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DINITROCHLOROBENZENE CONTACT SENSITIZATION IN PULMONARY TUBERCULOSIS

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SUMMARY

Skin sensitization with DNCB was carried out in forty-five untreated, 106 'on treatment' and fifteen fully treated patients with pulmonary tuberculosis along with fifty-five controls. Mantoux test with PPD was also carried out simultaneously. All the normal controls could be sensitized to DNCB and the degree of sensitization was 4+ in majority of the subjects. In contrast, in untreated patients only eighteen could be sensitized to DNCB and the degree of sensitivity did not reach 4+ in any patient. Similarly, fifty-five patients could not be sensitized with DNCB in the group of 106 subjects who were still suffering from active disease and were on therapy and none gave a 4+ response. The difference in the proportion of subjects who could be sensitized to DNCB in these two patient groups was significantly less in comparison to controls. Among fifteen patients who were fully treated and did not have active disease thirteen could be sensitized with DNCB, but the degree of response was again found to be less than the controls. All the patients as well as the controls gave a high proportion of Mantoux positivity. These findings indicate that there is a subtle degree of immunodeficiency in pulmonary tuberculosis which improves simultaneously with the clinical improvement.

INTRODUCTION

The presence of immunodeficiency secondary to chronic infections has been appreciated only recently (Bryceson, 1974; Bullock, 1974; Verrier-Jones & Talwar, 1974). Studies in leprosy have shown that in the lepromatous form there is a marked degree of specific as well as non-specific suppression of cell-mediated immune response (CMIR), the non-specific component of which recovers concomitantly with the diminution of the bacterial load on chemotherapy though the specific CMIR to *Mycobacterium leprae* remains depressed (Godal, 1974). There are indications that such a phenomenon may also be present in tuberculosis (Bryceson, 1974; Bullock, 1974) though the published data are sparse.

The present work is a part of a continued study on the immunological status in patients suffering from tuberculosis. Earlier reports from this laboratory indicated that patients with

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pulmonary tuberculosis show a poor ability to get sensitized to 1-chloro-2,4-dinitrobenzene (DNCB) (Malaviya, Kumar & Dingley, 1973). The data presented here confirm the earlier findings and adds information on the DNCB response in fully treated patients with pulmonary tuberculosis.

MATERIALS AND METHODS

Subjects. Patients were selected from the tuberculosis hospital and the out-patients department of the hospital of All-India Institute of Medical Sciences. All the patients were sputum-positive for acid-fast bacilli and active tuberculous lesion were demonstrated on chest X-rays some time during the course of their illness. Their age was forty-five years or less. As far as could be ascertained clinically, they did not have malnutrition and other conditions known to cause suppression of CMIR (Edwards, 1972). The patients were divided into the following three groups.

Patient group I: this group included forty-five freshly diagnosed patients who had not received any treatment in the past.

Patient group II: this group included 106 patients who were on anti-tuberculous therapy for 1 month or more. The therapy consisted of a combination of isonicotinic acid hydrazide, streptomycin, para-amino salicylic acid and thiacetazone.

Patient group III: fifteen fully treated subjects with inactive disease and negative sputum and who were clinically in normal health were included in this group. These patients had had 18 months to 2 years of anti-tuberculous therapy.

Fifty-five age- and sex-matched healthy controls with no past history of tuberculosis were taken from among the laboratory and hospital staff.

DNCB skin sensitization. This was carried out and interpreted according to the technique of Catalona (1972). In short, it consisted of applying 2000 μg of DNCB (Eastman Organic Chemicals, Rochester, New York) in 0·1 ml of acetone over a skin area of 3 sq cm on the volar surface of the forearm through a metal ring of 2 cm diameter. Simultaneously, 50 μg of DNCB in 0·1 ml of acetone was applied in a similar manner on the volar surface of the other forearm. Both sites were covered with 'Band-Aid' (Johnson & Johnson) for three days and washing was avoided. The site was observed for non-specific inflammatory response (NIR) for up to 3 days. The development of a 'spontaneous flare' was recorded up to day 14. If no spontaneous flare developed up to day 14 a challenge dose of 50 μg was applied in the same way as described. The site of application was observed up to 72 hr after the application for the development of induration and erythema. The interpretation of the results was as follows: 4+ if the spontaneous flare was observed at both 2000 and 50 μg DNCB application sites within 14 days; 3+ if the spontaneous flare occurred only at 2000 μg site; 2+ if the spontaneous flare did not occur by day 14 but there was an unequivocal reaction within 72 hr of the application of the challenge dose of 50 μg; the reaction was considered 1+ if the reaction was equivocal after the challenge dose and needed skin biopsy for the confirmation of the contact dermatitis (it was confirmed in the first three cases only with skin biopsy).

