

Studies in Stinging Insect Hypersensitivity: Postmortem Demonstration of Antivenom IgE Antibody in Possible Sting-Related Sudden Death

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The purpose of this study was to extend previous findings of elevated antivenom IgE antibodies in sera from persons experiencing fatal insect sting reactions. Elevated IgE antibodies to at least one venom were measured in sera from four of six witnessed fatal cases of sting anaphylaxis; in one of two unwitnessed cases of sudden, unexpected death in which the circumstances suggested possible sting anaphylaxis; and in one unwitnessed death where autopsy findings of massive swelling of the pharynx and larynx suggested anaphylaxis. Conversely, no elevated IgE antibodies were measured in serum from a boy who received more than 40 yellow jacket stings with subsequent cardiopulmonary arrest, from which he was successfully resuscitated. Measurement of IgE antibodies in postmortem sera may not only provide confirmatory evidence of fatal anaphylaxis following witnessed insect stings, but also may provide useful additional data in unwitnessed deaths where the clinical history or autopsy findings suggest unexplained anaphylaxis. (Key words: Insect allergy; Sudden death; Anaphylaxis; IgE) *Am J Clin Pathol* 1986; 85: 607-610

APPROXIMATELY 12% of the general population has been reported to exhibit positive skin tests to insect venoms, even in the absence of a history of sting reactions.⁵ This prevalence of venom skin test positivity may exceed 40% in patients who have been stung without adverse reactions within the preceding 12 months.¹³ The clinical significance of these positive skin tests is unknown.

In contrast, only about 1% of normal blood donors have elevated venom-specific IgE antibodies in their serum.¹³ It has previously been documented that postmortem demonstration of venom-specific IgE antibodies is feasible.^{7,11} During the last year, we had the opportunity to examine a number of postmortem sera from persons believed to have died as a consequence of Hymenoptera venom allergy, as well as serum from a near-fatal Hymenoptera sting case. The following report details the results of our experience.

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Materials and Methods

Study Sera

Postmortem sera were submitted by pathologists from nine cases suspected to have died as a result of insect sting anaphylaxis. Most specimens were submitted as a consequence of the pathologists' awareness of a previously published case history from our institutions.¹¹ In some cases, the sera had been stored for several months at 4 °C or -20 °C, and/or were heavily hemolyzed. These variables did not interfere with the measurement of IgE antibodies. One additional serum from a near fatal case was examined.

Solid-Phase Allergens

Honeybee venom (grade 1, Sigma Chemical Co., St. Louis, MO) and wasp venom (Dr. James Gillaspay, Kingsville, TX) were reacted overnight at 4 °C with CNBr-activated microcrystalline cellulose particles (Avicel, Brinkmann) at a ratio of 250 mg venom protein per g cellulose (honeybee) or 46 mg venom protein per g cellulose (wasp). The venom-cellulose was washed exhaustively with borate buffer until the spectrophotometric absorbance at 280 nm was <0.050, after which the venom-cellulose was resuspended at a concentration of 1 mg/mL in RAST diluent.¹

Yellow jacket, yellow hornet, and white-faced hornet venoms (Vespa Laboratories, Spring Mills, PA) were reconstituted in 0.1 M ammonium formate (pH 4.7) and separately gel filtered over a 4 × 16.5 cm calibrated Sephadex G-15[®] column to remove the beta alanine-con-

taining buffer. Tubes comprising the void volume peak were pooled, concentrated, lyophilized, and then reacted with CNBr-activated cellulose particles (10–26 mg dry weight per g).

IgE Antibody Assays

IgE antibodies to honeybee, wasp, yellow jacket, yellow hornet, and white-faced hornet venoms were tested by the mini-RAST procedure.⁴ Positive and negative control sera or test sera (0.05 mL) were diluted with 0.15 mL RAST diluent, after which was added 0.05 mL venom-cellulose suspension. After incubation overnight at room temperature with end-over-end rotation of tubes, the venom-cellulose-IgE antibody complex was washed three times with RAST wash,¹ after which was added 0.1 mL radio-labeled rabbit anti-IgE (50 ng/mL) plus 0.15 mL fetal calf serum diluent. After identical incubation and washing steps, the cellulose complex was counted in an automatic gamma spectrometer.

IgE antibodies to whole fire ant body were measured using fire ant allergens coupled to paper discs (MIAB, Knivsta, Sweden). The radioimmunoassay was performed as previously discussed, except that the first-stage incubation period was shortened to four hours.

Results from the IgE antibody assays were expressed as a percentage of radioactive binding produced by serum from a nonallergic individual; in our laboratory, values greater than 300% negative control are considered positive. In some cases (7 and 8), it was not possible to measure IgE antibodies to all venoms because of limited quantities of serum. In another case (9), IgE antibody assays were selected based on the clinical history given by friends of the decedent.

