

The use of radioactive isotopes for analysis of cutaneous allergic responses

K. JAN, C. NAHMIAS, G. COATES, F. E. HARGREAVE, C. DAVIS and J. DOLOVICH

Departments of Pediatrics, Nuclear Medicine and Medicine, McMaster University, Hamilton, Ontario, Canada

(Received 19 February 1979; accepted for publication 27 March 1979)

Summary

Local exudation at the sites of allergic and histamine-induced cutaneous reactions, was measured with an *in vivo* double-labelling procedure, based upon local serial simultaneous measurements of indium-113 m (^{113m}In) labelled transferrin and technetium-99m labelled red blood cells (^{99m}Tc RBC). Analyses depended upon the reaction index i.e. comparison of the ratio of $^{113m}\text{In}/^{99m}\text{Tc}$ counts at the reaction site with the ratio of $^{113m}\text{In}/^{99m}\text{Tc}$ at the corresponding unreacted site in the skin of the opposite arm.

Reaction indices were initially significantly elevated at the sites of histamine and allergic reactions. After the first hour, there was a progressive reduction at histamine sites indicating a tapering of the local accumulation of indium-labelled transferrin. By contrast allergic reaction sites showed a continuing increase in the reaction index after one hour. During the first hour the curves were comparable, but they diverge and were significantly different after one hour ($0.05 > P > 0.02$). This is consistent with the known occurrence of late cutaneous responses at the site of allergic but not histamine reactions.

Introduction

In sufficiently intense cutaneous allergic reactions induced by allergen in allergic individuals (McCarthy & Pepys, 1971; Dolovich *et al.*, 1973; Umemoto *et al.*, 1976), or by anti-IgE (Dolovich *et al.*, 1973; Poothullil *et al.*, 1976), the characteristic weal-and-flare response evolves into a more prolonged local response involving erythema, oedema and tenderness which has been termed the late cutaneous allergic response. These responses have previously received gross visual examination and also microscopic examination with histological and immunofluorescent methods (McCarthy Pepys, 1971; Dolovich *et al.*, 1973; Solley *et al.*, 1976). These methods demonstrate the

Correspondence: Dr J. Dolovich, Department of Pediatrics, McMaster University, Hamilton, Ontario, Canada.

morphology but do not measure the physiological processes involved in the evolution of the reactions.

Isotopic methods for the demonstration of increased vascular permeability have recently become available (Fishman, 1972; Robin *et al.*, 1972; Gorin *et al.*, 1978). Cutaneous allergic reactions are highly suited to the use of these methods as reactions in the skin are readily accessible and a number of sites can be studied at the same time. Moreover, the reactions can be induced at will and followed through their evolution.

The present report describes an isotopic demonstration of cutaneous reactions induced by allergen and anti-IgE (designated allergic reactions) and a comparison with the histamine reaction.

Materials and methods

Subjects

Subjects were allergic and non-allergic volunteers from a chest-allergy clinic. There were three male and three female subjects, all over the age of 45 yrs.

Reagents

Solutions of 5% w/v mixed grass pollen extract and 5% w/v *Dermatophagoides farinae* were purchased from Hollister-Stier Laboratories, Mississauga, Ontario. Sheep anti-serum to human IgE, which has undergone solid phase absorption to produce mono-specificity, was purchased from the Central Resource for Immunologic Reagents of McMaster University.

Indium-113m was eluted in dilute hydrochloric acid from a tin-113 generator supplied by Union Carbide. Technitium-99m was eluted in normal saline from a molybdenum-99 generator, manufactured by the McMaster Regional Radiopharmacy.

Skin test methods

Reactions were induced with allergen, or with anti-IgE and regarded as analogous to allergic reactions. The concentration of allergen or anti-IgE used in allergic reactions was initially determined with serial 1:10 dilutions of appropriate allergen in allergic individuals or serial 1:3 dilutions of a 1:10 dilution of anti-IgE in non-allergic subjects. The concentration of reagent which induced a reaction with a weal 2–3 mm in diameter in preliminary observations by the prick (puncture) test method was chosen.

Allergic reactions were induced by the intracutaneous injection of 0.05 ml of reactant. Histamine reactions were induced by the injection of 0.02 ml of undiluted histamine phosphate, 1 mg/ml. One allergic subject received grass pollen extract in a 1:10 dilution. A second allergic subject received the *D. farinae* extract undiluted. Anti-IgE in a dilution of 1:30 was used to induce reversed reaginic reactions (Ishizaka & Ishizaka, 1969) in four non-allergic subjects. In each subject, an allergic reaction was induced on the ventral surface of the left upper arm 2–3 hours before the intravenous injection of isotopes. A second allergic reaction was induced at a separate site on the left upper arm about 1 hr before the injection of isotopes. One-half hour after the injection of isotopes, a third allergic reaction was induced on the ventral surface of the left forearm, 5 cm below the crease of the elbow; at the same time, a reaction to histamine was induced on the ventral surface of the left forearm, 5 cm above the wrist crease.

