Delayed Hypersensitivity to Tuberculin and Other Antigens in a Hospital Population

R. S. WALLS

SUMMARY

Delayed hypersensitivity is a valuable index of cellular immune function, provided that the incidence of positive reactions in the population is known. One hundred and sixty-two hospital patients were examined, using tuberculin (PPD), streptokinase and antigens derived from *Candida albicans* and mumps. Whereas 75% of the group responded to *Candida*, 61,2% to mumps and 69,3% to streptokinase, only 30,8% showed positive Mantoux reactions. Elderly patients showed fewer reactions, but these were of similar size.

S. Afr. Med. J., **48**, 2073 (1974).

It is becoming increasingly important to identify patients with compromised immunological function, and assessment of delayed hypersensitivity forms an important part of this investigation. Antigens to which the subject is presumed to have been exposed previously are used. The probability of an antigen eliciting a positive reaction depends on a variety of factors, including the nature of the population group under study. The incidence of reactions in this population should be known, therefore, for proper interpretation of results.

Clinical Immunology Service, Department of Medicine, Groote Schuur Hospital and University of Cape Town

R. S. WALLS, M.B. CH.B., D.PHIL., F.C.P. (S.A.)

Date received: 18 June 1964.

The present study aimed at providing information about delayed hypersensitivity reactions in hospital patients, which would serve as a basis of comparison in assessment of individual cases. It would also help to establish the significance of positive skin reaction to tuberculin and other antigens.

PATIENTS AND METHODS

Patients had been admitted to a general medical ward or were referred for medical opinion. The nature and aims of the investigation were explained before obtaining consent. Ages of the 162 patients examined ranged from 12 to 81 years, and sexes were equally distributed. Ninety-three were Coloured, 53 Black and 16 White. Diagnoses are shown in Table I.

Delayed Hypersensitivity Tests

Antigens were injected intradermally in 0,1-ml volumes, using a disposable tuberculin syringe with a 26-gauge needle. Injections were given into the flexor surface of the forearm, starting 4 cm below the elbow crease, and spaced diagonally at least 4 cm apart. Injection sites were examined 24 and 48 hours later. Erythema was ignored and the diameter of induration was measured at right angles to the long axis of the arm. In assessing results

TABLE I. DIAGNOSES OF PATIENTS STUDIED

Diagnosis	No. of patients
Cardiac and cerebral vascular disease	24
Renal diseases including glomerulonephritis, pyelonephritis and nephrotic syndrome	9
Diabetes mellitus	9
Rheumatic and congenital heart disease	8
Bacterial endocarditis	4
Tuberculosis	19
Amoebiasis	3
Other infections (respiratory, septicaemia, meningitis, viral infections)	28
Malignant disease (carcinoma)	3
Malignant disease (leukaemia, lymphoma, myeloma)	7
Iron deficiency anaemia	7
Auto-immune and rheumatic disease	15
Sarcoidosis	3
Alcoholism	7
Liver and gastro-intestinal disease	8
Neurological (including Guillain-Barré, epilepsy, encephalitis)	3
Hypogammaglobulinaemia	2
Partial lipodystrophy	2
Vitamin deficiency	2
Miscellaneous group (one each of schizophrenia, postpartum fits, hilar adenopathy, eosinophilia, coma of unknown cause, aortic aneurysm, lumbar pain, porphyria, gout, Wegener's granulomatosis, monoclonal gammopathy, proptosis of unknown aetiology)	12

Where more than one diagnosis was made, each is recorded in the table.

the maximum diameter at either 24 or 48 hours was considered. Induration of 5 mm or more was considered to be a positive reaction. The following antigens were used:

1. Purified protein derivative of tuberculin (PPD)—intermediate strength containing 5 TU per 0,1-ml dose (Parke-Davis). In the first 69 patients tested, solutions were used within 2 weeks of reconstitution. Thereafter they were used within 30 minutes.