Report of Ten Cases

Case 1

A 63-year-old woman was found dead in her bathroom, where a box of bandages and a bottle of meat tenderizer were on the countertop of the sink. There was a past history of insect sting allergy, but no venom immunotherapy had been given. The patient had been mowing her lawn prior to her death, and there was a known insect nest (wasp or yellow jacket) in the area. Autopsy revealed moderate edema of the aryepiglottic folds, mucous plugging of the intrapulmonary bronchi, and hyperinflated lungs filled with foamy, blood-tinged fluid. Microscopic examination showed degranulating mast cells in the aryepiglottic folds. The cause of death was listed as allergic reaction to a Hymenoptera sting.

Case 2

A 25-year-old truck driver was stung by a yellow jacket on the wrist. Within 25 minutes he had collapsed, experienced a generalized tonic seizure, and was in cardiopulmonary arrest on arrival at the hospital. He was resuscitated and placed on life-support systems. Multiple serum samples were obtained after admission to the hospital and prior to his

death ten days later. The cause of death was listed as an anaphylactic reaction to insect sting with anoxic encephalopathy.

Case 3

A 37-year-old woman was stung by a wasp on her right leg. Fifteen minutes later, she experienced dizziness, collapsed, fell down four steps, and was pronounced dead at the scene. There was no prior history of Hymenoptera allergy. The insect that stung her was killed and positively identified as a wasp (*Polistes fuscatus*). Autopsy showed moderate expansion of the lungs, a galeal contusion, and loosening of the atlanto-occipital joint. The cause of death was listed as anaphylaxis caused by a wasp sting.

Case 4

A 36-year-old man was found to have died while trimming hedges; he was surrounded by a large number of bees when found. There was no past history of Hymenoptera sting allergy, but he had exhibited an irrational fear of bees, once jumping off a 14-foot ladder to escape a bee. Autopsy showed hemorrhagic pulmonary edema and congestion of the larynx and epiglottis; there were two punctate lesions consistent with insect stings, one on the chin and one on the left neck. The cause of death was listed as acute cardiac arrhythmia of undetermined etiology, perhaps caused by extreme fright.

Case 5

A 43-year-old man was stung by a bee while driving on an interstate highway. He had a prior history of Hymenoptera sting allergy, and his wife administered epinephrine to him without apparent benefit. On arrival at an emergency room, the patient was cyanotic without vital signs; ECG showed ventricular fibrillation. Resuscitation attempts were unsuccessful. Autopsy showed an insect sting site on the left forearm, pulmonary edema, laryngeal edema, and upper airway edema. The cause of death was listed as acute anaphylaxis resulting from Hymenoptera sensitivity.

Case 6

A 26-year-old mentally retarded man who lived in a group home was found dead in bed in the morning. Past medical history was unavailable. Autopsy showed extreme edema of the upper airway from the nasopharynx to the epiglottis, with obstructing edema of the larynx. The cause of death was listed as respiratory obstruction resulting from unknown causes.

Case 7

A 40-year-old woman sustained four insect stings while gathering wood for a picnic fire. She passed out a few minutes later and was pronounced dead on arrival at a nearby hospital. There was no history of insect sting allergy. Autopsy showed only four cutaneous lesions consistent with insect stings. The cause of death was listed as an anaphylactic reaction resulting from insect stings.

Case 8

A 77-year-old man was stung by a "bee" while mowing his lawn, and thereafter developed angioedema of the face and lips, followed by cardiopulmonary arrest. Autopsy showed minimal to moderate edema of the aryepiglottic and vestibular folds, vocal cords, and upper trachea, with multiple areas of mucosal hemorrhage, some of which were felt to represent intubation injury. The left anterior descending coronary artery showed moderate to severe atherosclerosis, with foci of greater than 50%

Table 1. Venom-Specific IgE Antibody Levels in Test Sera

Case Number	City	RAST (% Negative Control)					Fire Ant
		Honeybee	Wasp	Yellow Jacket	Yellow Hornet	White-Faced Hornet	
Fatalities							
1	Seattle, WA	200	133	1,450	867	342	ND
2	Youngstown, OH	<100	178	100	<100	<100	ND
		128	157	171	166	196	ND
		157	206	140	<100	220	ND
		169	196	166	<100	210	ND
3	Richmond, VA	471	1,400	693	495	360	ND
4	Providence, RI	<100	134	<100	<100	<100	ND
5	Billings, MT	399	1,067	1,882	762	522	ND
6	Billings, MT	569	1,362	1,201	782	677	ND
7	Washington, DC	<100	ND	208	ND	ND	ND
8	Washington, DC	<100	ND	358	ND	ND	ND
9	Miami, FL	ND	ND	ND	ND	ND	359
Nearfatality							
10	Cleveland, OH	<100	100	146	<100	111	ND

ND = not done.

obstruction. The cause of death was listed as anaphylactic shock secondary to bee sting, associated with occlusive coronary arteriosclerosis.