Mean diameters of weal and flare responses were determined at 15 min and mean diameters of late responses were determined at 6 hr by methods previously described (Dolovich *et al.*, 1973; Umemoto *et al.*, 1976; Poothullil *et al.*, 1976).

Isotopic methods

One-half hour after the injection for the second allergic reaction, 1 ml of 'cold' stannous pyrophosphate, 1 mg/ml, was injected intravenously. After a 30-min interval, 500 μ Ci of sodium ^{99m}Tc -pertechnetate, and 2 μ Ci of ^{113m}In chloride were injected intravenously, with a 5 min lag between the two isotope injections. There is a known 95% efficiency of *in vivo* labelling of circulating red blood cells in the first hour after pertechnetate injection (Pavel *et al.*, 1977). Indium binds almost exclusively to transferrin but not to RBC (Hosain *et al.*, 1969).

The isotope injections were immediately followed by sequential 1 min measurements of the count rates of ^{99m}Tc and ^{113m}In at each intracutaneous injection site and at the corresponding control site on the opposite arm. After one-half hour, the measurements were repeated at least every half-hour for up to 5 hr. The count rates were measured with a calibrated 2 inch thick by 2 inch diameter sodium iodide crystal interfaced to a scaler and a PDP 11/20 computer. The collimator bore was 3 cm. Separate single channel analysers were used to separate the ^{113m}In counts from the ^{99m}Tc counts. All the measurements were corrected for the contribution of the ^{113m}In Compton scatter peak into the ^{99m}Tc window. For each measurement, the patient's arm was raised to shoulder level and rested on a support. The detector was placed horizontally such that the centre of the collimator was at the centre of the skin response and as close to the arm as possible.

For each skin test site and control site and for each measurement, a ratio of the indium counts/technetium counts was calculated. The reaction index is the ratio of these two ratios:

$$\frac{\text{1 minute In counts at the reaction site/1 minute Tc counts at the reaction site}}{\text{1 minute In counts at control site/1 minute Tc counts at the control site.}}$$

A reaction index of 1 is expected where no reaction has taken place.

Statistical methods

A 'repeated measures analysis of variance' (Winer, 1962) was used to compare the different reaction sites with control sites on the opposite arm. To eliminate the exponential quality of the reaction index data obtained from ratios, a log transformation was initially applied to the data prior to this analysis.

Results

Mean weal diameters ranged from a minimum of 2.6 to a maximum of 6.2 cm. Late responses ranged from a minimum of 2.6 cm in mean diameter to a maximum, in one subject, which could not be accurately measured since the two late responses on the upper arm and the two late responses on the lower arm were sufficiently large to become confluent. There was no direct relationship between the diameters of the reactions and the reaction indices.

The mean reaction indices and the standard error of the mean in the six subjects for histamine reactions are illustrated in Fig 1 and for the allergic reactions, induced after isotope injection, are illustrated in Fig. 2. The mean weal diameters for the histamine

and the allergic reactions respectively are 4.8 and 4.5 cm. The data represented prior to time 0 indicate readings performed prior to the induction of the reactions. In the absence of reactions, it can be noted that the reaction indices fall close to the expected value of 1 with an actual mean value of 0.91 ± 0.37 at the sites which would subsequently be the location of histamine reactions and 1.04 ± 0.21 at the sites of subsequent allergic reactions. The mean values for histamine reactions (Fig. 1) and allergic reactions (Fig. 2) were comparable during the first hour but diverged after one hour with a diminution in the indices for histamine reactions and continued increase for allergic reactions. Repeated measurement analysis of variance confirms the absence of any significant difference between the curves in the first hr ($P=0.6$); in contrast, there is a significant difference between the two curves ($P=0.002$) after 1 hr, showing a relative persistence of the allergic reaction in comparison with the histamine reaction.

Reactions initiated 1 hr prior to the injection of isotope had a mean weal diameter of 4.9 cms and reactions initiated 2 or more hr prior to the injection of isotope had a mean weal diameter of 4.5 cms. Comparison of left (reaction) arm $^{113m}\text{In}/^{99m}\text{Tc}$ ratios with right (control) arm ratios by repeated measures analysis of variance indicated

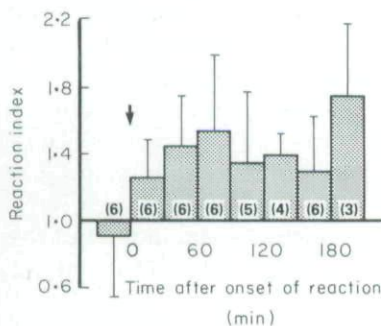


Fig. 1. Histamine-induced cutaneous reaction: the mean reaction indices and standard error of the mean are illustrated. The number of subjects is indicated in parentheses. The arrow indicates the time of injection of isotope.

