Clin. exp. Immunol. (1974) **16**, 23–30.

T AND B LYMPHOCYTES IN PATIENTS WITH LEPROMATOUS LEPROSY

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(*Received* 27 *March* 1973)

SUMMARY

The percentages of T and B peripheral blood lymphocytes were established in thirty-six patients with lepromatous leprosy. T lymphocytes were detected by rosette formation with sheep erythrocytes (E) and B lymphocytes were detected by rosette formation with human erythrocytes sensitized with antibody and complement (HEAC). The mean per cent values for both T and B lymphocytes were significantly lower in these patients as compared to mean values from thirty normal subjects ($0{\cdot}05 > P > 0{\cdot}01$). Lymph nodes sections treated *in vitro* with E or HEAC from two lepromatous patients which were examined, showed marked depletion of T cells in the paracortical areas and the follicles were slightly reduced in size, but still presenting B cells. Establishing the percentages of T and B peripheral blood lymphocytes and their distribution in lymph nodes may represent an additional method of evaluating the immunologic status of leprosy patients.

INTRODUCTION

In leprosy there are a wide variety of clinical forms which range from the tuberculoid to the lepromatous. The type of disease manifested is probably due to cellular immune mechanisms rather than circulating antibodies (Turk & Bryceson, 1971). A specific depression of cellular immunity to *Mycobacterium leprae* leading to the lepromatous form is a host-determined characteristic and is probably genetically determined (Newell, 1966). A variable proportion of lepromatous leprosy patients present impaired delayed hypersensitivity responses to other antigens (Bullock, 1968a; Shepard, 1968; Waldorf *et al.*, 1966; Job & Karat, cited by Hart & Rees, 1967). There is also evidence of depressed cellular immune responses in patients with tuberculoid leprosy although it is not as severe or manifested so consistently (Bullock, 1968a).

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Patients with lepromatous leprosy have been shown to have: (1) depressed delayed hypersensitivity reactions to common bacterial antigens (Bullock, 1968a); (2) negative intradermal reactions to lepromin (Shepard, 1968); (3) depressed capacity to develop contact sensitivity to picrylchloride (Bullock, 1968a) and dinitrochlorobenzene (Waldorf *et al.*, 1966); and (4) prolonged skin allograft survival (Job & Karat, cited by Hart & Rees, 1967). There have been contradictory reports on *in vitro* lymphocyte response to phytohaemagglutinin (PHA) in patients with leprosy (Dierks & Shepard, 1968; Sheagren *et al.*, 1969; Nelson *et al.*, 1971).

Lymph nodes from patients with lepromatous leprosy (Turk & Waters, 1968, 1971) and from mice infected with *Mycobacterium leprae murium* (Ptak *et al.*, 1970) show depletion of small lymphocytes in the paracortical (thymus-dependent) area that is populated by macrophages containing the microorganism.

Lepromatous patients have no apparent defect in their capacity to produce immunoglobulins (Bullock, Ho & Chen, 1970) and show high titres of antibody to mycobacterial antigens (Rees *et al.*, 1965).

The present investigation was undertaken to estimate the proportion of T and B peripheral blood lymphocytes in patients with lepromatous leprosy. In addition, the distribution of T and B lymphocytes in lymph node sections from two patients was also studied.

The methods used for these studies were based on the findings that human T and B lymphocytes can be distinguished by two different types of membrane receptors (Silveira, Mendes & Tolnai, 1972). T but not B lymphocytes bind untreated sheep erythrocytes (E) forming rosettes and B but not T lymphocytes have a receptor for a modified component of complement (C3) which can be detected by rosette formation with erythrocytes treated with antibody and complement (EAC) (Silveira *et al.*, 1972; Lay *et al.*, 1971). T and B human lymphocytes can also be differentially detected in tissue sections of lymphoid organs by adherence of E or EAC (Silveira *et al.*, 1972).

