

Leprosy XI. Evaluation of Thymus-Derived Lymphocytes by an Antihuman T-Lymphocyte Antiserum^{1,2}

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Patients with lepromatous leprosy are known to display various immunological abnormalities including deficiency of delayed hypersensitivity to skin antigens (²), difficulty of skin sensitization (¹⁸), delayed rejection of skin allografts (⁸), decreased antigen or mitogen-induced lymphocyte proliferation *in vitro* (⁴), decreased macrophage inhibition factor (MIF) production (¹¹), and depletion of lymphocytes in the deep cortical area of the lymph node (¹⁹).

This impairment of cell-mediated immunity could be due to a reduction of the thymus-dependent lymphocyte (T lymphocyte) population (¹⁹), to an inadequacy on the part of T lymphocytes to exert their function (⁹), or to humoral inhibitory factors (³), and/or to deficiencies of response of amplification or effector systems to T cells.

Recent studies have demonstrated that patients with lepromatous leprosy have a high proportion of circulating lymphocytes possessing membrane-bound immunoglobulins which may represent an accumulation of bone marrow derived lymphocytes (B lymphocytes) as a consequence of overcompensatory production in the presence of a deficiency of thymus-derived lymphocyte (T lymphocyte or T cell) (^{5,7}).

Also, the quantitation of T lymphocytes in leprosy patients by using the property of

T lymphocytes to form nonimmune rosettes with sheep red blood cell (SRBC) has been performed in our laboratories, and a low level of rosette-forming T lymphocytes (T-RFC) was demonstrated in both percentage and absolute number of T lymphocytes (¹³).

The purpose of this paper is to evaluate T lymphocytes in the peripheral blood of patients with various types of leprosy using a method based on the cytotoxic activity of an anti-T-cell serum prepared in rabbits against lymphocytes of patients with X-linked agammaglobulinemia.

MATERIALS AND METHODS

Patients. A total of 36 patients were studied: 20 had active lepromatous leprosy, 9 had inactive lepromatous leprosy, 4 had borderline leprosy and 3 indeterminate leprosy. The degree of activity and the form of the disease was established on the basis of clinical findings, histopathology and bacteriological studies including animal inoculation. The classification of each patient was based on the Ridley and Jopling scale (¹⁵). All patients had been treated with DDS, B663 and rifampicin and other specific drugs for varying periods before study. The duration of the disease and the racial background of the patients were heterogeneous. The constitution of patient material was described in detail elsewhere (¹³).

Twelve of twenty active lepromatous leprosy patients were drug (DDS) resistant, and seven of the cases with active lepromatous leprosy had *erythema nodosum leprosum* (ENL); five had received steroids (20 mg–40 mg prednisolone/day) for varying periods before the study.

Preparation of anti-T-cell serum. The preparation and the criteria for specificity of the antihuman T-cell serum (ATCS) are described elsewhere (¹⁷). Briefly, we immunized rabbits with blood lymphocytes from

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a patient with Bruton-type agammaglobulinemia and absorbed the de complemented antiserum with cultured B lymphoblasts.

Blood samples for lymphocytes. Peripheral blood (10 cc–20 cc) was drawn with sterile plastic syringes containing heparin and shipped to Minneapolis by direct air flight. Blood collected simultaneously from two to three healthy subjects living in the same area was handled in exactly the same way and shipped along with the samples from the leprosy patients and analyzed simultaneously as controls. Details were described in our previous publication (¹³).

Procedure. The technic used was that of two-stage microcytotoxicity as described by Amos *et al* (¹). Peripheral blood lymphocytes from patients or normal individuals were always separated on Ficoll-Hypaque gradient (¹⁶). Before proceeding with the experiment, the viability of isolated cells was tested by trypan blue dye exclusion method. Then a suspension of 2×10^6 cell/ml was incubated with antiserum in the well of a microtest tissue culture plate (Falcon Plastics, Los Angeles, California), washed, and rabbit complement (C) (Grand Island Biological Company, Grand Island, New York) was added in a dilution of 1:2. All samples were studied with several dilutions of ATCS. Every test was done in duplicate. The trypan blue exclusion method was used to evaluate the percentage of live and dead cells, 300 cells being counted in each well when the cytotoxic index was calculated.