2. *Candida albicans* 1/100 in buffered solution (Hollister-Stier).

3. Mumps skin test antigen (Eli Lilly). This antigen became available later in the study and was administered to 85 patients.

4. Streptokinase 5 IU with streptodornase 1,25 IU (SK) prepared by diluting Varidase Topical (Lederle) in sterile normal saline.

The chi-squared test was used to compare numbers of positive reactions between different groups. Student's *t*-test was employed in comparing mean diameters of reactions.

RESULTS

Numbers of patients responding to individual antigens are shown in Fig. 1. *Candida* antigen elicited the largest number of reactions. Only 30,8% of the population reacted to PPD. Ten patients out of 85 showed no

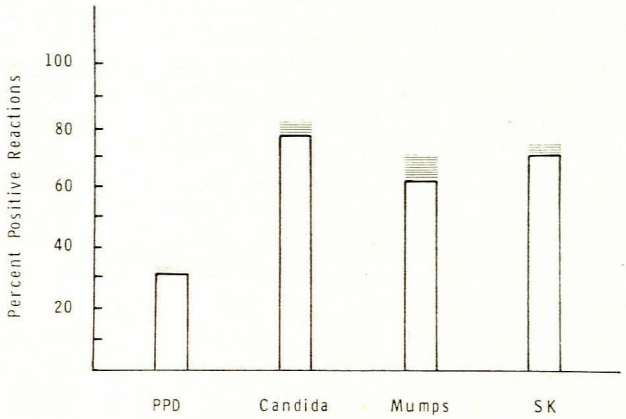

Fig. 1. Percentage of patients showing positive reactions to individual antigens. Eighty-five patients were injected with mumps and 162 with each of the other antigens. Open histograms represent the total population. Lined areas show additional percentage in a population from which 23 patients in respect of the mumps, and 26 in respect of other antigens, were excluded. Their diagnoses were systemic lupus erythematosus, sarcoidosis, lymphoma, Hodgkin's disease and uraemia—conditions known to be associated with impaired delayed hypersensitivity.

reactions to any of four antigens used together. Their diagnoses were systemic lupus erythematosus (SLE) in 3, Hodgkin's disease or stage IV lymphoma in 2, sarcoidosis in 2, chronic renal failure with uraemia in 1, and long-standing rheumatic heart disease in 2 patients who died (during that admission) in cardiac failure. Their blood ureas had been less than 100 mg/100 ml during their stay in hospital.

A further 10 of 77 patients tested with PPD, *Candida* and SK showed no reaction. Three of these died shortly after testing, 2 following prolonged coma (one of hypoglycaemia, the other of undetermined cause) and 1 with myocardial infarction and cerebrovascular accident. Other diagnoses were *Klebsiella* pneumonia (2), pneumonia with unexplained hepatomegaly, chronic alcoholism, recurrent staphylococcal abscesses, ichthyosis, and unexplained high ESR in a patient with lumbar pain. Impairment of delayed hypersensitivity has been documented in SLE,[1] sarcoidosis,[2] Hodgkin's disease and lymphoma,[3] and in uraemia.[4] When 26 patients with these conditions were excluded, the percentage of positive reactions increased (Fig. 1). Testing with four antigens together, 69% reacted to at least three antigens, 84% to two or more, and 2 patients (2,7%) with rheumatic heart disease alluded to previously, failed to react to any antigens.

Diameters of induration varied with the antigens being used (Table II). Of the 12 patients who reacted to only one antigen, 9 responded to *Candida* and their reactions were somewhat smaller than those of the group as a whole ($P<0,05$). The incidence of positive reactions to *Candida*, mumps and SK did not differ statistically in Coloureds, Blacks and Whites (Fig. 2), but Black patients showed the highest incidence of positive reactions to PPD ($P<0,01$).

