Transfer of Cell-Mediated Immunity in Leprosy by Transfer of Lymph Node Cells[1]

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Landsteiner and Chase ([7, 8]) demonstrated the transfer of cell-mediated immunity for tuberculosis by lymphocytes in inbred guinea pigs. The experiment could not be repeated in nonsyngeneic animals. They presumed that living cells were necessary for transfer of cell-mediated immunity (CMI).

Lawrence ([9]) demonstrated the transfer of CMI in tuberculosis in non-inbred guinea pigs. He and his associates further showed ([12]) that transfer of immunity could be achieved by using cellular extracts, and even by a dializable factor obtained from sensitized leukocytes. This factor is heat stable at 56°C for 30 minutes. It is a dializable moiety of <10,000 molecular weight which is not immunogenic nor immunoglobulin-like. Lawrence ([9]) demonstrated the practical use of the transfer factor (TF) in human tuberculosis by injecting subcutaneously 85×10^6 leukocytes from tuberculin positive to tuberculin negative persons. The degree of transferred sensitivity was measured *in vivo* by the tuberculin test and *in vitro* by lymphocyte transformation. The negative recipients became sensitized within 48 hours and sensitivity remained up to two years ([10]).

Lawrence ([11]) postulated the use of transfer factor in leprosy. De Bonaparte *et al* ([3, 5]) demonstrated such transfer in leprosy in 5 of 13 cases as measured by lepromin skin testing.

Dierks and Shepard ([4]) and Paradisi *et al* ([14]) have reported that lymphocytes from lepromatous leprosy patients do not undergo transformation as freely as those from normal individuals when phytohemagglutinin (PHA) and the mycobacterial antigens are added. The response to PHA was moderately depressed in tuberculoid leprosy. Similar results were obtained by Bullock and Fasal ([2]) who showed that the addition of PHA impaired the rate of DNA synthesis in leukocytes cultured from leprosy patients. Wong *et al* ([16]) similarly observed a depression in immunological response in leprosy.

In the present study an attempt has been made to transfer cell-mediated immunity from lepromin positive (tuberculoid) to lepromin negative (lepromatous) patients. This study reports the findings of lepromin testing and of lymphocyte transformation in seven lepromatous recipients before and after the transfer of lymph node cells from a tuberculoid donor.

MATERIALS AND METHODS

Selection of patients. Lymphocytes were obtained from the venous blood of the following three groups: 1) normal controls; 2) lepromatous leprosy; 3) tuberculoid leprosy at the Tata Department of Plastic Surgery, J.J. Hospital in Bombay. Brief history of the patients is summarized in Table 1.

Preparation of lymphocyte cultures. Ten milliliters of venous blood were collected in 4 ml of 6% Dextran citrate solution and allowed to sediment at room temperature (1-2 hrs), the supernatant fluid of leukocyte-rich plasma was withdrawn and an equal volume of Hank's BSS was added to the supernatant. Relatively rich, 73-75% lymphocyte culture was obtained. The cell suspension was centrifuged at 2,000 rpm for ten minutes and the resulting pellet was diluted in TC-199 (Difco) with 15% AB group human serum to a concentration of 1×10^6 cells/ml; penicillin 100 units/ml, and streptomycin 100 mg/ml were added. Two tubes from each patient containing 5 ml of the above cell suspension was incubated at 37°C in 3% CO_2. PHA-P (Difco) 1 μl/ml and lepromin (R. J. W. Rees, London) containing 1.1×10^7 *M. leprae* bacilli in 1.4×10^2 ml were added per ml of suspension and incubated for three days following which a morphological count was made of the cells to ascertain the percentage of lymphoblast transformation. The cells were washed in hypotonic

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TABLE 1. *Brief history of the patients.*

No.	Leprosy type[a]	Duration of leprosy (in years)	Treatment[b]	Lepromin test (3 week)
1	LL	6	±	—
2	LL	20	±	—
3	LL	22	+	—
4	LL	22	+	—
5	LL	17	+	—
6	LL	14	+	—
7	LL	22	+	—
8	TT	5	+	6 mm
9	TT	25	+	11 mm
10	TT	12	+	5 mm

[a] LL = lepromatous leprosy; TT = tuberculoid leprosy.
[b] + = regular; ± = irregular or doubtful.

