

Allergic reactions to the Australian paralysis tick, *Ixodes holocyclus*: diagnostic evaluation by skin test and radioimmunoassay

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Summary

Allergic reactions to *Ixodes holocyclus* are well recognized but poorly defined. Tick-bite reactions in 42 individuals in this study fell into six classes. Skin-prick tests and radioimmunoassay (RIA) indicated that all systemic hypersensitivity (class 3) and atypical reactions (class 4) were IgE-mediated. Some 73% of the large local reactions (class 2) and only 12.5% of the small local reactions (class 1) were associated with IgE specific for tick allergens. Subjects who reported heavy exposure to tick-bite were more likely to have positive RIA values ($P < 0.05$). There was an association between the individual's atopic status and tick allergy ($P > 0.05$).

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Introduction

The Australian paralysis tick, *Ixodes holocyclus* is widely distributed along the eastern coast of Australia. This arachnid requires a blood meal for its development from larval to nymph to adult stages as well as for egg formation by the gravid female [1]. Its principal hosts are the long-nosed or northern brown bandicoots, *Perameles nasuta* and *Isoodon macrourus* respectively. However, it also feeds on humans, domestic animals and livestock, and therefore constitutes a significant health and economic problem in tick-infested areas.

Tick-bite reactions include the effects of the paralyzing toxin from which it derives its common name [2–7], inflammatory and allergic reactions [8–11] and the transmission of rickettsial and other infections [12, 13].

Allergic reactions are well recognized but poorly defined because of small sample sizes [2–5, 8, 9, 12, 13] and, until recently, because of the lack of reliable diagnostic tests for tick allergy [14]. In this report, 42 volunteers with tick-bite reactions were assessed and the reactions classified into six classes based on clinical description, and into

allergic and non-allergic categories by means of a skin-prick test and radioimmunoassay (RIA) for IgE specific to the salivary gland extract (SGE) of *I. holocyclus* [14].

Subjects and methods

Subjects

A total of 42 volunteers was recruited from the south-east Queensland region following referral by their doctors or employers, or self-referral. Information on age, atopic status (familial pre-disposition for allergy, incidence of asthma, hay fever, eczema, rhinitis or other allergies), tick-exposure in relation to occupational, domestic or recreational proclivities, and full descriptions of tick-bite reactions were obtained. Ten millilitres of blood were drawn by venepuncture from each subject for RIA.

Preparation of tick allergens

Salivary glands were removed from unfed adult female *I. holocyclus*, homogenized in sterile 0.9% saline (20 glands/ml) at 4°C and centrifuged at $15\,000 \times g$. The supernatant was removed and the protein concentration of the SGE solution was estimated by the method of Bradford [15] and adjusted to 50 µg/ml. For skin testing, the allergen solution was sterilized by passage through 0.22-µm filters and UV irradiation. For RIA, SGE was

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prepared in a minimal volume of water and adjusted to 50 µg/ml final protein concentration with 0.1 M carbonate/bicarbonate buffer, pH 9.6 [16]. Aliquots of the bulk allergen solution were taken for individual skin tests, and potency was evaluated by measuring specific IgE binding by RIA.

Skin-prick test

All tests and categorizations were done blind without prior knowledge. A drop of SGE was placed on the forearm of each subject and pricked through once with a 27-gauge sterile needle. Control solutions included histamine (1 mg/ml) and sterile 0.9% saline. The induration was measured after 10 min on two axes and the average diameter was recorded. A reading of 2 mm or greater was taken as a positive reaction [14,17], 2 mm was used instead of 3 mm and 10 min instead of the standard 15 min, because of the greater reactivity induced by salivary gland extracts [14,16].

Radioimmunoassay (RIA)

Radioimmunoassay was performed as previously described [14]. Results were expressed as an RIA ratio.

$$\text{Ratio (\%)} = \frac{\text{Mean serum reading} \times 100}{\text{Pooled negative control reading}}$$

An RIA ratio of 400% or above was taken as a positive reading.

Results

Clinical classification

The clinical spectrum of tick-bite reactions of the 42 subjects fell into six classes that are summarized in Table 1. The first four classes were based on the stinging insect reaction categories described by Maguire & Geha [18]. No induration or weals were induced by saline control treatment and all positive reactions were very obvious with single tests.

Tick exposure and tick-bite reactions

The age, sex, tick exposure frequency and circumstances, type of tick-bite reaction, skin test and RIA reactivity of the 42 subjects are shown in Table 2.

