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Laboratory Investigation of Deaths Due to Anaphylaxis

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ABSTRACT: To establish a useful laboratory protocol to investigate possible cases of fatal anaphylaxis, we measured mast-cell-derived tryptase levels and allergen-specific immunoglobulin E (IgE) antibody levels in sera obtained prior to or within 24 h after death from 19 anaphylaxis victims. Elevated serum tryptase levels (range = 12 ng/mL to 150 μg/mL) were found in nine of nine Hymenoptera sting fatalities, six of eight food-induced fatalities, and two of two reactions to diagnostic/therapeutic agents. Tryptase levels were normal (<10 ng/mL) in 57 sequential sera obtained postmortem from six control patients. Tryptase could not be measured in pleural or pericardial fluids for technical reasons. Serum IgE antibodies were elevated in five of the nine Hymenoptera sting fatalities and in eight of the eight fatal food reactions; assays were unavailable for the two diagnostic/therapeutic agents. If elevated, the victim's serum IgE antibodies to food could be used to identify allergens in uneaten portions of foods consumed shortly before the anaphylactic event. IgE antibodies were moderately stable during storage in a variety of anticoagulants at room temperature for up to 11 weeks. Elevated mast-cell-derived tryptase levels in postmortem sera reflect antemortem mast cell activation and may be used as a marker for fatal anaphylaxis. If assays are available for IgE antibodies to relevant allergens, such assays provide evidence for antemortem sensitization; these assays may be modified to identify allergens in foods consumed by victims of food-induced anaphylaxis.

KEYWORDS: pathology and biology, anaphylaxis, immunoglobulins, death, tryptase, mast cell-derived tryptase

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The pathologic findings in cases of fatal anaphylaxis are nonspecific. Until now, the diagnosis of anaphylactic death was mainly one of exclusion, relying heavily on a unique clinical scenario and a collection of variable numbers of nonspecific morphologic findings, including visceral congestion, pulmonary edema, and no demonstration of an anatomic cause of death. Although angioedema of the larynx and pulmonary emphysema due to obstruction of the lower airways are sometimes noted [*1*], these two findings were present in only 15 and 11 cases, respectively, of the 43 cases of fatal anaphylaxis carefully studied by Delage and Irey [*2*].

The pathophysiology of human anaphylaxis involves the widespread release of histamine and other pro-inflammatory compounds from tissue mast cells. This mast cell activation is frequently triggered by the interaction of allergen and specific immunoglobulin E (IgE) antibody on the mast cell surface, but certain drugs and diagnostic agents can directly trigger mast cell histamine release in the absence of IgE antibody [*3*].

Tryptase is a neutral protease found in mast cell secretory granules [*4*], and sensitive immunoassays have been developed for its quantitation in serum [*5*]. Serum tryptase levels have been shown to be elevated 1 to 4 h after nonfatal anaphylaxis [*6*]. In contrast to plasma histamine levels, which peak at 5 to 10 min after the sting in Hymenoptera anaphylaxis, serum tryptase levels peak 1 to 2 h after the insect sting, then decline under apparent first-order kinetics with a $t_{1/2}$ of approximately 2 h [*7*].

Although there are sensitive radioenzymatic [*8*] and immunologic [*9*] assays for plasma histamine, the rapidity with which histamine is released and metabolized following anaphylaxis and its potential to be released by basophils during fluid processing complicates the interpretation of histamine levels for documenting cases of fatal anaphylaxis. Elevated levels of specific IgE antibodies have been measured in postmortem sera from victims of fatal anaphylaxis due to insect stings [*10,11*] or food allergy [*12*]. However, the presence of these antibodies merely documents previous sensitization, indicating an enhanced risk for having an anaphylactic event, but does not directly prove an anaphylactic event as the cause of death.

