Humoral Immunity in Leprosy: Immunoglobulin G and M Antibody Responses to *Mycobacterium leprae* in Relation to Various Disease Patterns

JACOB TOUW,[1]* ELS M. J. LANGENDIJK,[2] GERALD L. STONER,[1]† AND AYELE BELEHU[1]

Armauer Hansen Research Institute[1] and All Africa Leprosy and Rehabilitation Training Centre,[2] Addis Ababa, Ethiopia

A solid-phase radioimmunoassay, applying whole *Mycobacterium leprae* as antigen and radiolabeled protein A from *Staphylococcus aureus* as antibody-detecting reagent, was used for the determination of specific immunoglobulin G (IgG) and IgM antibody responses in leprosy patients. High IgG anti-*M. leprae* antibody levels were found in lepromatous leprosy patients, whereas the antibody response in tuberculoid leprosy patients varied from negative, i.e., comparable with responses measured in normal individuals, to strongly positive. In tuberculoid leprosy patients, a significant increase in IgG anti-*M. leprae* antibody levels was observed in the more widespread forms of the disease, but positive antibody responses were especially predominant among patients with active lesions. Lepromatous leprosy patients generally demonstrated high levels of both IgG and IgM anti-*M. leprae* antibodies, but no relation was found between the antibody responses and bacillary load or other clinical parameters. A marked decrease in specific IgG and IgM antibody levels was observed in lepromatous leprosy patients during their first year of treatment. Differences in mechanisms regulating the humoral immune response in tuberculoid and lepromatous leprosy patients were indicated, and the application of antibody assessments in leprosy control programs is discussed.

Immunological resistance to infection with *Mycobacterium leprae* and other obligate intracellular parasites is primarily dependent on mechanisms of cell-mediated immunity (32). The role of the humoral immune response in leprosy and especially its influence on the host's defense have not been defined. In general, high levels of antimycobacterial antibodies are found only in patients with the disseminated, multibacillary infection (lepromatous leprosy), although occasionally a strong antibody response may be associated with tuberculoid leprosy, the localized, paucibacillary form of the disease (1, 3, 11, 20, 29). After chemotherapy, a decrease in antibody levels has been demonstrated in both lepromatous and tuberculoid leprosy patients (19, 21, 33), whereas an increase in serum antibody activity has been noted in relapsed tuberculoid leprosy patients (33). These findings indicate the usefulness of antibody assessments in leprosy control programs, but much better descriptions of the classes and specificities of the anti-*M.*

leprae antibodies are needed. It is especially important to correlate the presence or absence of those antibodies with the various clinical manifestations of the disease, particularly in the tuberculoid region of the spectrum. Definition of these features will allow a judgement as to whether serial antibody assessments can provide information on the prognosis of the disease.

In this paper, three solid-phase radioimmunoassays for the quantitation of anti-*M. leprae* antibodies are described. In the first assay, human serum was incubated with whole *M. leprae* bacilli which were immobilized on the wells of a polyvinyl microtiter plate, and bound antibodies were detected with ^{125}I-labeled protein A from *Staphylococcus aureus*. In the second assay, an incubation with rabbit anti-human immunoglobulin G (IgG) antiserum was inserted to amplify the sensitivity of the assay. Third, a modification was presented for the detection of IgM antibodies reacting with *M. leprae*. These solid-phase radioimmunoassays were used for the quantitation of anti-*M. leprae* antibodies in sera from normal individuals and leprosy patients across the clinicohistopathological spectrum of the disease. The antibody levels were correlated with the various disease symptoms,

* Address correspondence to J. Touw, Armauer Hansen Research Institute, P.O. Box 1005, Addis Ababa, Ethiopia.
† Present address: National Institutes of Health, Bethesda, MD 20205.

and the effect of chemotherapy on the anti-*M. leprae* antibody response was assessed in lepromatous leprosy patients.

MATERIALS AND METHODS

Sera. Sera were obtained from leprosy patients attending the clinics of the All Africa Leprosy and Rehabilitation Training Centre, Addis Ababa, Ethiopia. Unless otherwise stated, the patients had not received any previous anti-leprosy treatment. Diagnosis and classification of leprosy were done by clinical and histological examinations (22, 23). As controls, sera from healthy individuals of similar ethnic and socioeconomic backgrounds were included. From 15 lepromatous leprosy patients, serum was collected at the start of treatment with 100 mg of diaminodiphenyl sulfone (DDS) daily, after 2 to 4 months of treatment, and after 10 to 14 months of treatment. All serum samples were stored at $-20°C$ until use. To minimize interassay variations, sera from different groups of patients were assayed in one experiment.

