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Variation of *in vitro* Lymphocyte Responses to *M. leprae* Antigen in Borderline Tuberculoid Leprosy Patients[1]

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Even before the discovery of the leprosy bacillus as the causative agent of leprosy, Danielssen and Boeck distinguished between two main groups of patients: "nodular" and "anesthetic" leprosy ([8]). Shortly after Hansen had described the bacillus ([13]), he showed that the lesions in "nodular" leprosy contained great numbers of bacilli while "anesthetic" leprosy patients had very few organisms in their lesions ([14]). This finding had, and still has, a great impact on the management of the patients and on leprosy control. When sulfones were introduced as specific chemotherapy, a spectrum of "nodular," or lepromatous leprosy became evident, now distinguishing between borderline lepromatous (BL), subpolar lepromatous (LLs) and lepromatous leprosy (LLp) ([22]).

A subdivision of tuberculoid leprosy (formerly called "anesthetic" leprosy) was based on the histopathological appearance of skin lesions ([21]). Lymphocyte infiltration and the presence of activated macrophages were presumed to reflect the degree of cell mediated immunity thought to be determinant for immune resistance.

The position of the different entities in a spectrum was later strengthened by results of *in vitro* tests for cell mediated immunity ([17]). Applying the morphological lymphocyte transformation test and the leukocyte migration inhibition test, the highest responses were found in polar tuberculoid patients, after which responses gradually decreased through TT/BT, BT, BB, to BL. Patients in the lepromatous part of the spectrum were virtually non-responsive, regardless of subclassification.

However, no clinical or bacteriological method exists to confirm that the spectrum of tuberculoid leprosy correlates to a spectrum of resistance ([20]). Responses in different immunological tests show a substantial individual variation between patients belonging to the same histopathological group. Lymphocytes from many patients with clinical and histopathological characteristics of borderline tuberculoid leprosy were found to show no response to *M. leprae* antigen *in vitro* ([4, 17]). Individual borderline tuberculoid leprosy patients could also have anti-*M. leprae* antibody titers higher than the average for lepromatous leprosy patients ([15, 24]). Thus the patient's immune status, described by *in vitro* lymphocyte responses and anti-*M. leprae* antibodies, did not seem to define the individual patient's position in the "immune spectrum of leprosy."

This paper is an attempt to discuss factors other than immune resistance that could influence patients' responses in one commonly used *in vitro* test for cell mediated immunity, namely the *in vitro* lymphocyte stimulation test with antigens from *Mycobacterium leprae*.

MATERIALS AND METHODS

Test subjects. All leprosy patients in this study were attending the All Africa Leprosy and Rehabilitation Training Center (ALERT) in Addis Ababa, Ethiopia. They were clinically diagnosed and classified by experienced leprologists; bacterial and morphological indexes (BI and MI) were determined by examination of skin smears from at least six sites in each patient ([19]) and the histological diagnosis and classification established in representative skin biopsies ([21]). For some of the experiments, a simplified classification has been used dividing the patients into tuberculoid (TT, TT/BT and BT together) and lepromatous

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(BL, LLs and LLp together). Mid-borderline leprosy patients (BB) are in these instances either excluded or referred to as a separate group named "borderline."

In lepromatous leprosy patients, there was a good agreement between histological and clinical classification. However, among patients with typical BT leprosy according to clinical criteria, about one third had a histological classification of either indeterminate (Idt) or early tuberculoid leprosy. This was particulary common in BT patients with flat hypopigmented skin lesions. Patients with a clinical diagnosis of BT are referred to as such even if the histological diagnosis was "indeterminate" or "early tuberculoid."

"Healthy contacts of leprosy patients" is a heterogeneous group consisting of household contacts who accompanied the patients to the clinic and professional contacts working in the ALERT clinic or the research laboratory next door, the Armauer Hansen Research Institute (AHRI), including both Ethiopians and expatriots. None of them had visible signs of leprosy, and they expressed physical well-being at the time of testing.

All subjects were bled by venipuncture between 8 and 10 a.m. and the blood processed immediately.

Preparation of antigens. Leprosy bacilli were prepared from non-ulcerated nodules of untreated lepromatous leprosy patients by homogenization in ice cold phosphate-buffered saline and differential centrifugation. The method is described in detail elsewhere ([4]). "Sonicated" antigens were produced by ultrasonification of bacillary suspensions at a concentration of 10^9 acid fast bacilli (AFB)/ml under constant cooling for 30 min, using a Branson B-12 Sonifier (Branson Sonic Power Company, Danburg, Connecticut, U.S.A.) with 80 W output on the medium-sized tip at which time no AFB could be seen in the suspensions. A 10^{-2} dilution of this sonicate is referred to as "equivalent dose" to 10^7 AFB/ml of whole bacilli.