PATIENTS AND METHODS

Patients

Thirty-six patients with lepromatous leprosy, at the Hospital Aimorés, São Paulo, Brasil, were studied. Their ages ranged from 23 to 60 years and the duration of the disease ranged from 2 to 37 years. All patients were lepromatous (LL) as defined by the Ridley–Waters classification (Ridley & Waters, 1969). All patients were under treatment with diaminodiphenylsulphone (DDS) for at least 1 year. None had received steroids or any immunosuppressive therapy at the time of the study. All had recently been skin-tested with lepromin and were found to be negative to it. Thirty healthy adults ranging in age from 20 to 55 years, from the same geographical area (São Paulo, Brasil) served as controls.

Separation of lymphocytes

Ten-millilitre samples of venous blood were drawn into sterile plastic syringes containing 150 units of heparin. Blood was transferred to 16×100 mm glass tubes and sedimented for 45 min at room temperature after which the leucocyte-rich plasma was drawn off. Cells were centrifuged at $200 \times g$ for 10 minutes, resuspended in 4 ml of Hanks's balanced salt solution (HBSS) (Grand Island Biological Company, Grand Island, New York) at pH 7·2, containing 50% of autologous plasma and layered over a Ficoll-Hypaque mixture of

density 1·076 and spun at $400 \times g$ for 30 minutes at 18°C, as described elsewhere (Thorsby & Bratlie, 1970). The lymphocyte suspension (98% pure) was washed three times in HBSS by centrifugation at $200 \times g$ and adjusted to 2×10^6 cells/ml in HBSS.

Detection of T lymphocytes by rosette formation with untreated sheep erythrocytes (E)

We have used the method previously described (Silveira *et al.*, 1972; Lay *et al.*, 1971), with minor modifications. One tenth of a millilitre of the lymphocyte suspensions containing 2×10^6cells/ml in HBSS were dispensed into 6 × 50 mm glass tubes and mixed with 0·1 ml of a 0·5% washed sheep erythrocyte suspension (E) in HBSS at room temperature. The mixture was centrifuged at $200 \times g$ for 5 minutes at room temperature and then incubated at 0°C for 1 hr in an ice-water bath. The cell button was then gently resuspended with a Pasteur pipette and the percentage of rosette-forming cells was determined microscopically in a haemocytometer. At least 300 lymphocytes were counted in triplicate tubes and only rosettes possessing three or more adherent erythrocytes were counted.

Detection of B lymphocytes by rosette formation with human erythrocytes sensitized with antibody and complement (HEAC)

We have used the method previously described (Silveira *et al.*, 1972), with some modifications. A 2·5% suspension of human erythrocytes in HBSS was incubated for 30 minutes at 37°C with equal volumes of a subagglutinating dilution of normal rabbit serum containing natural heterophile antibodies to human erythrocytes. One tenth of a millilitre of undiluted mouse complement was added to 2 ml of the sensitized erythrocyte suspension and incubated for an additional 30 min at 37°C. This mixture (HEAC) was then washed once with HBSS and adjusted to 0·5%. For rosette formation 0·1 ml of the lymphocyte suspension (2×10^6 cells/ml) were mixed at room temperature with equal volumes of HEAC in triplicate 6 × 50 mm glass tubes. The tubes were then immediately centrifuged at $200 \times g$ for 5 min at room temperature. After centrifugation, the cell button was gently resuspended with a Pasteur pipette and the percentage of rosette-forming cells was determined microscopically. The number of cells counted and the criteria used for determining a rosette were the same as described for E rosettes.

