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REDUCTION OF A SUBPOPULATION OF T LYMPHOCYTES IN LEPROMATOUS LEPROSY

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SUMMARY

A reduction in number of a subpopulation of T lymphocytes was noted in lepromatous leprosy cases with high bacillary load. Tuberculoid and treated lepromatous patients, who were bacillary negative had normal levels of these cells. B-cell numbers were high in lepromatous patients irrespective of treatment and bacillary load.

INTRODUCTION

Various studies have indicated that in leprosy the host resistance to the intracellular microorganisms is related to cell-mediated immunity (Bullock & Fasal, 1971; Turk & Bryceson, 1971). The clinical manifestations of the disease form a spectrum which supports this view (Ridley & Jopling, 1966; Turk & Bryceson, 1971). The lepromatous 'low resistance' form of the disease is associated with lack of delayed hypersensitivity skin reactions (Bullock, 1968; Turk & Waters, 1969), depleted paracortical areas of lymph nodes (Turk & Waters, 1971) and *in vitro* unresponsiveness of peripheral lymphocytes to mitogens such as phytohaemagglutinin and *Mycobacterium leprae* antigens (Dierks & Shepard, 1968; Godal *et al.*, 1971; Talwar *et al.*, 1972). Failure to produce macrophage inhibition factor (Katz, Debetz & Zaias, 1971) and inability to show leucocyte migration inhibition (Godal *et al.*, 1972) have also been noted in these patients.

Most of the *in vitro* tests of T-cell functions have been on peripheral lymphocytes. It is not clear therefore whether the functional depression noted by these tests is due to reduced numbers of circulating T cells or due to the inability of these lymphocytes to respond to mitogens and antigens. A recent report suggests that T-cell numbers are decreased in the circulation of lepromatous patients (Dwyer *et al.*, 1973). This communication describes the status of both T and B cells in the peripheral blood of untreated patients across the leprosy spectrum. As shifts in immunological reactions with chemotherapeutic treatment

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have been indicated (Mehra *et al.*, 1972), treated lepromatous patients were also studied. The relationship between antigenic load and B- and T-cell numbers has been analysed.

Recent reports indicate that T cells detected after a short incubation at 4°C are related to cell-mediated immune functions and those detected after 24 hr of incubation at 4°C reflected the total number of T cells (Wybran *et al.*, 1973). In our study we measured T cells at two different incubation times and found a reduction in the subpopulation detected at 2 hr.

PATIENTS AND METHODS

The study has been carried out on thirty-one untreated freshly detected cases of leprosy and nine lepromatous leprosy patients treated with 4:4-diaminodiphenyl sulphone (DDS) for variable time periods. The patients were graded on the basis of clinical status, bacteriological and morphological indices and on histopathological examination of biopsies across the Ridley Jopling Scale (Ridley & Jopling, 1966). As controls, laboratory staff members were studied. In view of the fact that these invariably belonged to a higher socio-economic group, another relevant control is constituted by the tuberculoid leprosy patients who had nutritional and ethnic background similar to those of the lepromatous leprosy patients.

Determination of B and T cells

Leucocyte-rich plasma was collected by gravity sedimentation of heparinized blood and purified on Ficoll–Hypaque gradients at 4°C (Böyum, 1968). The lymphocyte layer was removed and washed three times with minimum essential medium (MEM). Ninety to 95% pure lymphocytes with 100% viability were obtained.

T cells

For T cells duplicate samples of 1×10^6 lymphocytes in 0·5 ml of MEM were incubated with 0·5 ml of 0·5% freshly washed sheep red blood cells (SRBC) (from the same sheep) at 37°C for 10 min. After centrifugation at 200 *g* for 5 min at room temperature, the cells were left at 4°C for 2 and 24 hr. They were then gently resuspended in 1% glutaraldehyde in phosphate-buffered saline, pH 7·4. This procedure prevented the disruption of fragile rosettes and also permitted the reading of the tests at a later time. 500 lymphocytes or 200 rosettes with three or more SRBC were counted.

B cells

These were identified by two different procedures: (i) by the presence of C_3 receptors by erythrocyte–antibody–complement rosettes as detailed by Bianco, Patrick & Nussenzweig (1970); (ii) by immunoglobulin determinants on these cells localized by means of immunosorbent-pure anti-human immunoglobulins conjugated with peroxidase (Nath *et al.*, 1974).

RESULTS

T and B cells were determined in the same patients as far as possible.