In one experiment, six different preparations of *M. leprae* were produced from one subcutaneous nodule in an attempt to make the bacilli expose different antigenic properties. These six preparations were produced as follows:

a) *Whole washed bacilli* produced by homogenization and differential centrifugation as previously described ([4]). This preparation was used for the production of the other five preparations.

b) *Autoclaved bacilli* were whole washed *M. leprae* heated to 125°C for 15 min in an autoclave.

c) *Sonicated bacilli* were produced from whole washed bacilli by ultrasonification for 30 min using a Branson sonifier B12 with 80 W output on the medium sized tip under constant cooling in a volume of 5 ml containing 10^9 AFB/ml suspension. The sonicate was centrifuged and filtered through a Millipore 0.45 μ filter to obtain sterile, soluble antigen.

d) *Trypsin treated bacilli* were prepared by a light trypsinization according to Abe ([1]). A light trypsinization is supposed to expose *M. leprae* specific antigens on the surface of the bacilli. Five ml of a suspension of whole washed bacilli at a concentration of 10^9 AFB/ml in 0.1 M Tris-buffer of pH 7.8 were digested with 0.1% trypsin (Sigma) for 1 hour at 37°C and thereafter washed twice in cold saline. Since the spontaneous clumping of *M. leprae* after trypsinization was minimal, the washings were performed without human serum albumin (HSA) in the medium.

e) *KSCN-treated bacilli* were produced from whole washed bacilli according to Edgington ([11]) to get rid of possible host immunoglobulin affixed to the surface of the bacilli. Five ml of a 10^9 AFB/ml suspension were incubated in phosphate-buffered saline of pH 7.2 containing 2 M KSCN for 20 min at 22°C and thereafter washed twice in cold saline with 1% HSA.

f) *Dharmendra lepromin* was prepared from whole washed bacilli according to the method of Dharmendra ([9]). The saline suspended bacilli were mixed several times in a glass homogenizer with chloroform until all AFB were found in the chloroform phase. The chloroform was evaporated in a Rotavapor until almost completely dry and the bacilli resuspended in methyl ether. The bacilli were washed once in ether, and the ether thereafter fully

evaporated in a vacuum. One mg of the dry powder was ground in a mortar with 100 μl of 0.1 M NaOH, 10 ml of saline was added, and the pH adjusted to 7.5. The suspension was exposed for a few seconds to light sonification to disperse the material.

All the preparations were immediately subdivided into small aliquots and stored at −70°C until used so that all stimulants were frozen and thawed only once before they were used in the cultures.

M. bovis strain BCG was prepared from dried human vaccine (Glaxo Lab. Ltd., Greenford, England) and Purified Protein Derivative from *M. tuberculosis* (PPD) was purchased free of preservative from Statens Seruminstitut, Copenhagen, Denmark.

Phytohemagglutinin (PHA) (Reagent Grade, Wellcome Research Lab., Beckenham, England) was used in parallel with antigen stimulation in all experiments to check for cell viability and general culture conditions. Results from subjects whose lymphocytes did not show substantial stimulation by PHA were disregarded.

Antibody activity against *M. leprae* antigen 7. A specific radioimmunoassay for antibody activity against *M. leprae* antigen 7 ([16]) was used to semi-quantitate this antibody activity in patients' sera diluted 10^{-3} and 10^{-4}. This assay was based on the previously described assay for antibody activity against BCG antigen 60 ([15]). In short, *M. leprae* were prepared from infected armadillos by the method of Draper ([10]), ultrasonificated, centrifuged, and the supernatant labeled with ^{125}I. Antigen 7 shows a high affinity for ^{125}I in the electrolytic labeling procedure, and after dialysis and separation on Sephadex G 200, labeled antigen 7 was recovered virtually pure in the void volume. Staphylococci, strain Cowan 1, were used as the solid phase in the assay. A high titered anti-*M. leprae* antiserum produced in a rabbit served as a standard, and the maximal uptake of ^{125}I labeled antigen 7 by this serum was taken as 100%. Comparison of the percent binding in the two serum dilutions in each patient indicated that we were working on the descending part of the standard curve.

Serum source for the *in vitro* lymphocyte stimulation test. Since individual sera can show greatly different capacities to support an *in vitro* lymphocyte stimulation test, we used batches of pooled sera from about 20 healthy persons coming from leprosy nonendemic areas as standard serum in the tests. This decreased the batch-to-batch variation considerably. This serum was stored in small aliquots at −70°C until used. As autologous plasma, we used heparinized plasma collected from the top of the centrifuge tubes after Ficoll-Isopaque separation of whole blood. The presence of heparin ranging from 5 to 40 IU/ml did not influence the tests. Serum and plasma from the same individual provided the same supportive effect on the lymphocyte responses provided the serum was separated promptly.