In this study, we report 19 cases that meet the diagnostic profile on which the diagnosis of "anaphylactic death" has been made in the past, adding measurements of mast-cell-derived tryptase and allergen-specific IgE antibodies to the profile of criteria for the diagnosis of anaphylactic death. The specific objectives of the present studies were (1) to determine whether tryptase levels were elevated in sera or other body cavity fluids collected postmortem from victims of fatal anaphylaxis or other diseases, (2) to estimate the stability of specific IgE antibodies in serum and plasma samples obtained in a variety of collection tubes and stored for varying lengths of time at various temperatures, and, (3) to demonstrate how IgE antibody-containing sera obtained postmortem can be used to identify allergen-containing food eaten by victims of fatal food-induced anaphylaxis. We show that elevated serum levels of allergen-specific IgE antibodies and, in particular, mast-cell-derived tryptase, are important specific markers useful in making the diagnosis of anaphylactic death.

Materials and Methods

Test Samples

Single sera were obtained prior to or within 24 h after death from 19 anaphylaxis victims who reacted to either Hymenoptera stings (n = 9), foods (n = 8), or diagnostic/therapeutic agents (n = 2). The clinical histories of several of these cases have been presented previously [*11,12*]. These samples were stored for varying periods of time at 4 or −20°C prior to assay for tryptase. We were concerned that postmortem autolysis of subendocardial mast cells might be sufficient to elevate cardiac chamber tryptase levels spuriously.

Therefore, we obtained control sera from six persons dying of other causes; in these cases, sequential samples were withdrawn from both the heart and a femoral vessel 1 to 15 h after death. These sera were isolated by centrifugation and stored at −20°C until assay. Finally, we obtained single samples of atrial blood, pleural fluid, or pericardial fluid from 15 persons dying of nonanaphylactic causes whose bodies were not discovered until 6 h to 15 days after death; these samples were stored at 4°C until assay.

Tryptase Assay

Tryptase levels were measured by a sandwich enzyme-linked immunoabsorbent assay (ELISA) using murine monoclonal immunoglobulin G (IgG) antitryptase as the capture antibody and goat polyclonal IgG antitryptase as the detection antibody, as reported previously [*3*], except that 10 m*M* ethylenediaminetetraacetic acid (EDTA) was included to prevent coagulation. Each sample was filtered through a 0.22-μm filter to remove particulate material prior to assay. The sensitivity of the assay was 2.5 ng/mL; values >10 ng/mL were considered elevated.

Allergen-Specific IgE Antibodies

Allergen-specific IgE antibodies were measured by solid-phase radioimmunoassay [*13*]. Commercial allergen extracts, or phosphate-buffered saline extracts of uneaten foods contained in the meal consumed by food-sensitive persons just prior to death (see below) were reacted with cyanogen-bromide-activated microcrystalline particles or filter paper disks to prepare in-house solid-phase allergens. Positive and negative control serum samples were included in each assay. Results were expressed as the radioactive counts bound by the test serum samples divided by the radioactive counts bound by the negative control serum, multiplied by 100. Binding values greater than 300% relative to the negative control value were considered elevated; values greater than 5000% relative to the negative control were considered markedly elevated.

Identification of Allergens in Foods

In situations where food-specific IgE antibodies could be demonstrated in postmortem sera from victims of food-induced anaphylaxis, we studied samples of uneaten portions of food that had been consumed by the victim in the meal just prior to anaphylaxis. Extracts were prepared by mixing the food with phosphate-buffered saline (pH 7.4) (1:10 weight/volume) in a blender, then magnetically stirring the slurry overnight at 4°C. The extract was recovered and clarified by centrifugation. The extract was either coupled to solid-phase carriers for direct radioallergosorbent test (RAST) assay, as described above, or used as a fluid-phase inhibitor in RAST inhibition experiments employing known individual food extracts coupled to solid-phase carriers [*13*]. The RAST inhibition data were analyzed by a computerized version of the parallel line assay, as recommended by the U.S. Food and Drug Administration [*14*].