The absolute numbers of T cells were calculated from the total WBC count and the differential count.

RESULTS

First, we compared the normal range of T cells in the control samples for healthy persons. As shown in Table 1, the mean value (62.1%) for T cells shipped in the control group, which were obtained from 25 normal individuals at the University of Minnesota, fell within the normal range (49%–78%, mean 65.5%). Table 2 summarizes the result of T cells for each group of leprosy patients and the control subjects.

Both percentage and absolute numbers of T cells were very low in the blood of patients with active lepromatous type of leprosy. In the other types of leprosy (inactive lepromatous, borderline, and indeterminate types), the numbers of T cells fell within the normal range. The rank of the groups from low to high is as follows: 1) active lepromatous, 2) inactive lepromatous, 3) borderline, and 4) indeterminate leprosy.

Some of the active lepromatous patients (12 cases) have been proved to be drug resistant (DDS). Their T cell levels were studied and the results are summarized in Table 3. The absolute number of T cells of the drug resistant patients were remarkably low as compared to the group which was not drug resistant. The difference was statistically significant ($p < 0.05$).

Also, the active lepromatous leprosy cases were divided on the basis of complications with *erythema nodosum* reaction and the levels of T cells were analyzed (Table 4). The percentage of T cells in cases complicated by ENL was not different from the uncomplicated cases, but the absolute number of T cells in the complicated cases was sig-

TABLE 1. Value of T cell determined by cytotoxic test with anti-T-cell serum for control group.

| Subjects | Total WBC | Absolute no. lymphocytes | % lymphocytes | Absolute no. T cell | % of T cell |
|----------|-----------|--------------------------|---------------|---------------------|-------------|
| 1 | 4.440 | 2464 | 56 | 1229 | 51.03 |
| 2 | 7.000 | 1540 | 22 | 908 | 59.78 |
| 3 | 4.700 | 2162 | 46 | 1535 | 71.19 |
| 4 | 6.300 | 2079 | 33 | 1074 | 51.66 |
| 5 | 6.000 | 1920 | 32 | 1057 | 55.08 |
| 6 | 5.700 | 2162 | 44 | 1494 | 69.13 |
| 7 | 7.800 | 3900 | 50 | 2994 | 76.75 |
| Mean: | | | | 1470 | Mean: 62.1 |
| S.D.: | | | | 710 | S.D.: 10.2 |
| S.E.: | | | | 273 | S.E.: 3.9 |

TABLE 2. *Percentage and absolute number of peripheral T cells in patients with various forms of leprosy.*

| Group | No. cases | Absolute no. ATCS sensitive cells/mm ³ blood (Mean \pm S.E.) | % of ATCS-sensitive blood lymphocytes (Mean \pm S.E.) |
|---------------------|-----------|---|---|
| I Normal population | 25 | | 65.5 \pm 1.7 |
| II Control group | 7 | 1470 \pm 270 | 62.1 \pm 3.9 |
| III Active LL | 20 | 1062 \pm 130 | 52.3 \pm 2.0 |
| IV Inactive LL | 9 | 1207 \pm 112 | 64.2 \pm 2.8 |
| V BB group | 4 | 1266 \pm 218 | 62.2 \pm 2.2 |
| VI I group | 3 | 2159 \pm 408 | 60.8 \pm 0.9 |

ATCS = anti-T-cell serum.

LL = lepromatous leprosy.

BB = borderline leprosy.

I = indeterminate leprosy.

Statistical analysis of percentage by Student's test: significant difference between Group III and II ($p < 0.05$); III and IV ($p < 0.01$); III and I ($p < 0.001$); no significant difference between Groups I, II, IV, V and VI.