Case 9

A 69-year-old Brazilian woman, vacationing in Florida, was bitten on the left ankle by an "ant." Within 10 minutes she collapsed, and resuscitation was unsuccessful. She was known to be allergic to "insect bites" and had experienced anaphylaxis in the past. Her prior immunotherapy status was unknown. Autopsy showed moderate edema of the aryepiglottic folds without airway obstruction. The primary cause of death was listed as insect sting reaction.

Case 10

A 4½-year-old boy received multiple (>40) yellow jacket stings and shortly thereafter developed generalized urticaria, laryngeal and cutaneous angioedema, hypotension, and cardiopulmonary arrest. The responding emergency squad administered intracardiac epinephrine during resuscitation; after subsequent emergency room therapy with prednisone and Benadryl®, he was dismissed after several hours. There was no prior history of insect sting anaphylaxis. Venom skin tests performed approximately six weeks after the reaction showed an equivocal reaction to white-faced hornet venom at 1 µg/mL; all other venom skin tests were negative.

Results

Our findings further substantiate the value of attempting to search for postmortem evidence of venom-specific IgE to validate death from insect sting anaphylaxis. Six of the nine postmortem sera submitted (cases 1, 3, 5, 6, 8, and 9) contained significant amounts of venom-specific IgE (Table 1). Highest RAST levels were to wasp and yellow jacket venoms. Most sera contained elevated IgE antibodies to several venoms. Cross-reactivity occurs frequently among vespid venoms (yellow jacket and hornets)^{8,10} and between vespid and *Polistes* wasp venoms.⁶

RAST results from the near-fatal case involving a 4-year-old child are of interest in that no evidence of high-titer antivenom antibody was found. Other explanations may account for the near death, including a toxic reaction caused by an overdose of vasoactive amines present in the venom itself.

Discussion

The relative importance of insect sting hypersensitivity as a cause of death is uncertain, but it has been estimated that perhaps 30 deaths may occur in the United States each year that can be directly attributed to insect sting anaphylaxis.² These estimates have been based on clinical criteria; confirmatory morbid anatomic changes have not always been present, because the pathology of anaphylaxis is nonspecific.³ It now appears clear that venom-specific IgE antibodies can be successfully measured in sera from patients with this clinical disease.^{7,11} IgE antibody levels correlate with venom skin results in insect-venom-allergic patients,¹² and thus are useful in the diagnosis of venom allergy.

"Envenomation" has been used as the explanation for some cases of sting-related death as an alternative to true anaphylaxis. Our case 10, in which a young child suffered a cardiac arrest following multiple stings, may represent such a case, as the patient showed no skin test or RAST evidence of venom-specific antibody.

The presence of detectable venom-specific IgE antibodies without any clinical evidence of insect sting allergy has been reported in a significant number of cases,^{9,13} but their presence in otherwise healthy people, dying unexpectedly in situations where insect sting was likely, should not be dismissed as clinically insignificant. Elevated levels

of IgE antibody to several venoms may be the result of multiple previous sensitizations or may represent cross-reactivity to shared allergens, especially among vespid venoms.^{6,8,10} In testing postmortem sera for venom-specific IgE antibodies, the selection of venoms depends on several variables, including the geographic area, the circumstances surrounding the patient's death, and the quantity of serum available. A minimal screening effort would involve testing for IgE antibodies to honeybee and yellow jacket venom in the eastern, north-central, and western United States. In the southeastern and Gulf coast areas, testing for IgE antibodies to paper wasp venom and imported fire ant would perhaps be preferable, as the latter insects cause the majority of Hymenoptera sting reactions in these areas.

Further studies are in progress; we would be glad to examine serum samples from suspected cases. Send samples to Dr. John W. Yunginger, Allergic Diseases Research Laboratory, Room 406, Guggenheim Building, Mayo Medical School, Rochester, Minnesota 55905.

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Clinical Trial of a New Disposable Bleeding-Time Device

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A new disposable bleeding time device (Hemalet®) was tested in 20 normal individuals and 11 patients with various bleeding disorders. The results were compared with those of Simplate II®. The mean bleeding time for normal individuals was 5.4 ± 1.5 (mean $\pm$ 1 SD) minutes by Hemalet and 5.8 ± 1.4 (mean $\pm$ 1 SD) minutes by Simplate II, with good correlation between the results by the two devices ($r = 0.81$). The bleeding time in patients with various bleeding disorders were also comparably prolonged between the two devices. The new disposable bleeding time device with a disposable blade has quick release (penetration) into skin and retraction, and offers an alternative means of

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a bleeding time test. (Key words: Bleeding time; Hemostasis) *Am J Clin Pathol* 1986; 85: 610-613

THE BLEEDING TIME is a useful screening test for primary hemostasis, correlating well with both platelet number and function.³ The concept of measuring the bleeding time to evaluate the hemostatic effectiveness of platelets was introduced by Duke,² although the original method was not sufficiently sensitive or reliable for that purpose.

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