A STUDY OF BLASTOGENIC TRANSFORMATION UNDER PHYTOHEMAGGLUTININ (PHA) IN LEPROSY

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Summary

The present study was aimed to assess the cell mediated immune response under PHA stimulus in leprosy patients and healthy contacts. Twenty one cases suffering from leprosy were included in the study. They were diagnosed clinically, by skin biopsy, smear examination and lepromin reaction. Five cases had lepromatous, 7 borderline and 9 tuberculoid leprosy. Eleven were healthy contacts.

Lepromin reaction was negative in 5 patients in lepromatous group while it was positive in the other patients and healthy contacts. Blastogenic transformation was depressed only in patients with lepromatous leprosy. In tuberculoid and borderline cases, lymphocytes exhibited similar reactivity with PHA as was shown by the controls. Healthy contacts of lepromatous leprosy patients were lepromin positive and did not show any C.M.I. depression. It is suggested that depression of PHA response may develop after the onset of leprosy infection.

KEY WORDS : C. M. I. Response, leprosy patients, leprosy contacts.

Ridley and Jopling[1] described leprosy as a clinicopathological and immunological entity. The disease manifests in a spectrum ranging between two polar forms viz. tuberculoid and lepromatous leprosy, the place in the spectrum being dependant on the immunological status of a person. Immunological studies in vitro provide enough evidence to believe that there is impairment of cell mediated immunity in patients suffering from leprosy, especially of the lepromatous type[2-7]. The nature of impairment of cell mediated immunity is not clear. Whether this impairment is of a general type or limited to the mycobacterium leprae antigen or because of the bacterial load on the cell itself, is yet to be worked out. The role of heredity, genetics and hormones in determining the immunity state of an individual have also been considered[8].

Material and Methods

The study was conducted in the departments of Paediatrics and Dermatology, S. N. Medical College, Agra in collaboration with JALMA (Japanese Leprosy Mission for Asia) Institute, Agra. Twenty one untreated leprosy patients and eleven healthy children of parents suffering from leprosy contacts were examined. The patients were

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classified on the basis of clinical findings, skin smears and histological changes of skin. Biopsies were taken from active skin lesions and fixed in 10% formalin. Routine processing and staining with haemotoxylin-eosin and Ziehl-Neelsen techniques were carried out.

The patients were classified into five groups namely, (i) polar lepromatous (LL), (ii) polar tuberculoid (TT), (iii) borderline or dimorphous (BB), (iv) subpolar lepromatous (BL) and (v) tuberculoid (BT).

Lepromin Test

All patients were examined for reactivity to lepromin, prepared by the method of Dharmendra[8]. The source of lepromin was JALMA Institute, Agra. The concentration was adjusted to contain 160×10^6 bacilli per ml. The dose given was 0.1 ml. intradermally. Interpretation of results was made according to WHO recommendations.[9] The positive responses were recorded as 1+, 2+ and 3+ on the basis of the size of flare and induration.

Lymphocyte transformation

The technique used was a recent modification of Turk and Water[10].

Observations

All the cases were normal for their anthropometric measurements by Indian standards[11]. No evidence of malnutrition was detected in the children.

a) Out of twenty-one patients, five had lepromatous leprosy (LL) as judged by clinical features, bacteriological and morphological index. The histopathological changes of skin in two patients showed features of polar lepromatous leprosy, one had borderline lepromatous (BL) features, one had borderline lepromatous features with lepromatous preponderance (BL-LL) and another one had changes suggestive of borderline leprosy (BL-BB).

b) Three patients were clinically determined to have borderline leprosy (BB). Histopathological changes were consistent with the clinical classification in two of these while one had features of borderline tuberculoid.

(c) Four patients were clinically classified under the borderline tuberculoid (BT) group. Histopathological picture revealed variable preponderance of either borderline or tuberculoid leprosy features.

(d) Eight were clinically classified under the tuberculoid leprosy (TT) group and were bacteriologically negative. Histopathologically, five of them were classified as tuberculoid (TT), three in tuberculoid to borderline-tuberculoid (TT-BT) group.

(e) One case belonged to the indeterminate group (I).

