

Evaluation of partially purified salivary gland allergens from the Australian paralysis tick *Ixodes holocyclus* in diagnosis of allergy by RIA and skin prick test

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Two diagnostic assays that detect IgE specific to I. holocyclus were developed using purified sources of allergens. Salivary gland extract was superior to whole body extract in both the radioimmunoassay and the skin prick test. Of the partially purified salivary gland allergens, the 28-KD protein fraction gave the most promising results.

INTRODUCTION

The need to develop diagnostic tests to aid in the clinical diagnosis of allergy induced by the Australian paralysis tick, *Ixodes holocyclus* has been discussed elsewhere.¹ The tick occurs along the east coast of Australia wherever conditions are moist and humid² and may cause severe allergic responses in humans who have become sensitized.³⁻⁶ Two diagnostic tests to detect specific IgE, including a radioimmunoassay (RIA) and a skin prick test based on whole body extract (WBE) have been developed previously.¹ Whole body extract was used because it was not known whether the allergens occurred in the saliva or in the body of the tick. In a later study it was shown that the allergens were concentrated in the salivary glands,⁷ and probably released in the tick's saliva via the feeding lesion into the

host during early feeding. Therefore salivary gland extract (SGE) replaced WBE as an enriched source of allergens to coat microtitre plates used in the RIA for the detection of *I. holocyclus*-specific IgE in human serum.

The immunochemical and partial biochemical purification and isolation of five salivary gland allergens (SGA) have been reported in another study.⁷ It seemed that two common allergens, SGA1 and SGA2 of approximately 28 and 355 kD respectively were more likely candidates for *I. holocyclus*-induced allergy than SGA3, 4, and 5 (45, 50, and 55 kD, respectively) according to RIA and immunoblotting. Consequently, partially purified SGAs were incorporated into the RIA and skin prick test to improve the sensitivity of these assays. The results confirmed previous conclusions that SGE was superior to WBE in both diagnostic assays. Of the SGAs, SGA1 seemed the most promising candidate for IgE-binding activity.

MATERIALS AND METHODS

Recruitment of Volunteers

Eight allergic volunteers were selected who showed clinical effects such as generalized urticaria, angioedema, bronchospasm, hypoten-

sion, collapse and positive RIA values (data not shown). Four nonallergic adults were also included as controls. Blood was drawn by venipuncture from volunteers and sera prepared as outlined elsewhere.¹

Allergens

Whole body extract, SGE, and partially purified SGAs were prepared from unfed female *I. holocyclus* as described elsewhere.^{1,7} Salivary gland allergens 1 and 2 were combined in equimolar proportions as an additional allergen mixture. The final protein concentration of tick extracts was 50 µg/mL⁸ in 0.1 M carbonate buffer, pH 9.5 for RIA or normal saline for skin prick testing. Sterilization was by passage through a 0.22-µm filter and by UV irradiation according to standard procedures for virus inactivation.

Radioimmunoassay

A previously described method was used. Briefly, allergens were bound to flexible U-welled polyvinyl chloride microtitration plates and dilutions of sera in 1% bovine serum albumin in phosphate buffered saline applied to the wells. Following incubation for two hours at 37 °C and aspiration of serum, antihuman IgE iodinated with iodine 125 was added to each well and incubated for 18 hours at 4 °C. Radioactive uptake was expressed as counts per minute (cpm).

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Table 1. Allergic Activities of Whole Body Extract (WBE), Salivary Gland Extract (SGE), and Salivary Gland Allergens (SGA) in Skin Prick Testing* of Volunteers Undergoing Diagnosis for Allergic Reactions to the Australian Paralysis tick, *Ixodes holocyclus*

Volunteer	N/S†	HIST‡	WBE	SGE	SGA					Combined I and II
					I	II	III	IV	V	
Allergic										
AS	0 × 0	4 × 3	2 × 2	7 × 7	2 × 2	0 × 0	2 × 2	3 × 3	0 × 0	3 × 3
BJ	0 × 0	2 × 2	3 × 3	6 × 6	2 × 2	2 × 2	2 × 2	2 × 2	3 × 3	2 × 2
AY	0 × 0	2 × 2	1 × 1	4 × 4	1 × 1	1 × 1	1 × 1	2 × 2	1 × 1	1 × 1
RS	0 × 0	3 × 3	0 × 0	3 × 3	0 × 0	0 × 0	0 × 0	2 × 2	0 × 0	0 × 0
GS	0 × 0	3 × 3	2 × 2	6 × 6	4 × 4	6 × 6	2 × 2	0 × 0	4 × 4	6 × 6
GC	0 × 0	4 × 4	3 × 3	4 × 5	2 × 2	0 × 0	0 × 0	0 × 0	2 × 2	2 × 2
EM	0 × 0	3 × 3	0 × 0	3 × 3	0 × 0	0 × 0	2 × 3	0 × 0	0 × 0	0 × 0
BDr	0 × 0	2 × 2	0 × 0	6 × 6	3 × 2	0 × 0	0 × 0	0 × 0	0 × 0	3 × 3
Nonallergic										
MM	0 × 0	2 × 2	0 × 0	0 × 0	0 × 0	0 × 0	0 × 0	0 × 0	0 × 0	0 × 0
CG	0 × 0	3 × 3	0 × 0	0 × 0	0 × 0	0 × 0	0 × 0	0 × 0	0 × 0	0 × 0
TS	0 × 0	3 × 3	0 × 0	0 × 0	0 × 0	0 × 0	0 × 0	0 × 0	0 × 0	0 × 0
MB	0 × 0	3 × 3	0 × 0	0 × 0	0 × 0	0 × 0	0 × 0	0 × 0	0 × 0	0 × 0

* Induration in mm.

