

A Follow up of T-cell Subsets and of Anti-*M. leprae* Antibody Titer as Measured by the FLA-ABS Test in Melanesian Leprosy Patients Under Polychemotherapy¹

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The uncontrolled development of *Mycobacterium leprae* in multibacillary lepromatous leprosy elicits a strong antibody production against the bacillus, which is at variance with paucibacillary leprosy where smaller amounts of anti-*M. leprae* antibodies are produced (^{5, 9, 10}). Another consequence of the bacillary load is the nonspecific disturbance of T-cell functions often found in lepromatous leprosy patients (^{6, 9, 18}). Recently, imbalances of T-cell subsets as defined by the monoclonal antibodies, OKT4 and OKT8, have been described in peripheral blood with a decrease in the OKT4+ : OKT8+ ratio of multibacillary patients without erythema nodosum leprosum (ENL) reactions. There is a transient increase in the OKT4+ : OKT8+ ratio seen during ENL episodes in these patients (^{2, 16}). Similar patterns of T-cell subset distributions have been reported from the analysis of T-cell infiltrates of skin lesions (^{14, 17, 23, 25}). The degree of nonspecific T-cell disturbances has been disputed by some workers (^{9, 19}). This perhaps may vary according to techniques, ethnic background, socioeconomic environment, and with therapy (^{2, 18}).

Several workers have reported that treatment induces a decrease in anti-*M. leprae* antibody titers as well as a normalization of the T-cell subsets and function. However, no precise kinetic data were available in patients followed up from the initiation of

treatment, except that of Mshana, *et al.* (¹⁵). We have undertaken such a follow-up study in a group of Melanesian leprosy patients, all living in the same South Pacific area (New Caledonia), receiving polychemotherapy, and studied before and during treatment for T-cell subset distribution and anti-*M. leprae*-specific antibodies by the fluorescent leprosy antibody absorption test (FLA-ABS) of Abe, *et al.* (¹). We found abnormalities of T-cell subset distribution in untreated lepromatous patients similar to those previously described (^{2, 24}), and high anti-*M. leprae* antibody titers. During the course of therapy, T-cell subsets returned to normal values after about nine months of treatment with occasional transient disturbances linked to ENL episodes. Conversely, no significant decrease of the antibody titers by the FLA-ABS test could be detected after 12 months of treatment.

MATERIALS AND METHODS

Patients. Fifty-one recently diagnosed leprosy patients, 6 to 60 years old, who were followed in the Centre Raoul Follereau in Nouméa, entered this study. They were classified along the leprosy spectrum according to the Ridley-Jopling clinical, histological, and bacteriological criteria (²¹) as tuberculoid (TT/BT) or lepromatous (LL/BL) patients. Among the tuberculoid group, 19 patients had never been treated when studied for the first time, and 5 of them had received therapy for three months. Among the lepromatous group, 22 patients had never been treated and one patient had been treated for less than four months. Four patients who had received irregular treatment for various periods of time and presented with clinical and bacteriological relapse were considered as untreated.

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All patients under study thereafter received polychemotherapy, either daily or bimonthly. Thus, among tuberculoid patients, one group received rifampin (600 mg) and dapsone (DDS; 100 mg) daily for six months, and another group received rifampin (600 mg) plus clofazimine (100 mg) plus ethionamide (500 mg) bimonthly for three months. Among the lepromatous patients, one group was treated with rifampin (600 mg), clofazimine (100 mg), and DDS (100 mg) daily for two years; another group received rifampin (600 mg), clofazimine (100 mg), and ethionamide (500 mg) bimonthly for two years.

Patients were followed every three months for T-cell subset enumeration and detection of anti-*M. leprae* antibodies. For lepromatous patients, data collected during the first year of this follow-up study are presented in this paper. For tuberculoid patients, whose treatment was stopped after three months, only the data recorded before and at the end of therapy were available in sufficient number. Since no difference was apparent between intermittent and daily therapy in any of the immunological parameters tested in this study, the results from both groups have been pooled.

Sixty-four healthy household contacts of the leprosy patients were also studied for the presence of anti-*M. leprae* antibodies. Forty-six healthy Melanesian subjects served as controls for T-cell subset studies. Sera from 15 normal subjects recently arrived from Europe were used as negative controls for the FLA-ABS test.