Mantoux test. Mantoux test was performed with all the precautions to avoid a false negative test (Edwards, 1972) using 1 unit of purified protein derivative of tuberculin (PPD, B.C.G. Laboratories, Guindy, Madras). Induration of 10×10 mm or more at the end of 72 hr was considered a positive Mantoux reaction. If the test was negative it was repeated with five units of PPD and the same criteria for the positivity was used.

RESULTS

The mean age of the forty-five patients in group I was 31·2 years with a range of 16–45 years. There were thirty-seven males and eight females. In group II of 106 patients the mean age was 33 years with a range of 16–45 years. There were ninety males and sixteen females. In group III of fifteen patients the age ranged from 18 to 43 years with a mean of 29·8 years. There were twelve males and three females. These figures were comparable to that of fifty-five controls where the age range was 19–50 years with a mean of 28 years. There were fifty males and five females.

TABLE 1. DNCB and PPD response in pulmonary tuberculosis

Subjects		DNCB						PPD-positive
		NIR* positive	Contact sensitization					
			Positive				Negative	
			4+	3+	2+	1+		
Controls	(55)	55	34	9	10	2	0	34
Untreated	(45)	40	0	2	9	7	27	45
On treatment	(106)	95	0	11	26	18	51	91
Treated	(15)	14	0	2	9	2	2	14

* Non-specific inflammatory response.

The results of non-specific inflammatory response (NIR) to irritant dose (2000 μg) of DNCB (3-day response) and DNCB contact sensitization is given in Table 1. The proportion of subjects developing contact sensitivity to DNCB in groups I and II was significantly less than that of the control group ($P<0{\cdot}01$) and the patient group III ($P<0{\cdot}01$). The latter group was not different in DNCB response from the controls ($P>0{\cdot}05$). Subjects showing negative NIR were also consistently negative for contact sensitization.

The mean age of the group of patients who gave a negative response to DNCB was comparable to that of the patients who gave a positive response (thirty-one years to thirty-two years respectively).

The correlation of Mantoux test with DNCB response is given in Table 2. In patient groups I and II a number of subjects could not be sensitized to DNCB though they were Mantoux-positive. In the control group as well as in patient group III the majority were both DNCB as well as Mantoux-positive.

TABLE 2. Correlation of DNCB and PPD response

	Control (55)	Group I (45)	Group II (106)	Group III (15)
DNCB+, PPD+	34	18	46	12
DNCB+, PPD−	21	0	9	1
DNCB−, PPD+	0	27	45	2
DNCB−, PPD−	0	0	6	0

DISCUSSION

The present study showed that in patients with pulmonary tuberculosis the delayed hypersensitivity to 'recall' antigen PPD was intact. In contrast, the deliberate sensitization with DNCB failed to elicit contact hypersensitivity in a significant proportion of untreated patients and patients 'on treatment'. But, the same procedure done in fully treated patients resulted in DNCB sensitization in proportions similar to that in the normal population.

These observations indicate that the DNCB unresponsiveness improved and reached normal proportions simultaneously with clinical improvement.

There are several non-specific causes of negative delayed hypersensitivity reactions. The most important are advancing age and malnutrition (Waldorf, Wilkins & Decker, 1968; Roberts-Thomson *et al.*, 1974). In the present work the age factor was carefully controlled and patients above the age of 45 years were not studied. Similarly, subjects with clinically apparent malnutrition were excluded though no detailed serum protein estimations were performed to exclude subclinical malnutrition.

There are only a few other reports available on DNCB contact sensitization in pulmonary tuberculosis. Thus, Gross (1965) and Gorodezky (1974) reported that approximately one-half of their patients did not develop sensitivity to DNCB. Their results are not very different from the data presented in this paper. Anergy to DNCB was also found by Tugwell & Tomkins (cited by Bryceson, 1974) in their patients with tuberculosis. However, in addition, they also found anergy to PPD. These authors concluded that the anergy in tuberculosis was related to decreased ability to mount an inflammatory response rather than to any basic immunological defect. They also noted a close correlation of anergy to serum transferrin levels, strongly suggesting that malnutrition was an underlying defect. In the present study the non-specific response of the skin was not found to be impaired in the majority of DNCB negative patients. Moreover, malnutrition, if present, was not clinically obvious.

Improvement in the proportion of DNCB responders among the fully treated patients may indicate that the defect was a secondary immunodeficiency related to the disease. Such a defect is documented in leprosy (Godal, 1974) and in one case of miliary tuberculosis (Waksman & Lockshin, 1973). However, improvement in the state of nutrition during treatment cannot be excluded.

It is of interest to note that though the ability to be sensitized to DNCB had recovered in fully treated patients, the degree of reactivity, as measured by Catalona's (1972) scoring method, remained low. The majority of the normals were 4+ responders while the fully treated patients were mostly 2+ responders. Whether this subtle degree of immunodeficiency contributed towards the development of post-primary tuberculosis in these individuals remains to be seen. Alternatively, they might not have as yet fully recovered from the immunodeficiency induced by the infection.

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