two patients (Nos. 2101 and 9105) demonstrated significantly high percent RAST counts comparable to patients with BTT-induced anaphylaxis. Assuming the percent RAST count of 3% or more as a true positive, the sensitivity of the prick test was as low as 13.6%, as presented in Table V. However, one patient (No. 11) with BTT-induced anaphylactic shock, and another patient (No. 21) with occupational asthma and rhinitis, were also positive for the prick test at this concentration of BTT. These results suggest that the prick test with 1000 U/ml of BTT is sensitive enough to detect such highly sensitized patients. In fact, no adverse reactions occurred on the application of BTT in patients who had been negative for the pretreatment skin tests, suggesting a very rare false negative reaction.

The other two patients with a positive prick test demonstrated normal percent RAST counts. Assuming the percent RAST count of 3% or more as a true positive, the specificity of the prick test was 98.9% (Table V). Since one patient (No. 9701) of the two patients with normal percent RAST counts received BTT without any adverse reaction, the positive prick test reactions observed in this patient is most likely a false positive. The incidence of false positive reactions is not negligible but can be permissible, considering the severity of anaphylactic reaction that may occur.

Furthermore, the prick test with 1000 U/ml of BTT by itself caused no systemic adverse reactions, even in patients with BTT-induced anaphylactic shock or BTT-induced occupational rhinitis and asthma, suggesting the safety of the test. In addition, 1000 U/ml of solution can easily be prepared from the powder in a commercial vial.

Taken together, the prick test at 1000 U/ml appears to be a useful, safe, and practical method to detect highly sensitive patients who may develop anaphylactic shock on topical application of BTT. However, the exact predictive value of the prick test in the prick

test-positive group remains to be confirmed further. Bielory et al.¹² also recommended a similar prick test as a predictive test for equine, antithymocyte, globulin-induced hypersensitivity reactions.

The prick test and the RAST were conducted 1 month after the administration of BTT to assess the immunogenicity of BTT. The posttreatment prick test was positive in one patient. The mean percent RAST counts 1 month after the treatment was significantly higher than counts of the normal control subjects. Twenty of 182 patients demonstrated percent RAST counts of 3% or more, although only two of these 20 patients had a history of allergy. Although blood samples were not obtained before BTT therapy in the present study, these findings appear to suggest that a certain proportion of patients could be sensitized or the reactions could be accelerated by the single treatment. Repeated administration of BTT may further increase the anti-BTT IgE levels in these patients.

We thank Mrs. Matsuko Fujita, Mrs. Keiko Onodera, and Miss Hiroko Ichijo for their technical and secretarial assistance. We are indebted to Dr. Katsutada Imai and Dr. Toshinori Saito of Sendai Municipal Hospital, Dr. Masami Ohsawa in the Department of Anesthesiology, University of Kyoto, and Dr. Toshikazu Usuda of Social Insurance Chukyo Hospital, for allowing us to study the patients under their care. We also appreciate the collaboration of the doctors of 28 institutions in Japan. We are also grateful to Dr. Michio Matsuda for the preparation of BTT fractions, to Dr. Yukio Fukuda and Dr. Toshinari Kamakura for the help in the statistical analysis, and to Warner-Lambert K.K. for the supply of BTT as well as the collaboration in the multicenter monitoring.

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Episodic eosinophilia-myalgia-like syndrome in a patient without L-tryptophan use: Association with eosinophil activation and increased serum levels of granulocyte-macrophage colony-stimulating factor

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We have studied a patient with recurrent bouts of angioedema, myalgia, and eosinophilia that was not due to L-tryptophan ingestion. Peripheral blood eosinophils (EOSs) during exacerbations of his illness displayed characteristics of "activation," including hypodense phenotype and increased responsiveness to platelet-activating factor (PAF) in vitro with respect to expression of CD11b surface adhesion proteins. Elevated serum levels of granulocyte-macrophage colony-stimulating factor (GM-CSF) bioactivity were also detected, whereas interleukin-3 and interleukin-5 levels were not increased. During treatment with glucocorticoids, all clinical symptoms resolved, EOSs decreased in number and became normodense, PAF responsiveness diminished, and GM-CSF levels returned to normal. During glucocorticoid tapering, a subsequent clinical relapse was again associated with EOS hypodensity, increased PAF responsiveness, and increased serum GM-CSF levels. Although this patient satisfies the diagnostic criteria for eosinophilia-myalgia syndrome, the episodic and profound nature of exacerbations and response to therapy in the absence of L-tryptophan usage suggests a possible overlap with the syndrome of episodic angioedema and eosinophilia. In vitro studies suggest that GM-CSF may play a role in the eosinophilia, EOS activation, and pathophysiology of disease in this patient and demonstrate resolution of these abnormalities during glucocorticoid therapy. The efficacy of glucocorticoid therapy in some hypereosinophilic states may therefore be mediated, at least in part, via reduction of GM-CSF production and/or EOS activation. (J ALLERGY CLIN IMMUNOL 1991;88:629-36.)

Key words: Eosinophil activation, myalgia, GM-CSF

From the Department of Medicine, Division of Clinical Immunology, Johns Hopkins Asthma and Allergy Center, Baltimore, Md.

Supported by National Institutes of Health Grants AI27429, AI08270, and AI20136.

Received for publication Jan. 30, 1991.

Revised May 6, 1991.

Accepted for publication May 22, 1991.

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Publication No. 058 from Johns Hopkins Asthma and Allergy Center.

Dr. Bruce S. Bochner was supported in part by a New Investigator Award from the American Lung Association.

Dr. Lawrence M. Lichtenstein was a recipient of a Pfizer Biomedical Award.

1/1/31238