TABLE 2. *Lymphocyte transformation in normal controls.*

No.	PHA-P%	Lepromin %	Lepromin skin test (3 week)
1	70.1	3.1	6 mm
2	72.6	4.1	8 mm
3	80.0	7.1	10 mm
4	72.2	2.6	11 mm
5	61.4	1.8	11 mm
6	60.0	1.1	5 mm
7	62.4	3.4	4 mm
Average	68.4	3.3	7.8 mm

saline solution, fixed in ethanol and glacial acetic acid (3:1 v/v) with final suspension in 0.2 ml fixative and a smear of the suspension was stained with Leishman's stain. The slides were mounted and 1,000 to 1,500 cells were scored from each culture. The number of spontaneously transformed cells in the control culture was subtracted from the number of cells transformed by the antigens in each case. We have generally followed the method described by Dierks and Shepherd (4). Lymphocyte transformation was studied before, and 48 hours and 21 days after injecting the lymphoid donor cells into the lepromatous patients.

Inguinal lymph nodes surgically removed from the 11 mm lepromin positive donor No. 9 were collected and teased in 5 ml of normal saline. The cell suspension was allowed to stand for 15 minutes to settle the debris and the supernatant was pipetted off and washed twice with normal saline. The cells were counted and one milliliter of cell suspension containing 120×10^6 cells (80% viable by the erythrocin B exclusion test) was injected subcutaneously in the deltoid region in each of the seven lepromin negative recipients. After 48 hours and 21 days, lepromin was injected intradermally over the site of the previous deltoid injection as well as into the opposite forearm. The lepromin test was read at 48 hours and 21 days. Blood was collected for the study of lymphoblast transformation with PHA and lepromin after 48 hours and 21 days.

RESULTS

Controls. The response of lymphocytes collected from seven normal controls was similar to that seen in other studies (4, 15). The average response to PHA was 68.4% and to lepromin was 3.3%. The results are summarized in Table 2. All normal volunteers were lepromin positive on skin testing, the average being a 7.8 mm response, which was read after three weeks.

TABLE 3. *Percent lymphocyte transformation in lepromatous leprosy patients before and after tuberculoid cell transfer.*

No.	PHA-P (% transformation)			Lepromin (% transformation)			
	Before cell transfer	48 hours	21 days	Before cell transfer	48 hours	21 days	Lepromin test
1	53.2	65	40	3.2	6.6	3.1	—
2	64	67	47.4	4.2	12.8	7.8	—
3	61.3	49.7	36	3.2	1.3	2.6	—
4	38.5	33.8	15.4	4.7	1.6	2.8	—
5	21.2	63.7	38.1	4.2	30.7	4.0	—
6	28.2	50.2	24.0	1.1	1.2	7.4	—
7	38.0	40.0	ND[a]	1.7	2.0	ND[a]	—
Average	43.5	52.7	33.5	3.3	8.1	4.6	

[a] ND = not done.

TABLE 4. *Lymphocyte transformation in tuberculoid leprosy.*

No.	PHA-P % transformation	Lepromin % transformation	Lepromin skin test (3 week)
1	45.7	10.5	6 mm
2	53.2	7.0	11 mm
3	38.7	3.7	5 mm
Average	45.9	7.1	7.3 mm

Lepromatous leprosy. This group, consisting of seven lepromin negative lepromatous leprosy patients (Table 3), showed a significant increase in lymphoblast transformation after cell transfer. The transformation was greater for the specific antigen (lepromin) than for nonspecific antigen (PHA) and in both cases was greater at 48 hours after cell transfer than after 21 days by which period the PHA transformation was less than before the cell transfer, while that of the response to lepromin remained higher. Despite the rise in rate of lymphoblast transformation following cell transfer the lepromin skin test at 48 hours and 21 days remained negative.