Stability of Allergen-Specific IgE Antibodies

To test the stability of IgE antibodies under a variety of storage conditions and temperatures, we drew peripheral blood from three living persons who were allergic to ragweed, peanuts, or guinea pigs. A portion of the blood was allowed to clot at room temperature for 1 to 2 h, after which the serum was harvested and stored at −20°C until assay. The remainder of the blood sample was distributed into evacuated glass tubes (Venoject®, Terumo Medical Corp., Elkton, Maryland) containing sodium citrate, EDTA,

sodium fluoride/heparin, or heparin alone; these tubes were kept at room temperature for 10 to 11 weeks. At one- or two-week intervals, the tubes were mixed, an aliquot was withdrawn, and a plasma sample was obtained by centrifugation and stored at −20°C. All samples were thawed and tested for the respective IgE antibody in a subsequent single RAST assay. IgE antibody levels in the various plasmas were expressed as a percentage of the IgE antibody level in the serum sample frozen on the day of collection; the level in the latter sample was assigned a value of 100%.

Selected Case Histories

Case No. 1

A 75-year-old man with a history of dementia had attempted suicide on four previous occasions. He disappeared from home and was found dead the following day adjacent to a fire ant (*Solenopsis invicta*) mound. He had been stung previously by fire ants without untoward reaction. The body was covered with hundreds of pustules typical of fire ant stings. The body was too decomposed to appreciate any laryngeal edema. The autopsy revealed only cardiomegaly and left ventricular hypertrophy, histologic changes in the brain typical of Alzheimer's disease, and no evidence of trauma. The pleural fluid samples had low levels of alprazolam, flurazepam, desalkylflurazepam, and nordiazepam. The heart blood ethanol concentration was 0.04%. Heart blood collected at autopsy into a tube containing sodium fluoride/potassium oxalate had elevated IgE antibodies to *Solenopsis invicta* (908% relative to the negative control) and strikingly elevated levels of tryptase (700 ng/mL). Based on these findings, the cause of death was listed as arthropod hypersensitivity reaction (*Solenopsis invicta*).

Case No. 10

A 10-year-old girl with a long-standing history of asthma and multiple food allergies had experienced a nonfatal cardiovascular collapse three years previously after eating a cookie containing peanuts. While attending a school outing, she ate a meal including french fries, sausage/cheese pizza, and a cola beverage. Shortly thereafter, she collapsed with respiratory distress and could not be resuscitated. An autopsy performed the following day showed doughy lungs, with patchy atelectasis and mucous plugging. No mucosal edema was present. Serum obtained postmortem showed strikingly elevated levels of IgE antibody to peanut (21 937% relative to the negative control) and soybean (7674% relative to the negative control), while the serum tryptase level was minimally elevated (12 ng/mL). Field investigation revealed that the french fries had been cooked in soybean oil and that the sausage pizza was fortified with soy protein. Using food samples collected at the fatality scene, we demonstrated elevated serum IgE antibodies to an extract of the pizza sausage (5180% relative to the negative control), but a normal IgE antibody level to an extract of the french fries (193% relative to the negative control). By RAST inhibition studies we demonstrated that her IgE antibodies were directed to the soybean component of the sausage (see below). Based on these findings, the cause of death was listed as asthma attack, with anaphylaxis due to food allergy.

Results

Tryptase Levels in Postmortem Samples

In Table 1 are displayed the tryptase levels and the specific IgE antibody levels in postmortem sera from the 19 anaphylaxis victims. Elevated tryptase levels were noted

TABLE 1—*Tryptase levels and specific IgE antibody levels in postmortem sera from 19 anaphylaxis victims.*[a]

Case No.	Tryptase, ng/mL	Specific IgE Antibody	
		Allergen	% of Negative Control
		HYMENOPTERA FATALITIES	
1	700	fire ant	908
2	50	yellow jacket	1 450
3	494	yellow jacket	1 882
4	55	yellow jacket	208
5	125	yellow jacket	100
6	41	white-faced hornet	1 300
7	150 000	yellow jacket	122
8	131	fire ant	145
9	5 041	honeybee	697
		FOOD FATALITIES	
10	12	soybean	7 674
11	74	crab	1 618
12	<2.5	peanut	12 300
13	<3.4	pecan	2 514
14	17	codfish	4 377
15	29	peanut	529
16	61	peanut	8 323
17	159	codfish	369
		DIAGNOSTIC/THERAPEUTIC AGENT FATALITIES	
18	1 038	Hexabrix®	NA[b]
19	6 000	Sotradecol®	NA

[a]The clinical histories and IgE antibody levels for cases 2 through 5 [*11*] and Cases 11 through 16 [12] have been published previously.
[b]NA = not available.

in 9 of the 9 insect fatalities, in 6 of the 8 food fatalities, and in both diagnostic/therapeutic agent fatalities.