† Normal saline.

‡ Histamine (1 mg/mL).

Diagnostic Tests

Skin testing and RIA were performed as discussed elsewhere.¹ For skin testing, histamine (1 mg/mL) and normal saline were used as positive and negative controls, respectively. A skin prick test was scored as positive if the induration of both axes was 2 mm or greater, ten minutes after the prick.

Radioimmunoassay readings were considered positive if the ratio percent of allergic to nonallergic sera was 400% or greater, borderline if it was between 250% and 400%, and negative if it was below 250%.⁹

Ratio (%)

$$= \frac{\text{mean serum reading} \times 100}{\text{pooled negative control}}$$

RESULTS

Biologic Activity of Tick Extracts in Skin Prick Testing

Salivary gland extract gave positive results in all eight allergic volunteers whereas four false negative results occurred with WBE (Table 1). No false positive results occurred among control subjects with either extract. Of the partially purified allergens, SGA1 resulted in the highest number of positive tests. Combining SGA2 with SGA1 did not have an additive effect. Overall, SGE appeared to be a superior source of allergens for skin testing than SGA1.

Table 2. Comparison of RIA Ratios (%)* for Salivary Gland Extract (SGE) and in Salivary Gland Allergens (SGAs) in Radioimmunoassay of Volunteers Undergoing Diagnosis for Allergic Reactions to the Australian Paralysis tick, *Ixodes holocyclus*

Volunteer	SGE	SGA					Combined I and II
		I	II	III	IV	V	
AS	1415	1444	398	222	286	251	830
BJ	2158	865	344	229	241	81	319
GC	2585	4200	3349	111	1081	742	2812
GS	3997	1095	1216	402	430	362	654
AY	1600	802	856	422	359	225	1048
IC	2585	1267	1381	597	608	450	1048
CW	4313	1675	1831	754	810	600	2333
JS	1583	865	344	229	241	81	673
Cont†	100	100	100	100	100	100	100

* Ratio (%) = $\frac{\text{mean serum reading} \times 100}{\text{mean control reading}}$; Mean readings $\pm$ SE omitted to simplify the Table 1.

† Four nonallergic control adults provided sera which was pooled. Their ratio (%) necessarily became 100.

IgE-Binding Capacities of Tick Extracts on RIA

Salivary gland extract and SGA1 were good indicators of *I. holocyclus*-induced allergy and both gave strongly positive results with all eight allergic subjects (Table 2). Salivary gland extract gave higher values than SGA1 while SGA2 gave five positive and three near positive, borderline results. SGA3, 4, and 5 were poor indicators of tick allergy. Positive values were under 1000% except in one instance. SGA1 and 2 combined gave one borderline result and in six out of eight cases gave lower values than SGA1 alone.

DISCUSSION

In this report WBE, SGE, and partially purified SGAs were compared for biologic activities by skin testing, and SGE and SGAs were compared by RIA for IgE-binding capacities.

In both tests the best results were achieved when SGE was used as the antigen and this supports the previous observation that SGE is superior to WBE on RIA.⁷ In general RIA is a better diagnostic test than the skin test. For example, SGA1 gave positive results on all eight allergic subjects whereas three false negative results were achieved using the same allergen in skin prick testing. This finding confirms the conclusion that RIA is a more reliable assay for specific IgE to this allergen than the skin prick test.¹

Salivary gland allergens 1 and 2 are major allergens that bind specific IgE in the sera of most allergic individuals on Western blots⁷ and give the highest counts on RIA. They are believed to play an important role in tick allergy. The SGA1 band, however, was more intense than the SGA2 band.⁷ This suggests

that SGA1 may cause the production of more specific IgE than SGA2, which may account for the lower skin reactivity of SGA2.

Salivary gland extract was superior to SGA1 on both RIA and skin test; however, immunoblots showed that, whereas SGA1 was immunologically pure, SGE contained many minor allergens including SGAs 3, 4, and 5.⁷ Most of these minor allergens showed low IgE-binding capacities on RIA. It is suggested that the purification process reduced the activities of the important allergens. This is not unexpected as the partially purified allergens were subjected to possible denaturation by heating at 95 °C and dissociation by sodium dodecyl sulphate into their constituent polypeptide chains. It is possible that during this process, the structures of the allergens were modified so as to render the allergenic determinants less accessible for IgE binding in RIA or skin tests. There is an extensive literature that discusses the effects of heat and sodium dodecyl sulphate on antigenicity which would support this conclusion.¹⁰⁻¹⁴

Research on insect bite allergies^{9,15} has provided many parallel instances to this necessarily preliminary study of tick allergies. Of particular interest has been the analogous effect of the blood-sucking bug *Triatoma protracta*.^{16,17} Studies have included the identification and partial purification of SGAs¹⁶ and successful immunotherapy for anaphylaxis induced by this bug.¹⁷

In summary, we have presented data on the superiority of SGE to WBE in the diagnostic assessment of tick allergy. The incorporation of the partially-purified SGA1, which appears to play an important role in *I. holocyclus*-induced allergy, to further improve the specificities of the diagnostic tests looks promising; however, a method of isolating

SGA1 without denaturation is required. Such studies would also be necessary if partially or wholly purified SGA1 is used for specific immunotherapy.

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