A positive reference serum pool was prepared by pooling 15 sera of patients with lepromatous leprosy, and a negative reference serum pool was prepared by combining 8 sera from healthy individuals living in Norway.

Antigens. Freeze-dried, armadillo-derived *M. leprae* bacilli (batch AB40) were provided by R. J. W. Rees, National Institute for Medical Research, London, England, through the Immunology of Leprosy section of the UNDP/World Bank/WHO Special Program for Research and Training in Tropical Diseases. A stock solution of *M. leprae* was prepared by reconstituting the bacilli in 0.9% NaCl at a concentration of 10 mg/ml, followed by brief sonication (Branson sonifier, 50 W for 5 s) to obtain a homogeneous suspension.

Radiolabeling. Protein A (250 μg), purchased from Pharmacia Fine Chemicals, Uppsala, Sweden, was iodinated with 2 mCi of carrier-free $Na^{125}I$ (Radiochemical Centre, Amersham, U.K.), using the chloramine T method (10). The labeled protein was separated from the unreacted iodide by filtration through a Sephadex G-25 column which was equilibrated with phosphate-buffered saline containing 0.2% human serum albumin (AB Kabi, Stockholm, Sweden). The first radioactive peak eluted was pooled and stored in aliquots at $-20°C$ until use. The specific activity of the labeled product varied from 12,000 to 17,000 counts per minute (cpm)/ng of protein A.

Radioimmunoassay. The following procedure for the immobilization of *M. leprae* bacilli on the wells of polyvinyl microtiter plates (no. 1-220-29, Dynatech Laboratories Inc., Kloten, Switzerland) was used in routine tests. A fresh suspension of *M. leprae* in 0.2% NaCl was prepared from the stock, and 25-μl volumes containing 2 μg of bacilli were placed in the wells of a microtiter plate. The plates were placed on a microshaker (Dynatech), shaken for 1 min, and left to dry at 37°C (2 h) or at room temperature (overnight). After drying, 75 μl of a 1% glutaraldehyde solution was added to each well. The plates were left for 15 min at room temperature, washed three times with phosphate-buffered saline with 0.1% Tween 20 (PBST), and left overnight after addition of 200 μl of RIA buffer (phosphate-buffered saline [pH 7.4] with 0.03 M NaN_3, 0.001 M EDTA, and 0.2% human serum albumin). The

antigen-coated plates were used for the three types of radioimmunoassays described below.

(i) Direct assay. The coated plates were washed twice with PBST, and 150 μl of serum (dilutions of 1:100 and 1:1,000 in RIA buffer) was added. The plates were incubated overnight at 4°C. Unbound antibody was removed by three washings with PBST. Radiolabeled protein A (100 μl containing 125,000 cpm), diluted in RIA buffer, was added, and after an incubation of 6 h at room temperature, the plates were washed three times with PBST and air dried. The wells were cut off, and bound radioactivity was measured in a gamma counter (1270 Rackgamma II; LKB, Turku, Finland).

(ii) Indirect assay for IgG antibodies. Amounts (150 μl) of serum diluted 1:1,000 and 1:10,000 in RIA buffer were placed into antigen-coated wells and left overnight at 4°C. After the plates were washed with PBST, 150 μl of a 1:200 dilution of rabbit anti-human IgG antiserum (DAKO immunoglobulins A/S, Copenhagen, Denmark) in RIA buffer was added, and the plates were incubated for 5 h. After three washings with PBST, 100 μl of protein A (125,000 cpm) was added and incubated for 6 h. Bound radioactivity was determined as described above.

(iii) Indirect assay for IgM antibodies. All sera were adsorbed first with *Staphylococcus aureus* Cowan type I, which was kindly provided by G. Kronvall, Department of Medical Microbiology, University of Lund, Sweden. The bacteria were grown on CCY medium and, after harvest, were heat killed, Formalin treated, and stored at 4°C as a 10% suspension in phosphate-buffered saline containing 0.05 M NaN_3 (15). Before use, the staphylococci were washed twice with PBST and adjusted to a 5% (vol/vol) suspension in PBST. For the adsorption of sera, aliquots (1 ml) were centrifuged at 2,000 × g for 30 min in plastic conical tubes (no. 72.690; Sarstedt, Nuembrecht, West Germany) giving a bacterial pellet of 50 μl. The supernatant was discarded, and 500 μl of test serum, diluted 1:100 in RIA buffer, was added. After suspending the bacteria by using a Vortex mixer, the suspension was incubated for 2 h at room temperature. The supernatant obtained after centrifugation at 2,000 × g for 45 min was assayed for antibody activity.

