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Rosette-forming Cells in Patients with Treated Leprosy[1]

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It has been suggested that patients with leprosy, especially lepromatous leprosy, suffer from defects in their cellular immune responses. Evidence for such an impairment has been obtained by measuring different parameters of cellular immunity: delayed-type hypersensitivity skin reactions ([2]), inductions of contact sensitization ([14, 19, 22]), skin allograft survivals ([5]), reconstitutions by normal lymphocytes ([18, 19]), lymphocyte transformation tests *in vitro* with nonspecific mitogens ([2, 5, 9, 11, 12, 17, 21]) and antigens ([2, 4, 6]), or in mixed leukocyte cultures ([7]). Some changes in the proportions of T and B lymphocytes in the peripheral blood of leprosy patients have also been reported, but the results obtained have not been consistent. In some studies, a decrease in T cells and an increase in B cells were found ([10, 16]), while in another study, on a similar group of patients, a decrease in B cells was found ([15, 20]). In some reports, the decrease in total T cells was found only in patients with untreated lepromatous leprosy who had a high mycobacterial load in the skin, and not in patients who had been treated with dapsone (DDS) ([16]). Recently, disturbances in cell cooperation, for example in T-macrophage interaction or in the effects of T suppressors on immune responses, have been reported in patients with leprosy ([8, 13]).

In the present study, we have investigated differences in the peripheral blood lymphocytes between controls and patients with either lepromatous leprosy or borderline leprosy. The cell populations we have measured include total leukocytes, total lymphocytes (by counts and morphology on stained smears), and subpopulations of lymphocytes defined by formation of rosettes under different conditions. Cells forming rosettes in a standard assay with sheep red blood cells or SRBC (ERFC) are considered to be total T cells. Rosette formation with SRBC coated with antibody and complement (EAC RFC) was used to detect non-T cells (mainly B cells, some monocytes, and null cells with Fc receptors). In studies on other kinds of patients, such as cancer patients, total ERFC often did not detect differences between patients and controls although disturbances in normal cell populations were suspected ([3, 24]). Modifications of the ERFC tests may be used which detect more subtle differences. Therefore in this study we identified subpopulations of T cells in two ways: "active" ERFC, cells forming rosettes immediately with SRBC, thought to represent recently activated T cells ([24]) and "high affinity" RFC, able to form rosettes at 29°C instead of the usual 4°C ([3, 25]). The results reveal some qualitative differences between control and patient peripheral blood lymphocytes.

MATERIALS AND METHODS

Patients. All patients had a clinical diagnosis of either lepromatous (LL) or borderline (BT, BB, BL) leprosy in the leprosy therapy center of Ha-Son-Binh province. All had been affected for more than three years and had been treated with DDS for long periods. The LL patients were all bacteriologically positive.

Lymphocyte preparation. Heparinized blood was separated over Ficoll-Isopaque, and the cells from the interface resuspended at 2×10^6/ml in Hanks' solution.

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TABLE 1. *Leukocyte and lymphocyte numbers in normal and patients' blood (Mean ± S.D.).*

Subjects	Leukocytes[a]	% Lymphocytes	Lymphocytes[a]
Normal (N = 30)	6477 ± 1594	30.60 ± 11.37	1945 ± 731
LL (N = 14)	4971 ± 1307[b]	40.21 ± 10.32[b]	1998 ± 773
Borderline (N = 14)	5657 ± 1906	35.64 ± 12.95	1910 ± 754

[a] Number of cells per mm^3 in peripheral blood.
[b] Significantly different from normal controls, $p < 0.01$, t test.

SRBC preparation. Sheep erythrocytes (always from the same sheep) were stored in Alsever's solution for up to ten days. They were washed thrice in phosphate buffered saline (PBS) and resuspended in Hanks' for E, 29° and active rosette assays. EAC were prepared by mixing equal volumes of SRBC (10^9/ml) with rabbit anti-SRBC serum diluted to a non-agglutinating titer. After 30 min incubation at 37°C and washing to remove excess antibody, the EA were mixed with an equal volume of mouse serum diluted 10× in Hanks'. After further incubation (30 min at 37°C) and washing, these were resuspended at 2×10^8/ml.

Rosette assays. For the standard (4°C) and the 29°C assays, equal volumes of lymphocytes (2×10^6/ml) and SRBC (2×10^8/ml) were incubated together at 37°C for 5 min then centrifuged at $150 \times g$ for 5 min. Replicate tubes were placed at 4°C overnight (standard ERFC assay) or at 29°C for 1 hr before counting the percentages of rosette-forming cells. For the "active" rosette assay, one volume of the lymphocyte suspension was first incubated at 37°C for 1 hr with an equal volume of fetal calf serum. This mixture was then added to two volumes of SRBC (16×10^6/ml) and centrifuged at $150 \times g$ for 5 min; the rosette-forming cells were counted immediately. For the EAC assay, equal volumes of lymphocytes and EAC supension were centrifuged together at $150 \times g$ for 5 min, then incubated for 30 min at 37°C before counting rosettes.