***In vitro* lymphocyte stimulation test.** The method has been described in detail previously ([4,6]). In brief, peripheral blood was anticoagulated in the syringe with preservative-free heparin and centrifuged at 400 × g for 30 min on a layer of Ficoll-Isopaque (sp. gr. 1.077) at ambient temperature ([5]). The cells obtained from the interface were washed three times in ice cold Hanks' balanced salt solution and cultured at a concentration of 0.5×10^6 mononuclear cells per ml. Medium TC 199 (Flow Laboratories, Irvine, Scotland) supplemented with sodium bicarbonate, penicillin-streptomycin, fresh glutamine, and either 10% standard serum or 10% fresh autologous plasma, was used as culture medium. The cells were cultured in 0.25 ml volumes as triplicates, with or without antigen present, in flat bottomed wells of microtiter trays (Linbro Chemical Comp., New Haven, Connecticut, U.S.A.) for seven days at 37°C and 100% humidity in a 5% CO_2-in-air atmosphere. Sixteen hours before harvesting, 0.5 μCi of ^3H-thymidine (specific activity 2.0 Ci/mmol) were added to the cultures. The cells were harvested on glass fiber filters and rinsed with distilled water. The filters were dried, placed in separate vials with scintillation fluid, and counted in a liquid scintillation counter.

Results of stimulation with antigen are expressed as mean counts per min in triplicate stimulated cultures minus mean counts per min in triplicates without any stimulant added (Δcpm).

Statistical evaluations. Statistical evaluations of differences between groups were calculated with the Wilcoxon Matched-

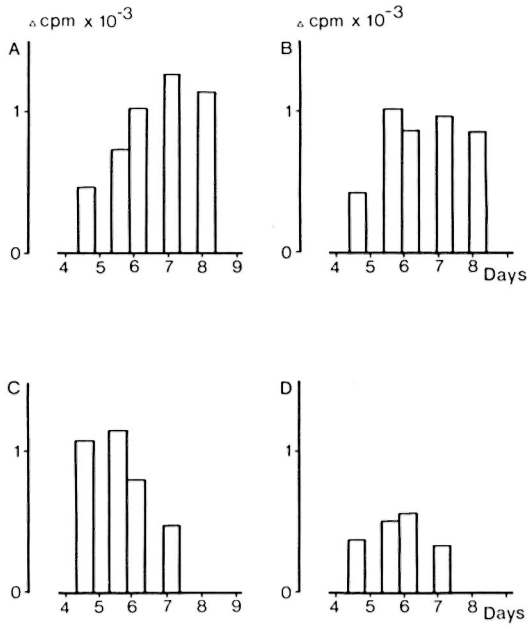

FIG. 1. The kinetics of *in vitro* lymphocyte responses to some mycobacterial antigens in the same BT patient. ^{3}H-thymidine was added to the cultures by the start of each bar, and the cultures harvested 12 hours later. A: whole *M. leprae* 10^{7} AFB/ml, B: sonicated *M. leprae* equivalent dose, C: BCG 10^{7} AFB/ml, and D: sonicated BCG equivalent dose.

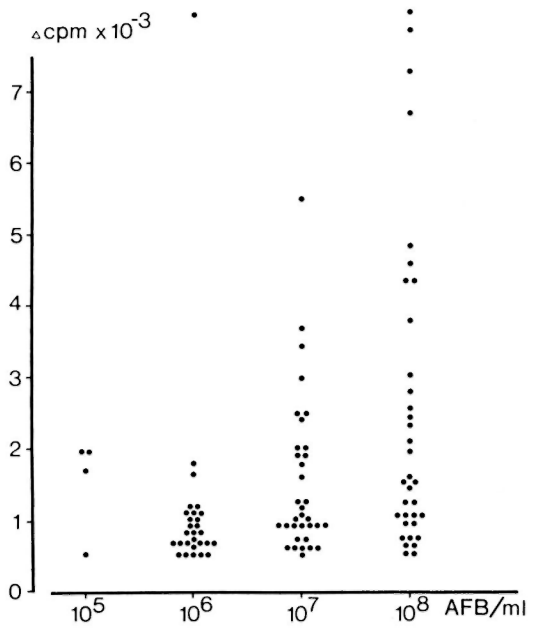

FIG. 2. Peak lymphocyte responses to whole *M. leprae* related to the dose of antigen giving the optimal stimulation in 102 tuberculoid leprosy patients. Of 157 tuberculoid leprosy patients tested, 55 did not show responses of Δcpm >500 to any of the antigen concentrations used, and the results with these patients are not shown in the plot.

Pairs-Signed-Ranks-Test when the observations were paired and by the Kolmogorov-Smirnov Two-Sample-Test ([23]) when the observations were not paired. In the bar graphs the medians are indicated for each group. Correlations were examined by the lesser quadrant method for linear regression when the correlation plot did not indicate a regression line diverging from a straight line. Plottings and calculations were done on a Hewlett-Packard 9820 A computer. The method of Hald ([12]) was used to test for significance of slopes differing from 1.

