

An anomalous effect of allergen concentration on weal size elicited by skin testing for immediate hypersensitivity

K. G. HUGGINS and J. BROSTOFF

Department of Immunology, Middlesex Hospital Medical School, London

(Received 27 June 1978; accepted for publication 8 August 1978)

Summary

We report the finding of anomalous responses in the skin of atopic individuals when prick tested with serial dilutions of cocksfoot grass pollen extracts. Each pollen extract was standardized using RAST inhibition activity related to the WHO standard for IgE. 75% of the patients gave a larger weal area with an allergen concentration of $1:10^5$ than with the next higher concentration, i.e. $1:10^4$.

Possible mechanisms whereby a larger skin weal is elicited with a lower concentration of allergen are discussed. This is not a rare finding and can be observed in patients undergoing routine skin testing in the clinic, even where extracts are diluted over a relatively narrow concentration range.

Introduction

Skin testing is used as a means of diagnosing atopy and more recently has been applied to the estimation of the potency of allergen extracts (Pepys *et al.*, 1975; Aas & Belin, 1972). By testing the skin response to serial dilutions of an allergen extract and observing the concentration which produces a desired weal size or which fails to give a response (end-point titration), quantitative data is obtained regarding the activity of the extract (Gleich *et al.*, 1974). Such titration methods appear to provide a convenient means of assessing allergen extracts and are being widely used. It is therefore with surprise that we report the finding of anomalous responses on skin testing with serial dilutions of pollen allergen.

A series of twelve hay fever patients were skin tested with six ten-fold dilutions of five different cocksfoot pollen extracts. Nearly 75% of these patients gave larger weals to a dilution of $1:10^5$ of the extracts than to the higher corresponding extract concentration.

Although similar findings have been reported by Rinkel (1949) they appear to have been generally ignored. In an attempt to substantiate the original observations

Correspondence: Dr K. G. Huggins, Miles Laboratories Limited, Stoke Court, Stoke Poges, Slough, Buckinghamshire.

and extend them, we have carefully conducted a study using RAST standardized allergen extracts (Huggins *et al.*, 1978).

Patients, materials and methods

Patients

Twenty-two patients presenting with a history of hay fever and found to give positive skin reactions to grass pollen allergens were prick tested with six ten-fold serial dilutions of cocksfoot (*Dactylis glomerata*) pollen extracts.

The first group of eleven hay fever patients were skin tested with four extracts (Nos 1 to 4 inclusive) in a series of dilutions; the second group of eleven patients being tested with a further two extracts (Nos 5 and 6), similarly diluted.

Standardization of pollen extracts

The six extracts were examined by standardized RAST inhibition employing the procedure of Huggins *et al.* (1978). In brief, the activity of an extract is expressed in terms of its ability to inhibit the binding of a specified weight of allergen-specific IgE antibody; this weight being directly related to the WHO standard for IgE.

The extracts were serially diluted in 50% glycerol-saline over eight ten-fold dilution ranges and stored at 4°C before use. In order to avoid 'carry-over' all dilutions were made by using a fresh, clean pipette at each transfer stage. An identical technique was used in diluting a solution containing a radioactive compound and a linear dilution pattern was obtained.

Skin tests

The diluted extracts were randomly allocated a code number and tested under blind conditions. Skin testing was performed by the prick method on the volar surface of the forearm. In three patients a latin square was employed on the back, the results obtained being similar to those obtained on the arm. After 20 min the weal outlines were drawn and transferred to clear adhesive tape. The weal areas were measured by the same operator using an electronic planimeter.

Results

RAST inhibition activity of the grass pollen extracts

The activity of the extracts as measured by the standardized RAST procedure are given in Table 1. It can be seen that these six extracts have a wide range of activity. Their *in vitro* activities did not correlate with their ability to elicit anomalous skin responses.

Skin tests

Our original observation of the anomalous response on skin testing with serial dilutions of allergen extracts is illustrated in Fig. 1. The results of skin testing on two patients with dilutions of five different extracts show that the weals obtained at a dilution of 1:10⁵ were frequently larger than those seen at the higher allergen concentration.

