V EVALUATION OF CELL MEDIATED IMMUNITY IN THE HISTOPATHOLOGIC SPECTRUM OF LEPROSY USING LYMPHOCYTE TRANSFORMATION TEST

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In a number of studies it has been shown that there is a generalized impairment of cell mediated immunity in lepromatous patients (1, 2, 3, 4, 5). These include experiments wherein considerable lengthening of the survival time of homologous skin grafts in lepromatous patients has been demonstrated (6, 7). It has also been shown that the transformation of lymphocytes in response to phytohemagglutinin (PHA) is much reduced in leprosy patients as compared to normal individuals (8, 9, 10). However, there are other reports which do not support these findings and have failed to find any difference in the response of lymphocytes of patients and normal individuals (11, 12).

Further, it is reported that the immune responsiveness of lymphocytes to <u>M. leprae</u> is highest in tuberculoid patients and it gradually decreases towards the lepromatous patients and reaches a point of negative response at the lepromatous end of the spectrum (13). This coincides very well with lepromin reaction which is strongly positive at the tuberculoid end, variable in the borderline groups, and totally negative at the lepromatous end of the spectrum (14, 15). No study has been done yet to confirm the spectral responsiveness of lymphocytes from leprosy patients to the antigenic stimulus of M. leprae.

Although the suppression of impairment of the cell mediated immune response of lepromatous patients appears to be generalized as evidenced by its reaction to PHA, it has been brought out by several workers that the defect in the cell mediated immunity is highly specific to <u>M. leprae</u>. Lepromin negative lepromatous patients are responsive to skin tests with mycobacterial antigens other than <u>M. leprae</u> (4, 13, 15, 16, 17, 18). Total absence of response of lymphocytes to <u>M. leprae</u> seems to be the defect in lepromatous patients.

In this investigation it has been aimed to study the response of all varieties of leprosy patients of Indian origin (skin smear positive and

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negative, PPD positive and negative) to antigens such as PHA, <u>M. leprae</u> and BCG and to compare their responses with those of normal controls, and to verify and confirm the findings of earlier studies.

## MATERIALS AND METHODS

The patients chosen were from Schieffelin Leprosy Research Center, Karigiri, Tamil Nadu, in South India. The lesions representative of the disease from all patients under study were biopsied and classified according to Ridley and Jopling (19), by one of us with no reference to the clinical picture. Thirty-five patients were polar lepromatous (LL); of these, 24 were smear positive, 11 smear negative. Sixteen were polar tuberculoid (TT), 10 borderline lepromatous (BL), and 10 borderline tuberculoid (BT). The mid-borderline (BB) patients were extremely rare in our experience and were not included in the study. The normal controls were 10 healthy staff members who volunterred for the study from the leprosy center.

All the patients had antileprosy treatment with DDS varying from 4 months to 20 years. All of them and the normal controls had a skin smear test, lepromin test  $(1.6 \times 10^8/\text{ml})$  and PPD test (10 T.U. per dose) done on them. The PPD test was read at the end of 48 hours. The lepromin test was read at the end of 21 days and the skin nodule was measured in millimeters.

The lymphocyte transformation test (LTT) was carried out according to the method adopted by Dr. Ravinder Maini of the Kennedy Institute, London (personal communication). It is a modified micromethod for LTT.

Venous blood was drawn in a heparinized, siliconised tube containing 0.01 ml of heparin/ml of blood. The blood was mixed well with heparin. The cultures were done in quadruplicate containing 0.9 ml of Eagles medium, 0.1 ml of heparinized blood, and the antigen in the required concentration was incorporated in the culture. The antigens used per culture were 50  $\mu$ g/ml, phytohemagglutinin, and 1.0 x 10<sup>7</sup> BCG, and 1.0 x 10<sup>7</sup> M. <u>leprae</u>. Autoclaved suspension of M. leprae and live BCG were used in the study.

The cultures were incubated at 37°C in the presence of 5% carbon dioxide for a period of 4 days in cultures stimulated with phytohemagglutinin and 6 days for other antigens used. Eighteen hours before the harvesting of cells, the cultures were pulsed with 2  $\mu$ C of 3H-thymidine in 0.1 ml of the culture medium. During harvesting, the culture media was separated out, the red blood corpuscles were lysed with glacial acetic acid and the white cells were washed with saline several times. The DNA was precipitated with 5% trichlor acetic acid and washed with methanol until it was free of water. The DNA was later dissolved in 0.5 ml of hyamine hydroxide at 56°C for 20 minutes and transferred to the counting vials with scintillation fluid consisting of 10 ml of toluene phosphor. Beckman's liquid scintillation counting chamber was used for counting. The results were obtained as counts per minute (CPM/Min). The later results were expressed as ratio of test and control.

### RESULTS

PPD Test. The control subjects were all PPD positive (Table 1). Of the 16 TT patients, 10 were PPD positive. Of the 10 BT and 10 BL patients, 4 from each group were PPD positive. In the LL group, 8 of the 24 smear-positive patients, and none of the 11 smear-negative patients were PPD positive.