After 2 hr of incubation at 4°C with SRBC, an average of $47{\cdot}3 \pm 1{\cdot}4\%$ of normal peripheral lymphocytes formed spontaneous rosettes (2-hr T cells). This number increased to $76{\cdot}9 \pm 1{\cdot}2\%$ after 24 hr of incubation (Table 1) and was considered to represent the total circulating T-cell population. Similar numbers of total and 2-hr T cells were seen in the

tuberculoid and in treated lepromatous patients (Tables 1 and 3). In untreated lepromatous patients there was a notable reduction of 2-hr T cells, although total T cells ranged from 65 to 81% (compared with a normal range of 72–86%). In individual cases B- + T-cell numbers were 90–100%.

It is noteworthy that in seven of the treated cases of lepromatous leprosy the 2-hr T-cell values are in the normal range. These patients had been under prolonged chemotherapeutic treatment and had become bacillary negative. In two cases which showed persistent

TABLE 1. The number of T lymphocytes in peripheral circulation in normal subjects and in patients suffering from leprosy

Subjects	Total numbers of T cells per ml ($\times 10^3$) (mean ± s.d.)		Percentage of T cells (mean ± s.d.)	
	2 hr	24 hr	2 hr	24 hr
Normal	1069 ± 66 n = 13	1728 ± 84 n = 13	47·3 ± 1·4 n = 13	76·9 ± 1.2 n = 13
Untreated tuberculoid	1218 ± 484 n = 8	1696 ± 290 n = 8	53·6 ± 4·2 n = 8	74·2 ± 3·7 n = 8
Untreated lepromatous	580 ± 118 n = 10	1921 ± 216 n = 6	22·1 ± 3·9 n = 10	71·7 ± 2·2 n = 6
Treated lepromatous bacillary negative	1301 ± 164 n = 7	1666 ± 157 n = 7	52·1 ± 4·9 n = 7	66·8 ± 2·5 n = 7
Treated lepromatous bacillary positive	(304 and 533) n = 2	(1289 and 1464) n = 2	(12·7 and 22·2) n = 2	(53·7 and 61·0) n = 2

n = Number of subjects studied.

TABLE 2. Two-hour and 24-hr rosette-forming T lymphocytes in untreated lepromatous leprosy subjects

Patient number	Clinical grading	Biopsy grading	Bacterio-logical index	Morpho-logical index (%)	Duration of symptoms (years)	Total lymphocytes in blood per mm³ ($\times 10^3$)	Percentage of T cells	
							2 hr	24 hr
85	LL	LL	4+	30	2.0	2·4	10·7	67·7
84	LL	N.D.	4+	35	3·0	2·0	12·2	64·8
68	LL	LL	4+	30	6·0	3·4	16·0	71·0
95	LL	LL	3+	25	2·0	2·8	37·0	80·6
100	LL	N.D.	5+	30	10·0	2·0	34·5	72·0
51	BL	LL	3+	35	1·0	3·2	34·8	N.D.
52	LL	LL	5+	40	0·5	3·0	20·0	N.D.
34	LL	LL	6+	35	2·0	1·7	9·0	N.D.
55	LL	BL	5+	40	1·0	2·2	10·0	N.D.

N.D. = Not determined.

TABLE 3. Two-hour and 24-hr rosette-forming T lymphocytes in DDS-treated leprosy subjects

Patient number	Bacteriological index	Morphological index (%)	Duration of symptoms (years)	Duration of DDS therapy (years)	Total lymphocytes in blood per mm³ ($\times 10^3$)	Percentage of T cells	
						2 hr	24 hr
102	0	0	12	7	2·4	40·5	72·3
103	0	0	5	3	2·4	48·3	55·7
105	0	0	5	3	1·6	41·7	60·6
111	0	0	10	6	2·6	75·5	70·0
112	0	0	4	2·5	2·2	57·0	73·0
113	0	0	10	6·0	3·4	41·3	65·0
114	0	0	8	5·0	2·8	60·3	71·0
106	2+	6	6	1·5	2·4	12·7	53·7
107	3+	7	2	1·5	2·4	22·2	61·0