The number of "non-rosetting" cells is the difference between 100% and the sum of the percentages of ERFC and EAC RFC.

Rosettes were counted by staining the gently resuspended cells with acridine orange to identify lymphocytes in the centers of rosettes which were defined as lymphocytes binding three or more erythrocytes. Each assay was carried out in triplicate and the mean of the three counts (of 100 lymphocytes each) calculated. Differences between control and patient groups were analyzed with a t test for significance.

RESULTS

The numbers of peripheral blood leukocytes and the percentages and absolute numbers of lymphocytes are shown in Table 1. The numbers of leukocytes in both the group with treated LL and that with borderline leprosy were lower than in the control group; the differences were significant only for the LL group. The increase in the percentage of lymphocytes in the LL group was also significant but not in the borderline group. It is interesting that the absolute number of peripheral blood lymphocytes was about the same in all three groups.

The percentages of ERFC from the standard assay, the "active" assay, and the 29°C "high affinity" assay are shown in Table 2. In the case of the standard ERFC, or total T cells, there was a significant decrease in the LL patients compared to the controls. Although the count for borderline patients was somewhat lower than for the controls, the difference was not significant. The same was true for both "active" and "high affinity" RFC: in both tests the LL group had significantly lower numbers of positive cells than the controls; the borderline group's scores, while apparently lower, were not significantly so.

In contrast to these T cell tests, in the case of EAC RFC, the percentages of positive cells in both LL and borderline groups were significantly higher than in the controls. When the proportion of cells not accounted for by either type of rosette assay was calculated, a significantly higher proportion of these was found in the LL group; the proportion in the borderline group was not only much lower than this, but slightly (although

TABLE 2. *Rosette-forming cells in normal and patients' blood (Mean ± S.D.).*

Subjects	% Rosette-forming cells				
	4°C ERFC	Active ERFC	29°C ERFC	EAC RFC	Non-rosetting
Normal	59.06 ± 7.27 (N = 30)	25.35 ± 8.57 (N = 23)	24.94 ± 9.23 (N = 18)	26.83 ± 8.30 (N = 30)	13.77 ± 11.65 (N = 30)
LL	42.00 ± 11.44[a] (N = 14)	14.29 ± 6.99[a] (N = 14)	10.71 ± 7.03[a] (N = 9)	34.29 ± 9.13[a] (N = 14)	23.71 ± 16.75[a] (N = 14)
Borderline	53.07 ± 12.66 (N = 14)	20.71 ± 7.50 (N = 14)	19.00 ± 10.23 (N = 7)	35.14 ± 9.29 (N = 14)	10.85 ± 12.64 (N = 14)

[a] Significantly different from normal controls, $p < 0.01$, t test.

not significantly) lower than the control group.

DISCUSSION

In this study a decrease in leukocyte numbers was found in leprosy patients with LL; there was also a decrease in the borderline group but this was not statistically significant. Since the absolute numbers of lymphocytes were not reduced, this may reflect changes in the numbers, distribution, or formation of granulocytes, due either to the infection with *Mycobacterium leprae* or to long treatment with DDS. Production and mobilization of lymphocytes into the circulation seems to be normal in these patients. However, some differences in the makeup of the population of lymphocytes could be demonstrated.

The proportion of cells forming rosettes with untreated sheep red blood cells under standard conditions was reduced in the LL patients but not in the borderline patients, and this was true also when two other kinds of T-rosette tests were used. These have been used in groups of patients of other kinds to pick up alterations in lymphocyte populations which were not evident when the standard test was used. Quan and Burtin ([23]) used the 29°C rosette assay to evaluate the immune status of cancer patients. Patients bearing tumors tended to have higher percentages of such RFC than did those with no evidence of disease. Although it is not yet known precisely which T cell subpopulations these tests can detect, they do seem to be able to detect alterations in the membrane/binding properties of lymphocytes, or in the makeup of the cell population with respect to these properties. "Active" rosettes have been shown to be increased after immunization and are thought to represent recently activated circulating T cells ([24]). The high affinity RFC have been shown to be distinct from the remainder of the ERFC with low affinity, able only to form rosettes with high numbers of SRBC at low temperature. The latter, low affinity RFC, includes the ERFC having receptors for IgG Fc, which is thought to include the supressor population ([23]). Thus, a decrease in high affinity RFC, relative to a minor decrease in total RFC, may indicate an increased number of supressor cells. Our results showing clear differences between patients and controls in both of these tests may then be taken as additional evidence for qualitative changes in the peripheral lymphocyte populations in the patients. These differences were less evident in the borderline patients, whose scores were generally lower than those of controls but not significantly so.