Lepromin reaction

All the five cases of lepromatous leprosy group (LL) showed negative lepromin reaction. The lepromin reaction was weakly positive in 3 cases of borderline leprosy (BB). The tuberculoid (TT) and borderline Tuberculoid (BT) patients (8 cases) had moderate to strong positive reaction with lepromin.

Four out of 11 healthy contacts reacted strongly (3+) to lepromin antigen while seven showed moderate reaction.

Blast cell transformation

Blast cell transformation with PHA antigen was studied in these cases. All the 5 patients with lepromatous and borderline lepromatous (LL-BL) group

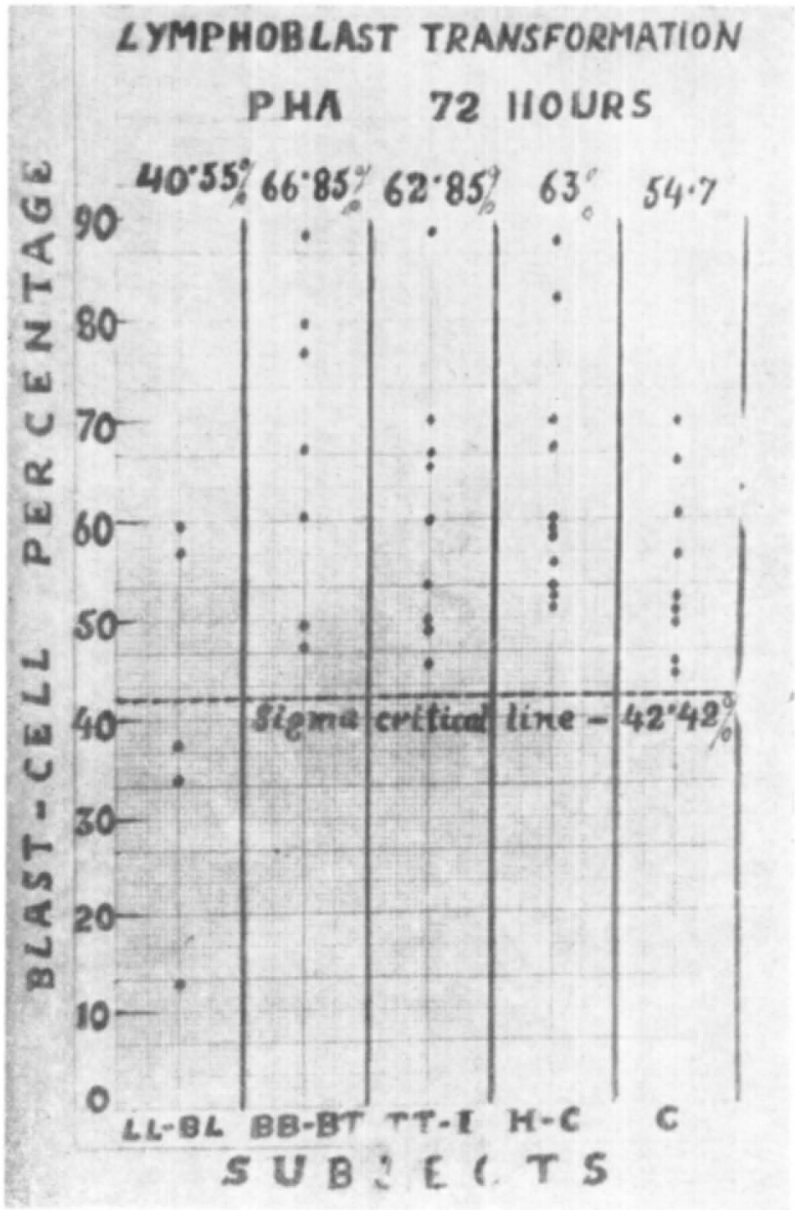
TABLE 1

Blast Cell Transformation with Phytohaemagglutinin

Age in years	Sex	Clinical Status	Histopathological Diagnosis	Bacterial Index	Lepromin test Early mm.	Lepromin test Late mm.	Percentage of Blast Cells Patients	Percentage of Blast Cells Control	
9	F	BL	BL,LL	+++	-ve	-ve	13.87	48.63	t=2.0697, p>.05
14	M	LL	LL	+++++	-ve	-ve	37.62	53.50	
17	M	BL	BL	+++	-ve	-ve	34.25	60.50	
10	M	LL	LL	+++++	-ve	-ve	59.75	66.33	
16	M	BL	BL,BB	+++	-ve	-ve	57.25	69.38	
13	M	BB	BB	+++	5	4	88.25	51.37	t=1.4359, p>.05
14	F	BB	BB	+	44	4	67.37	66.33	
15	M	BB	BB-BT	++	5	5	79.87	69.38	
12	F	BT	BT-TT	+	3	5	60.125	51.37	
14	M	BT	BT-TT	—	5	6	47.5	51.12	
14	F	BT	BT	+	36	6	77.62	69.38	
6	M	TT	TT	-ve	5	9	65.50	51.37	t=2.3061, p>.05
9	M	TT	TT	-ve	11	6	70.0	51.37	
13	F	TT	TT	-ve	12	12	59.87	51.37	
14	M	TT	TT-BT	-ve	7	12	46.50	44.00	
15	F	TT	TT-BT	-ve	6	7	49.62	48.63	
8	M	I	I	-ve	5	6	49.75	44.19	
14	M	TT	TT	-ve	7	8	53.50	44.19	
13	M	TT	TT	-ve	12	10	88.62	56.50	
16	M	TT	TT-BT	-ve	7	7	67.25	56.60	

showed depression of blast cell transformation as compared to their controls. Out of these five patients, three showed marked depression of blast cell response and their values were below sigma critical level while rest of the two patients also had low response but the values were above sigma critical level 42.42% (Table 1). On statistical analyses, the difference was insignificant ($t = 2.0697$, $p > .05$)

In seven patients with borderline (BB) and borderline tuberculoid (BT) leprosy, the blast cell transformation showed good response. The average blast cell response was 66.46% which is quite above the sigma critical level.