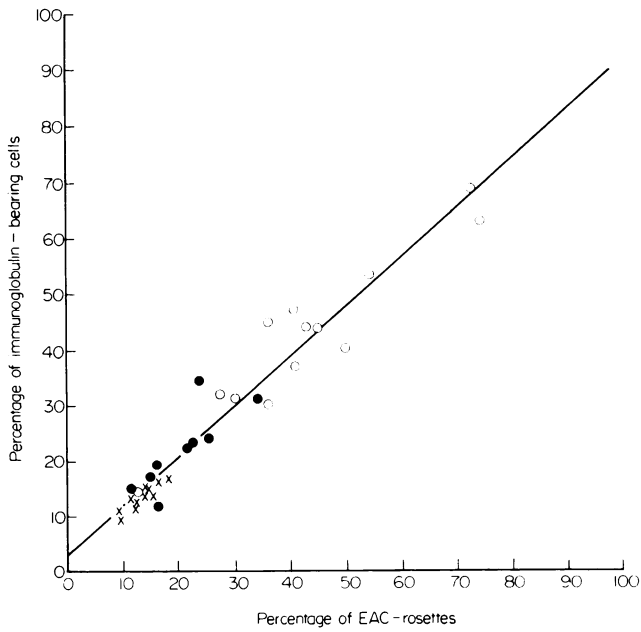

FIG. 1. Correlation of the percentage of circulating lymphocytes forming EAC-rosettes with the percentage of lymphocytes with surface immunoglobulin in normal subjects (×), leprosy patients (●) and patients of CLL (○). $r = 0{\cdot}9698$. $P<0{\cdot}001$. $b = 0{\cdot}9$. $y = 2{\cdot}8+0{\cdot}9x$.

bacillary load, the 2-hr T cells were low, and similar to the untreated cases (Tables 2 and 3).

The absolute numbers of circulating T cells reflected the same changes in the various groups of patients studied (Table 1).

B cells were identified by their surface immunoglobulin determinants and by their ability to form complement rosettes. In our hands, there was a highly significant correlation of the

percentage of peripheral lymphocytes identified as B cells by these two methods (Fig. 1). The data in Table 4 are the mean values of immunoglobulin-bearing and complement receptor-bearing cells. B-cell numbers in tuberculoid leprosy patients were normal. The mean number of B cells in lepromatous cases was approximately double that of normal and tuberculoid patients (Table 4). In four out of twenty-three cases of lepromatous leprosy the B-cell numbers were in the normal range. The increase in B cells in lepromatous patients persisted after treatment with DDS up to 7 years and even when bacterial load was not detectable. The total lymphocyte numbers were in the normal range and there was an increase in the absolute numbers of circulating B cells in these patients.

TABLE 4. The number of B lymphocytes in peripheral circulation in normal subjects and patients suffering from leprosy

Subjects	Total numbers of B cells per ml ($\times 10^3$) (mean $\pm$ s.d.) (range)	Percentage of B cells (mean $\pm$ s.d.) (range)
Normal	371 ± 51 (86–1010) n = 18	$14{\cdot}4 \pm 0{\cdot}6$ (9·0–18·5) n = 23
Untreated tuberculoid	364 ± 39 (200–700) n = 15	$14{\cdot}2 \pm 1{\cdot}7$ (10·0–22·5) n = 17
Untreated lepromatous	759 ± 103 (350–1344) n = 11	$27{\cdot}5 \pm 2{\cdot}0$ (14·0–42·0) n = 14
Treated lepromatous	612 ± 71 (339–991) n = 9	$25{\cdot}0 \pm 2{\cdot}8$ (15·5–41·0) n = 9

n = Number of subjects studied.

DISCUSSION

The most significant alteration in untreated cases of lepromatous leprosy is the reduction in the number of 2-hr rosette-forming T cells without a significant change in the 24-hr rosette-forming cells. The difference between these two populations of lymphocytes is not clearly known. By virtue of the readiness with which the rosettes are formed by the former group, it may be surmised that they have high affinity receptors for sheep erythrocytes. Wybran & Fudenberg (1973) suggest that these lymphocytes may correlate with cell-mediated immune functions. Our study shows that this subpopulation of high affinity T cells is not fully expressed in freshly detected cases of lepromatous leprosy.

The low 2-hour T-cell values correlate well with the bacillary load in the leprosy patients. In the untreated lepromatous cases with a high bacterial index these cells are in the low range (Tables 1 and 2). Tuberculoid and treated lepromatous patients with undetectable bacterial load in the skin or nose have normal numbers of these cells (Tables 1 and 3). The total

circulating T cells as measured by spontaneous rosettes at 24 hr appear to be in the normal range, in all the cases across the leprosy spectrum (Table 1). When individual lepromatous cases are studied, those with high B cells show a corresponding reduction of total T cells maintaining the T- + B-cell numbers at 80–100%. An observation in conflict with the above data is that of Dwyer *et al.* (1973), who found reduced numbers of cells forming spontaneous rosettes at 24 hr in lepromatous cases.

While the correlation between 2-hr T cells and bacterial load is evident, it is not clear whether the depression of 2-hr T cells is a consequence or the cause of high bacterial load. Turk & Waters (1971) noted that the depleted T-cell areas of lymph nodes in two treated cases of lepromatous leprosy were repopulated when the bacterial index fell to low levels. Our findings of the return of normal 2-hr T cells into the circulation of treated patients is similar to the above phenomenon.

