

31435

## Detection in Allergic Individuals of IgE Specific for the Australian Paralysis Tick, *Ixodes holocyclus*

M. Gauci<sup>a</sup>, B.F. Stone<sup>a</sup>, Y.H. Thong<sup>b</sup>

<sup>a</sup> CSIRO, Division of Tropical Animal Science, Long Pocket Laboratories, Indooroopilly, Qld;

<sup>b</sup> Immunobiology Laboratory, University Department of Child Health, Mater Children's Hospital, South Brisbane, Qld, Australia

**Abstract.** Three techniques were developed to detect IgE specific to *Ixodes holocyclus*. The radioimmunoassay (RIA) was found to be superior to both radioallergosorbent test and enzyme immunoassay techniques. Validation of the RIA technique was provided by dilution experiments and by a blind study involving 13 volunteers; the RIA graph was linear over a wide range of concentrations. The RIA ratio clearly discriminated between those volunteers with and without systemic allergic reactions and helped to identify borderline cases showing local or cutaneous allergic reactions. It appears to be suitable for diagnostic and research applications.

### Introduction

The Australian paralysis tick, *Ixodes holocyclus*, has been recorded from the eastern coast of Australia from Normanton in north Queensland to Lakes Entrance in Victoria and affects humans as well as domestic pets and livestock [1]. In humans, *I. holocyclus* has caused neuromuscular paralysis [2-5], transmitted rickettsial diseases [6, 7] and induced allergic reactions. Severity of allergic responses in humans ranges from mild irritation to life-threatening anaphylaxis [8-11]. Workers in forestry reserves and in national parks as well as campers and inhabitants of tick-infested areas may be put at risk by *I. holocyclus* attachment. An assay detecting and semi-quantitating tick-specific IgE could be used to identify residents and workers in rural and forest areas who are susceptible to allergy and may prevent potentially lethal responses. Allergic individuals thus identified could either avoid tick-infested areas, undergo desensitisation therapy or take precautionary measures before entering such areas.

Three in vitro assays were developed to detect specific IgE in the sera of allergic individuals. These assays were radioimmunoassay (RIA), radioallergosorbent test (RAST) and enzyme immunoassay (EIA). Results of RIA and skin tests were compared in a 'blind' study designed to investigate the relationships

between antibody titres, skin prick tests, exposure to ticks and allergic symptoms in reactive subjects. Currently, a skin prick test is the only method available to detect allergy to ticks.

### Materials and Methods

#### Preparation of Tick Allergens

Unfed female *I. holocyclus* from northern New South Wales were ground in a pool of liquid nitrogen into a coarse 'powder' of frozen fragments in a mortar and pestle and held at -80°C until required. Unfed females were used because allergies are usually detected shortly after the commencement of feeding. Whole-body extract was used in this preliminary study to ensure that the total range of salivary gland and body allergens would be included in the allergen 'cocktail' and because the commercial desensitising preparation was made this way. The 'powder' was suspended in a small volume of 0.1 M carbonate buffer, pH 9.6, and diluted with the same buffer for RIA, RAST and EIA or with saline for skin prick testing. Protein content was determined by the method of Bradford [12]. All allergen concentrations were expressed as µg protein/ml.

#### Sera

Sera were prepared from 50 volunteers. Some had been exposed to *I. holocyclus* and exhibited allergic symptoms, some showed no symptoms and some had never been exposed.

#### Detection of Specific IgE

Three methods of detecting *I. holocyclus*-specific IgE were attempted: EIA, RAST and RIA.

**Table I.** Comparison of three methods for the detection of specific IgE to *I. holocyclus*

| Volunteer      | EIA               |          | RAST        |          | RIA         |          |
|----------------|-------------------|----------|-------------|----------|-------------|----------|
|                | OD <sub>405</sub> | ratio, % | cpm         | ratio, % | cpm         | ratio, % |
| P <sub>1</sub> | 0.700 ± 0.016     | 142      | 6,417 ± 674 | 2,153    | 7,147 ± 238 | 5,179    |
| P <sub>2</sub> | 0.760 ± 0.028     | 154      | 8,078 ± 272 | 2,710    | 7,853 ± 509 | 5,691    |
| N <sub>1</sub> | 0.492 ± 0.014     | 100      | 298 ± 38    | 100      | 138 ± 6     | 100      |

Results expressed in counts per minute (cpm) and as a ratio. Each value is the mean of 3 readings ± SE. P<sub>1</sub>, P<sub>2</sub> are volunteers with allergic symptoms, exposed to *I. holocyclus*. N<sub>1</sub>, N<sub>2</sub> are volunteers who had not been exposed to *I. holocyclus*.