RESULTS

Variations in the day of peak response. Figure 1 shows a typical example of variation in ^{3}H-thymidine incorporation with time. The pulse in this experiment was 12 hours in each case. The antigens, *M. leprae* and BCG, were used at a concentration of approximately 10^{7} AFB/ml in the culture (or as a 10^{-2} dilution of 10^{9} sonicated AFB). Testing of five different borderline tuberculoid patients gave no indication of great variability in the day of peak response to the same antigen, and since the peak is broad with all mycobacterial preparations used, wrong timing of the harvest is an unlikely explanation for negative responses to *M. leprae* antigens in tuberculoid leprosy patients. A pulse of 16 hours and harvest at 7 days (168 hours) was used as a routine in all the following experiments. This was done because we were primarily interested in the responses to *M. leprae* and not to BCG, which showed an earlier peak.

Variations in antigen dose-response curve. One hundred and fifty-seven patients with tuberculoid leprosy (TT/BT, BT and Idt by histological classification) were tested *in vitro* with four different doses of whole *M. leprae* bacilli (10^{5},10^{6},10^{7}, and 10^{8} AFB/ml). One hundred and two of these patients showed responses of >500 Δcpm to one or more of the antigen concentrations used. Fifty-five (35%) did not show significant responses to any of the concentrations used. Among these there was only

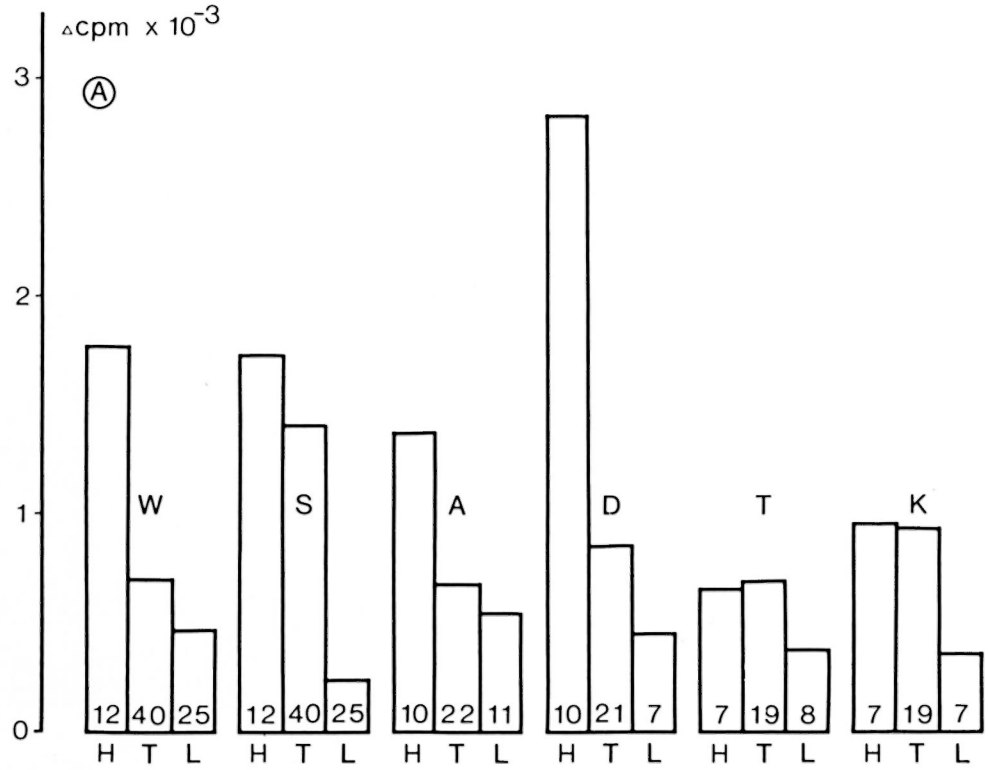

FIG. 3a. The median responses of lymphocytes *in vitro* to six different preparations of *M. leprae* antigens from the same source. W: whole washed bacilli, S: sonicated bacilli, A: autoclaved bacilli, D: bacilli prepared by the method of Dharmendra, T: trypsinized bacilli, and K: bacilli treated with KSCN. All test subjects were tested with four different concentrations of each antigen preparation, and the optimal responses, disregarding antigen dose, are given in the figure. H: healthy contacts to leprosy patients, T: patients with clinical classification BT (histologically TT/BT, BT and Idt), and L: patients with lepromatous leprosy (BL, LLs and LLp). The numbers at the bottom of each bar indicate the number of persons tested. (Four patients with BB leprosy are included in the T group.)

one patient with histological classification TT/BT. Eleven were BT, and the remaining 43 were Idt by histology. This means that patients with clinical BT but an Idt histology were more common among the non-responders (43/55) than among the responders (10/102).

The optimal responses of the 102 patients are plotted in Fig. 2. As can be seen in the figure, 70% of the cases responded most strongly to 10^8 or 10^7 AFB/ml. The remaining 30% showed optimal responses to 10^6 or 10^5 AFB/ml. These responses were generally lower than those of the patients responding optimally to high concentrations of bacilli, and most often patients with optimal responses to the lower antigen concentrations showed no significant responses to the higher antigen concentrations. This implies that about 20% of tuberculoid patients with insignificant responses to the routinely used doses of leprosy bacilli were in fact positive responders but with optimal stimulation by lower doses of *M. leprae* antigen.

