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# Thymus-Dependent Lymphocytes of Peripheral Blood in Leprosy Patients

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Study of the numbers of thymus-derived lymphocytes by the rosette assay (T-RFC) in patients with leprosy reveals that lower than normal numbers of T-RFC are regularly seen in those patients with the active lepromatous form of this disease. Essentially normal numbers of T-RFC were found in inactive lepromatous, borderline, and indeterminate types of leprosy. The lowest percentages and lowest absolute numbers of T-RFC were encountered in patients with lepromatous leprosy resistant to chemotherapy. Patients with lepromatous leprosy complicated by erythema nodosum leprosum show numbers of T-RFC that are more nearly normal than the numbers of T-RFC in patients with uncomplicated lepromatous leprosy. These findings are discussed with respect to the pathogenesis of lepromatous leprosy and the T-RFC deficiency demonstrated in this disease. The possibility that transient defects in T-RFC numbers or function may predispose to lepromatous leprosy is proposed.

The immunological phenomena in leprosy were recently studied by several workers (2, 3, 5, 12, 13, 15, 18, 27-30). It is now well documented that patients with leprosy, especially the lepromatous type, have abnormalities of cellular immunity. These abnormalities are reflected as (i) depression of delayed skin reactivity to various cutaneous test antigens (2), (ii) difficulty of sensitization with skin-sensitizing agents as compared to normal subjects (28, 29), (iii) delayed rejection of skin allografts in patients with either lepromatous or tuberculoid types of leprosy (this abnormality, which is most pronounced in lepromatous leprosy, may be encountered) (12), (iv) depressed mitogen-induced transformation of peripheral blood lymphocytes (3, 5, 13, 18, 30) and failure to produce migration inhibitory factor (MIF) after stimulation with certain antigens in vitro (15), and (v) gross depletion of the lymphocyte population in the deep cortical areas of lymph nodes (27).

Some authors (13, 27) have suggested that these abnormalities may be due to reduction of the thymus-derived lymphocyte (T lymphocyte) population in the lepromatous form of leprosy. However, Gaafar and Turk (8) have indicated that the histological findings of reduced lymphocyte population in lymph nodes are not necessarily an indication of deficiency of T lymphocytes since such histological patterns could be caused by a mechanical obstruction to the circulation of lymphocytes through the lymph node.

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). It has been proposed that such increases of B lymphocyte numbers may represent overcompensation for a deficiency of T lymphocytes (or T cell) since a deficiency of T lymphocytes in patients with lepromatous leprosy was reported (6, 9).

The reaction between a certain population of human peripheral blood lymphocytes and sheep red blood cells (SRBC) results in "rosette" formation which has been shown to be an excellent assay for T lymphocytes (1, 4). These rosettes differ from immune rosettes, especially in that the formation process requires incubation at 37 C (14, 31). The numbers of these human rosette-forming cells (T-RFC) are not related to hemagglutinin titers, and the cells do not possess readily demonstrable surface immunoglobulins such as those that characterize antibody-producing B lymphocytes (1, 14).

In this paper, we report direct evaluation of

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the number of circulating T-RFC in various forms of leprosy. In addition we discuss the relation between these T-RFC and the various types of leprosy. (Our observations were originally presented at the 2nd International Workshop meeting on immunodeficiency diseases in man, February, 1973, and at the American Federation for Clinical Research meetings, 28, April, 1973.)

## MATERIALS AND METHODS

**Patients.** A total of 36 patients having various types of leprosy were studied; 20 had the most polar form of active lepromatous leprosy (LL), 9 had inactive LL, 4 had borderline leprosy (BB), and 3 had indeterminate leprosy. The disease activity or inactivity was determined by clinical manifestations and by histological and bacteriological study. The inactive LL showed that bacterial indexes in both smear and biopsy were 0. The classification of each patient was based on the Ridley and Jopling scale (22). All patients had been treated with diaminodiphenyl sulfone (Dapsone) or a phenazine dye, B663 (Clofazimine) or rifampin, for varying durations before blood was collected. The duration of the disease up to the time of study varied from 1 to 45 years. Racial backgrounds included white Caucasian, Mexican-American, Filipino, Samoan, Hawaiian, Black, Chinese, and persons of mixed origins.