A total of 57 sequential samples were obtained from the cardiac chambers and femoral vessels of the 6 persons dying of causes other than anaphylaxis. The tryptase levels were <2.5 ng/mL in 50 of the 57 samples and ≤10 ng/mL in 57 of the 57 samples (data not shown).

We were unable to measure tryptase in pleural fluid samples from 8 subjects or in pericardial fluid from one subject because of technical problems. In some cases, these samples were too viscous to process, and in many cases, they yielded a high background (data not shown).

Stability of Specific IgE Antibodies

The plasmas were heavily hemolyzed in all tubes after several weeks. There was a falloff in specific IgE antibody in plasma stored for 10 to 11 weeks at room temperature; the values are shown in Fig. 1. In most cases the antibody levels declined slowly during the first few weeks, reaching a plateau after 5 to 7 weeks of storage. A notable exception involved the guinea-pig-specific IgE antibody titer in fluoride/heparin tubes, which declined rapidly after only one week of storage. Antibody titers declined to a greater degree

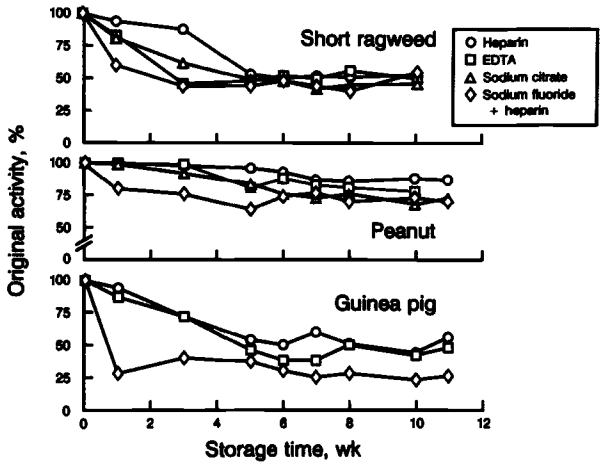

FIG. 1—*Changes in IgE antibodies to short ragweed, peanut, and guinea pig during storage at 22°C in various anticoagulants. The time zero IgE antibody levels were 1714%, 17 364%, and 1722% relative to the negative control level, respectively.*

in fluoride/heparin tubes and to a lesser degree in tubes containing heparin alone. In no case was the decline sufficient to produce a false negative result, however.

Identification of Allergen-Containing Food

Using postmortem sera from Case 10, which we knew contained IgE antibodies to both soy protein and an extract of the pizza sausage, we used RAST inhibition assays to verify that her IgE antibodies were directed to the soy protein in the pizza sausage, not to the meat protein in the sausage. Addition of either soybean extract or pizza sausage extract produced dose-dependent inhibition of either the soybean RAST or the pizza sausage RAST (Fig. 2). The slopes of the two inhibition curves in both assays did not differ significantly, indicating that the allergenic determinants in the soybean extract and in the sausage extract were qualitatively identical.

Discussion

Our findings indicate that elevated mast-cell-derived tryptase levels in postmortem sera reflect antemortem mast cell activation, which is compatible with an anaphylactic reaction. We have previously shown that tryptase in serum is most stable when stored at −20°C [7], but >50% of endogenous tryptase can be detected even after four days of storage at room temperature. Because elevations in serum tryptase may not be detected during the first 15 to 30 min after the onset of an anaphylactic event [7], persons experiencing sudden anaphylaxis that is rapidly fatal may not have elevated tryptase levels in their postmortem sera. This may have been the situation in Cases 12 and 13, where the postmortem tryptase values were in the normal range. The finding of a normal tryptase level in Case 12 was unexpected, however, since the interval between the development of symptoms and death was >1 h. Conversely, tryptase levels may have returned to normal in blood drawn several hours or more after an anaphylactic event but prior to death. From the records provided it was not possible to determine accurately the interval between the onset of the allergic reaction and the time when the serum sample was obtained in most cases.