Classification	PPD positive	PPD negative	Total
Control	10	Nil	10
Tuberculoid (TT)	10	6	16
Borderline tuberculoid (BT)	4	6	10
Borderline lepromatous (BL) Lepromatous leprosy (LL)	4	6	10
smear positive Lepromatous leprosy (LL)	8	16	24
smear negative	Nil	11	11

TABLE 1. TUBERCULIN REACTION

Lepromin Test. Lepromin nodules were above 5 mm in diameter in all controls except two which were 4 mm in diameter. A lepromin nodule was present in all TT and BT patients. In three of the TT patients and seven of the BT patients they were only 4 mm in diameter. In all others they were above 5 mm in diameter. The lepromin test was negative in all LL patients. In 4 of the 10 BL patients, lepromin nodules were present, of which 2 were below 3 mm and in 2 above 5 mm in diameter.

TABLE	2.	LEPROMIN	REACTION

Classification	Lepromin positive < 5 mm	Lepromin positive > 5 mm	Lepromin negative	Total	
Control	8	2	Ni1	10	
Tuberculoid (TT)	13	3	Ni1	16	
Borderline tuberculoid (BT)	3	7	Ni1	10	
Borderline lepromatous (BL) Lepromatous leprosy (LL)	2	2	6	10	
smear positive Lepromatous leprosy (LL)	Ni1	Ni1	24	24	
smear negative	Ni1	Ni1	11	11	

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Lymphocyte Transformation Test. The control cultures of blood from patients with no mitogens or antigens showed little incorporation of radioactive thymidine and CPM were mostly below the value of 100, whereas the control cultures from a large majority of the normal control subjects were higher than 100 and in one it reached over 300.

Response to PHA. The values of tritiated thymidine labelled DNA synthesized after PHA stimulation is found in Fig. 1. The response of lymphocytes was expressed as the ratio between the average of 3H-thymidine uptake in antigen containing cultures and that in control cultures.

FIG. 1. LYMPHOCYTE RESPONSE TO PHA



It was found that the response of lymphocytes from control subjects to PHA was considerable and was far above that of all types of patients. There was no statistically significant difference in the PHA responses among the different types of leprosy patients. They were all uniformly low. It was also found that there was no statistically significant difference between lepromatous smear negative and smear positive patients in their response to PHA or any other antigens used (Fig. 2).

## FIG. 2. LYMPHOCYTE RESPONSE TO PHA, BCG AND M. leprae TO SMEAR POSITIVE AND SMEAR NEGATIVE LEPROSY PATIENTS



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Response to M. leprae. 3H-thymidine uptake of lymphocytes was the highest in the polar TT patients and was gradually reduced towards the lepromatous end and was the lowest in the lepromatous patients (Fig. 3). Although the response of control subjects was less than that of TT patients, the difference was not statistically significant.

There was steady fall in the responsiveness of lymphocytes to <u>M</u>. <u>lep-rae</u> from TT towards LL (Fig. 4). However, the response of only LL patients showed statistically significant difference from that of normal control subjects (P < 0.05).



FIGS. 3 and 4. LYMPHOCYTE RESPONSE TO M. leprae.





Response to BCG. The response of the lymphocytes to stimulation by BCG is shown in Fig. 5. There was no correlation between response to BCG and classification of leprosy. However there was statistically significant difference between the response of PPD-negative patients and PPD-positive patients to BCG in all the different types of leprosy patients (P < 0.05) (Fig. 6).

FIG. 6. LYMPHOCYTE RESPONSE TO BCG OF PPD-POSITIVE AND PPD-NEGATIVE LEPROSY PATIENTS.



The high response in the LTT to BCG correlated very well with the positive reaction to PPD.

## DISCUSSION

In our study, the lymphocyte transformation to PHA has shown a significant reduction in all forms of leprosy patients both lepromatous and nonlepromatous. Nelson et al (10) have found depression of PHA-stimulated transformation of lymphocytes of all patients classified as stable compared with normal cells. There is much evidence to support the view that there is a generalized depression of cell mediated immunity in leprosy patients (1, 8, 9, 20). A more severe depression in lepromatous leprosy patients than in other forms is also shown. Even among them, there seems to be a difference between patients who are highly bacillated and who are negative for acid-fast bacilli (8). In our

experience there is no difference in the responsiveness between active lepromatous patients with positive skin smears and resolving ones with negative bacteriologic findings.

Myrvang et al (13) in an original and masterly study have very clearly demonstrated the spectral concept of leprosy using lymphocyte transformation and leucocytic migration inhibition tests. In our study, it is possible to confirm their findings that the immune responsiveness of lymphocytes to M. leprae gradually increases from a poor response in LL patients towards TT patients in whom the response is the highest even higher than lepromin-positive normal controls.

It is interesting to note that the response to PIIA in normals is far higher than the TT patients, although response to M. leprae in them is comparable. The LTT is certainly of great value in evaluating the immune responsiveness to M. leprae of leprosy patients, and is comparable to the lepromin test.