Seven cases of active LL (cases 1, 2, 6, 8, 11, 15, and 17) were complicated by erythema nodosum leprosum (ENL). Twelve of twenty cases (cases 2, 3, 5–7, 9, 12–16, and 20) were drug resistant (to diaminodiphenylsulfone [DDS]). Five had received various doses of steroid (20 to 40 mg of prednisolone per day) for varying periods before study. Three were being treated with steroid at the time of study.

**Lymphocytes.** Samples of peripheral venous blood were drawn with sterile plastic syringes containing heparin and were transported by airplane from the leprosarium (Public Health Service Hospital, Carville, La., and San Pedro, Calif., Public Health Service Hospital outpatient clinics) to the University of Minnesota. As a control on each occasion, blood was simultaneously collected from two or three healthy persons and handled in the same way.

**SRBC.** SRBC were stored at 4 C in Alsever solution (50% vol/vol). Before use, the cells were washed twice and adjusted to a 0.5% suspension in RPMI-1640 (approximately $80 \times 10^6$/ml).

The T-RFC were assayed by a modification of the method of Jondal et al. (14). Briefly, lymphocytes were isolated from the patient's peripheral blood by Ficoll-Hypaque gradients (24) and washed three times in serum-free RPMI-1640 tissue culture medium. The viability of the cells was then determined by trypan blue dye exclusion method (each sample transported from Carville, La., and San Pedro, Calif., to Minneapolis, Minn. showed more than 98% viability). The cell concentration was adjusted to $5 \times 10^6$/ml and mixed with an equal volume of 0.5% SRBC to give a final ratio of 10 SRBC per lymphocyte. The mixture was incubated at 37 C for 5 min, centrifuged at $200 \times g$ for 5 min to optimize cell contact, and then incubated again at 4 C for 60 min. After gentle resuspension of the pellet with a Pasteur pipette, one drop of the cell suspension was mounted on a hemocytometer and the T-RFC were counted. The percentage of T-RFC among 500 or more lymphocytes counted was calculated, and the absolute numbers were calculated from the total white blood cell and differential counts.

## RESULTS

T lymphocytes in peripheral blood of patients with leprosy were assayed by counting the number of rosette-forming cells. As shown in Table 1, the mean value for T-RFC in a control group of seven healthy individuals whose blood was shipped with that of the patients was 40%. A group of 40 normal controls studied previously at the University of Minnesota by the same assay procedure had a mean of 46% with a range of 34 to 62% and a standard deviation of 6%. The shipped control samples were therefore slightly low in their content of T-RFC.

The number of T-RFC for various types of leprosy is shown in Table 2. Both percentages and absolute numbers of T-RFC were low in active LL, whereas in the other types of

TABLE 1. *Percentage and absolute number of T-RFC in the blood of healthy human subjects*[a]

| Subjects | Absolute no. of lymphocytes/mm³ | T-RFC (% of total lymphocytes)[b] | Absolute no. of T-RFC[c] |
|---|---|---|---|
| 1 | 2,464 | 40.0 | 985 |
| 2 | 1,540 | 35.9 | 568 |
| 3 | 2,162 | 39.7 | 858 |
| 4 | 2,508 | 43.7 | 1,095 |
| 5 | 3,900 | 47.1 | 1,836 |
| 6 | 2,079 | 44.1 | 916 |
| 7 | 1,920 | 34.1 | 654 |

[a] Absolute values designate the numbers of cells per cubic millimeter of blood.

[b] Mean = 40.8; standard deviation (SD) = 4.1; standard error (SE) = 1.5.