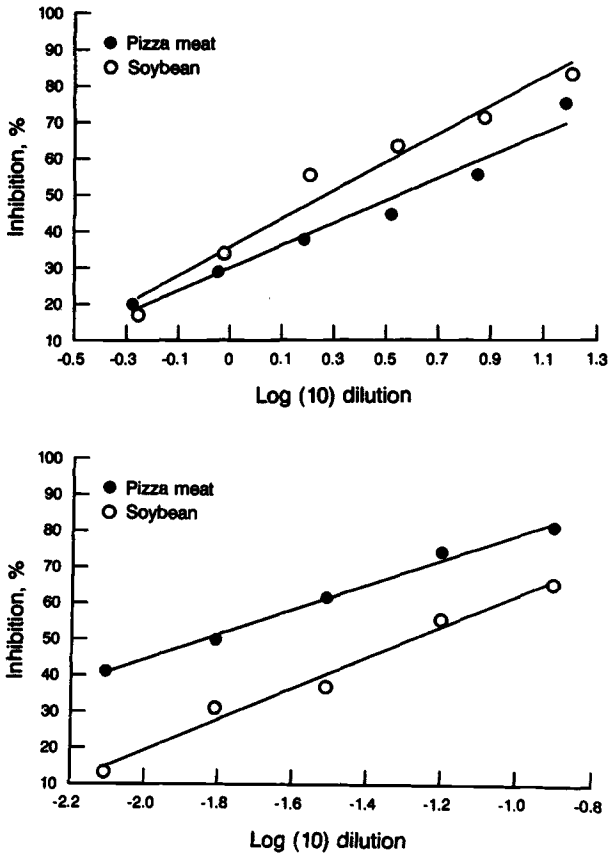

FIG. 2—*Allergenic activity of soybean extract and pizza meat extract as measured by* (top) *soybean inhibition immunoassay and* (bottom) *pizza meat inhibition immunoassay.*

Of interest was the finding of somewhat higher serum tryptase levels in persons who had encountered their lethal allergen parenterally (in Hymenoptera venoms and injectable agents) rather than orally (in foods). It is possible that parenterally administered allergens preferentially trigger human tryptase- and chymase-containing (TC) mast cells, the type of mast cell that predominates in skin and that contains approximately threefold more tryptase than human tryptase-containing (T) mast cells [*15*], which are found in small bowel mucosa and in the lungs [*16*].

Although elevated allergen-specific IgE antibodies were present in most of the anaphylaxis victims, this was not the situation in Cases 4, 5, 7, and 8. This is not surprising, because elevations in venom-specific IgE antibodies are present in only 80% of persons sensitive to Hymenoptera stings who also have compelling clinical histories of sting-induced anaphylaxis and positive venom skin tests [*17*].

The IgE antibody levels in 17 of the anaphylaxis victims covered a wide range, similar to that seen in living sensitized persons. Therefore, the risk of fatal anaphylaxis is not determined solely by the magnitude of the IgE antibody elevation. Other variables, such as the quantity of allergen delivered, the distribution of allergen and sensitized mast cells, the degree of mast cell and target tissue responsiveness, and the promptness with which medical therapy is administered, may each influence the lethality of an anaphylactic event.

Based on these experimental findings, in cases of suspected anaphylaxis, one or more serum samples should be obtained antemortem (if possible) and postmortem for quantitation of tryptase and allergen-specific IgE antibodies. Sera should be frozen and stored at −20°C until assay. Blood samples obtained 1 to 3 h after the onset of the reaction are most apt to have elevated tryptase levels. Pleural fluids or pericardial fluids, at present, are unsatisfactory sources for quantitation of tryptase for technical reasons. The family members, friends, and physicians of anaphylaxis victims should be queried regarding previous allergen-induced reactions, and appropriate specific IgE antibody levels should be checked. Unconsumed portions of foods eaten by the victim shortly before the anaphylaxis episode should be saved, because extracts of these foods may be used to create "custom" RAST reagents to search for food-specific IgE antibodies. Finally, RAST inhibition assays may be used successfully to identify specific allergens in foods causing fatal food-induced anaphylaxis.

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