Antigenic specificity of the lymphocyte responses. Since the quantities of *M. leprae* that can be extracted from human tissues are insufficient for purification of several separate antigens to be used in the lymphocyte stimulation test, we tried instead by different treatment of leprosy bacilli prepared from one single subcutaneous nodule to make preparations with different antigenic properties.

Thirteen healthy persons with different degrees of exposure to leprosy patients, 16 leprosy patients with the tuberculoid form

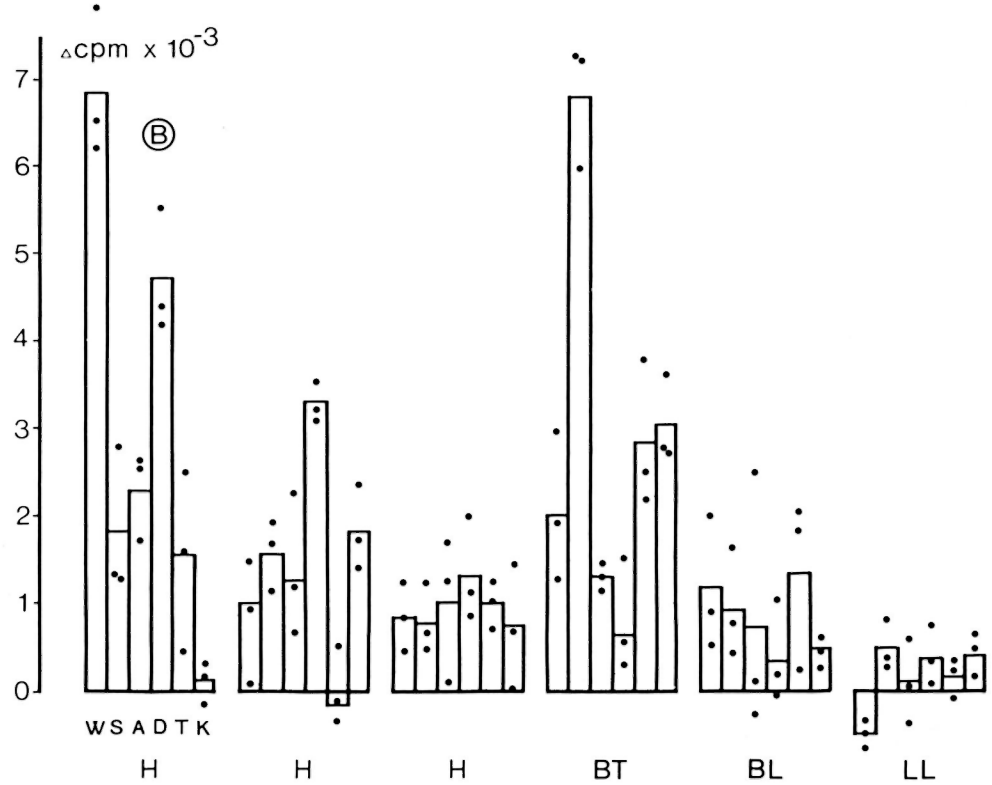

FIG. 3b. The optimal lymphocyte responses to the six *M. leprae* preparations used in six individual subjects The dots indicate the values obtained with the individual cultures.

of the disease (TT, TT/BT, BT and Idt histologically), five patients with borderline leprosy (BB), and 10 patients with lepromatous leprosy (BL, LLs and LLp) were tested *in vitro* with four different concentrations of six *M. leprae* preparations. The results are shown in Fig. 3a and 3b.

Four of the six *M. leprae* preparations gave a stronger response in healthy contacts than in leprosy patients. Also BCG and *M. duvalii* gave stronger responses in healthy contacts than in leprosy patients (results not shown). The responses to Dharmendra lepromin were particularly strong in this group. Trypsinized and KSCN-treated bacilli were less potent as stimulators in the test in contacts than the other preparations. Although KSCN-treated bacilli, which are cleared of antibodies from their surface, gave low responses in lepromatous patients, two polar lepromatous patients with no responses to the other *M. leprae* preparations showed responses of more than 1000 Δcpm to KSCN-treated bacilli.

Of particular interest are the great differences in the responses to the individual preparations recorded in the same test subject. Six examples are given in Fig. 3b. In contact one, responses to whole bacilli and Dharmendra antigen are significantly higher than to all the other preparations and the response to KSCN treated bacilli entirely negative. In contact two, the response to trypsinized bacilli is negative and the response to Dharmendra antigen significantly higher than the other responses. In the BT patient, who had a neuritis at the time of testing, the response to sonicated bacilli is dominating. These differences clearly demonstrate that the *in vitro* lymphocyte responses showed specificity to different *M. leprae* antigens in individual patients.