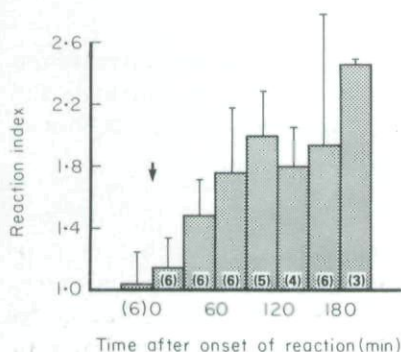


Fig. 2. Cutaneous allergic reaction: the mean reaction indices and standard error of the mean are illustrated. The number of subjects is indicated in parentheses. The arrow indicates the time of injection of isotope. The values for these reactions do not differ from those of the histamine-induced reactions illustrated in Fig. 1 during the first hr; however, after the first hr, these reactions were significantly greater than the histamine-induced reactions.

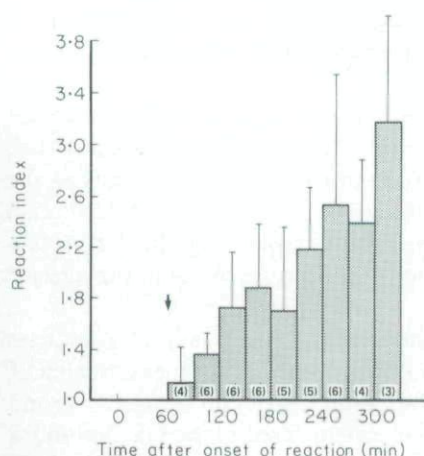


Fig. 3. Cutaneous allergic reaction: the mean reaction indices and standard error of the mean are illustrated. The number of subjects is indicated in parentheses. The arrow indicates the times of injection of isotope. The data are for reactions initiated 1 hr before the injection of isotope. There was a highly significant increase in the In-transferrin/Tc-RBC ratio in the reaction arm by comparison with the control arm. The accumulation of In-transferrin, indicated by these data coincides with the late cutaneous allergic response which is observed morphologically to emerge rapidly at this time.

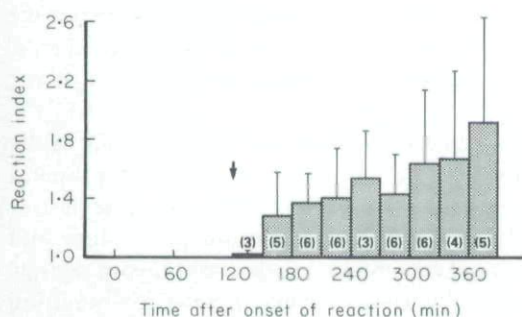


Fig. 4. Cutaneous allergic reaction: the mean reaction indices and standard error of the mean are illustrated. The number of subjects is indicated in parentheses. The arrow indicates the time of injection of isotope. The data are for reactions which are initiated 2 or more hr prior to the injection of isotope. There was, nevertheless, a significant increase in the reaction arm ratio, by comparison with the control arm ratio, indicating significant continuing protein (transferrin) accumulation in the reactions.

significant accumulation of protein at both the '1 hour' ($P < 0.001$) and the '2 or more hr' ($P < 0.05$) reactions.

Discussion

The isotopic methods used here permit a direct examination of exudation at the sites of cutaneous reactions. The examination of counts of In-transferrin in relation to counts for Tc-RBC eliminates the possibility of an artefact due merely to changes in blood volume. Over the period of the study, ^{99m}Tc does elute from the red cells and ^{113m}In does dissociate from the transferrin. In terms of the analyses, effects from these changes of concentrations of isotopes in the vascular pool are eliminated by use of the measure-

ments on the control limb. If there had been no extravascular accumulation of ^{113m}In -transferrin in the reaction sites, the reaction indices would have stayed at 1 as they were before the reactions were induced.

The anti-IgE reversed reaginic reaction can be considered to be analogous to the reaction induced by the interaction of antigen with antibody of the IgE class. The reversed reaginic reaction *in vitro* is subject to the same control mechanisms as the antigen-induced IgE-dependent reaction (Lichtenstein *et al.*, 1970). The *in vivo* reaction induced by anti-IgE involves early weal and flare responses followed by late cutaneous allergic responses comparable to antigen-induced reactions in the allergic individual (Dolovich *et al.*, 1973).

The absence of a direct relationship between the size of cutaneous allergic responses and the magnitude of reaction indices possibly reflects the method of measurement of the isotope. The diameter of reactions is due to a radial dispersion of reactants and products in the skin. The local radioactivity measurements used in the reaction index depend upon sampling with a probe of a fixed diameter smaller than the diameter of the reactions. It is probable that a probe sufficiently large to encompass the total diameters of reactions would provide data more directly related to the measured diameters of reactions.

