

Allergy to snake venom: the use of radioimmunoassay for the detection of IgE antibodies against antigens not suitable for cutaneous tests

JOSEPH F. KELLY *and* ROY PATTERSON

Section of Allergy and Immunology, Department of Medicine, Northwestern University Medical School, Chicago, Illinois

Summary

A case of a severe immediate-type respiratory reaction to rattlesnake venom is reported. Reaginic antibody to the snake venom was demonstrated in the patient's serum by long latency passive cutaneous anaphylaxis in a monkey. IgE antibody against the venom was demonstrated in the patient's serum by a solid phase radioimmunoassay.

This report confirms the immediate-type respiratory reactions that have been observed by others. The study also confirms that these reactions are associated with reaginic antibodies against the venom and that these antibodies are probably of the IgE class. It thus appears most likely that this patient's reaction was due to IgE reaginic antibody.

In vitro assays for specific IgE activity may be particularly valuable in cases where antigen may be toxic for skin testing.

Introduction

Anaphylaxis and immediate-type respiratory reactions to snake venom have been reported previously (Parrish, Watt & Arnold, 1957; Ellis & Smith, 1965; Mendes, 1960; Stanic, 1956; Parrish, 1957). Skin testing with snake venom to demonstrate specific reaginic antibody has been complicated by local haemorrhagic (Peck, 1933; Golan & Hill, 1940) and non-specific weal and erythema responses (Parrish *et al.*, 1957; Parrish, 1957; Peck, 1933; Golan & Hill, 1940) in both normals and venom sensitive individuals, presumably caused by the enzymatic and toxic activity of these agents. The following is a case report of an IgE mediated response to snake venom. An *in vitro* solid phase radioimmunoassay (RIA) was used to demonstrate the presence of IgE antibodies against the snake venom.

Case report

The patient is a 32-year-old white male chemistry student who, while pouring powdered rattlesnake venom (*Crotalus adamanteus*) into a beaker, had the acute onset of nasal

Correspondence: Dr Roy Patterson, Section of Allergy and Immunology, 303 E. Chicago Avenue, Chicago, Illinois, 60611, U.S.A.

congestion, sneezing, ocular itching, severe cough, wheezing and chest tightness associated with a faint feeling. The symptoms decreased over the next hour although mild wheezing persisted for 2 hr. Nasal congestion persisted for 3 days and prompted the patient to seek medical attention. The patient was first exposed to snake venom 5 years prior to evaluation. The venom was used in the laboratory as a source of various enzymes. The usual exposure was 5 min every 2–3 months while weighing out the venom and preparing solutions. Two years before evaluation the patient had first noted mild rhinitis upon exposure to the powdered venom and since then has been symptomatic with each exposure. The patient never was bitten by a snake and had no personal history of atopic disease, although a brother had asthma. The physical examination was normal except for pallor and oedema of the nasal mucosa. An oral antihistamine was prescribed, and the patient was observed. All symptoms abated in 48 hr. The patient was not skin tested with the venom because of its inherent toxicity and the possible occurrence of anaphylaxis with an antigen that is not used in common clinical diagnostic procedures.

Materials and Methods

Specific IgE binding of snake venom by the patient's serum was detected by an *in vitro*, solid phase radioimmunoassay. This assay was based on methods previously described (Salmon, Mackey & Fudenberg, 1969) and has been used to demonstrate IgE antibodies against insulin (Patterson, Mellies & Roberts, 1973) and parathyroid hormone (O'Rourke, Booth & Patterson, 1973) and to quantitate IgE antibodies against ragweed antigen E (Zeiss *et al.*, 1973). The assay has been adapted to detect IgE antibodies against snake venom.