[c] Mean = 987; SD = 385; SE = 148.

TABLE 2. *Percentage and absolute number of T-RFC in the peripheral blood lymphocytes of the patients with various types of leprosy*

| Group[a] | No. of subjects | Percent of RFC (mean ± SE) | Absolute no. of RFC/mm³ (mean ± SE) |
|---|---|---|---|
| Control | 7 | 40.8 ± 1.5 | 987 ± 148 |
| Active LL | 20 | 30.9 ± 1.5 | 613 ± 66 |
| Inactive LL | 9 | 48.8 ± 1.7 | 902 ± 68 |
| BB | 4 | 51.2 ± 3.5 | 1,101 ± 232 |
| IL | 3 | 44.3 ± 4.5 | 1,502 ± 165 |

[a] LL, Most polar form of lepromatous leprosy; BB, borderline leprosy; IL, indeterminate leprosy.

leprosy (inactive lepromatous, BB, and indeterminate), the numbers of T-RFC were either normal or possibly higher than normal. The latter differences were not statistically significant. It is very interesting to see that a spectrum of the number of T-RFC corresponds with the clinical spectrum of the diseases. The rank order of the groups, from low to high, is as follows: (i) active LL, (ii) inactive lepromatous, (iii) BB, and (iv) indeterminate leprosy.

Active LL was further studied as described below. The number of T-RFC in the blood of patients with this type of disease is shown in Table 3. The mean values for both the percentages and absolute numbers of T-RFC are low compared with the values obtained for the control group ($P < 0.01$ for percentage, $P < 0.05$ for absolute number as determined by Student's $t$ test). Fifteen of 20 patients with LL showed levels lower than the mean control values both with respect to percentages and absolute numbers of T-RFC. Seven patients showed very low values (below 30% and an absolute number below 600) and an additional five patients showed either percentages or absolute numbers of T-RFC in this very low range. The percentage of the other four cases (cases 4, 8, 11, 19) fell within the normal range. Two of these, however, had absolute numbers of T-RFC lower than the mean normal value. Except for these two patients, a good correlation between the percentages and absolute numbers was found in most instances. A notable exception is seen in patient 1, who had a very low percentage of T-RFC but a high total lymphocyte count, and consequently a high value of numbers of T-RFC.

Some of the patients with active LL had

TABLE 3. *Active polar form of lepromatous leprosy*[a]

| Patient no. | Age | Sex | Race | Bacterial index | Biopsy index or MI[b] | Treatment[c] | | DDS resistant | ENL | T-RFC (%)[a] | Absolute no. T-RFC[e] | Remarks |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | Antileprosy chemotherapy | Drug treatment | | | | | |
| 1 | 22 | o | Mexican | 6+ | 3.0 | | Steroid | | + | 20.0 | 1040 | Since 1968 |
| 2 | 44 | o | Hawaiian | 4+ | 1.5 | DDS | Steroid | + | + | 17.5 | 260 | 1943 |
| 3 | 48 | o | Samoan | 5+ | 2.4 | B663 | | + | | 24.6 | 791 | 1931 |
| 4 | 68 | o | Caucasian | 4+ | 3.0 | DDS | | | | 43.6 | 529 | Several yr |
| 5 | 74 | o | Caucasian | 5+ | 2.0 | B663 | Hydrochlorothiazide (for blood pressure) | + | | 25.4 | 316 | 1943 |
| 6 | 44 | o | Mexi-A | 4+ | 2.4 | DDS | Steroid | + | + | 28.0 | 289 | 2 yr |
| 7 | 69 | o | Mexi-A | 4+ | ND | Sulphetrone | Oretic | | | | | |
| | | | | | | | Aldomet | + | | 32.0 | 541 | 1950 |
| 8 | 43 | o | Cuban | 4+ | | DDS | Thalidomide | | + | 38.5 | 1002 | 1943 |
| 9 | 54 | o | Caucasian A | 5+ | | Rifampin | Valium, meprobamate | + | | 24.0 | 375 | 1939 |
| 10 | 38 | o | Mexi-A | 5+ | | DDS | Steroid (for neuritis) | | | 29.9 | 828 | Unknown |
| 11 | 25 | o | Filipino | 5+ | | B663 | Steroid | | + | 45.2 | 654 | 2 yr |
| 12 | 52 | o | Chinese | 2+ | | B663 | | + | | 33.3 | 692 | 1941 |
| 13 | 50 | o | Caucasian | 3+ | | B663 | Atromid-S | + | | 29.2 | 555 | 1947 |
| 14 | 69 | o | Caucasian | 4+ | | B663 | Vasodilan Diamox. | + | | 27.0 | 229 | 1933 |
| 15 | 57 | o | Filipino | 4+ | | B663 | Thalidomide | + | + | 33.5 | 906 | 1932 |
| 16 | 62 | o | Caucasian | 4+ | | B663 | Sinequan. meprobamate | + | | 33.9 | 445 | 1938 |
| 17 | 47 | o | Cuban | 4+ | | B663 | Thalidomide | | + | 29.8 | 871 | 1945 |
| 18 | 50 | o | | 2+MI | 0/28 | DDS | | | | 28.0 | 328 | 3 yr |
| 19 | | o | | 5+MI | 0/100 | DDS | | | | 39.0 | 1259 | 2 yr |
| 20 | | o | | 6+MI | 2/100 | Rifampin | | + | | 35.0 | 352 | 1957 |

