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THE POSSIBLE ROLE OF SOLUBLE MATERIAL FROM MACROPHAGES IN CELL MEDIATED IMMUNITY IN PULMONARY TUBERCULOSIS

RAJI SWAMY, R. PRABHAKAR, AND P.R. NARAYANAN

Summary : Lymphocytes from pulmonary tuberculosis patients and healthy controls showed identical proliferative responses to mitogen (PHA) and antigen (PPD) on Day 'O' (DO), Day-2 (D2) and Day-7 (D7) of culture. Also, there was no suppression of PHA induced lymphocyte proliferation in the presence of culture supernatants of pulmonary tuberculosis patient's mononuclear cells.

Introduction

Immunosuppression by macrophages has been reported in tuberculosis (Elinor, 1978) and sarcoidosis (Goodwin, 1977, 1979). Though the precise mechanism of the immunosuppression is poorly understood, several macrophage mediators have been identified, such as thymidine (Opitz et al., 1975), Prostaglandins (Morley, 1974), Oxygen metabolites (Grey et al. 1979), Arginase (Kung et al., 1977), Complement components (Allison, 1978). The specificity and in vivo role of these mediators in immunosuppression are yet to be determined.

While investigating the immunosuppression in chronic fungal infection, Stobo (1977) has shown that patients' macrophages liberated, *in vitro,* a soluble material (SM) capable of inhibiting the blastogenic response of normal T-cell to mitogens and antigens. However, not all T-cells were equally susceptible to its suppressive effect. Only the short-lived, low-density T-cells in fresh peripheral blood mononuclear cells (PBMC) could be suppressed by the SM. Hence, incubation of PBMC for seven days in culture fluid could result in a functional deletion of the short lived low density T-cells and yielded high density T-cells whose reactivity could not be suppressed by SM.

In this report, we have looked for the possible existence of a similar suppressive mechanism in pulmonary tuberculosis.

Material and Methods

Subjects : The patient group consisted of 12 pretreatment pulmonary tuberculosis patients admitted to the controlled clinical trial of the Tuberculosis Research Centre. The sputum of all the patients was positive by smear a Twelve healthy blood bank volunteers served as controls.

Twenty millilitres of blood was drawn from each individual in a heparinised container and PBMC separated on Ficoll hypaque (Boyum, 1968). The cells were washed three times with Hank's balanced salt solution (HBSS) and suspended at a concentration of 0.75 X 10^6 cells per ml. in RPMI-1640, containing 2 mM Glutamine, 80 ug/ml gentamycin and 10% pooled heat-inactivated human AB serum (CRPMI) and placed in plastic tissue culture flasks at 37°C in a humidified atmosphere of 95% air and 5% CO_2. On O, 2 and 7 days the cultures were terminated by centrifugation. The cells were washed, resuspended at 0.5x 10^6 cells per ml in fresh CRPMI and a lymphocyte proliferation test was performed. Triplicates of 0.2 ml of cells were placed in a 96 well microculture plate and the cultures were stimulated with either 5 ug/ml Phytohemagglutinin (PHA) or 50 ug/ml PPD and maintained for 4 and 6 days, respectively. Eighteen hours before harvesting, 1 uCi ^{3}H-thymidine was added to each well and the cells were harvested in a semi-automated cell harvester on fibre glass filter paper discs. Radioactivity of the discs was measured in a liquid scintillation counter. The results were expressed as stimulation index (S.I.).

$$\text{S.I.} = \frac{\text{Mean log count stimulated culture}}{\text{Mean long count control culture}}$$

In addition, the supernatant culture fluid obtained on day-2 and day-7 of the PBMC was added in a final concentration of 25% to 2 normal PBMC suspended in CRPMI and the PHA reactivity of this mixture tested. Stimulation index was calculated as described above.

Address for Correspondence : Dr. P.R. Narayanan, Deputy Director (Immunology), Tuberculosis Research Centre, Spur Tank Road, Chetput, Madras - 600 031, INDIA.

Address for reprints : Dr. R. Prabhakar, Director, Tuberculosis Research Centre, Spur Tank Road, Chetput Madras - 600 031, INDIA.

Results and Discussion

Stimulation Index for PHA and PPD induced lymphocyte proliferation on day O, 2 and 1

PBMC were incubated for seven days in plastic tissue culture flasks and lymphocyte proliferation tests were done on days 0, 2 and 7. The results of these tests are presented in Table 1. Increasing duration of incubation did not result in increased stimulation of the patients' lymphocytes. In Stobo's work (1977) there was an eight-fold rise in the proliferative response of patients' lymphocytes on day 7 which was attributed to the functional deletion of low density, short—lived T-lymphocytes. These lymphocytes which are susceptible to the suppressive SM liberated by the patients' macrophages were responsible for the inhibition of lymphocyte proliferation on day 'O'. Since lymphocytes from tuberculosis patients have not shown any enhancement of stimulation, either by antigen or by mitogen, it may be concluded that tuberculous macrophages do not produce the suppressive material as macrophages from chronic fungal infection do. The results of the second experiment also suggest the same.

Stimulation Index for normal PBMC in the presence of 25% *culture supernatant of control and tuberculous PBMC and* 5 *ug/ml PHA*

A lymphocyte proliferation test for PHA (5ug/ml) was done on two healthy blood bank volunteers' lymphocytes in the presence of 25 % culture supernatant collected on day 2 and day 7 of control and tuberculous PBMC cultures. The results are shown in Table 2. Tuberculous PBMC supernatant have failed to show any

TABLE 1

Mean ± S.D. of Stimulation Index of lymphocytes stimulated with 5 ug/ml PHA or 50 *ug/ml PPD on Day 'O', 2 and 7 of culture*

Days	Control (12)		Patient (12)	
	(PHA)	(PPD)	(PHA)	(PPD)
D—0	1.53 ± 0.09	1.12 ± 0.11	1.53 ± 0.17	1.13 ± 0.12
D—2	1.56 ± 0.12	1.13 ± 0.08	1.51 ± 0.13	1.10 ± 0.11
D—7	1.37 ± 0.13	1.16 ± 0.15	1.37 ± 0.18	1.18 ± 0.18

N.B. ; Numbers in parantheses indicate number of individuals tested.

TABLE 2

Mean ± S.D. of Stimulation Index for PHA (5 ug/ml) induced lymphocyte proliferation in the presence of 25 % *culture supernatants on two normal lymphocytes*

No.	Control SN (10)		Patient SN (10)		SN
	Day 2	Day 7	Day 2	Day 7	0%
1.	1.37 ± 0.05	1.39 ± 0.09	1.33 ± 0.11	1.38 ± 0.06	1.33
2.	1.33 ± 0.09	1.32 ± 0.06	1.26 ± 0.09	1.32 ± 0.09	1.28

N.B. : Numbers in parantheses indicate number of individuals tested.

SN = Supernatant.

suppression of the SI of normal lymphocytes. The supernatant from the control group also gave similar results.

To conclude, the present results suggest that tuberculous macrophages may not be producing suppressive soluble material as was reported for chronic fungal infection. According to Ellner (1978), circulating suppressor monocytes are responsible for the diminished response to PPD induced lymphocyte blast transformation in low responders in tuberculosis and was able to show a 24-fold enhancement of lymphocyte proliferation in these patients by depletion of adherent monocytes. Further, it was also observed that this effect could not be mediated through monocyte cell supernatants.

Thus, the results of the experiment described above suggest that the possible approach to the defect in cell mediated immuity in tuberculosis could be studies related to evaluation of microbicidal properties of macrophages stimulated with appropriate lymphokines.

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