The following materials were sequentially added to polystyrene tubes with incubation and washing between each step: (1) 2.0 ml of *Crotalus adamanteus* venom (Sigma Chemical Co., Lot No. 42C-3630) containing 2.0 mg/ml, (2) 1.0 ml of 5% human serum albumen, (3) 1.0 ml of test serum, (4) 1.0 ml of rabbit anti-human IgE (Patterson, Talbot & Roberts, 1972), and (5) 1.0 ml of ^{125}I -IgE containing 7 units/ml. After washing, the counts per minute (cpm) bound to the tube were determined. Specificity of IgE antibody for the snake venom was demonstrated by specific inhibition of IgE binding after preincubation of the serum with snake venom and appropriate negative results with normal human serum. The specificity of the radioimmunoassay (RIA) for IgE antibodies was shown by inhibition of the RIA after the addition of excess unlabelled IgE (8000 units) at step 4.

Passive transfer of reaginic activity was demonstrated by a long latency passive cutaneous anaphylaxis (PCA) in a monkey (Patterson, 1969). One-tenth ml of serial two-fold dilutions of control and patient sera were implanted intradermally in a monkey (*Macaca mulatta*). Forty-eight hours later, after the administration of sodium pentobarbital anaesthesia and Evans blue dye, the test sites were challenged with 1.0 ng of the venom. Definite blueing at the test site within 30 min is considered a positive response. Normal monkey skin challenged with 1.0 ng and 10 ng of the venom caused no blueing.

Results

The PCA was positive in the monkey at 1:16 dilution of the patient's serum when 1.0 ng of venom was used as the challenge dose. This titre demonstrates the transfer of significant specific reaginic activity to the snake venom. The control serum sites were negative when challenged with the venom.

The results of the RIA are shown in Table 1. The RIA with the patient's serum gave 10 times more counts per minute than the RIA with serum from a control who had had no previous exposure to snake venom. Marked inhibition of binding occurred when the patient's serum was incubated with venom before the RIA and demonstrates the specificity for the venom. The inhibition of the assay by addition of excess unlabelled IgE demonstrates that the radioactivity bound was radioactive IgE.

Discussion

The ability of *in vitro* immunologic assays to demonstrate specific IgE activity has been documented (Zeiss *et al.*, 1973; Wide, Bennich & Johansson, 1967; Coombs *et al.*, 1968; Ishizaka, Ishizaka & Hornbrook, 1967), although the exact role that these assays will play in clinical practice has not been defined. The skin test remains a highly sensitive, inexpensive and rapid procedure for evaluation of IgE mediated reactions. Passive transfer studies in humans carry the significant risk of hepatitis whereas studies in subhuman primates lack the sensitivity of human studies, are expensive and are inconvenient.

Table 1. *In vitro* demonstration of IgE antibody against snake venom by a solid phase radioimmunoassay. The venom bound to polystyrene is reacted sequentially in a 'sandwich' technique with test serum, anti-IgE and ^{125}I -IgE

Assay	Radioactivity bound* (cpm)
Patient serum	4640
Patient serum + preincubation with venom†	217
Patient serum + unlabelled IgE‡	50

* Recorded as counts per minute (cpm) of ^{125}I -IgE bound minus cpm bound by control human serum (457 cpm).

† Inhibition of binding by preincubation of patient's serum with 18 μg venom.

‡ Inhibition of binding by addition of excess IgE.

One potential value of *in vitro* assays for IgE antibodies is demonstrated in this patient. The clinical history and the reaginic activity of the patient's serum suggest that the patient presented here had an IgE mediated response to venom exposure. As noted, direct skin testing did not seem warranted because of difficulty in interpretation, inherent toxicity of the venom, and possibility of anaphylaxis in a highly sensitive patient. Passive transfer to either human or sub-human primates does not remove the problem of inherent local or systemic toxicity of antigen or the difficulty in interpretation. In this study, care was used in limiting the total dose of venom given to the monkey, and was only done to confirm the *in vitro* assay. The solid phase assay demonstrated IgE activity specific for the venom and eliminated the need (and risks) of direct skin testing.