Effect of autologous plasma on the *in vitro* lymphocyte responses to *M. leprae*. The non-specific effect of autologous plasma on PHA responses of peripheral blood lymphocytes from leprosy patients has been described in a previous publication ([3]). To

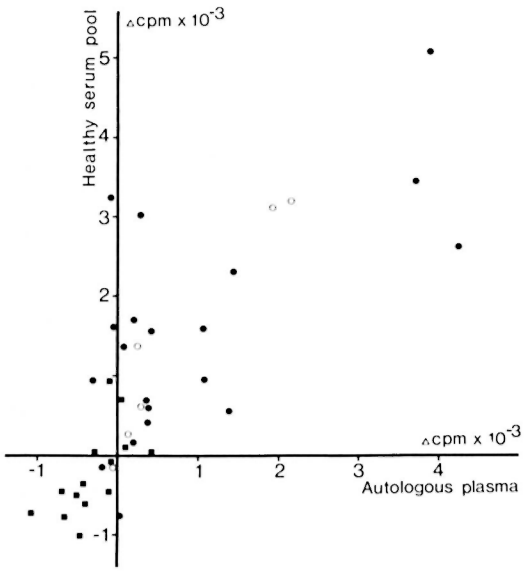

FIG. 4. *In vitro* lymphocyte responses to *M. leprae* whole bacilli, 10^7 AFB/ml in 14 patients with lepromatous leprosy: ■, 20 patients with tuberculoid leprosy: ●, and six healthy contacts: ○. The responses were recorded simultaneously in 10% autologous plasma and in 10% pooled serum from healthy persons living in leprosy non-endemic areas.

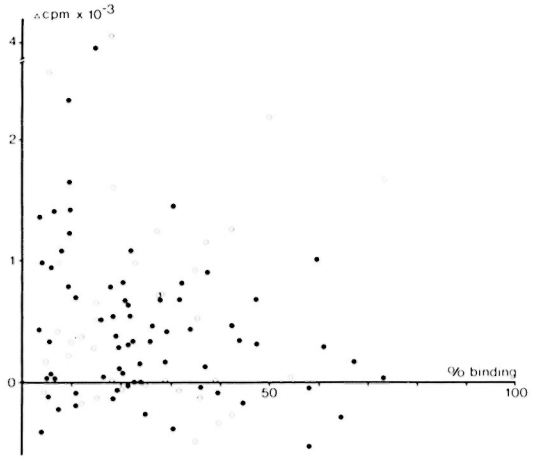

FIG. 5. Correlation plot between *in vitro* lymphocyte responses to *M. leprae* sonicate diluted 10^{-2} from a stock containing 10^9 sonicated AFB/ml and antibody activity against *M. leprae* antigen 7 measured as % binding of ^{125}I-labeled antigen 7 in sera diluted 10^{-4}. The experiment included 69 patients with tuberculoid leprosy without evidence of present or recent reversal action (●) and 36 tuberculoid patients tested during or within six months after a reversal reaction (○).

test for possible effects of autologous plasma on *in vitro* stimulation with *M. leprae* antigens, we studied 40 subjects with different forms of the disease. Fourteen patients had lepromatous leprosy (BL, LLs and LLp), 20 patients were classified as tuberculoid leprosy (TT, TT/BT and BT), and six subjects were healthy contacts of leprosy patients. The cultures were tested with two concentrations of *M. leprae* (whole washed bacilli) in the presence of 10% pooled serum from healthy persons living in leprosy non-endemic areas or 10% fresh autologous plasma in the same trays. The correlation plot for responses to 10^7 AFB/ml in the presence of autologous plasma and pooled serum is shown in Fig. 4. Overall, the responses in standard serum and autologous plasma showed a highly significant correlation ($r = 0.71$, $p < 0.025$). In 30 of the test subjects, a good correlation between the two responses was seen. In 10 subjects, however, the responses in pooled serum were more than 500 Δcpm while the simultaneous responses in autologous plasma were less than 20% of that in pooled serum. Among these 10 subjects, one was a healthy contact, two were LLs patients in ENL reaction (erythema nodosum leprosum), and the remaining seven were borderline tuberculoid patients, four of whom had just recovered from a reversal reaction. This implies that a fair number of patients with tuberculoid leprosy would show insignificant responses to *M. leprae* when tested in autologous plasma, while the same lymphocytes would respond well in standard serum.

Effect of anti-mycobacterial antibodies on the *in vitro* lymphocyte responses to *M. leprae*. Lymphocytes from 145 leprosy patients were tested *in vitro* with two concentrations of whole and sonicated *M. leprae* and with BCG in the presence of 10% standard serum. The patients' sera were taken at the same time as the lymphocyte stimulation test was performed and tested in a radioimmunoassay for anti-*M. leprae* antigen 7 activity.