In contrast to the T cells, the numbers of EAC RFC were significantly increased in both patient groups with respect to the controls. Both of these patient groups are known to have high antibody levels and high levels of immune complexes; the higher numbers of B cells may be related to these phenomena. It may also, in the case of the borderline group, be only a compensatory increase due to the decrease in T cells.

The percentages of non-rosetting cells was significantly increased in the LL patients. These may be true "null cells" (including K, NK, and perhaps other cell types) or perhaps altered T cells which are no longer able to form rosettes. They may, indeed, represent the low affinity T RFC, thus T cells with receptors for IgG Fc. In contrast, these non-rosetting cells were not only much less frequent in the borderline group than in the

LL patients, they were even less frequent in the borderline patients than in the control group (although not significantly so).

Our observations, using modified rosette tests to detect subpopulations of peripheral blood lymphocytes, provide further evidence for qualitative differences in the makeup of the pool of circulating lymphocytes in lepromatous leprosy patients. Patients with borderline leprosy did not show most of these differences. A quantitative change in total leukocytes was also seen. Since these patients had longstanding infections and had been treated for years with DDS, it is not clear whether the differences were related to their susceptibility to the disease or were due to the course of infection or treatment. Further investigation as to the functions of these cells in the patients may yield more information.

SUMMARY

White blood cell counts and the percentages and absolute numbers of lymphocytes in the peripheral blood of active lepromatous (BT, BB, BL) leprosy patients, patients with borderline leprosy, and normal controls were determined. Lepromatous patients showed decreased leukocyte counts and elevated percentages of lymphocytes, resulting in normal absolute lymphocyte counts. The proportion of peripheral blood mononuclear cells forming "active" rosettes, standard (4°C overnight) rosettes, and "high affinity" (29°C for 1 hr) rosettes with sheep erythrocytes, and rosettes with EAC were determined. Lepromatous patients, compared with normal controls, had decreased "active" rosettes, standard rosettes, and "high affinity" rosettes with sheep erythrocytes with an increase in the nonrosetting proportion. Both lepromatous and borderline leprosy patients showed increased percentages of EAC rosettes compared with normal controls.

RESUMEN

Se determinaron las cifras de leucocitos y los porcentajes y números absolutos de linfocitos en la sangre periférica de pacientes con lepra lepromatosa activa o con lepra intermedia (BT, BB, BL) y de controles normales. Los pacientes lepromatosos presentaron cuentas disminuídas de leucocitos y porcentajes elevados de linfocitos, dando como resultado cuentas absolutas normales de linfocitos. También se determinó la proporción de células mononucleares sanguíneas formadoras de rosetas "activas," de rosetas estándar (4°C, toda la noche) y de rosetas de "alta afinidad" (29°C por 1 hora) con eritrocitos de carnero, y de rosetas EAC. Comparados con los controles, los pacientes lepromatosos tuvieron disminuídas sus rosetas "activas," sus rosetas estándar y sus rosetas de "alta afinidad" con eritrocitos de carnero además de un incremento en la proporción de células no formadoras de rosetas. Comparados con controles normales, los pacientes con lepra lepromatosa y los pacientes con lepra intermedia mostraron porcentajes incrementados de rosetas EAC.

RÉSUMÉ

On a procédé à des numérations cellulaires des globules blancs, et à la détermination des pourcentages de lymphocytes et du nombre absolu de ces cellules, dans le sang périphérique de malades atteints de lèpre lépromateuse active, de malades souffrant de lèpre dimorphe (BT, BB, BL), et de témoins normaux. Les malades lépromateux ont révélé une diminution du nombre des leucocytes, et une élévation du pourcentage des lymphocytes, ce qui a pour conséquence des chiffres normaux pour la numération du nombre total de lymphocytes. La proportion des cellules mononucléaires du sang périphérique formant des rosettes actives, des rosettes standards (4°C en une nuit), et des rosettes de haute affinité (29°C après 1 heure), avec des érythrocytes de moutons, et des rosettes avec EAC ont été déterminées. Quand on compare les malades lépromateux avec des témoins normaux, on constate chez ceux-là une diminution des rosettes actives, des rosettes standards et des rosettes à haute affinité pour les érythrocytes de moutons; par ailleurs, on constate une augmentation dans la proportion de cellules ne formant pas de rosettes. Les malades lépromateux et les malades atteints de lèpre dimorphe ont montré des pourcentages accrus de rosettes EAC par rapport aux témoins normaux.

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