TABLE 3. *Percentage and absolute number of peripheral T cells in leprosy patients drug (DDS) resistant and nondrug-resistant patients.*

| Subject | No. cases | Absolute no. ATCS-sensitive cells/mm ³ blood (Mean \pm S.E.) | % of ATCS-sensitive blood lymphocytes (Mean \pm S.E.) |
|-------------------------|-----------|---|---|
| Drug (DDS) resistant | 12 | 832 \pm 105 | 52 \pm 3.3 |
| Nondrug (DDS) resistant | 8 | 1342 \pm 215 | 52 \pm 2.6 |

TABLE 4. *Percentage and absolute number of peripheral T cells in leprosy patients with ENL and without ENL.*

| Subject | No. cases | Absolute no. ATCS-sensitive cells/mm ³ blood (Mean \pm S.E.) | % of ATCS-sensitive blood lymphocytes (Mean \pm S.E.) |
|-----------------------|-----------|---|---|
| ENL complicated group | 7 | 1315 \pm 290 | 52 \pm 3.9 |
| Without ENL group | 13 | 951 \pm 170 | 51 \pm 3.8 |

TABLE 5. *Percentage and absolute number of peripheral T cells in leprosy patients who received steroid and nonsteroid-treated patients.*

| Subject | No. cases | Absolute no. ATCS-sensitive cells/mm ³ blood (Mean \pm S.E.) | % of ATCS-sensitive blood lymphocytes (Mean \pm S.E.) |
|---------------------|-----------|---|---|
| Steroid Tx group | 5 | 1216 \pm 406 | 50 \pm 6.3 |
| Nonsteroid Tx group | 15 | 976 \pm 215 | 52 \pm 4.7 |

nificantly higher than that of the uncomplicated cases.

Five of the active lepromatous cases had received steroid treatment which might have suppressed the number of T cells. When these five cases were compared to the others (Table 5), no meaningful difference in the number of T cells, as compared with those who had not received steroid treatment (15 cases), was observed.

DISCUSSION

The evidence for specificity of ATCS has been described in a previous publication (¹⁷). The incidence of T cells, as evaluated by ATCS, in peripheral blood of normal persons is in agreement with the results of other methods for evaluation of T cell numbers; for instance, the percentage of T-RFC in normal human peripheral blood has been found to be as high as 52%–81% by some authors (¹⁰). But the percentage of T-RFC in normal human peripheral blood evaluated by our institute showed values a bit lower than that observed by others: range is 34%–62% (¹²).

An average of 65.5% (range 49%–78%) T cells determined by ATCS in normals fell between these two ranges and mean values.

The lowest values of both percentage and absolute numbers of T cells, as evaluated by ATCS, in active lepromatous leprosy agrees with the results obtained by the rosette-forming technic (^{5,13}). It is also consistent with the observation of a complementary increase in the surface immunoglobulin-bearing lymphocytes as revealed by analyses using fluorescein-labeled antihuman immunoglobulin serum (^{5,7}).

Other types of leprosy (inactive lepromatous, borderline, and indeterminate types) showed a normal range of T cells. This finding, too, agreed well with findings obtained using the rosette-forming technic (¹³). It is also very interesting that the spectrum of absolute T cell numbers corresponds with the clinical spectrum of the diseases (¹³).

In further study of active lepromatous leprosy, it was noted that a difference exists in the number of T cells between 12 drug (DDS) resistant active lepromatous leprosy cases, and the 8 nondrug (DDS) resistant active lepromatous patients. When one compared drug resistant (DDS) and nondrug resistant patients with lepromatous leprosy

by percentage of T lymphocytes enumerated by the cytotoxicity test, one did not observe significant differences in percentage of T lymphocytes. When T lymphocytes were evaluated in terms of absolute numbers, however, a most significant difference in absolute numbers of T lymphocytes between the two groups was observed ($p < 0.05$). From this finding we believe that the enumeration of the percentage of T lymphocytes may be insufficient to evaluate the adequacy or inadequacy of this population of cells in the peripheral blood. It may suggest that a marked deficiency of the capacity for cellular immunity exists in these drug (DDS) resistant patients. At the present, it is not clear how certain patients gain drug resistance during antileprosy chemotherapy. Moreover, it is not known whether or not development of drug resistance is related to the immunologic status of these patients. However, our results suggest that severe T cell deficiency may in some way influence or be a consequence of the propensity to develop resistance to chemotherapy.