When antibody levels, measured as the % binding in sera diluted 10^{-4}, were compared to the lymphocyte responses to *M. leprae* sonicate equivalent to 10^7 AFB/ml, no significant correlation was noted ($r = -0.33$, $p > 0.1$). The tendency for individuals with high antibody activity to have low

lymphocyte responses was mainly a reflection of the basic differences between lepromatous and tuberculoid patients. When non-reactional tuberculoid patients were studied separately (Fig. 5), there was similarly no correlation ($r = -0.24$). Reactional and post reactional tuberculoid patients also showed no correlation between lymphocyte responses *in vitro* and antibody activity when analyzed separately.

Lymphocyte responses of >500 Δcpm were, however, more commonly recorded in non-reactional tuberculoid patients with low antibody levels (<10% binding of ^{125}I *M. leprae* antigen 7) than in patients with high antibody levels (>40% binding) ($p = 0.036$ using the Fisher Exact Test for calculation).

DISCUSSION

In patients with tuberculoid leprosy, bacilli are scanty, and the lesions generally contain large numbers of lymphocytes and macrophages with signs of activation. This histological appearance of the lesions is considered as evidence for a cell mediated immune response to *M. leprae*. Both *in vitro* and *in vivo* tests for cell mediated immunity (CMI) to *M. leprae* antigens, however, are not always positive in these patients. This paper discusses some of the factors that may explain the great variability in responses to *M. leprae* seen in one of the commonly employed tests for CMI, the *in vitro* lymphocyte stimulation test.

The kinetics of the lymphocyte responses to *M. leprae* antigens did not show individual variation large enough to explain the great spread of tuberculoid patients' responses (Fig. 1). The optimal antigen dose for stimulation, however, was so different in different subjects that about 20% of those who failed to respond to the standard doses had measurable responses to lower doses of *M. leprae* (Fig. 2). A full dose-response curve is therefore necessary to determine whether a given patient is responding in the test.

Experiments with six preparations of *M. leprae* modified by different types of treatment showed clearly that a particular treatment could cause a complete loss of stimulating capacity for a given preparation in one subject while the same preparation could retain its stimulating capacity fully in another individual (Fig. 3b). The most likely interpretation of this finding is that leprosy patients show different antigenic specificites in their CMI responses to *M. leprae*. A systematic pattern in this antigenic heterogeneity could be found in reversal reactions where patients with acute neuritis had higher responses to a sonicate of *M. leprae* while patients with acute inflammation only in their skin lesions responded most strongly to whole washed bacilli ([2]). One single preparation of *M. leprae* is therefore insufficient to elucidate a patient's *in vitro* response to *M. leprae* antigens.

We have earlier described the suppressive effect of plasma from untreated leprosy patients on their lymphocyte responses to PHA ([4]) in agreement with the findings of several other authors ([18]). During reversal reactions, autologous plasma augmented the PHA response ([3]). These non-specific effects of autologous plasma might also affect the *M. leprae* specific responses. In 30 of 40 test subjects, the responses to *M. leprae* in 10% autologous plasma correlated well with the simultaneous responses in 10% pooled serum from healthy individuals (Fig. 4). It should be pointed out that this does not exclude the possibility of humoral factors modulating the lymphocyte responses in these patients since such factors could be produced *in vitro* by the lymphocytes or monocytes present. On the other hand, in 10 subjects there were significant responses to whole *M. leprae* in pooled healthy serum and no response to the same antigen in autologous plasma. This suppression in autologous plasma was not overcome by using the same antigen in a higher dose.

The humoral factor(s) responsible for this suppression might be anti-*M. leprae* antibodies. The antibody activity against *M. leprae* antigen no. 7 was semi-quantitated in a radioimmunoassay. This antigen specificity was chosen since purified *M. leprae* antigen 7, which elicits good lymphocyte responses, seems to be an antigen of major importance in the *in vitro* lymphocyte stimulation test with peripheral blood lymphocytes from leprosy patients ([7]).

Tuberculoid patients showed a great individual variation in anti-*M. leprae* 7 activity ([16]), and there was no correlation be-

tween the antibody activity and *in vitro* lymphocyte responses to sonicated leprosy bacilli (Fig. 5). Still, antibodies to *M. leprae* might be an important modulating factor for the CMI response *in vivo*. The lack of correlation only signifies that the antibody response to this particular antigen did not influence the lymphocyte responses to the antigenic determinants eliciting the *in vitro* responses. Presently, our knowledge of the specificity of the *in vitro* lymphocyte response is incomplete, and we are still not able to evaluate the (various) cell mediated immune reactions that occur *in vivo* and the degree of protective immunity from *in vitro* tests. The test is highly influenced by the degree of delayed type hypersensitivity to *M. leprae*, which does not always correlate with protective immunity ([4]), and the responses are dependent upon dose of antigen, antigen presentation, and humoral factors in the test.