The response to BCG has no correlation at all with the different forms of leprosy but there is statistically significant correlation with PPD positive patients even within each group of leprosy patients. Although there are antigens in M. leprae common to BCG, the immune response of lymphocytes to BCG is specifically confined largely to those in whom PPD is positive. It is interesting to note that PPD positive LL patients showed responsiveness to BCG comparable to normal controls and other forms of leprosy. Therefore it is reasonable to conclude that the defective immune response to M. leprae in lepromatous patients is specific.

### SUMMARY

Lymphocyte transformation in response to PHA, <u>M. leprae</u> and BCG with the use of measurements of 3H-thymidine uptake, was studied in 10 normal controls, and 71 leprosy patients of different types histologically classified according to the Ridley-Jopling scale. Lepromin and PPD tests were done in each one of them. It was found that there was a generalized depression of immune responsiveness to PHA in all forms of leprosy. The immune responsiveness of lymphocytes to <u>M. leprae</u> continuously and gradually increases from the lepromatous (LL) towards the tuberculoid (TT) end of the spectrum. The loss of immune response of lymphocytes from lepromatous patients to <u>M. leprae</u> is specific as evidenced by their capacity to respond selectively to BCG when PPD-positive, irrespective of their place in the leprosy spectrum.

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### REFERENCES

- Bullock, W. E., (1968): Studies of immune mechanisms in leprosy.

   Depression of delayed allergic response to skin test antigens.
   New Engl. J. Med. 278:298.
- 2. Turk, J. L. and Waters, M. F. R., (1968): Immunological basis for depression of cellular immunity and the delayed allergic response in patients with lepromatous leprosy. Lancet ii:436.
- 3. Turk, J. L. and Waters, M. F. R., (1969): Cell mediated immunity in patients with leprosy. Lancet ii:243.
- Godal, T., Myklestad, B., Samuel, D. R. and Myrvang, B., (1971): Characterization of the cellular immune defect in lepromatous leprosy: a specific lack of circulating <u>Mycobacterium</u> <u>leprae</u>-reactive lymphocytes. Clin. Exp. Immunol. 9:821.
- 5. Turk, J. L. and Bryceson, A. D. M., (1971): Immunological phenomena in leprosy and related diseases. Advanc. Immunol. 13:209.
- 6. Job, C. K. and Karat, A. B. A., (1967): Personal observations.
- 7. Han, S. H., Weiser, R. S. and Kau, S. T., (1971): Prolonged survival of skin allografts in leprosy patients. Int. J. Leprosy 39:1.
- 8. Dierks, R. E. and Shepard, C. C., (1968): Effect of phytohemagglutinin and various mycobacterial antigens on lymphocyte cultures from leprosy patients. Proc. Soc. Exp. Biol. (N.Y.) 127:391.
- 9. Bullock, W. E. and Fasal, P., (1971): Studies of immune mechanism in leprosy. III. The role of cellular and humoral factors in impairment of the in vitro immune response. J. Immunol. 106:888.
- Nelson, D. S., Nelson, M., Thurston, J. M., Waters, M. F. R. and Pearson, J. M. H., (1971): Phytohemagglutinin-induced lymphocyte transformation in leprosy. Clin. Exp. Immunol. 9:33.
- 11. Sheagren, J. N., Block, J. B., Trautman, J. R. and Wolff, S. M., (1967): Immunologic reactivity in leprosy. Clin. Res. 15:300.
- Sheagren, J. N., Block, J. B., Trautman, J. R. and Wolff, S. M., (1969): Immunologic reactivity in patients with leprosy. Ann. Int. Med. 70:295.
- Myrvang, B., Godal, T., Ridley, D. S., Froland, S. S. and Song, Y. K., (1973): Immune responsiveness to <u>Mycobacterium leprae</u> and other mycobacterial antigens throughout the clinical and histopathological spectrum of leprosy. Clin. Exp. Immunol. 14:541.

- 14. Dharmendra, (1948): The Lepromin Test. Belcra Medical Series, No. 1. London.
- Rees, R. J. W., (1964): The significance of the lepromin reaction in man. Prog. Allergy 8:224.
- Job, C. K., (1969): Isopathic phenomenon in lepromatous leprosy. A reappraisal. Int. J. Leprosy 37:365.
- Guinto, R. S., (1968): Skin tests in leprosy. Ann. NY Acad. Sci. 154:149.
- 18. Godal, T., Myrvang, B., Froland, S. S., Shao, J. and Melaku, G., (1972): Evidence that the mechanism of immunological tolerance (central failure) is operative in the lack of host resistance in lepromatous leprosy. Scand. J. Immunol. 1:311.
- 19. Ridley, D. S. and Jopling, W. H., (1966): Classification of leprosy according to immunity. A five-group system. Int. J. Leprosy 34:255.
- Waldorf, D. S., Sheagren, J. N., Trautman, J. R. and Block, J. B., (1966): Impaired delayed hypersensitivity in patients with lepromatous leprosy. Lancet 2:773.