TABLE II. MEAN DIAMETERS ± 1 SD OF POSITIVE REACTIONS TO FOUR ANTIGENS (NUMBERS OF PATIENTS TESTED IN BRACKETS)

Age group (years)	PPD	*Candida*	Mumps	SK
All ages	9,7 ± 3,8 (49)	12,9 ± 5,6 (121)	8,2 ± 4,1 (52)	13,1 ± 5,1 (112)
12–	7,4 ± 2,8 (7)	13,4 ± 5,8 (16)	6,7 ± 2,1 (7)	14,2 ± 6,4 (15)
20–	11,1 ± 3,1 (8)	15,3 ± 8,1 (20)	9,4 ± 7,1 (8)	14,0 ± 5,4 (21)
30–	9,9 ± 3,8 (7)	12,0 ± 4,4 (21)	7,5 ± 3,5 (10)	13,7 ± 5,6 (22)
40–	9,3 ± 3,8 (15)	13,3 ± 5,0 (26)	9,4 ± 4,3 (14)	12,7 ± 4,3 (28)
50–	10,5 ± 5,4 (6)	11,6 ± 5,0 (18)	9,0 ± 3,6 (7)	12,6 ± 3,5 (15)
60–	10,5 ± 3,7 (4)	12,9 ± 6,1 (14)	7,0 ± 2,6 (6)	9,6 ± 3,1 (7)
70–	5 (2)	9,8 ± 4,0 (6)	Nil	13,3 ± 5,6 (4)

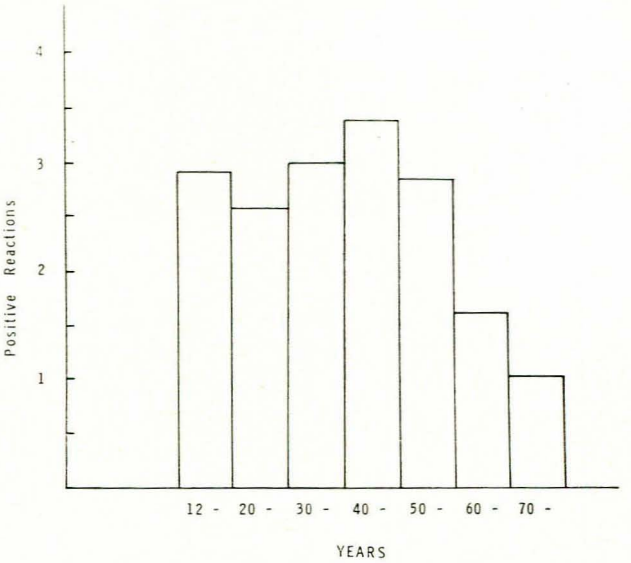

Fig. 3. Mean numbers of reactions to four antigens administered together in each 10-year age group.

Influence of Age

Fig. 3 shows that the mean number of positive reactions dropped sharply in the 60-year-olds. Of 72 patients, 58 (80,5%) under the age of 60 years reacted to at least two of four antigens, compared with only 5 of 13 (37,5%) older patients ($P = 0{,}003$ by exact test for 2 × 2 contingency table).[5] This pattern was reflected particularly in reactions to SK and to a lesser extent to PPD. Responsiveness to *Candida* antigen and mumps showed less fall-off. There appeared to be no change in size of reactions with advancing years (Table II).

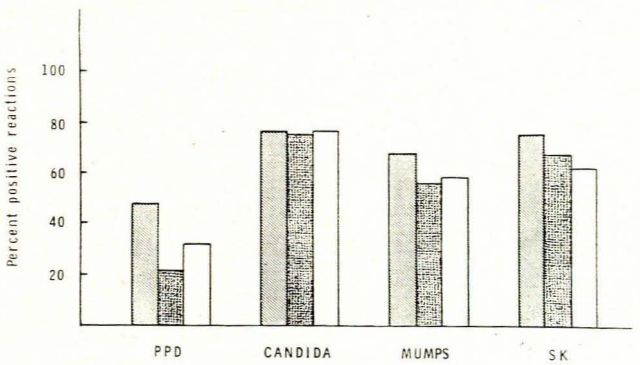

Fig. 2. Percentage of patients in each race showing reactions to individual antigens. Cross-hatched bars denote Black patients, speckled bars Coloureds and open bars Whites.