The direct comparison of histamine-induced reactions with allergic reactions initiated after the injection of isotopes shows a significant difference in the responses after 1 hr with a tapering off of the histamine but not the allergic reaction. In morphological terms, the weal and flare reaction tends to subside after 1 hr but the allergic reaction then evolves into a late cutaneous allergic response. The allergic reactions induced prior to the injection of isotope provide a separate demonstration of these prolonged responses.

It is thought that the previously reported accumulation of fibrin at the site of the late cutaneous allergic response (de Shazo *et al.*, 1978) with its hygroscopic property might contribute to the local swelling characteristic of late cutaneous allergic responses. This would imply a role for the reduction in the drainage of fluid and presumably solute, at least in part, in the fluid accumulation in late cutaneous allergic responses. The present data do not permit a conclusion as to whether the accumulation of In-transferrin is due to an increased rate of local exudation or a reduced rate of removal or both. Nevertheless, isotopic methods should make possible the examination of this question.

Acknowledgments

The authors wish to thank Gary Anderson and Gerry Chan of the Department of Epidemiology and Biostatistics of McMaster University for advice and assistance in the statistical analyses. We wish also to express thanks to Jerry Watson of the Department of Nuclear Medicine for the isotope injection and Mrs Joan English for secretarial assistance in the preparation of the manuscript. Kang Jan, a medical student, performed this work as a summer project. This study was supported by the Medical Research Council of Canada.

References

- DE SHAZO, R., DVORAK, H., DAVIS, R., EVANS, R. & LEVINSON, A. (1978) The late phase skin reaction (abstract). *Journal of Allergy and Clinical Immunology*, **61**, 186.
- DOLOVICH, J., HARGREAVE, F.E., CHALMERS, R., SHIER, K.J., GAULDIE, J. & BIENENSTOCK, J. (1973) Late

- cutaneous allergic responses in isolated IgE-dependent reactions. *Journal of Allergy and Clinical Immunology*, **52**, 38.
- FISHMAN, A.P. (1972) Pulmonary edema; the water exchanging function of the lung. *Circulation*, **47**, 390.
- GORIN, A.B., WEIDNER, W.J., DEMLING, R.H. & STAUB, N.C. (1978) The non-invasive measurement of pulmonary transvascular protein flux in sheep. *The Journal of Applied Physiology*, **45**, 225.
- HOSAIN, F., MCINTYRE, P.A., POULOSE, K., STERN, H.S. & WAGNER, H.J. JR (1969) Binding of trace amounts of tonic indium-113m to plasma transferrin. *Clinica chimica acta*, **24**, 69.
- ISHIZAKA, K. & ISHIZAKA, T. (1969) Immune mechanisms of reversed type reaginic hypersensitivity. *Journal of Immunology*, **103**, 588.
- LICHTENSTEIN, L.M., LEVY, D.A. & ISHIZAKA, K. (1970) *In vitro* reversed anaphylaxis: characteristics of anti-IgE mediate histamine release. *Immunology*, **19**, 831.
- MCCARTHY, D.S. & PEPYS, J. (1971) Allergic broncho-pulmonary aspergillosis. Clinical immunology: (2) skin, nasal and bronchial tests. *Clinical Allergy*, **1**, 415.
- PAVEL, D.G., ZIMMER, A.M. & PATTERSON, V.N. (1977) *In vivo* labelling of red blood cells with ^{99m}Tc: a new approach to blood pool visualizations. *Journal of Nuclear Medicine*, **18**, 305.
- POOTHULLIL, J., UMEMOTO, L., DOLOVICH, J., HARGREAVE, F.E. & DAY, R.P. (1976) Inhibition by prednisone of late cutaneous allergic responses induced by antiserum to human IgE. *Journal of Allergy and Clinical Immunology*, **57**, 164.
- ROBIN, E.D., CAREY, L.C., GLAVSER, F. & GAUDIO, R. (1972) Capillary leak syndrome with pulmonary edema. *Archives of Internal Medicine*, **130**, 66.
- SOLLEY, G.O., GLEICH, G.J., JORDON, R.E. & SCHROETER, A.L. (1976) The late phase of the immediate weal and flare skin reaction. *Journal of Clinical Investigation*, **58**, 408.
- UMEMOTO, L., POOTHULLIL, J., DOLOVICH, J. & HARGREAVE, F.E. (1976) Factors which influence late cutaneous allergic responses. *Journal of Allergy and Clinical Immunology*, **58**, 60.
- WINER, B.J. (1962) Statistical Principles in Experimental Design. p. 105. McGraw-Hill, New York.

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.