Tuberculoid leprosy. The group of tuberculoid leprosy patients (Table 4) had low transformation response to PHA (average 45.9%) as compared with the controls (68.4%) but about the same as lepromin negative patients (43.5%). The response to lepromin (7.1%) was significantly higher than the controls (3.3%) and that of the lepromin negative patients before cell transfer (3.2%).

DISCUSSION

De Bonaparte and associates ([3]) stated that it is possible that preparation of transfer factor from lepromin sensitive donors would also function as a prompt therapeutic immunizing agent and convert the progressive lepromatous type of leprosy to the more benign tuberculoid type.

Immunity in mycobacterial disease is mediated by lymphocytes which can be studied by observing the transformation of circulating lymphocytes into blast cells by PHA ([6, 13]). Mitosis and DNA synthesis have been studied by using tritiated thymidine ([1, 2, 16]) and the authors observed depression in the immunologic response in leprosy patients.

Our findings in the control healthy subjects yielded similar findings to those of other studies, e.g., Dierks and Shepard ([4]). After transferring viable lymph node cells, the lepromin negative patients did not become lepromin positive on skin testing, but the more sensitive *in vitro* test of lymphocyte transformation showed a moderate transformation with PHA and a marked increase

with lepromin at 48 hours. At 21 days the transformation rate had returned to below the initial level while that with lepromin still remained higher than the initial level at 48 hours.

Thus, this study provides several interrelated conclusions. In both lepromatous and tuberculoid leprosy patients there is depression in immunological response to the nonspecific antigen PHA as compared to controls. In both controls and lepromatous leprosy, blast transformation to lepromin is similar but in tuberculoid leprosy it is significantly higher. Transfer of lymph node cells from a tuberculoid donor to lepromatous recipients produces in 48 hours, an increase in blast transformation in response to both nonspecific (PHA) and specific (lepromin) antigens; this increase being much greater with lepromin. At 21 days the transformation rate for both PHA and lepromin showed a decrease. For PHA the rate was lower than the initial level while for lepromin, though decreased significantly below the 48 hour level, it was still higher than before the transfer of cells. Despite the significant though temporary change in immunological status produced by transfer of lymph node cells as shown by this *in vitro* test, there was no change in cutaneous sensitivity to lepromin. The feasibility of transfer of specific CMI in leprosy has been demonstrated. The difficulty of obtaining a large source of sensitized leukocytes has been overcome by the use of lymph node cells obtained by inguinal lymph node biopsy, a procedure more acceptable to our donors.

SUMMARY

Seven lepromin-negative lepromatous leprosy patients were given 120×10^6 lymph node cells from a lepromin positive tuberculoid donor. The CMI of the recipients was studied by skin testing with lepromin and *in vitro* lymphoblast transformation before and after transfer of the cells and compared with the findings of the controls.

The possibility of transfer of CMI in this disease has been demonstrated.

RESUMEN

A siete pacientes con lepra lepromatosa lepromino-negativos se les administró 120×10^6 células de ganglios linfáticos de un donante tuberculoide lepromino-positivo. La IC de los recipientes se estudió por medio de pruebas intradérmicas con lepromina y transformacion linfoblástica *in vitro* antes y después de la transferencia de células, comparándose los hallazgos con los controles.

Se ha demostrado la posibilidad de transferir la IC en esta enfermedad.

RÉSUMÉ

On à administré à sept malades atteint de lèpre lépromateuse négative à la lépromine, 120×10^6 cellules provenant d'un ganglion lymphatique d'un donneur tuberculoïde positif à la lépromine. Le CMI des individus ayant reçu ces cellules a été étudié au moyen d'épreuves cutanées à la lépromine, et par une épreuve de transformation lymphoblastique *in vitro* avant et après transfert des cellules. Ces résultats ont été comparés avec les observations faites chez les témoins.

La possibilité d'un transfert de CMI dans la maladie a été démontrée.

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