Morbidity of Tests

Little discomfort was experienced at the time of injection. Three patients developed weal and flare reactions of immediate hypersensitivity to *Candida* and 1 developed generalised urticaria which lasted 24 hours. He had no history of allergy. Symptoms were easily controlled with oral promethazine. In another patient a large painful Arthus reaction developed. She had partial lipodystrophy, chronically depressed levels of C3 and a tendency to recurrent infection. In another patient with polyarthritis, recurrent infection and low serum levels of IgA, large reactions to SK and *Candida* developed after 24 hours. Topical betamethasone reduced the intensity of inflammation and swelling.

DISCUSSION

The immunological system has been arbitrarily divided into humoral and cellular effector limbs.[6] Humoral immunity is mediated by antibody while cellular immunity requires the participation of thymus-processed lymphocytes. Assessment of immunological status requires investigation of both these components of specific immunity in addition to other aspects such as complement and neutrophil function.

Several means are available for investigating cell-mediated immunity, including the use of primary immunising agents such as 2:4 dinitrochlorobenzene (DNCB), assessment of skin graft survival, and *in vitro* assays of lymphocyte function such as phytohaemagglutinin (PHA)-induced transformation and cytotoxicity, and leucocyte migration inhibition (LMI). *In vitro* tests need sophisticated laboratory facilities, while skin grafting is inconvenient and may lead to undesirable immunisation with foreign tissue. Assessment of delayed hypersensitivity is a standard technique for measuring cell-mediated immunity. Common antigens are used, to which the person is likely to have established immunity, and their choice in the present study was governed by the frequency of reactions to them in published series.[1,7] Testing with a larger

number of antigens may be thought to increase the percentage of people showing positive reactions, but Forbes[7] found that there was little advantage in increasing the number of antigens beyond SK, *Candida* and mumps.

The Mantoux reaction is widely considered to be a useful index of intact immune function, since tuberculosis is still a common disease in South Africa. In the municipal area of Cape Town, with a total population of 752 460 inhabitants, 1 399 new cases of pulmonary tuberculosis were notified during 1972.[8] The low incidence of positive reactions was, therefore, quite unexpected. Attention has already been drawn to the incidence of negative results in tuberculosis[9] and it has been suggested that the PPD may be at fault. Liquid tuberculin containing Tween 80 elicits a higher incidence of positives than does a reconstituted dry tablet, although the latter preparation gives satisfactory results if used within 30 minutes of reconstitution.[10] This precaution did not appear to affect the number of positives in the present study. Furthermore, these arguments cannot account for the fact that a proportion of the population reacts satisfactorily. The large number of negative reactions is not because of anergy, since 74% of the population reacted to at least three antigens. Nor was it likely to be the result of faulty technique, since results with other antigens correspond with experience elsewhere.[1,7] It was surprising to find no difference in incidence of reactions to PPD between Coloureds and Whites, in view of the fact that the notification rate of tuberculosis for Coloureds in Cape Town during 1972 was 1,61 per 1 000 population, against 0,22 per 1 000 for Whites.[8] The rate for Blacks was 7,47 per 1 000.

