Clin. exp. Immunol. (1975) **22,** 404–408.

SECONDARY IMMUNODEFICIENCY IN MILIARY TUBERCULOSIS

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(*Received* 30 *April* 1975)

SUMMARY

Cell-mediated immune response was investigated in fifteen patients with miliary tuberculosis. Delayed hypersensitivity skin test with 'recall' antigens PPD and SK–SD was positive in two and one patients respectively. An irritant dose of DNCB failed to induce non-specific inflammatory response in the skin of thirteen patients and the same patients also did not develop contact sensitivity to DNCB. Leucocyte migration test in the presence of *Mycobacterium tuberculosis* was also negative in eight of eleven patients studied. The proportion of E rosette-forming cells was found to be significantly depressed, though the proportion of EAC rosette-forming cells did not show any abnormality. On repeat skin tests in five patients after 3 months of chemotherapy and clinical improvement four showed a positive PPD and DNCB response. It was concluded that there is a marked degree of secondary immunodeficiency in miliary tuberculosis.

INTRODUCTION

In a study of cell-mediated immune response (CMIR) in patients with pulmonary tuberculosis, a mild degree of immunodeficiency was observed (Malaviya, Kumar & Dingley, 1973; Malaviya *et al.*, 1975). It was noted that a large proportion of these patients had a poor response to DNCB sensitization but their response to 'recall' antigens purified protein derivative (PPD) and streptokinase–streptodornase (SK–SD) was intact. The proportion of T cells in peripheral blood was also normal. With treatment and clinical improvement the proportion of patients able to be sensitized to 1-chloro-2,4-dinitrobenzene (DNCB) increased but the degree of response remained subnormal. It was concluded that tuberculous infection induced a subtle degree of immunodeficiency. The present work was undertaken to extend the study in patients with miliary tuberculosis which is a more severe and generalized form of the disease. The results show a marked degree of immune unresponsiveness in these patients.

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MATERIALS AND METHODS

The study included fifteen patients with miliary tuberculosis. The criteria for the diagnosis included 'miliary' infiltrate visible in chest X-ray or a miliary pattern of tubercles visualized in multiple organs at surgical examination along with bacteriological or histological evidence and/or dramatic chemotherapeutic response confirming the diagnosis. Fifteen age- and sex-matched controls were selected from among the students, faculty members and the nurses of this institute. However, twenty healthy controls were also studied to establish the normal range for E and EAC rosette-forming lymphocytes in the peripheral blood.

The CMIR was investigated with the following tests.

Delayed hypersensitivity skin tests. PPD (BCG Laboratory, Guindy, Madras) and SK–SD ('Varidase', Lederle, Pearl River, New York) were used as 'recall' antigens. The Mantoux test, using five units of PPD, was performed simultaneously with other skin tests. All precautions were taken to avoid a false negative result as recommended by Edwards (1972). Induration of 10 mm or more developing within 72 hr was regarded as positive. SK–SD skin test was performed with five units of the antigen in 0·1 ml of diluent given intradermally. An induration of 5 mm or more at the end of 72 hr was considered positive. If negative, the test was repeated with fifty units of the antigen in 0·1 ml and interpreted similarly. DNCB (Eastman Organic Chemicals, U.S.A.) skin sensitization was carried out according to the method of Catalona (1972) described briefly in the accompanying paper (Malaviya *et al.*, 1975).

Leucocyte migration test (*LMT*) The technique of Søborg & Bendixen (1967) was used with modifications. Leucocyte-rich plasma was separated from 8–10 ml heparinized blood (50 u/ml) using equal volumes of 2% gelatin (E. Merck, Germany) and 45 min sedimentation at 37°C to remove the red cells. The leucocytes were washed three times in Eagle's minimum essential medium (MEM) and resuspended to a strength of 30–40 $\times 10^6$ cells per millilitre. The leucocytes were packed in 37-mm capillary tubes (Gelman-Hawksley, U.K.) with internal diameter of 1 mm by centrifugation at 200 *g* for 5 min. The capillaries were cut at the cell–fluid interface and the cell containing capillary stub was placed in a perspex migration chamber. The chambers were filled with MEM containing 10% foetal calf serum (Difco, U.S.A.) with or without antigen for test and control migrations respectively. A 15 μg amount per millilitre of heat-killed and ultrasonically lysed *Mycobacterium tuberculosis* $H_{37}Ra$ (kindly supplied by Dr B. Bloom, New York) served as the antigen. Antigen toxicity for leucocytes was tested by the trypan blue dye exclusion test and found to be nontoxic at that dose level. In each test a set of four capillaries were set up with and four without antigen. The chambers were incubated horizontally for 20 hr at 37°C. The area of migration was determined by projection and planimetry. The area of migration in the controls was compared with that of the tests for each set of tests separately using Student's *t*-test of significance and probability calculated. A significant difference in the migration area of control and test was considered positive.