The mean numbers of T cells in seven cases of *erythema nodosum leprosum* (ENL) are higher than that of other cases with active lepromatous leprosy patients who did not have this complication. Even though this finding is not statistically significant, it shows a similarity with results obtained in the study of T lymphocytes by the rosette-forming technic in these two groups.

The T cell numbers of five cases of active lepromatous leprosy who had received steroid treatment did not differ significantly from the T cell numbers of those patients with lepromatous leprosy who had not received steroid. This result indicates that the low dose of steroid (20 mg of prednisolone) used in chronic treatment of these cases, does not profoundly influence the T cell numbers in peripheral blood. This fact is consistent with the results obtained by others (^{6,14}).

All of the findings of this study, including the decreased number of T cells in active lepromatous leprosy and in drug (DDS) resistant patients, were similar. Therefore, the evaluation of T lymphocytes using a heterologous antiserum apparently specific for T cells may be taken as a method valid for findings based on study of T-RFC which we and others have reported elsewhere. Means

of evaluating T cell numbers yield essentially the same information as is obtained from a study of the numbers of so-called T-rosette-forming cells. In this study no advantage of one method for the other was observed.

SUMMARY

T lymphocytes were evaluated in the peripheral blood of patients with various forms of leprosy, using a heterologous antiserum specific for human T cells. A significant decrease in T lymphocyte numbers was observed in cases of active lepromatous leprosy but not in the inactive lepromatous, borderline or indeterminate forms of the disease.

Patients with lepromatous leprosy resistant to chemotherapy showed a lower level of T lymphocytes than did drug sensitive patients, while patients with lepromatous leprosy complicated by *erythema nodosum leprosum* showed higher levels than did those with uncomplicated lepromatous leprosy. Evaluation of T lymphocytes by microcytotoxicity test with the anti-T-cell serum used in this study proved to be as accurate as the nonimmune or spontaneous formation of rosettes with the sheep red blood cells after incubation at 37°C.

RESUMEN

Se evaluaron los linfocitos T en la sangre periférica de pacientes con diversas formas de lepra, usando un antisuero heterólogo específico para células T humanas. Se observó una disminución significativa del número de linfocitos T en los casos con lepra lepromatosa activa, pero no en los casos con lepra lepromatosa inactiva o con la forma borderline o la forma indeterminada de la enfermedad.

Los pacientes con lepra lepromatosa que eran resistentes a la quimioterapia mostraron un nivel más bajo de linfocitos T que los pacientes sensibles a drogas, mientras que los pacientes con lepra lepromatosa complicada por eritema nodoso leproso mostraron niveles más altos que los con lepra lepromatosa no complicada. La evaluación de los linfocitos T por medio de la prueba de microtoxicidad con el suero anti-células T utilizado en este estudio, demostró ser tan exacta como con el método no inmune, o de formación espontánea de rosetas con glóbulos rojos de carnero, después de incubación a 37°C.

RÉSUMÉ

On a eu recours à un anti-sérum hétérologue

spécifique pour des cellules-T humaines, pour évaluer les lymphocytes-T dans le sang périphérique de malades atteints de diverses formes de lèpre. Une diminution significative dans le nombre des T-lymphocytes a été constatée dans les cas de lèpre lépromateuse active; cela n'a pas été observé dans les formes de lèpre lépromateuse inactive, borderline ou indéterminée.

Les malades présentant une lèpre lépromateuse résistante à la chimiothérapie ont présenté un niveau plus bas de T-lymphocytes que les malades sensibles à la thérapeutique. Par ailleurs, les patients souffrant d'une lèpre lépromateuse compliquée par un *érythème noueux lépreux* montraient des niveaux plus élevés que ceux atteints de lèpre lépromateuse non compliquée. L'évaluation des T-lymphocytes par une épreuve de microcytotoxicité au moyen d'un sérum préparé contre les cellules-T, telle qu'elle a été pratiquée dans cette étude, s'est révélée aussi précise que la méthode basée sur la formation non immune ou spontanée de rosettes avec érythrocytes de moutons incubés à 37°C.

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