In the current controlled series of skin tests nineteen out of twenty-two patients tested showed this anomalous response with at least one pollen extract, thus confirm-

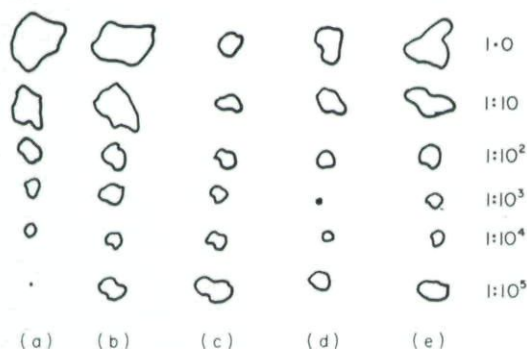
Table 1. Standardized RAST inhibition activities of the six cocksfoot pollen extracts used in this study.

Allergen numbers	Activity (standard RAST units per ml)
1	12.6
2	54.7
3	9.7×10^{-2}
4	4.6×10^{-2}
5	11.4
6	8.4

ing our original observation. Extract No. 3 failed to elicit the effect in any patient and extract No. 6 showed the effect in three out of the eleven patients tested. Both these extracts were tested over the equivalent standardized RAST unit ranges as those other extracts eliciting the anomalous effects.

The results of the mean weal areas in eleven patients tested with dilutions of extract No. 5 are shown in Fig. 2. The second peak in weal area can be seen at a dilution of about 10^{-4} standardized RAST units per ml. In contrast, the skin tests elicited

(S.W.)



(M.R.)

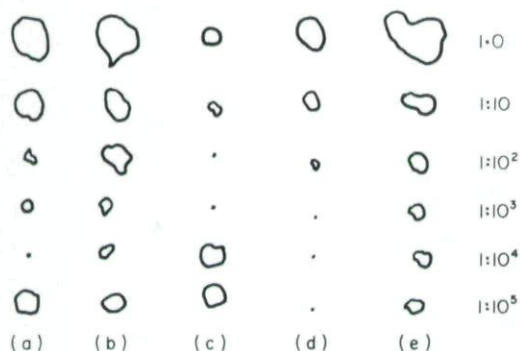


Fig. 1. A typical series of skin tests showing the increase in weal area with reduction of allergen concentration. Skin responses of the six ten-fold serial dilutions of five cocksfoot extracts (a to e) in the skins of patients S.W. and M.R.

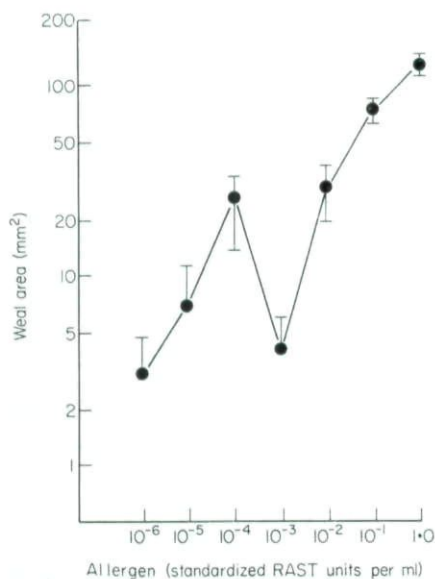


Fig. 2. Mean ($\pm$ s.e.m.) weal areas of skin test on eleven patients using extract No. 5. The weal area of the skin test using the allergen at a concentration of 10^{-4} standardized RAST units per ml is larger than that for the higher concentration of 10^{-3} units per ml ($P < 0.02$).

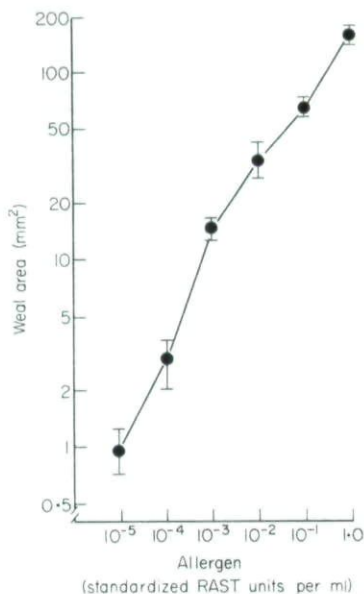


Fig. 3. Mean ($\pm$ s.e.m.) weal areas using extract No. 6. The weal sizes titrate out in a linear manner. In this case the area for an extract concentration of 10^{-4} units per ml is less than that for the 10^{-3} units per ml.

with extract 6 in the same patients showed no second peak at a lower allergen concentration. The mean weal areas with the extract in the same eleven patients are shown in Fig. 4.