T-cell typing. T cells were typed using the OKT series of anti-T-cell monoclonal antibodies: OKT3 (anti-pan T cells), OKT4 (anti-helper T cells), OKT8 (anti-suppressor/cytotoxic T cells); the specificities of these monoclonal antibodies have been described elsewhere (²⁰). T-cell typing was performed as already described (²). In brief, lymphocytes were obtained from peripheral venous blood collected in the morning in heparinized tubes. Blood was centrifuged on a Ficoll-Triosil gradient. Monoclonal antibodies (Ortho Pharmaceuticals, Raritan, New Jersey, U.S.A.) were added at optimal concentrations: 5 µg/ml for OKT3 and OKT8, and 10 µg/ml for OKT4 in 50 µl Hanks' balanced solution. After a 30-min incubation at 4°C, the cells were washed

twice in Hanks' medium containing 5% irradiated fetal calf serum and 0.2% sodium azide. Fifty µl fluorescein conjugated F(ab')₂ fragments of goat anti-mouse IgG antibodies (Cappel Laboratories, Downingtown, Pennsylvania, U.S.A.) was then added. After a 30-min incubation at 4°C, the cells were washed three times with Hanks' buffer. The percentages of labeled cells were evaluated using fluorescence microscopy.

Detection of anti-*M. leprae* antibodies. The fluorescent leprosy antibody absorption test (FLA-ABS) described by Abe, *et al.* (¹) was used. In brief, whole *M. leprae*, provided by the *M. leprae* bank of the Pasteur Institute (Paris), was spread onto slides which were then successively treated by CCl₄ and a trypsin solution. The 50 µl volumes of sera to be tested were each mixed in a glass tube with 50 µl of a suspension of BCG (Institut Pasteur Production, Marnes la Coquette, France) and of *M. vaccae* (kindly given by Dr. Abe, National Institute for Leprosy Research, Tokyo, Japan) and 350 µl of diluent A (composed of 1 volume of 0.04% alcoholic cardiolipin-lecithin solution plus 19 volumes of phosphate buffered saline). The absorbed sera were then centrifuged, and the supernatants diluted to obtain 1:40, 1:60, 1:640, and 1:2560 final dilutions in diluent B (1 volume of a 1% bovine serum albumin solution plus 9 volumes of diluent A). A drop of each serum dilution was added to antigen smears for 1 hr at 37°C in a moist chamber. After washing, the smears were reacted with FITC-labeled goat anti-human F(ab')₂ fragments antibodies (Cappel), previously absorbed with BCG and diluted 1:40, overnight at 4°C. The slides were then washed and mounted in buffered glycerol, and observed under a fluorescent microscope (Labor Lux, Leitz, West Germany).

As described by Abe, *et al.*, smears were considered as positive when a majority of the bacilli appeared fluorescent. The titer is taken as the log₄ of the reciprocal of the highest dilution (divided by ten) giving positive smears (¹). Titers of 1 or more were considered as positive, since all sera of normal subjects with no known exposure to *M. leprae* had anti-*M. leprae* antibody titers below that value.

Statistics. Student's *t* test was used for all statistical analyses.

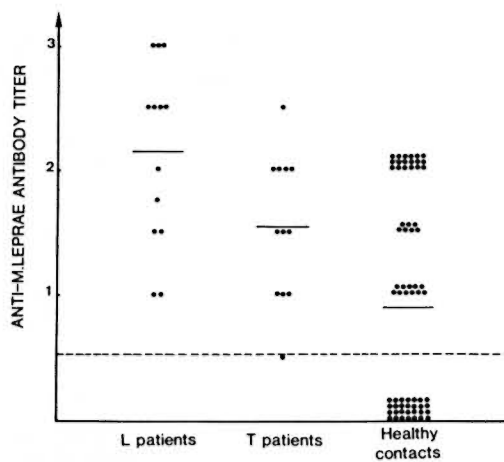


FIG. 1. Anti-*M. leprae* antibody titers as measured by the FLA-ABS test in leprosy patients and their healthy household contacts. Antibody titer is the \log_4 of one tenth of the reciprocal of the highest serum dilution giving a positive fluorescence in the FLA-ABS test (1). (—) = mean of antibody titers for each group of subjects; (---) = upper limit of normal values.

RESULTS

T-cell subset analysis in untreated patients (Table 1). Since lepromatous patients suffering ENL were previously reported to display peculiar T-cell subset disturbances, data obtained from these patients will be presented separately (see below). A significant decrease of the percentage of OKT3+ and OKT4+ cells, and of the OKT4:OKT8 ratio was noted in lepromatous patients as compared to the controls ($p < 0.05$). Tuberculoid patients exhibited normal T-cell numbers and subset distributions.