[a] Patient information and percentage and absolute number of T-RFC/mm³ of blood from patients with active lepromatous leprosy.

[b] ND, Not done.

[c] DDS, Diaminodiphenylsulfone; B663, riminophenazine derivatives; rifampin, 3-(4-methyl-piperazinyl-iminomethyl); sulphetrone, diamino par 3-phynol 1-3 disulphopropylaminosulfone. Oretic, hydrochlorothiazide; Aldomet, methyldopate hydrochloride, MSD; Valium, diazepan; Atromid-S, clofibrate; Diamox, acetazolamide; Sinequan, doxepin HCl; Vasodilan, isoxsuprine HCl.

[d] Mean = 30.9; SD = 6.9; SE = 1.5.

[e] Mean = 613; SD = 291; SE = 66.

received steroid treatment which might have suppressed the number of T-RFC. However, when five patients who had received steroid treatment were studied (Table 4), there was no meaningful difference in the number of T-RFC as compared with those not receiving steroid treatment (15 cases). In other words, whether steroid was given or not, T-RFC were regularly low in patients with active LL.

The patients with active LL were divided on the basis of whether or not their disease was complicated by ENL, and the levels of T-RFC were analyzed (Table 4). The percentages of T-RFC in patients whose disease was complicated by ENL (seven cases) were not different from the values obtained for the uncomplicated group, but the absolute number of T-RFC in the complicated cases was significantly higher (by Student's $t$ test) than that of uncomplicated cases ($P < 0.05$). The absolute number of T lymphocytes, however, was still significantly below normal.

At present, one of the most difficult problems in therapeutic management of leprosy is the problem presented by patients who seem to be drug resistant. It is not known whether or not development of drug resistance is related to immunological status of these patients. Hence, we studied the T-RFC levels in the drug-resistant patients. Both percentages and absolute numbers of T-RFC in the patients harboring drug-resistant organisms were low compared with the values for the patients whose organisms were not drug resistant (Table 5). This difference was statistically significant ($P < 0.05$, Student's $t$ test).