Detection of T and B lymphocytes in lymph node sections

Inguinal lymph nodes were obtained surgically from two lepromatous patients after consent and studied histologically for the distribution of T and B lymphocytes. We have used the method that we described in detail elsewhere (Silveira *et al.*, 1972). Six micra-thick sections were cut on an International Harris Cryostat. The slides were allowed to dry at room temperature under ventilation, washed in cold HBSS and then covered with 1 ml of either E or HEAC 1% suspensions. In order to detect T cells, we incubated the sections at 37°C for 1hr and then at 0°C for 1 hr. The slides were then washed by dipping in cold HBSS until E no longer floated off. The washed slides were fixed with 4% formaldehyde in phosphate-buffered saline at pH 7·2 for 1 hr, washed in HBSS, stained with Leishman's and examined under the light microscope. To study the distribution of B cells, we incubated the sections covered with HEAC at 37°C for 30 min, then washed, fixed and stained them as above.

RESULTS

The percentages of T and B lymphocytes detected by rosette formation with E and HEAC in patients with lepromatous leprosy and in normal subjects are summarized in Fig. 1. There was a significant difference ($0{\cdot}05 > P > 0{\cdot}01$) for both T and B peripheral blood lymphocytes mean values between the populations of lepromatous leprosy patients and normal controls, by the *t*-test. The mean per cent values of T cells detected by rosette formation with E was

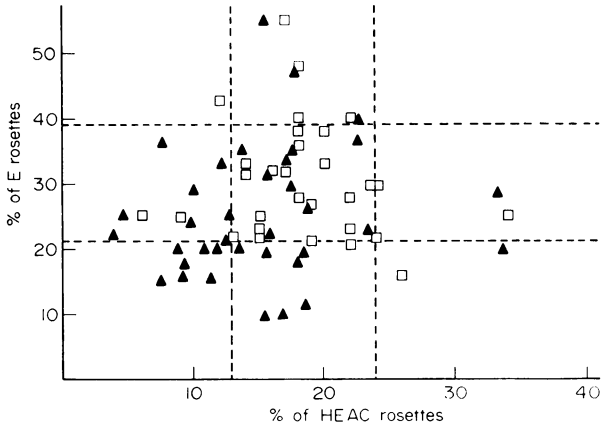

FIG. 1. Percentages of rosettes with E and HEAC in patients with (△) lepromatous leprosy and (□) in normal subjects. Dotted lines represent standard deviations (SD) of the normal values. The mean percentage of E rosettes was $30{\cdot}4 \pm 8{\cdot}8$ (SD) in the control group, compared to $25{\cdot}2 \pm 10{\cdot}0$ (SD) in the lepromatous group (*t* observed = 2·220). The mean percentage of HEAC rosettes was $18{\cdot}4 \pm 5{\cdot}4$ (SD) in the control group and $15{\cdot}1 \pm 6{\cdot}5$ (SD) in the lepromatous group (*t* observed = 2·189).

FIG. 2. Lymph node section from a patient with lepromatous leprosy showing no T cells detectable by adherence to E. The paracortical area is occupied by macrophages (×80).

30·4±8·8 (standard deviation, SD) in the control group, compared to 25·2±10·0 (SD) in the lepromatous leprosy group (t = 2·220). The mean per cent values of HEAC rosette-forming cells (B lymphocytes) were 18·4±5·4 (SD) in the control group and 15·1±6·5 in the lepromatous leprosy group (t = 2·189). As can be seen in Fig. 1, some patients have per cent values of E and HEAC rosette-forming cells included in the central quadrant delineated by the dotted lines, which represent graphically the standard deviations of the normal values. Most of the patients show reduced values for one or both lymphocyte populations detected by this method and only a few show high values.

The lymph nodes which were examined showed almost no T cells in the paracortical area as judged by adherence to E (Fig. 2). This area was occupied by macrophages containing bacilli. The follicles were slightly reduced in size but still presented B lymphocytes which could be detected by selective adherence to HEAC (Fig. 3).