Anti-*M. leprae* antibodies in untreated patients and their healthy contacts (Fig. 1). Out of 13 untreated lepromatous patients tested by the FLA-ABS test, 100% proved to be positive with a mean titer of 2.13 (S.D. = 0.72). Among the group of tuberculoid patients, 11 out of 12 (92%) were positive (mean titer = 1.54, S.D. = 0.58). Fifty-six percent of the healthy contacts (36 out of 64) were also found positive (mean titer = 0.89, S.D. = 0.86).

Follow up of T-cell subsets and anti-*M. leprae* antibodies in patients under polychemotherapy. Nonreactional lepromatous patients exhibited a progressive normalization of the T-cell subset balance: after the ninth month of therapy, abnormalities in the OKT4:OKT8 ratio (Fig. 2) and in the percentages of OKT3+, OKT4+, and OKT8+ cells (data not shown) could no longer be detected. In contrast, no significant modification of the anti-*M. leprae* antibody titer could be seen during the first year of therapy (Fig. 2).

In tuberculoid patients, T-cell number, subset distribution and anti-*M. leprae* antibody titers remained unaltered after three months of therapy (Table 2).

T-cell subsets during ENL. Nine lepromatous patients could be studied while suffering an episode of ENL. A noticeable decrease in the percentage of OKT8+ cells with a proportional increase of the OKT4:OKT8 ratio was observed in this group of patients when compared to the group of healthy controls ($p < 0.05$) (Fig. 3A). Five patients who could be studied before, during, and after ENL exhibited a transient in-

TABLE 1. *T-cell subsets in untreated leprosy patients.*

Subjects ^a	No.	% positive cells among peripheral blood mononuclear cells with ^b			OKT4+:OKT8+ ratio
		OKT3	OKT4	OKT8	
LL/BL patients	19	59 ± 2 ^c	31 ± 2 ^c	30 ± 2	1.04 ± 0.07 ^c
TT/BT patients	19	63 ± 2	37 ± 2	29 ± 1	1.35 ± 0.09
Melanesian healthy controls	46	66 ± 2	41 ± 2	30 ± 2	1.42 ± 0.13

^a All subjects and patients were Melanesian.

^b Values are given as mean ± S.E.M.

^c Values significantly different from control values, $p < 0.05$, Student's *t* test.

TABLE 2. Effect of treatment on T-cell subsets and anti-*M. leprae* antibodies in tuberculoid patients.^a

Subjects	% positive cells among peripheral blood mononuclear cells with			OKT4+ : OKT8+ ratio	Anti- <i>M. leprae</i> antibody titer
	OKT3	OKT4	OKT8		
Untreated tuberculoid patients	63 ± 2 (19)	37 ± 2 (19)	29 ± 1 (19)	1.35 ± 0.09 (19)	1.54 ± 0.16 (12)
2-4 Months' treated tuberculoid patients	62 ± 2 (11)	38 ± 2 (11)	27 ± 2 (11)	1.48 ± 0.13 (11)	1.78 ± 0.14 (7)
Healthy controls	66 ± 2 (46)	41 ± 2 (46)	30 ± 2 (46)	1.42 ± 0.13 (46)	<1 (46)

^a Values are given as mean ± S.E.M. (N).

crease of the OKT4 : OKT8 ratio during the reaction (Fig. 3B).

DISCUSSION

Our present study of Melanesian leprosy patients living in New Caledonia (South Pacific) showed abnormalities of monoclonal antibody-defined, T-cell subsets similar to those previously reported by us (^{2, 24}) and

by Mshana, *et al.* in their study of Ethiopian patients (^{15, 16}). Whereas tuberculoid patients displayed a normal T-cell subset number and distribution, untreated lepromatous patients exhibited a decrease of the OKT3+ and OKT4+ cell percentage, and a consequential decrease of the OKT4 : OKT8 ratio. At variance with our previous report (²⁴), only a minor increase in the per-