TABLE 4. *Comparison of T-RFC in patients with and without ENL and with and without steroid treatment*

| Group | No. of pateints | Percent of T-RFC (mean ± SE) | Absolute no. of T-RFC (mean ± SE) |
|---|---|---|---|
| ENL complicated | 7 | 30.3 ± 3.4 | 717 ± 116 |
| Without ENL | 13 | 31.2 ± 1.5 | 556 ± 74 |
| Steroid T* | 5 | 28.1 ± 4.4 | 614 ± 137 |
| No steroid | 15 | 31.9 ± 1.4 | 612 ± 82 |

TABLE 5. *Comparison of T-RFC: active lepromatous leprosy patients resistant and patients not resistant to drug (DDS)*

| Group | Cases | Percent of T-RFC (mean ± SE) | Absolute no. of T-RFC (mean ± SE) |
|---|---|---|---|
| Total of active LL | 20 | 30.9 ± 1.5 | 613 ± 66 |
| Drug (DDS) resistant | 12 | 28.7 ± 1.4 | 479 ± 62 |
| Non-drug (DDS) resistant | 8 | 34.2 ± 2.8 | 813 ± 100 |

## DISCUSSION

The identification of human T-RFC as T lymphocytes is now well documented (4, 31), and the use of the rosette assay has already proven of value for T cell quantitation in primary immunodeficiencies, transplant-related immunosuppression, and malignancy (16; D. F. Kiszkiss, Y. S. Choi, and R. A. Good, in press). This report indicates a further application of this assay, and the data herein indicate that deficiency of T-RFC numbers may be an important factor in active LL. The main finding of this study is that active cases of LL have low numbers of T-RFC as regards both percentage and absolute numbers. The quantitation on the basis of absolute numbers of T-RFC were needed, because there are cases (e.g., case 1 of active LL) where comparison on the basis of the percentage of T-RFC alone can be misleading.

Other types of leprosy investigated, including inactive lepromatous, indeterminate, and BB types, appear to have normal or higher than normal numbers of circulating T-RFC both with respect to percentage of the total lymphocyte population and absolute numbers. In fact, a consideration of the absolute numbers of T-RFC for patients of each of the groups studied here indicated that a broad spectrum of T cell numbers occurs in patients with leprosy. Patients with active LL, which is clinically the most serious type of infection, show the lowest numbers of T-RFC. T-RFC numbers in BB cases of leprosy show values intermediate between those seen in patients with active LL and patients with the indeterminate type of leprosy. Patients with inactive LL have T-RFC levels higher than patients with active LL but apparently lower than the levels seen in patients with BB.

The T-RFC levels in five of the patients with active LL who had received steroid treatment did not differ significantly from those of patients of other groups that had not received steroids. Thus, our results are consistent with the results of others (7; A. S. Fauci, personal communication), which indicate that there was no significant change of the T-RFC level by the doses of steroid used (20 to 80 mg of prednisolone). This is interesting in light of the known immunosuppressive influence of steroids.

The mean value of T-RFC in seven patients

whose disease was complicated by ENL is higher than the mean value obtained for patients with active LL who did not have the complication of ENL. This finding is consistent with the view that ENL may be a reaction that reflects good prognosis from the immunological standpoint (20). However, up to the present, we cannot establish a clear relationship between ENL per se, the number of T lymphocytes, and the prognosis. Some cases complicated by ENL are clinically quite troublesome, and their symptoms can last several years.

The patients with active LL who were refractory to chemotherapy with DDS had T-RFC levels lower as a group than patients who were responsive to DDS therapy. This difference is particularly apparent in terms of absolute numbers of T-RFC and suggests that a marked deficiency in cellular immunity exists in patients who are refractory to chemotherapy. It is tempting to speculate on the relationship between refractoriness to chemotherapy and the lowered T-RFC levels. At present it is not clear how certain patients become refractory to chemotherapy during treatment. It has been postulated that inadequate (low or irregular) doses of drug may produce drug resistance of the infecting organisms (19, 25). However, our results suggest another possibility, namely that severe T-RFC deficiency may in some way contribute to the tendency to become refractory to chemotherapy. Both could be related to the number of organisms with which the body must deal.