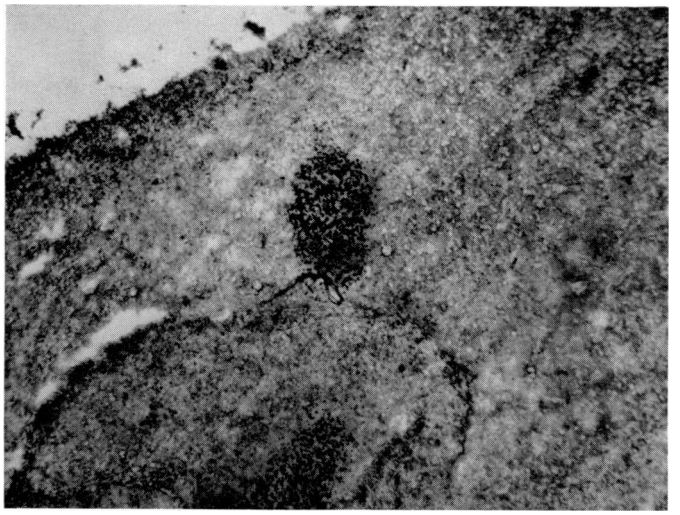

FIG. 3. Lymph node section from a patient with lepromatous leprosy showing adherence of HEAC to B cells in the follicles which seem to be slightly reduced in size (×80).

DISCUSSION

The significant decrease in the proportion of T cells that we observed in the peripheral blood and the depletion of T lymphocytes in the paracortical areas of involved lymph nodes are in agreement with the impaired cell-mediated immunity and lymph node lesions described in lepromatous leprosy patients (Turk & Waters, 1968, 1971) and experimentally infected animals (Ptak *et al.*, 1970).

The replacement of lymphocytes in the paracortical areas by abnormal tissue has been studied by Turk and Waters (1968, 1971). A similar depression of delayed hypersensitivity associated with depletion of lymphocytes in the paracortical areas can be seen in cases of Hodgkin's disease (Brown *et al.*, 1967), sarcoidosis (Turk & Oort, 1971) and South American blastomycosis (Mendes *et al.*, 1971). The mechanism of loss of T lymphocytes in the paracortical areas is unknown. The function of T lymphocytes in lepromatous leprosy may also

be influenced by depressive humoral factors as has already been suggested (Bullock, 1968b). These factors have also been shown to depress PHA-induced transformation of lymphocytes *in vitro* (Nelson *et al.*, 1971).

Mice experimentally infected with *Mycobacterium leprae murium*, besides showing marked lesions in thymus-dependent areas of lymph nodes, also show progressive disorganization of germinal centres and 3 months after the infection the cortex is devoid of germinal centres, though small follicles remain at the periphery (Ptak *et al.*, 1970). The lymph nodes from patients with lepromatous leprosy that we examined also have had follicles which were slightly reduced in size but still presented B cells, as judged by HEAC adherence. We also found that patients with lepromatous leprosy had statistically significant decrease in the proportion of peripheral blood B lymphocytes as compared to controls. However, as in experimentally infected mice (Ptak *et al.*, 1970), the humoral immune response in lepromatous patients seems to be normal in spite of the lymph node lesions and significant HEAC rosette-forming cell depletion in peripheral blood. The reduction in the proportion of B cells determined by rosette formation with HEAC probably does not interfere significantly with their immune functions, when evaluated by immunoglobulin levels (Bullock *et al.*, 1970) or antibody levels against mycobacterial antigens (Rees *et al.*, 1965). An interesting hypothesis which might be considered is that cells having receptors for HEAC could bind immune complexes from the plasma *in vivo* and in this situation part of the membrane receptors for C3 would be occupied, interfering with rosette formation. Evidence for the presence of immune complexes in leprosy patients has recently been reported (Moran *et al.*, 1972).

Establishing the percentages of B and T peripheral blood lymphocytes may represent an additional method of evaluating the immunologic status of leprosy patients. The possible changes in these proportions during the course of the disease, their relationship between the duration of the disease and therapy remain to be clarified.

ACKNOWLEDGMENTS

We are indebted to Dr D. Opromola and Dr J. J. G. Lima for making available the patients studied and Dr F. W. Rodrigues for the statistical analysis.

This work was supported by Fundação de Amparo à Pesquisa do Estado de São-Paulo.

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