E rosettes. The technique of Jondal, Holm & Wigzell (1972) was used with minor modifications. One per cent, thrice-washed sheep red cells (SRC), not more than one week old, were mixed with a suspension of gelatin-separated, thrice-washed leucocytes at a concentration of 30–40 $\times 10^6$ leucocytes per millilitre in MEM-containing 10% foetal calf serum. After 15 min incubation at 37°C the mixture was sedimented at 200 *g* for 5 min and then incubated in crushed ice for 20 hr. The cell button was gently resuspended, stained with 0·5% methylene blue and a coverslip preparation made. It was sealed with nail-polish and 200 lymphocytes counted to calculate the percentage of rosette-forming cells.

EAC rosettes. The technique of Bianco, Patrick & Nussenzweig (1970) was used with some minor modifications. Five per cent SRC in Hanks's balanced salt solution were sensitized with equal volume of a subagglutinating dilution (1 : 100 for the batch used) of haemolysin (Grand Island Biologicals, U.S.A.) at 37°C for 15 min. After three washes the cells were incubated with 1 : 5 dilutions of fresh mouse serum for 45 min and washed twice. The final suspension of 1% sensitized and complement-coated SRC was made. These cells were mixed with 3–4 $\times 10^6$ cells per millilitre strength of leucocytes separated from blood as for E rosettes. After 15 min incubation at 37°C the suspension was vigorously shaken on a 'vortex mixer' and then stained, prepared and read for rosettes as in the E-rosette test.

RESULTS

Among the patients there were ten females and five males. The age range was eighteen to forty-two years with a mean of 26·5 years. Thirteen patients showed a miliary pattern on

chest X-ray and the other two showed miliary granulomas in the liver and by bone marrow biopsy. Four patients had sputum and cerebrospinal fluid positive for acid-fast bacilli. All the patients were on anti-tuberculous drugs but none were on corticosteroids at the time of the study. Seven patients were put on steroids later in the course of their illness. Among the controls there were nine females and six males. Their age ranged from nineteen to thirty-nine years with a mean of 25·2 years.

Delayed hypersensitivity skin tests

Results are given in Table 1. PPD skin test was positive in two and SK–SD in one of the fifteen patients respectively. In controls these figures were ten and thirteen respectively. Only

TABLE 1. Skin tests in miliary tuberculosis

Subjects	NIR	DNCB Contact sensitization					PPD-positive	SK–SD-positive
		Positive				Negative		
		4+	3+	2+	1+			
Patients (15)	2	0	1	1	0	13	2	1
Control (15)	15	9	2	4	0	0	10	13

two patients (the same who gave positive PPD reactions) could be sensitized to DNCB in contrast to controls where all the subjects were sensitized. The degree of sensitivity in two DNCB positive patients was 3+ and 2+ respectively as compared with the controls who were mostly 4+. All the fifteen controls developed strong nonspecific inflammatory response in the skin at the site of 2000 μg of DNCB application by day 3 but only two of the patients showed such a response and they were the same who gave a positive PPD and DNCB contact sensitization.

Leucocyte migration tests

The results of this test along with its correlation with Mantoux test is given in Table 2. There was good correlation between these two parameters of CMIR except in two normal individuals one of whom was PPD-positive but leucocyte migration test-negative and in the other *vice versa.*

E rosettes

The range of E rosettes in twenty controls was 58–80% with a mean of 68% (standard error of mean 1·17). In the patients the range was 31–78% with a mean of 60% (standard error of mean 3·45%). The difference was found to be statistically significant ($P<0·05$).