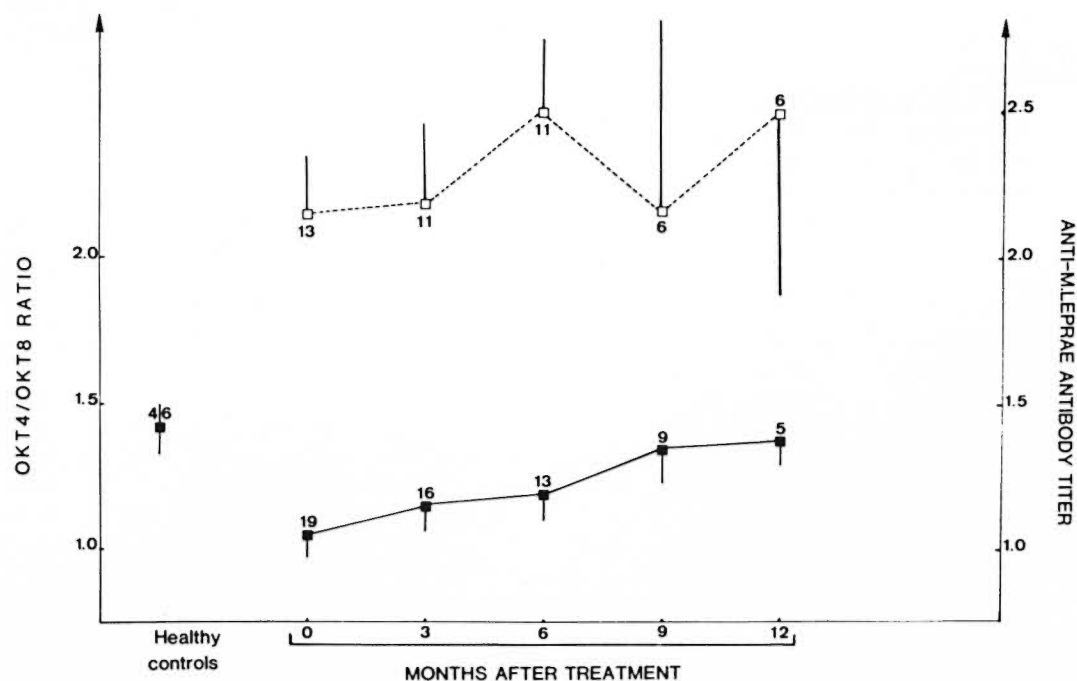


FIG. 2. OKT4:OKT8 ratio and anti-*M. leprae* antibody titer in lepromatous patients during the course of therapy. Each point indicates the mean (±S.E.M.) of the results from the number of patients indicated beside each point. —■— = OKT4:OKT8 ratio; --□-- = anti-*M. leprae* antibody titer, as measured by the FLA-ABS test.

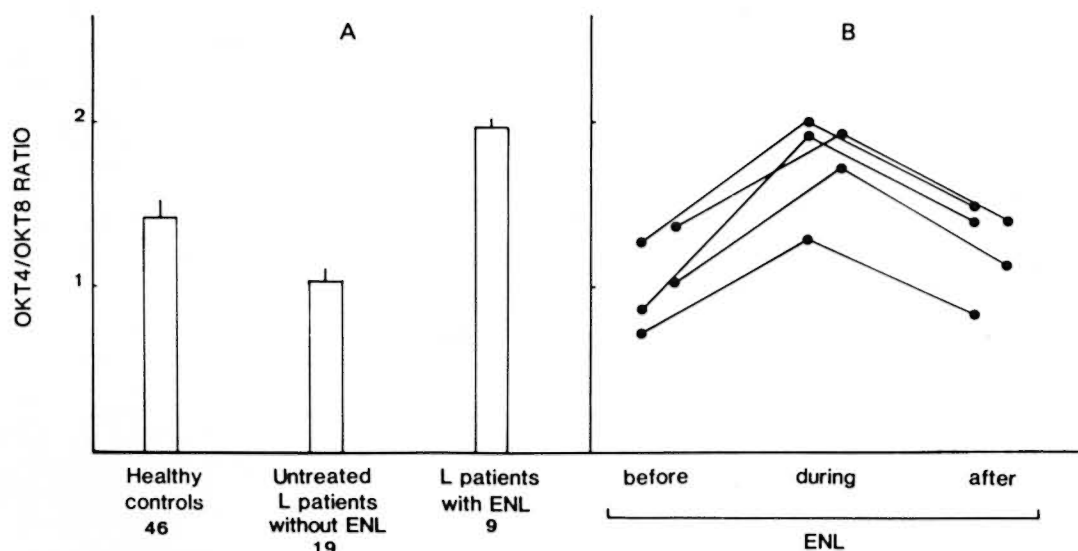


FIG. 3. OKT4:OKT8 ratio in lepromatous patients during ENL. A) columns indicate the mean \pm S.E.M. of OKT4:OKT8 ratios; B) OKT4:OKT8 ratios of 5 individual patients before, during, and after ENL.

centage of OKT8+ cells was observed in the present study. Bullock, *et al.* (7) and Rea, *et al.* (19), studying a group of patients in the U.S.A., reached a different conclusion since they found only a decrease of the OKT3+ cell absolute number and percentage without a disturbance of the T-cell subset distribution.