Table 1. Summary of tick-bite reactions caused by the Australian paralysis tick, *Ixodes holocyclus*

Class	Tick-bite reactions	Clinical description
1	Small local (<i>n</i> = 8)	Painful, pruritic, erythematous and papular lesion at bite site (minutes to hours) persisting for up to 3 days. Itchy spot at site for several weeks.
2	Large local (<i>n</i> = 15)	Painful, erythematous lesion (minutes to hours) generally greater than 50 mm diameter and persisting for 1 week or more. Swelling and erythema contiguous with bite site. Some patients incapacitated by intense inflammation and pain.
3	Anaphylactic (<i>n</i> = 12)	Mild: urticaria, erythema and pruritis distant from bite site: not life-threatening. Severe: (<i>n</i> = 7): generalized urticaria*, angio-oedema*, laryngeal-oedema, bronchospasm (wheezing, coughing, dyspnoea) colic, hypotension (pallor, dizziness), collapse and loss of consciousness† within 1 hr after tick bite (usually following physical removal of tick): potentially life-threatening.
4	Atypical (<i>n</i> = 5)	Large local reaction as for class 2, followed by headache, nausea, lethargy and arthralgia 12–24 hr after removal of tick, persisting for 1 week or more.
5	Tick paralysis‡ (<i>n</i> = 1)	Neuromuscular paralysis, myocarditis and respiratory failure—2.5 year old boy; tick attached for 1 week; 12 hr after removal developed ataxic gait and widespread lower motor neuron weakness; ventilatory support required; complete recovery after tick paralysis antiserum administered.
6	Tick-borne infection‡ (<i>n</i> = 1)	Macular rash, lymphadenopathy, spiking fevers, arthralgia—42 year old man; symptoms persisted for 1 week.

n = Number of subjects.

* Four patients.

† Four patients had postural hypotension, two had dyspnoea and wheezing and one with respiratory difficulties and loss of consciousness.

‡ Not allergic in nature but patients were not specifically tested for allergies.

Table 2. Correlation between tick exposure and allergy to the Australian paralysis tick, *I. holocyclus*

Subjects	Age (years)	Sex	Exposure		Skin test (mm)	RIA ratio‡
			Frequency*	Circumstances†		
Class 1, small local reaction						
1	21	M	L	R	3	493
2	38	M	L	O	0	119
3	26	F	H	O	0	80
4	66	F	L	D	0	129
5	65	M	H	ODR	0	83
6	9	M	M	R	0	210
7	41	M	L	D	0	114
8	55	M	H	IDR	0	232
Class 2, large local reaction						
9	58	F	L	R	0	83
10	46	M	L	OD	0	85
11	53	F	H	OR	0	302
12	51	F	H	ODR	0	83
13	48	M	H	OR	6	2158
14	67	M	H	ODR	6	3997
15	76	F	H	DR	2	637
16	61	M	H	ODR	4	1118
17	61	M	H	ODR	2	781
18	63	M	H	OD	2	1245
19	35	M	H	ODR	3	3342
20	43	F	L	DR	2	771
21	62	F	H	ODR	3	9236
22	61	M	H	ODR	5	859
23	83	F	H	ODR	1	1264
Class 3, anaphylactic reaction						
24	31	F	H	ODR	3	4186
25	48	F	H	DR	1	749
26	66	M	H	ODR	5	16114
27	65	F	H	ODR	4	11076
28	58	M	H	ODR	0	1657
29	35	F	H	ODR	2	8395
30	51	F	H	ODR	4	3128
31	69	F	H	ODR	3	19365
32	59	M	L	D	3	9525
33	56	M	H	ODR	3	844
34	46	M	H	ODR	7	2142
35	49	F	H	R	6	3844
Class 4, atypical reaction						
36	50	F	H	ODR	2	3382
37	64	M	H	ODR	4	784
38	56	F	H	ODR	1	8883
39	63	F	M	ODR	5	1936
40	65	F	H	ODR	6	8411
Class 5, tick paralysis						
41	2	M	L	R	0	229
Class 6, tick-borne infection						
42	47	M	H	ODR	0	76

* Frequency of exposure was coded as light (L) if the subject had experienced between one and five tick bites; moderate (M, 6–10 tick bites) and heavy (H, > 10 tick bites).

† Circumstances of exposure were listed as occupational (O), domestic (D) or recreational (R).

‡ RIA ratio (%) = $\frac{\text{mean serum reading} \times 100}{\text{pooled negative control reading}}$.

There was a significant correlation between heavy exposure to tick bites and positive RIA (Chi-square = 6.61, d.f. = 2, $P < 0.05$).

Table 3. Sensitivity and specificity of skin-prick test and RIA for diagnosis of allergy to the Australian paralysis tick, *I. holocyclus*

Class	Syndrome	Total No.	Skin test, No. positive (%)	RIA No. positive (%)
1	Small local reaction	8	1 (12.5)	1 (12.5)
2	Large local reaction	15	10 (66.6)	11 (73.3)
3	Systemic hypersensitivity	12	10 (83.3)	12 (100)
4	Atypical reaction	5	4 (80)	5 (100)
5	Tick paralysis	1	0	0
6	Tick-borne infection	1	0	0

RIA vs Skin test

A comparison of the two diagnostic tests for tick allergy is presented in Table 3. The RIA was superior in terms of diagnostic accuracy; all 12 patients (100%) in the systemic hypersensitivity group were diagnosed as having tick allergy by RIA, compared with 83% by the skin-prick test.