For EIA [13], flat-well polystyrene microtitration plates (Dynatech, Plodringen, FRG) were incubated with 100 µl of 50-µg/ml allergen solution for 3 h at 37°C. Unbound sites on the solid phase were blocked with 1% horse serum in phosphate-buffered saline (PBS), pH 7.4, for 1 h at 37°C, with 200 µl/well. Plates were washed with 0.05% Tween 20 in PBS, then 100 µl of serum was added diluted one-half in 1% horse serum. 'Positive' sera were from volunteers with reactions to *I. holocyclus*, 'negative' sera were from volunteers with no reactions. Goat anti-human IgE (5 µg/ml; Bio-Rad, Richmond, Calif., USA), then rabbit anti-goat IgG [10 µg/ml; Commonwealth Serum Laboratories (CSL), Melbourne, Australia] followed by goat anti-rabbit IgG-horseradish peroxidase conjugate (CSL; 5 µg/ml in 1% horse serum) were added and incubated for 2 h at 37°C. All volumes were 100 µl. The substrate added was 2,2-azino-di-3 ethylbenzthiazoline sulphonic acid, 0.55 mg/ml in phosphate citrate buffer, pH 4.3, containing 0.002% H<sub>2</sub>O<sub>2</sub>. Absorbance was read at 405 nm.

RAST was performed by a modification of the method of Walsh et al. [14]. Nitrocellulose discs of 5 mm diameter were prepared with an office punch and immersed in 100 µl of a 50-µg/ml allergen solution for 3 h in a polystyrene microtitration plate, 1 disc per well. Discs were air-dried, then placed in another plate containing 200 µl blocking solution [3% bovine serum albumin (BSA) in PBS] for 1 h at 37°C. The blocking solution was aspirated off and replaced with 'positive' or 'negative' sera diluted in 3% BSA and incubated 2 h at 37°C after washing with 0.05% Tween 20. Anti-human IgE iodinated with <sup>125</sup>I (Pharmacia, Uppsala, Sweden) was added to each well containing a disc, 25 µl/well, and incubated for 18 h at 4°C. Radioactive uptake was expressed as counts per minute.

RIA [15] was carried out in a similar manner to RAST except that allergen was bound directly onto a flexible U-welled polyvinyl chloride plate (Flow Laboratories) and dilutions were in 1% BSA in PBS.

#### Calculations

EIA, RAST and RIA ratios were determined by the method of Homburger and Jacob [16] and results were classified by their method as negative (< 250% of control), borderline (250–400% of control) or positive ratio (> 400% of control), where

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

#### Blind Study

Sera were prepared from 13 volunteers participating in a blind study. Their histories of tick allergy, including the frequency of exposure to *I. holocyclus* and the occurrence of local or systemic reactions were recorded. They were ranked in order of exposure to tick bite – heavy, moderate, light or none – and the presence or absence of local and systemic allergic reactions. Coded serum samples were assayed by RIA for specific IgE reaction to *I. holocyclus* and by the IgE RIA 100 kit (Pharmacia) for total IgE. Pooled sera from three known non-allergic volunteers were used as the negative control. Skin tests were performed on all 13 volunteers using allergen extract prepared in normal saline, sterilised by passage through a 0.22-µm filter (Millipore, Bedford, Mass., USA) and irradiated with UV light according to standard procedure for virus inactivation. An induration of 3 mm was used as the cut-off in accordance with the study of Squire et al. [15].

#### Statistical Analyses

The RAST, RIA and EIA data were transformed by log, double-square root and no transformation, respectively, and differences were tested by analysis of variance.

## Results

#### Comparison of EIA, RAST and RIA

The RAST and RIA assays clearly distinguished between 'positive' and 'negative' sera ( $p < 0.05$ ), whereas the EIA did not (table I). The EIA also showed more non-specific binding than RAST or RIA.

With the RIA, dilution experiments using known 'positive' sera showed that after log transformation the binding curves were linear over a wide range of antibody concentrations (table II). 'Negative' sera did not show linear binding curves.