SUMMARY

Out of 157 patients with a clinical classification of borderline tuberculoid leprosy, 88 did not respond significantly in the *in vitro* lymphocyte stimulation test to the commonly used doses of leprosy bacilli. A large group of "non-responders" in this test is a usual finding in spite of clinical and histological evidence of good cell mediated immune response to *Mycobacterium leprae* in borderline tuberculoid patients. Some of the factors which might explain failure to respond in this test were evaluated. About 20% of BT patients who did not respond to the commonly used doses of *M. leprae* did respond significantly to lower doses. Variation in antigen specificity of the *in vitro* lymphocyte responses, together with variation in antigenic presentation in different preparations of *M. leprae* antigens, caused some individuals to be non-responsive to one preparation and at the same time respond strongly to another preparation. Some of the patients had factor(s) in their plasma which caused a severe suppression of the response to *M. leprae* in the test, while the same cells responded well when they were cultured in sera from persons not exposed to leprosy. There was no clear negative correlation between antibody responses to one important *M. leprae* antigen and the *in vitro* responses to *M. leprae* preparations which exposed the same antigen. The kinetics of the responding cells did not show individual variation great enough to explain a lack of *in vitro* response when the cultures were harvested on day six or seven. It is concluded that the *in vitro* lymphocyte stimulation test in its present form gives inconclusive results for evaluating the patients' immune resistance to the disease.

RESÚMEN

Se estudió la respuesta *in vitro* de los linfocitos de pacientes con lepra cercana a la tuberculoide (borderline tuberculoide) a la estimulación con bacilos de la lepra. De los 157 pacientes estudiados, 88 no respondieron significativamente a las dosis usuales del *M. leprae*. En los pacientes "borderline tuberculoides" es frecuente encontrar un gran número de "no respondedores" en esta prueba, no obstante las evidencias clínicas e histológicas que sugieren una buena respuesta immune celular al *M. leprae*. En este trabajo se evaluaron algunos de los factores que podrían explicar la falta de reactividad de estos pacientes en la prueba. Aproximadamente el 20% de los pacientes BT que no respondieron a las dosis usuales del *M. leprae*, respondieron significativamente a dosis menores. Variaciones en la especificidad del antígeno, junto con las variaciones en la presentación antigénica de las diferentes preparaciones del *M. leprae*, ocasionaron que algunos individuos fueran no respondedores a una preparación y al mismo tiempo fuertemente respondedores a otra preparación. Algunos pacientes tuvieron factores en su plasma que causaron una severa depresión de la respuesta al *M. leprae* en la prueba *in vitro*, mientras que las mismas células respondieron bien cuando se cultivaron en presencia del suero de personas no expuestas a la lepra. No se observó una clara correlación negativa entre la respuesta humoral hacia un antígeno importante del *M. leprae* y la respuesta *in vitro* hacia las preparaciones del *M. leprae* conteniendo el mismo antígeno. Las cinéticas de las células respondedoras no mostraron variaciones individuales lo suficientemente grandes como para explicar una falta de respuesta *in vitro* cuando los cultivos se cosecharon en el día 6 ó 7. Se concluye que la prueba de la estimulación *in vitro* de los linfocitos, en su forma actual, da resultados que no son conclusivos para la evaluación de la resistencia inmune de los pacientes a la enfermedad.

RÉSUMÉ

Parmi 157 malades présentant une forme de lèpre classée cliniquement comme borderline tuberculoïde, 88 ne montraient pas de réponse significative à l'épreuve de stimulation lymphocytaire *in vitro*, avec les doses communément utilisées de bacilles de la lèpre. Il est commun de trouver une grande proportion de malades qui ne répondent pas à cette épreuve

malgré des signes cliniques et histologiques nets témoignant d'une réponse cellulaire immunitaire suffisante à *Mycobacterium leprae* chez les malades souffrant de lèpre borderline tuberculoïde. On a évalué certains des facteurs qui pourraient expliquer cette absence de réponse lors de la pratique de ce test. Environ 20% des malades BT qui ne répondaient pas au doses habituelles de *M. leprae*, montraient cependant une réponse significative à des doses plus faibles. Par suite d'une variation dans la spécificité antigénique pour la réponse lymphocytaire *in vitro*, et par suite également de variations dans la présentation antigénique de différentes préparations d'antigènes de *M. leprae*, certains individus peuvent ne pas répondre à une préparation, alors qu'ils répondent fortement à une autre. Certains des malades étudiés présentaient dans le plasma des facteurs qui provoquaient une suppression très prononcée de la réponse à *M. leprae*, lors de ce test, alors que les mêmes cellules répondaient bien lorsqu'elles étaient cultivées dans du sérum provenant d'individus non exposés à la lèpre. Il n'a été observé aucune corrélation négative entre les réponses d'anticorps à un antigène important de *M. leprae*, et les réponses *in vitro* à des préparations de *M. leprae* qui avaient été exposées au même antigène. L'étude de la cinétique des cellules qui répondait à ce test n'a pas révélé de variations individuelles suffisamment grandes pour expliquer une absence de réponse *in vitro* lorsque les cultures étaient récoltées au sixième ou au septième jour. On en conclut que l'épreuve de stimulation lymphocytaire *in vitro* dans sa forme actuelle fournit des résultats qui ne sont pas concluants pour évaluer la résistance immunitaire des malades à la maladie.

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