There is some disagreement regarding T lymphocyte levels and cellular immune status in leprosy patients. For example, some investigators (8, 13) argue for an actual T lymphocyte deficiency, whereas Gaafar and Turk (8) argue that the deficiency of lymphocytes in the deep cortical areas of the lymph nodes is consequent to inadequate lymphocyte circulation caused by macrophage accumulation. Our data suggest that, at least in the active LL, an actual T-RFC deficiency exists. One can then speculate that the T-RFC deficiency is intimately related to development of LL. This is consistent with the experimental findings of Rees (21) and others (10, 11) who have shown that inoculation of thymectomized and antilymphocyte serum-treated mice with *Mycobacterium leprae* results in a disseminated infection in mice over 6 months of age, whereas a similar inoculation in normal mice causes a localized tuberculoid lesion (23).

It is, of course, possible that the congestion of the T-dependent regions of lymphoid organs with histiocytes secondary to infection with *Mycobacterium leprae* interferes with normal traffic and proliferation of T lymphocytes. Thus, the deficit of T lymphocytes reflected by the low numbers in the circulation shown in this study may be consequent to the histiocytic accumulation at sites where T lymphocytes normally proliferate. However, we think that a transient T-RFC deficiency may occur which permits initiation of proliferation of *Mycobacterium leprae* which could in turn lead to further cellular immune dysfunction. Such transient T lymphocyte deficiency may not be recognized in retrospect. Indeed, it may be only identifiable in the experimental animals in which T lymphocyte deficiency can be artificially induced by appropriate manipulation, e.g., antilymphocyte serum or thymectomy (10, 11, 21).

At present, we do not think that T lymphocyte deficiency is a genetic defect because the number of T-RFC returns to normal levels after leukocyte infusion therapy or effective chemotherapy (17, 26). However, further study will be required to clarify when, what, and how the deficit of T-RFC develops in the host which expresses acquired LL.

## ADDENDUM

While this paper was in preparation data somewhat similar to those presented here for lepromatous leprosy were published by Dwyer et al. (N. Engl. J. Med. **288**:1036-1039, 1973).

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## LITERATURE CITED

1. Brain, P., J. Gordon, and W. A. Willets. 1970. Rosette formation by peripheral lymphocytes. Clin. Exp. Immunol. **6**:681-688.
2. Bullock, W. E. 1968. Studies of immune mechanisms in leprosy. I. Depression of delayed allergic response to skin test antigens. N. Engl. J. Med. **278**:298-304.
3. Bullock, W. E., Jr., and P. Fasal. 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. **100**:888-899.
4. Coombs, R. R. A., B. W. Gurner, A. B. Wilson. 1970. Rosette-formation between human lymphocytes and sheep red cells not involving immunoglobulin receptors. Int. Arch. Allerg. **39**:658-663.