#### Blind Study

All three subjects with heavy exposure and systemic reactions to tick bites had an RIA ratio of 400

**Table II.** The effect of serial dilution of serum on *I. holocyclus*-specific IgE levels of exposed and unexposed volunteers using RIA

| Volunteer      | Serum dilution |              |             |              |            |              |          |              |
|----------------|----------------|--------------|-------------|--------------|------------|--------------|----------|--------------|
|                | 1:5            |              | 1:25        |              | 1:125      |              | 1:625    |              |
|                | cpm            | RIA ratio, % | cpm         | RIA ratio, % | cpm        | RIA ratio, % | cpm      | RIA ratio, % |
| P <sub>1</sub> | 4,722 ± 21     | 3,524        | 2,219 ± 53  | 1,656        | 1,068 ± 25 | 797          | 283 ± 11 | 211          |
| P <sub>2</sub> | 6,293 ± 39     | 4,696        | 4,224 ± 127 | 3,152        | 1,777 ± 93 | 1,326        | 698 ± 50 | 521          |
| N <sub>1</sub> | 389 ± 42       | 290          | 175 ± 30    | 131          | 156 ± 10   | 116          | 151 ± 20 | 113          |
| N <sub>2</sub> | 144 ± 12       | 107          | 218 ± 14    | 163          | 121 ± 5    | 90           | 112 ± 6  | 84           |

For explanations, see table I.

**Table III.** Blind study comparing histories, skin test, specific IgE and total IgE to *I. holocyclus* in 13 volunteers

| Volunteer No. | History  |           |          | Skin test mm | Specific IgE cPM | RIA ratio % | Total IgE kU/l |
|---------------|----------|-----------|----------|--------------|------------------|-------------|----------------|
|               | exposure | reactions |          |              |                  |             |                |
|               |          | local     | systemic |              |                  |             |                |
| 13            | heavy    | +         | +        | 4            | 3,919            | 2,925       | > 1,000        |
| 11            | heavy    | +         | +        | 7            | 746              | 557         | 129            |
| 4             | heavy    | +         | +        | 3            | 3,298            | 2,461       | 552            |
| 12            | heavy    | +         | —        | 5            | 392              | 293         | 40             |
| 10            | moderate | +         | —        | 0            | 228              | 170         | 23             |
| 1             | light    | —         | —        | 2            | 282              | 210         | 85             |
| 2             | light    | —         | —        | 2            | 204              | 152         | 16             |
| 3             | light    | +         | —        | 2            | 415              | 310         | 38             |
| 7             | light    | —         | —        | 2            | 233              | 170         | 301            |
| 5             | none     | —         | —        | 0            | 212              | 160         | 70             |
| 6             | none     | —         | —        | 0            | 233              | 174         | 148            |
| 8             | none     | —         | —        | 0            | 309              | 234         | 34             |
| 9             | none     | —         | —        | 1            | 226              | 169         | 35             |

Exposure: heavy = > 10 ticks; moderate = 6–10 ticks; light = 1–5 ticks. Allergic response local = oedema, erythema and pruritis at the site of tick attachment; systemic = generalised urticaria, angioedema, hypotension and bronchospasm. Skin test: the diameter of the largest induration is recorded. Specific IgE determined by RIA.

or greater, and all 10 subjects without vascular or respiratory reactions had an RIA ratio of less than 400. Two subjects with both local and/or cutaneous reactions and skin test readings of 2 and 5 mm, respectively, had an RIA between 250 and 400 (table III).

## Discussion

Of the three techniques developed in our laboratory for the detection of specific IgE to allergens of

the Australian paralysis tick *I. holocyclus*, the RIA was preferred to RAST because it was simpler and quicker to perform and preferred to EIA because of greater sensitivity (see Results).

Validation of the RIA technique was achieved by dilution experiments which showed proportionate reduction in titre with sequential dilutions of sera. Conclusive validation was achieved by a blind study involving 13 volunteers of which three had systemic vascular/respiratory reactions and 10 had none. Homburger and Jacob [16] advocated a borderline

category and attributed their very low frequency of frank misclassifications to the use of a scheme that incorporated this category. They also demonstrated minimal false borderline results by using a range of ratios of 250–400%. A similar approach was adopted in the present investigation and the borderline category accommodated a possible false-positive response to the skin prick test (table III; volunteer 12) who has, at times, suffered moderately large local reactions and some possible cutaneous systemic reactions. Thus, the cut-off point of 400% for the RIA ratio clearly distinguishes systemic allergic from non-systemic allergic subjects and from borderline subjects. This cut-off point of 400% accords with the recommendations of Homburger and Jacob [16] for tests of specific IgE to Hymenoptera (wasp, hornet and bee) venoms and other allergens.

In comparison, the skin prick test was less discriminating. Possibly because of the presence of vasoactive and chemotactic substances and irritants in tick preparations which can produce non-specific reactions [17], one individual without a history of systemic allergic reactions had a positive prick test (table III). Although these cutaneous reactions may be due to the direct action of tick oral secretions at the site of attachment, they may also be later phase type I and thus IgE-mediated or type IV cell-mediated, as discussed by Schmidt [18] in relation to Hymenoptera cutaneous reactions.