Atopic status and tick allergy

Whether atopic individuals are more prone to develop tick allergy is not known. Of our 42 subjects, 15 were atopic while 27 were not; of 28 individuals allergic to ticks, 13 were atopic, and of 14 not allergic to ticks only two were atopic. If Yates' correction for continuity is not applied, Chi-square analysis shows an association between atopy and tick-allergy ($P < 0.05$) as indicated in a 2×2 contingency table.

		Atopic		Total
		+	-	
Allergic	+	13	15	28
	-	2	14	16
Total		15	27	42

Discussion

Allergic reactions to *I. holocyclus* have not been studied previously in a systematic manner. Current information is based on individual case reports and casual observations [2, 6, 8, 10, 12, 13]. In the present study, 42 subjects with alleged tick allergy were assessed in terms of clinical presentation, skin reactivity and the presence of specific IgE.

Clinical classification

The results showed that tick-bite reactions fell into six general categories (Table 1). Small local reactions were usually non-allergic in nature, while large local reactions were often associated with a positive skin test and RIA reactivity, and therefore included a significant proportion of tick-allergic patients. The results also confirmed clinical impressions that systemic or atypical reactions were associated with tick allergy. Non-allergic reactions included tick paralysis and tick-borne infection.

Tick exposure and tick-bite reactions

It can be seen (Table 2) that subjects who reported heavy exposure in all three activities (occupational, domestic or recreational) were more likely to experience class 2–4 tick-bite reactions, and were more likely to have positive RIA reactivity (Chi-square = 6.61, d.f. = 2, $P < 0.05$).

Clinical and laboratory diagnosis of tick allergy

From clinical experience, subjects with small local reactions are unlikely to have tick allergy. This was supported by negative skin-prick tests and RIA reactivity in seven of the eight subjects in this category. However, one of the eight subjects (No. 1, Table 2) had positive reactions to both the skin test and RIA, suggesting that he may be allergic to tick bite. Thus, diagnosis by history alone may result in a false-negative diagnosis. The converse interpretation, that skin tests and RIA may give false-negative results is less likely, but the issue cannot be settled until the patient concerned is challenged with another tick bite.

The allergic status of subjects with large local reactions was unclear. The results showed that four of the 15 subjects in this category had a negative skin test and RIA reactivity, another one had a negative skin test but positive RIA, while the remainder showed positive reactions to both tests (Table 2). Thus, two-thirds of subjects with large local reactions had positive test results.

In clinical practice, subjects with systemic hypersensitivity reactions are presumed to be allergic to ticks. It is therefore not surprising that all 12 individuals in this category had positive RIAs (Table 2). However, two of the 12 had a negative skin test reactivity, which suggests that the RIA may be more reliable than the skin test for diagnosis of tick-bite allergy, but the two tests are complementary.

The allergic state of subjects with atypical reactions was also unclear. Data showed that four out of five subjects had a positive skin test, and all five had positive RIAs (Table 2), which suggests that these reactions have an allergic association.

Patients with tick paralysis or tick-borne infection as

their only reaction to tick bite, are unlikely to be allergic and the results (Table 2) show negative reactions to both the skin test and RIA.

If we assume that the RIA is a reliable and reproducible test of tick allergy [14], it can be deduced that all patients with atypical reactions can be classified in the tick allergy category, compared with 73% of patients with large local reactions, and only 12% of patients with small local reactions (Table 3).

In terms of diagnostic efficacy, the RIA appeared to be superior to the skin-prick tests. Neither of the tests gave rise to false-positive results, but the skin-prick test had a false-negative rate of 33% for class 2, 26% for class 2 and 3, and 25% for class 2–4. In clinical practice both tests should be used together to improve the reliability for assessment of the first four categories of tick-bite reactions; namely small local, large local, systemic and atypical.

With regard to the large local reactions, it is not clear why patients fall into both allergic and non-allergic categories. The pathogenesis may involve delayed hypersensitivity or late-phase IgE responses in sensitized individuals [17], or the direct pharmacological activity of tick secretions or other arthropod salivary reactions in non-sensitized individuals [7, 19].

An explanation for the atypical reactions is also not readily forthcoming, but may involve immune-complex mechanisms. All subjects in this category have been sensitized to tick allergens, as shown by the positive RIA reactivities, but evidence of immune-complex mediation would require the demonstration of tick-specific IgG antibodies, as was shown with hymenopteran reactions [17].

An alternative explanation for atypical reactions is the possibility of a direct toxic effect of tick saliva. This is, however, less likely because the symptomatology does not resemble the known effect of the toxin which produces a syndrome of flaccid paralysis, myocarditis, hypothermia and respiratory failure at least 4–5 days after tick attachment [2–7]. Neither is it very likely to be associated with an infectious organism because the symptoms recur in a similar fashion with each tick bite and do not resemble the typical features of tick-borne infections [12–13].

Finally, it may be concluded that reactions in classes 2 and 3 (and possibly 4) are probably the Type I reaction of Coombs & Gell [20].

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