B-cell numbers were found to be normal in the blood of tuberculoid patients. There was a significant increase in both the percentage and absolute numbers of circulating B cells in most cases of lepromatous leprosy. This increase was noted in cells bearing both complement receptors and immunoglobulin determinants. In our hands, there was a good correlation of B-cell numbers as determined by peroxidase-coupled antisera and complement rosettes. The increase in B cells noted in lepromatous cases was long-lasting and persisted in spite of treatment and in the absence of detectable bacillary load. These findings are in accordance with the observations of other workers (Gajl-Peczalska *et al.*, 1973; Dwyer *et al.*, 1973).

Longitudinal studies on the time of onset of B-cell increase and the relationship between the bacterial load and the return of 2-hr T cells into circulation are necessary for further elucidation of these phenomena. It is essential to clarify the role of 2-hour T cells in relation to cell-mediated immune functions as this study gives evidence that the two are related in lepromatous leprosy.

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REFERENCES

BIANCO, C., PATRICK, R. & NUSSENZWEIG, V. (1970) A population of lymphocytes bearing a membrane receptor for antigen–antibody–complement complexes. I. Separation and characterisation. *J. exp. Med.* **132,** 702.

BOYUM, A. (1968) Separation of leucocytes from blood and bone marrow. *Scand. J. clin. Lab. Invest.* **21,** 77.

BULLOCK, W.E. (1968) Studies on immune mechanisms in leprosy. I. Depression of delayed allergic responses to skin test antigens. *New Engl. J. Med.* **278,** 298.

BULLOCK, W.E. & FASAL, P. (1971) Studies of immune mechanisms in leprosy. III. The role of cellular and humoral factors in impairment of the *in vitro* immune response. *J. Immunol.* **106,** 888.

DIERKS, R.E. & 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.

DWYER, J.M., BULLOCK, W.E. & FIELDS, J.P. (1973) Disturbance of the blood T:B lymphocyte ratio in lepromatous leprosy. *New Engl. J. Med.* **288,** 1036.

GAJL-PECZALSKA, K.J., LIM, S.D., JACKSON, R.R. & GOOD R.A. (1973) B lymphocytes in lepromatous leprosy. *New Engl. J. Med.* **288,** 1033.

GODAL, T., MYKLESTAD, B., SAMUEL, D.R. & MYRVANG, B. (1971) Characterization of the cellular immune defect in lepromatous leprosy. *Clin. exp. Immunol.* **9,** 821.

GODAL, T., MYRVANG, B., FROLAND, S.S., SHAO, J. & 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.

KATZ, S.I., DEBETZ, B.H. & ZAIAS, N. (1971) Production of macrophage inhibitory factor by patients with leprosy. *Arch. Derm.* **103,** 358.

MEHRA, V.I., TALWAR, G.P., BALAKRISHNAN, K. & BHUTANI, L.K. (1972) Influence of chemotherapy and serum factors on the mitogenic response of peripheral leucocytes of leprosy patients to phytohaem-agglutinin. *Clin. exp. Immunol.* **12,** 205.

NATH, I., CURTIS, J., MANGALIK, A. & TALWAR, G.P. (1974) Presence of lymphocytes devoid of conventional T and B cell markers in chronic lymphatic leukemia. (Submitted for publication.)

RIDLEY, D.S. & JOPLING, W.H. (1966) Classification of leprosy according to immunity. A five-group system. *Int. J. Leprosy*, **34,** 255.

TALWAR, G.P., KRISHNAN, A.D., MEHRA, V.L., BLUM, E.A. & PEARSON, J.M.H. (1972) Evaluation of cell mediated immune responses in untreated cases of leprosy. *Clin. exp. Immunol.* **12,** 195.

TURK, J.L. & BRYCESON, A.D.M. (1971) Immunological phenomena in leprosy and related diseases. *Advanc. Immunol.* **13,** 209.

TURK, J.L. & WATERS, M.F.R. (1969) Cell-mediated immunity in patients with leprosy. *Lancet*, **ii,** 243.

TURK, J.L. & WATERS, M.F.R. (1971) Immunological significance of changes in lymph nodes across the leprosy spectrum. *Clin. exp. Immunol.* **8,** 363.

WYBRAN, J. & FUDENBERG, H.H. (1973) Thymus derived rosette forming cells. *New Engl. J. Med.* **288,** 1072.

WYBRAN, J., LEVIN, A.S., SPITLER, L.E. & FUDENBERG, H.H. (1973) Rosette-forming cells, immunologic diseases and transfer factor. *New Engl. J. Med.* **288,** 710.