Discrepancies might be due to the different techniques used to score percentages of fluorescent cells (microscopy in our studies and that of Mshana, *et al.*, flow cytometry in the studies of Bullock, *et al.*, and Rea, *et al.*). Geographic and ethnic factors may also play a role. In this respect, it is very interesting to note that healthy Melanesian controls in this study presented a significant difference of T-cell subset distribution compared to normal European subjects living in Paris, concurrently studied by one of us using the same technique. Thus, Melanesian subjects exhibited a significantly lower OKT4:OKT8 ratio (1.4) than did Parisian subjects (1.8) ($p < 0.05$) with a significantly higher OKT8+ cell percentage (30 versus 25, $p < 0.02$). Besides the ethnic factors, environmental factors are likely to influence T-cell subset distribution, since it was recently demonstrated that sun exposure could quite significantly reduce the OKT4:OKT8 ratio (11).

In our present work, we could follow the

effect of polychemotherapy on the T-cell subset balance, which became normal again in lepromatous patients by nine months of treatment. Such an effect is likely to result from the reduction of the bacillary load rather than from a direct influence of the treatment on T-cells, since the T-cell subset distribution of tuberculoid patients, which was normal before treatment, was not altered by therapy. Our data are in agreement with previous reports of the normalization of mitogen-induced T-cell proliferation in treated lepromatous patients (18). A few lepromatous patients suffered ENL in the course of therapy, and showed transiently at that time a decrease of the OKT8+ cell percentage with an increase of the OKT4:OKT8 ratio, as also reported previously (2, 15).

We also studied the influence of treatment of anti-*M. leprae* antibodies, as measured by the FLA-ABS test (1). Applying this assay to sera of untreated lepromatous and tuberculoid patients and to household contacts, our results were in agreement with those reported by Abe, *et al.* (1) and more recently by Ji, *et al.* (12) in terms of antibody titers and of percentages of positive cases among these three groups. After one year of treatment, no significant change in antibody titer could be noted in lepromatous patients, nor after three months in tuberculoid pa-

tients, which suggests that anti-*M. leprae* antibody production as measured by this assay may be maintained at high levels by a small antigenic load. This explains the relatively higher proportion of positive sera observed among contacts and tuberculoid patients with the FLA-ABS test when compared with the results obtained by others using radioimmunoassays or ELISA techniques with whole *M. leprae* (²²), *M. leprae* sonicate (¹³), or the phenolic glycolipid-I of *M. leprae* (^{4, 5, 8, 26}) as antigens. Although antibody titers recorded with these assays were generally lower in long-term treated lepromatous patients than in untreated ones (^{4, 8, 10, 13}), kinetic data concerning the first year of treatment have rarely been reported. In the only available study, Touw, *et al.* (²²), using whole *M. leprae* as antigen in a radioimmunoassay, noticed a decrease in anti-*M. leprae* antibody titers in lepromatous patients after one year of treatment with dapsone.

In conclusion, our studies show that efficient therapy normalizes within a few months the T-cell subset abnormalities in nonreactional lepromatous patients without significantly affecting the anti-*M. leprae* antibody titers as detected with the FLA-ABS test. These results indicate that another serological assay should be used as an early indicator of treatment efficacy. The phenolic glycolipid-I of *M. leprae* (³), a species-specific antigen, can now be used as an antigen in immunoassays (^{4, 8, 26}), and this may provide a better quantitative evaluation of anti-*M. leprae* antibodies. On the other hand, whole *M. leprae* extract may also serve as antigen, since species specificity is not an absolute requirement for the purpose of following treatment efficacy. Such studies are in progress in our laboratory.