EAC rosettes

The range of EAC rosettes in twenty controls was 8–28% with a mean of 16% (standard error of mean 0·71). EAC rosette results were only available in thirteen patients. The range

TABLE 2. Correlation of PPD skin reaction with LMT using *M. tuberculosis* $H_{37}Ra$

	Leucocyte migration test	
	Positive	Negative
Patients		
PPD+	2	0
PPD−	1	8
Controls		
PPD+	8	1
PPD−	1*	5

* See text for details.

was 8–38% with a mean of 20% (standard error of mean 1·95%). This was not found to be statistically different from the controls ($P > 0{\cdot}05$).

Skin tests during follow up.

Five initially PPD- and DNCB-negative patients were retested after 3 months of chemotherapy and clinical improvement. They were given 50 μg of DNCB and five units of PPD and the results read after of 72 hr. Four of these patients gave a positive response to both of these antigens.

In the patient group there was one subject who was PPD negative but in LMT her leucocytes migrated neither in control nor in culture with antigen, repeated three times. She was 7 months pregnant and 3 months later she died suddenly in coma with hemiplegia. It was considered that she might have had a circulating leucocyte migration inhibition factor but this could not be pursued further. In Table 2 she has been placed in the PPD−, LMT+column.

DISCUSSION

These patients with miliary tuberculosis showed marked anergy to 'recall' antigens' In addition they could not be sensitized to a skin sensitizer. The negative leucocyte migration in the presence of the specific antigen confirmed the skin test results. The derangement in the T-cell population in peripheral blood pointed further towards disturbance in cell-mediated immune functions of these patients. These findings indicate much more impressive changes in the immune system of these patients than was observed in pulmonary tuberculosis (Malaviya *et al.*, 1975).

In one other published report on the study of immune response in a single patient with miliary tuberculosis (Waxman & Lockshin, 1973) the patient lacked the capacity to mount an inflammatory response, could not be sensitized to DNCB, had negative delayed hypersensitivity response to PPD and SK–SD and did not show migration inhibition with PPD. These findings are consistent with the present observations. However, these workers found a positive LMT which is not consistent with the results of the present study. In this regard it

may be pertinent to note that Waxman & Lockshin (1973) used 300 μg/ml of PPD for LMT, a concentration which could have been toxic to leucocytes.

The present work does not clarify the basic nature of the immunological defect in miliary tuberculosis. Inability to mount a non-specific inflammatory response alone might explain the negative delayed hypersensitivity skin reactions. In several conditions with skin anergy a serum factor which inhibits chemotaxis has been demonstrated (Dennis, Palmer & Williams, 1974). However, this cannot explain all the findings of the present study. The negative LMT indicates a defect either in lymphocytes or the indicator cells. The low proportion of T cells in the peripheral blood also suggests a defect in the central component of the immune response.

So far only five patients have been retested in the follow-up study. In four of them PPD reaction became positive. It is interesting that the same four patients also showed a positive response to DNCB within 72 hr of the challenge dose. This period is not sufficient for sensitization to take place. Obviously these persons were sensitized during the earlier application of DNCB indicating that at the time of initial testing the afferent limb and central component of the immune response were intact.

These results suggest that there is a major defect in the expression of the immune response in miliary tuberculosis. In addition there may be a qualitative and/or quantitative abnormality in peripheral blood T cells to be sensitized as shown by the negative LMT with specific antigen. A change in the T cell peripheral blood population also points to such a defect. An intact central component with a derangement in the proportion of sensitized peripheral blood T cells could result from an abnormal traffic pattern of T lymphocytes as has been shown in experimental leprosy by Bullock (1974).

The defect seems to be related to the tuberculous infection itself as in pulmonary tuberculosis (Malaviya *et al.*, 1975). Whether the more marked changes in miliary tuberculosis indicate merely a quantitative change or also a qualitative changes remains to be elucidated.

We thank Prof. Sneh Bhargava for the interpretation of the chest roentogenograms and Mr R. L. Taneja, Mr R. Dhan and Mr S. Ram for excellent technical help.

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