These results indicate that the RIA technique is a sensitive and reliable assay for use in the diagnosis of tick allergy. No false-negatives occurred with this method. However, skin prick tests would be useful for confirmation, particularly of borderline cases. The RIA should also prove useful for selecting positive sera required for the immunopurification of allergens.

### Acknowledgements

We would like to thank Drs Sullivan, Nicolaides and Partners, pathologists, for blood collection and sera preparation. We gratefully acknowledge the assistance of the staff of the Mater Misericordiae Hospital, Brisbane, for the preparation of pooled fetal cord sera and determination of total IgE, and Mr John Kerr of CSIRO, Long Pocket Laboratories, for performing statistical analyses. This research was supported by a grant to CSIRO from the National Health and Medical Research Council, Australia, and by a grant to the University of Queensland from the Mayne Bequest Fund.

### References

- 1 Roberts, F.H.S.: Australian ticks (CSIRO, Melbourne 1970).
- 2 Ferguson, E.W.: Deaths from tick paralysis in human beings. *Med. J. Aust. ii*: 346–348 (1924).
- 3 Hamilton, D.G.: Tick paralysis: a dangerous disease in children. *Med. J. Aust. i*: 759–765 (1940).
- 4 Pearn, J.H.: A case of tick paralysis with myocarditis. *Med. J. Aust. i*: 629–630 (1966).
- 5 Pearn, J.H.: The clinical features of tick bites. *Med. J. Aust. ii*: 313–318 (1977).
- 6 Andrew, R.; Bonnin, J.M.; Williams, S.: Tick typhus in north Queensland. *Med. J. Aust. ii*: 253–258 (1946).
- 7 Domrow, R.; Derrick, E.H.: *Ixodes holocyclus*, the man-biting tick in S.E. Queensland. *Aust. J. Sci.* 27: 234–235 (1965).
- 8 Trinca, J.C.: Insect allergy in Australia: results of a five year survey. *Med. J. Aust. ii*: 659–663 (1964).
- 9 Banfield, J.F.: Tick bites in man. *Med. J. Aust. ii*: 600–601 (1966).
- 10 Sutherst, R.W.; Moorhouse, D.E.: *Ixodes holocyclus* larvae and 'scrub-itch' in southeast Queensland. *Southeast Asian trop. Med. publ. Hlth* 2: 82–83 (1971).
- 11 Bagnall, B.: Cutaneous immunity to the tick *Ixodes holocyclus*; PhD thesis, University of Sydney (1975).
- 12 Bradford, M.M.: A rapid and sensitive method for the quantitation of microgram quantities of protein utilising the principle of protein-dye binding. *Analyt. Biochem.* 72: 248–254 (1976).
- 13 Wisdom, B.G.: Enzyme-immunoassay. *Clin. Chem.* 22: 1243–1255 (1976).
- 14 Walsh, B.J.; Wrigley, C.W.; Musk, A.W.; Baldo, B.A.: A comparison of the binding of IgE in the sera of patients with bakers asthma to soluble and insoluble wheat grain proteins. *J. Allergy clin. Immunol.* 76: 23–28 (1985).
- 15 Squire, E.N., Jr.; McBride, D.C.; Ledoux, R.; Nelson, H.S.: Assessment of IgE-mediated sensitivity during immunotherapy: comparison of the RAST with and without adsorption of IgG to titrated prick skin tests. *Ann. Allergy* 56: 22–27 (1986).
- 16 Homburger, H.A.; Jacob, G.L.: Analytic accuracy of specific immunoglobulin E antibody results determined by a blind proficiency survey. *J. Allergy clin. Immunol.* 70: 474–480 (1982).
- 17 Stone, B.F.; Doube, B.M.; Binnington, K.C.; Goodger, B.V.: Toxins of the Australian paralysis tick *Ixodes holocyclus*; in Rodriquez, Recent advances in acarology, vol. 1, pp. 347–356 (Academic Press, New York 1979).
- 18 Schmidt, J.O.: Allergy to Hymenoptera venoms; in Piek, Venoms of the Hymenoptera, pp. 509–546 (Academic Press, London 1986).

Received: June 20, 1987

Correspondence to: Dr. B.F. Stone  
CSIRO, Division of Tropical Animal Science  
Long Pocket Laboratories  
Private Bag No. 3  
Indooroopilly, Qld 4068 (Australia)