Exposure to snakes is not common, but venoms are now commonly used in biochemical, diagnostic and therapeutic laboratory studies. Thus, the incidence of allergic reactions to snake venoms may increase. The use of *in vitro* assays for specific IgE activity may provide a means of evaluating suspected allergic reactions and may serve as a screening procedure in high risk groups.

Acknowledgments

Supported by U.S.P.H.S. Training Grant 5TOI A1-00057 from the National Institute of Allergy and Infectious Diseases and the Ernest S. Bazley Grant to Northwestern Memorial Hospital.

References

- COOMBS, R.R.A., HUNGER, A., JONAS, W.E., BENNICH, H., JOHANSSON, S.G.O. & PANZANI, R. (1968) Detection of IgE (IgND) specific antibody (probably reagin) to castorbean allergen by the red-cell-linked antigen-antiglobulin reaction. *Lancet*, **i**, 1115.
- ELLIS, E.T. & SMITH, F. (1965) Systemic anaphylaxis after rattlesnake bite. *Journal of the American Medical Association*, **193**, 401.
- GOLAN, H.G. & HILL, R. (1940) Snake venom and allergy. *Journal of Allergy*, **12**, 11.
- ISHIZAKA, K., ISHIZAKA, T. & HORN BROOK, M.M. (1967) Allergen binding activity of γ E, γ G and γ A antibodies in sera from atopic patients: *in vitro* measurement of reaginic antibody. *Journal of Immunology*, **98**, 490.
- MENDES, E. (1960) Allergy to snake venom. *Journal of Allergy*, **31**, 68.
- O'ROURKE, J.N., BOOTH, B.H. & PATTERSON, R. (1973) An anaphylactic reaction to parathyroid hormone. *Journal of Allergy and Clinical Immunology*, (in press).
- PARRISH, H.M. (1957) Survey of human allergy to North American snake venoms. *Arizona Medicine*, **14**, 461.
- PARRISH, H.M., WATT, H.F. & ARNOLD, J.D. (1957) Human allergy resulting from North American snake venoms. *Journal of the Florida Medical Association*, **43**, 1116.
- PATTERSON, R. (1969) Laboratory models of reaginic allergy. *Progress in Allergy*, **13**, 332.
- PATTERSON, R., MELLIES, C.J. & ROBERTS, M. (1973) Immunologic reactions against insulin. II. IgE anti-insulin, insulin allergy and combined IgE and IgG immunologic insulin resistance. *Journal of Immunology*, **110**, (in press).
- PATTERSON, R., TALBOT, C. & ROBERTS, M. (1972) Reverse passive respiratory reactions to anti-IgE in rhesus monkeys. *Clinical and Experimental Immunology*, **10**, 267.
- PECK, S.M. (1933) Sensitization and desensitization in man with snake venom (*Ancistrodon piscivorus*). *Archives of Dermatology and Syphilology*, **27**, 312.
- SALMON, S.E., MACKAY, G. & FUDENBERG, H.H. (1969) "Sandwich" solid phase radioimmunoassay for the quantitative determination of human immunoglobulins. *Journal of Immunology*, **103**, 129.
- STANIC, M. (1956) Allergenic properties of venom hypersensitiveness in man and animals. In: *Venoms* (Ed. by E. Buckley and N. Pargès) American Association for the Advancement of Science, Washington.
- WIDE, L., BENNICH, H. & JOHANSSON, S.G.O. (1967) Diagnosis of allergy by an *in vitro* test for allergen antibodies. *Lancet*, **ii**, 1105.
- ZEISS, C.R., PRUZANSKY, J.J., PATTERSON, R. & ROBERTS, M. (1973) A solid phase radioimmunoassay for the quantitation of human reaginic antibody against ragweed antigen E. *Journal of Immunology*, **110**, 414.

Copyright of Clinical Allergy: Journal of the British Allergy Society is the property of Blackwell Publishing Limited and its content may not be copied or emailed to multiple sites or posted to a listserv without the copyright holder's express written permission. However, users may print, download, or email articles for individual use.