SUMMARY

Melanesian leprosy patients from New Caledonia were studied for the following parameters during the course of polychemotherapy: peripheral blood T-cell subsets, as identified in an immunofluorescence assay with monoclonal antibodies OKT3 ("pan-T"), OKT4 ("helper/inducer"), and OKT8 ("cytotoxic-suppressor"), and anti-*Mycobacterium leprae* antibodies in the se-

rum, as measured by the fluorescent leprosy antibody absorption test. A group of Melanesian healthy subjects with no known exposure to *M. leprae* served as controls. Healthy contacts of leprosy patients were also studied for the presence of anti-*M. leprae* antibodies. Untreated, nonreactional lepromatous patients displayed moderate but significant T-cell abnormalities, consisting of a decrease in the percentage of OKT3+ and OKT4+ cells with a decrease in the OKT4:OKT8 ratio. These abnormalities disappeared within nine months of treatment. A transient decrease in the percentage of OKT8+ cells with an increase in the OKT4:OKT8 ratio was seen in patients suffering erythema nodosum leprosum (ENL). Tuberculoid patients, whether treated or not, did not show any T-cell marker disturbances. Positive serological tests for anti-*M. leprae* antibodies were found in 100% of lepromatous patients, 92% of tuberculoid patients, and 56% of healthy contacts. No significant decline in the antibody titer was observed with treatment during the survey period.

RESUMEN

En un grupo de pacientes melaneses de la Nueva Caledonia con lepra sujetos a tratamiento con poliquimioterapia, se estudiaron las subclases de sus linfocitos T usando inmunofluorescencia y anticuerpos monoclonales contra OKT3 (pan T), OKT4 (cooperadores/inductores) y OKT8 (supresores/citotóxicos), y los niveles de anticuerpos anti-*Mycobacterium leprae* por la prueba de absorción del anticuerpo fluorescente. Como controles, se incluyeron individuos melaneses sanos sin exposición conocida al *M. leprae*. Se encontró que los pacientes lepromatosos no tratados y no reaccionales mostraron moderadas pero significativas anomalías en sus células T, consistentes en una disminución en el porcentaje de las células OKT3+ y OKT4+ y una disminución en la relación OKT4+:OKT8+. Estas anomalías desaparecieron en 9 meses de tratamiento. Los pacientes con eritema nodoso leproso (ENL) mostraron disminución alguna en sus porcentajes de células OKT8+ y el consecuente incremento en la relación OKT4+:OKT8+. Los pacientes tuberculoideos, tratados o no, no mostraron ninguna alteración en los marcadores fenotípicos de sus células T. En cuanto a las pruebas serológicas, se encontraron anticuerpos anti-*M. leprae* en el 100% de los pacientes lepromatosos, en el 92% de los pacientes tuberculoideos, y en el 56% de los contactos sanos. No se observó una disminución significativa en los títulos de anticuerpo durante el tiempo que duró el estudio.

RÉSUMÉ

On a étudié des malades de la lèpre mélanésien, de Nouvelle-Calédonie, en vue de mesurer une série de paramètres au cours de la polychimiothérapie. Ces paramètres étaient: les sous-groupes des lymphocytes T du sang périphérique, mis en évidence par une épreuve d'immuno-fluorescence utilisant des anticorps monoclonaux OKT3 (pan-T), OKT4 (auxiliaire/stimulant), et OKT8 (cytotoxique-suppresseur), et les anticorps anti-*Mycobacterium leprae* du serum, tels qu'on peut les mesurer par l'épreuve d'absorption des anticorps antiléproux fluorescents. Un groupe de sujets mélanésien en bonne santé, sans aucun antécédent connu d'exposition à *M. leprae*, a servi de témoin. Les contacts en bonne santé de malades de la lèpre ont également été étudiés en vue de mettre en évidence des anticorps anti-*M. leprae*. Les malades lépromateux non traités, ne souffrant pas de réaction ont témoigné d'anomalies modérées mais significatives des cellules-T. Ces anomalies consistaient en une diminution du pourcentage des cellules OKT3+ et OKT4+, avec un abaissement du ratio OKT4:OKT8. Ces anomalies disparaissaient après neuf mois de traitement. Un déclin transitoire du pourcentage des cellules OKT8+, accompagné d'une augmentation dans le ratio OKT4:OKT8, été observée chez les malades souffrant d'érythème noueux lépreux (ENL). Les malades tuberculoides, tant traités que non traités, ne présentaient aucune perturbation des lymphocytes-T servant de marqueur. Les épreuves sérologiques concernant les anticorps anti-*M. leprae* se sont révélées positives chez 100% des malades lépromateux, 92% des malades tuberculoides, et 56% des contacts sains. Aucune diminution significative du titre d'anticorps n'a été observée en rapport avec le traitement durant la période d'enquête.

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