Similarly, average response in tuberculoid and indeterminate leprosy group was 62.85%. In healthy contacts of leprosy patients the average blast cell response observed was 63% and this value was also above the sigma critical line. Obviously, none of the patients in these groups showed depression in blast cell response (Table I and Graph I), and the difference with the controls was statistically insignificant ($t = 1.4359$ and 2.3061; $p > .05$).

Discussion

Acquired cellular immunity is currently viewed to be responsible for immunity in mycobacterial infection as exemplified in tuberculosis[12,13]. This is mediated by macrophages and lymphocytes and can be studied by observing the transformation of circulating lymphocytes into "blast cells" by stimulation with non specific mitogen P.H.A[14].

Various authors [2-5,7] have suggested that there is an altered immunological response in lepromatous leprosy patients. In our study we observed that there is depression in the blastogenic response under PHA in the lepromatous (LL) leprosy patients. However, the depression in blast cell response was not unifrom in all cases of lepromatous group, as two of them did not reveal significant depression and the difference was statistically insignificant. The lymphocytes from patients of lepromin positive BB and TT group revealed no statistical difference of blast cell formation as compared to normal control. Similar results had been shown by Rodriguez Paradisi et al[16].

In our study, five patients with lepromatous leprosy(LL) had shown CMI depression as evidenced by depressed response of blastogenic transformation under PHA. None of these had clinical signs of malnutrition. It appears that the depression of CMI is dependent on bacterial load rather than nutritional status. Jayalakshmi and Gopalan[17], Harland[18], Lloyd[18], Smythe et al[19] and Jose and Good[20] conducted studies on severely malnourished individuals and reported that CMI depression was independent of nutritional status or hereditary factor.

As suggested by the present study that lymphocyte transformation in TT and BB leprosy patients is more than the sigma critical line and cannot be differentiated from normal individual, it cannot be predicted, as to which healthy person is going to suffer from TT or BB form of the leprosy. Secondly, in majority of LL patients, there is depression of lymphoblast formation but a minority show variable results, and the difference is statistically insignificant, thus making it difficult to predict LL form of leprosy.

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