Discussion

We are satisfied that we are seeing a real property of the reaction between allergen-specific IgE and the mast cell in these anomalous skin responses. Indeed, when one is specifically aware, they can be observed in patients undergoing routine skin testing in the clinic, even where the extracts are diluted over a relatively narrow concentration range. While similar dose-response curves are seen for the allergen-induced release of histamine from human basophils or passively sensitized chopped lung (Sheldon & Hammond, personal communication), the exact mechanism for these anomalous effects is obscure and one can only speculate upon possible causes.

It is known that the bridging of two IgE molecules by allergen is necessary for triggering of the mast cell (Ishizaka & Ishizaka, 1968) and that in certain conditions allergen in a monomer form can block histamine release (De Weck & Schneider, 1968). Our crude allergen extracts must contain a mixture of molecules ranging in size from monomers through to polymers and large aggregates. A competition between small and large molecular entities for IgE antibody sites on the mast cell may exist at high concentrations of allergen, with some inhibition of mast cell degranulation by the smaller species. As dilution occurs the competition becomes less but inhibition still continues, although less marked. At some low concentration the 'balance' is tipped and the larger molecules, having a greater epitope concentration per molecule, can exhibit their full stimulating potential.

Alternatively, we could be seeing degranulation resulting from more than one mechanism. The possible involvement of immunological components of complement should be considered. It has been reported that some allergens can stimulate complement reactions *in vitro* (Berrens, 1974). Non-immunological second mechanisms may also play their role in these observations. The release of histamine from mast cells is under the influence of such biochemical mediators as Ca^{2+} and cyclic nucleotide phosphates (Orange, 1976). With reports of reciprocal relationships between the concentrations and effects of GMP and cyclic AMP (Triner *et al.*, 1972), it is tempting to speculate on whether our observations may also reflect such biochemical phenomena taking place at the level of the mast cell membrane in human skin.

References

- AAS, K. & BELIN, L. (1972) Standardization of diagnostic work in allergy. *Acta Allergologica*, **27**, 439.
- BERRENS, L. (1974) Inhalant allergens in human atopic disease: their chemistry and mode of action. *Annals of the New York Academy of Sciences*, **221**, 183.
- GLEICH, G.L., LARSON, J.B., JONES, R.T. & BAER, H. (1974) Measurement of potency of allergen extracts by their inhibitory capacities in the radioallergosorbent test. *Journal of Allergy and Clinical Immunology*, **53**, 158.
- HUGGINS, K.G., ROITT, I.M., BROSTOFF, J. & TAYLOR, W.A. (1978) Standardization of radioallergosorbent test (RAST) and allergen extracts in terms of the WHO standard for IgE. *International Archives of Allergy and Applied Immunology*. (In press).
- ISHIZAKA, K. & ISHIZAKA, T. (1968) Human reaginic antibodies and immunoglobulin E. *Journal of Allergy*, **42**, 330.
- ORANGE, R.P. (1976) The immunological release of chemical mediators from human lung: approaches to pharmacological antagonism. *Role of Immunological Functions in Infectious, Allergic and Autoimmune Processes* (eds R. F. Beers and E. G. Bassett), p. 223. Raven Press, New York.

- PEPYS, J., TANGE, O., PERKINS, F.T., TATE, H. & BRIGHTON, W.D. (1975) Examination of cocksfoot pollen (*Dactylis glomerata*) extracts by skin testing in man, RAST-based allergen assay and protein content. *Developments Biological Standardization*, **29**, 284.
- RINKEL, H.J. (1949) Inhalant allergy. I. The whealing response of the skin to serial dilution testing. *Annals of Allergy*, **7**, 625.
- TRINER, L., VULIEMOY, Y., VEROSKY, M. & NAHA, G.G. (1972) Acetylcholine and the cyclic AMP system in smooth muscle. *Biochemistry and Biophysics Research Communications*, **46**, 1866.
- WECK, A.L. DE & SCHNEIDER, C.H. (1968) Immune and non-immune responses to monovalent low molecular weight penicilloyl-polylysines and penicilloyl-bacitracin in rabbits and guinea-pigs. *Immunology*, **14**, 457.

This document is a scanned copy of a printed document. No warranty is given about the accuracy of the copy. Users should refer to the original published version of the material.