5. Dierks, R. E., and C. C. Shepard. 1968. Effect of phytohemagglutinin and various mycobacterial antigens in lymphocyte cultures from leprosy patients. Proc. Soc. Exp. Biol. Med. **127**:391-395.
6. Dwyer, J. M., W. E. Bullock, and J. P. Fields. 1973. Disturbance of the blood T:B lymphocyte ratio in lepromatous leprosy. N. Engl. J. Med. **288**:1036-1039.
7. Fauci, A. S., and D. C. Dale. 1973. The effect of in vivo hydrocortisone on in vitro lymphocyte function in humans. Clin. Res. **21**:578.
8. Gaafar, S. M., and J. L. Turk. 1970. Granuloma formation in lymph nodes. J. Pathol. **100**:9-20.
9. Gajl-Peczalska, K. J., S. D. Lim, R. R. Jacobson, and R. A. Good. 1973. B-lymphocytes in lepromatous leprosy. N. Engl. J. Med. **288**:1032-1035.
10. Gaugas, J. M. 1968. Enhancing effect of antilymphocytic globulin on human leprosy infection in thymectomized mice. Nature (London) **220**:1246-1248.
11. Gaugas, J. M., and R. J. W. Rees. 1968. Enhancing effect of antilymphocytic serum on mycobacterial infections in mice. Nature (London) **219**:408-409.
12. Han, S. H., R. S. Weiser, and S. T. Kau. 1971. Prolonged survival of skin allografts in leprosy patients. Int. J. Leprosy **39**:1-6.
13. Han, S. H., R. S. Weiser, and Y. S. Lim. 1971. Transformation of leprous lymphocytes by leprolin, tuberculin and phytohemagglutinin. Int. J. Leprosy **40**:789-795.
14. Jondal, M., G. Holm, and H. Wigzell. 1972. Surface markers on human T and B lymphocytes. J. Exp. Med. **136**:207-215.
15. Katz, S. I., B. H. DeBetz, and H. Zaias. 1971. Production of macrophage inhibitory factor by patients with leprosy. Arch. Dermatol. **103**:358-361.
16. Lay, W. H., and N. F. Mendes. 1971. Binding of sheep red blood cells to a large population of human lymphocytes. Nature (London) **230**:531-532.
17. Lim, S. D., R. M. Fusaro, and R. A. Good. 1972. Leprosy. VI. The treatment of leprosy patients with intravenous infusions of leukocytes from normal persons. Clin. Immunol. Immunopathol. **1**:122-139.
18. Nelson, D. S., M. Nelson, and J. M. Thurston. 1971. Phytohaemagglutinin-induced lymphocyte transformation in leprosy. Clin. Exp. Immunol. **9**:33-43.
19. Pettit, J. H. S., R. J. W. Rees, and D. S. Ridley. 1966. Studies on sulfone resistance in leprosy. 1. Detection of cases. Int. J. Leprosy **34**:375-389.
20. Pettit, J. H. S., and M. F. R. Waters. 1967. The etiology of erythema nodosum leprosum. Int. J. Leprosy **35**:1-10.
21. Rees, J. W., M. F. R. Waters, and A. G. M. Weddell. 1967. Experimental lepromatous leprosy. Nature (London) **215**:599-602.
22. Ridley, D. S., and W. H. Jopling. 1966. Classification of leprosy according to immunity. A five group system. Int. J. Leprosy **34**:255-273.
23. Shepard, C. C. 1960. The experimental disease that follows the injection of human leprosy bacilli into foot-pads of mice. J. Exp. Med. **112**:445-454.
24. Thorsby, E., and A. Bratle. 1970. A rapid method for preparation of pure lymphocyte suspensions, p. 655. *In* P. I. Terasaki (ed.), Histocompatibility testing. Munksgaard, Copenhagen.
25. Trautman, J. R., and C. D. Enna. 1970. Leprosy: TICE's Practice of Medicine, vol. 2, p. 27-28.
26. Turk, J. L., and A. D. M. Bryceson. 1972. Immunologic phenomena in leprosy. Advan. Immunol. **19**:209-237.
27. Turk, J. L., and M. F. R. Waters. 1968. Immunological basis for depression of cellular immunity and the delayed allergic response in patients with lepromatous leprosy. Lancet **2**:436-438.
28. Turk, J. L., and M. F. R. Waters. 1969. Cell-mediated immunity in patients with leprosy. Lancet **2**:243-246.
29. Waldorf, D. S., J. H. Sheagran, J. R. Trautman, and J. B. Block. 1966. Impaired delayed hypersensitivity in patient with lepromatous leprosy. Lancet **2**:773.
30. Wong, P. C., C. H. Chan-Teoh, and S. Wu. 1971. Transformation of lymphocytes by phytohemagglutinin in leprosy sera. Int. J. Leprosy **39**:7-12.
31. Wybran, J., M. C. Carr, and H. H. Fudenberg. 1972. The human rosette-forming cell as a marker of a population of thymus-derived cells. J. Clin. Invest. **510**:2537-2542.