There is still controversy as to whether skin reactivity is reduced in old age.[1,11] In the present study, there was a sharp drop in numbers of positive reactions in the sixties and seventies, rather than a linear decrease in reactivity with increasing age. Most patients had serious illnesses necessitating their admission to a medical ward, and yet 88,3% reacted to one or more antigens. Only 2 patients without diseases recognised as being associated with anergy failed to respond to any of four antigens, and both died shortly afterwards. General illness appeared to have little non-specific depressant effect on skin reactivity. Claims that iron deficiency anaemia is associated with impaired delayed hypersensitivity responses[12] could not be substantiated, although numbers were small. Seven patients were tested, 3 with all four antigens and 4 with PPD, *Candida* and SK. The latter all reacted to at least two antigens. Of the others, 2 patients reacted to three antigens and 1 to SK only. No systematic attempt was made to determine the effects of therapy on delayed hypersensitivity responses. It has been reported that administration of steroids has no effect,[1] and this was in keeping with present experience.

There is no information on the degree of impairment of cellular immunity which results in failure to mount delayed hypersensitivity responses, nor on whether the ability to mount fewer responses than the mean for the whole population is of any significance. *Candida* antigen was responsible for most single reactions. Whether these patients had partial impairment of immune competence is not clear. It is interesting that the size of their reactions was smaller than that of patients with several positives.

Investigation of delayed hypersensitivity is but a part of the assessment of cellular immunity. Both lymphocyte transformation to PHA[13] and leucocyte migration inhibition (LMI)[14] correlate with positive skin tests, although there is no relationship between the size of reaction and the degree of LMI.[15] Reports have described impaired delayed hypersensitivity with normal lymphocyte transformation to PHA.[7] This is understandable, since the latter tests a single step in the response, whereas delayed hypersensitivity depends on a complex integrated sequence of events, any component of which may be defective. The presence of delayed hypersensitivity reactions does not preclude a degree of impairment of cellular immunity since it does not test the ability of the subject to react to new antigens. This may be studied conveniently by attempting to sensitise subjects to 2:4 dinitrochlorobenzene.[16] Evaluation of delayed hypersensitivity remains a valuable investigation, but to make a meaningful assessment, the likelihood of response in a particular population should be known.

I wish to thank Dr H. A. Brown and others for permission to study their patients.

REFERENCES

1. Toh, B. H., Roberts-Thomson, I. C., Mathews, J. D., Whittingham, S. and Mackay, I. R. (1973): Clin. Exp. Immunol., **14**, 193.
2. Citron, K. M. (1959): Tubercle (Lond.), **38**, 33.
3. Aisenberg, A. C. (1973): New Engl. J. Med., **288**, 883.
4. Kirkpatrick, C. H., Wilson, W. E. G. and Talmage, D. W. (1964): J. Exp. Med., **119**, 727.
5. Bailey, N. T. J. (1961): *Statistical Methods in Biology*, 2nd ed. p. 63. London: English Universities Press.
6. Roitt, I. M., Greaves, M. F., Torrigiani, G., Brostoff, J. and Playfair, J. H. L. (1969): Lancet, **2**, 367.
7. Forbes, I. J. (1971): Aust. N.Z. J. Med., **1**, 160.
8. Annual Report of the Medical Officer of Health for the year 1972, City of Cape Town.
9. Holden, M., Dubin, M. R. and Diamond, P. H. (1971): New Engl. J. Med., **285**, 1506.
10. Edwards, P. Q. (1972): *Ibid.*, **286**, 373.
11. Grossman, J., Baum, J., Gluckman, J., Fusner, J. and Condemi, J. J. (1973): J. Allergy Clin. Immunol., **51**, 127.
12. Joynson, D. H. M., Jacobs, A., Walker, D. M. and Dolby, A. E. (1972): Lancet, **2**, 1058.
13. Oppenheim, J. J. (1968): Fed. Proc., **27**, 21.
14. Rocklin, R. E., Rosen, F. S. and David, J. R. (1970): New Engl. J. Med., **282**, 1340.
15. Mitchell, C. G., Smith, M. G. M., Golding, P. L., Eddleston, A. L. W. F. and Williams, R. (1972): Clin. Exp. Immunol., **11**, 535.
16. Catalona, W. J., Taylor, P. T., Rabson, A. S. and Chretien, P. B. (1972): New Engl. J. Med., **286**, 399.