Amounts (150 μl) of the supernatant were pipetted into an antigen-coated well and left overnight at 4°C. The unbound antibody was washed off with PBST, and 150 μl of a 1:200 dilution of rabbit anti-human IgM antiserum (DAKO immunoglobulins A/S) was added. After an incubation of 5 h at room temperature, the plates were washed three times with PBST and incubated with protein A (125,000 cpm). Bound radioactivity was determined as described above.

Evaluation. All determinations were done in duplicate, and mean values with background subtracted were calculated. The antibody content of the test serum was compared with the activity of the positive reference serum pool. Results were expressed as follows.

(i) Percent cpm. The bound cpm obtained with the test serum was expressed as a percentage of the bound cpm measured with the positive reference serum pool at the same dilution. For the direct assay and the indirect assay for IgM antibodies, the percentages were calculated from the results obtained with a 1:100
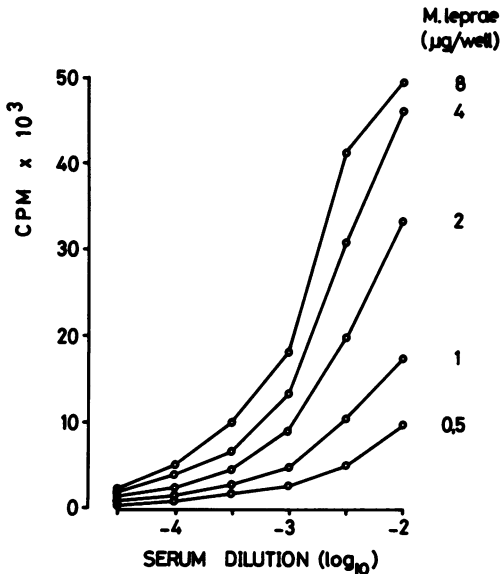
FIG. 1. Effect of varying the amount of immobilized *M. leprae* on the antibody binding at several dilutions of the positive reference serum pool. Bound radioactivity was measured after incubation with ^{125}I-protein A (125,000 cpm per well).
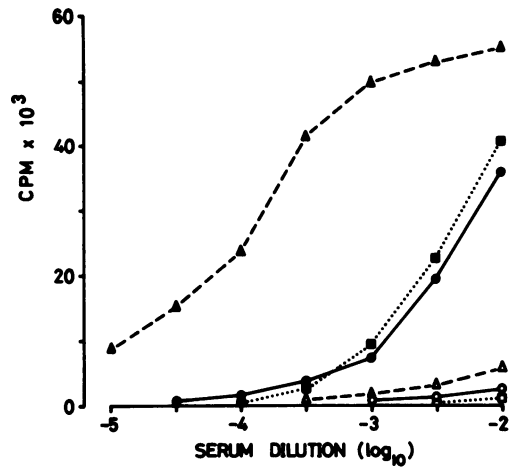

FIG. 2. Binding curves obtained with the positive reference serum pool (closed symbols) and the negative reference serum pool (open symbols) in the direct assay (circles), indirect assay for IgG antibodies (triangles), and indirect assay for IgM antibodies (squares).

serum dilution, whereas a 1:1,000 serum dilution was used for the indirect assay for IgG antibodies.

(ii) **Percent activity.** Several dilutions of the positive reference serum pool were included in each experiment to construct a standard binding curve (bound cpm versus serum dilution). With this curve, the bound cpm obtained with the test sample was converted to a serum dilution of the positive reference serum pool and expressed as a percentage of 10^{-2} (1:100 test serum dilution) or as a percentage of 10^{-3} (1:1,000 test serum dilution). The percent activity of the positive reference represents the average of percentages determined for two serum dilutions. Serum samples which gave values outside the range of the standard binding curve were reassessed at adjusted dilutions.

Differences between groups were assessed for significance with the Student's t test, using transformed data when appropriate or the chi-square test (27).

RESULTS

Radioimmunoassay conditions. The binding curves for the positive reference serum pool, obtained in the direct assay with increasing amounts of *M. leprae* fixed to the wells, are shown in Fig. 1. With the negative reference serum pool at a 1:100 dilution, the radioactivity bound to the wells increased from 958 to 1,505 cpm. A dose of 2 μg of *M. leprae* was chosen for routine use because it was sufficient for an excellent discrimination between the positive and negative reference serum pools and conserved the limited supply of antigenic material available. By using this concentration of *M. leprae* and a 1:100 dilution of the positive reference serum pool, the addition of ^{125}I-protein A giving 125,000 cpm resulted in apparent saturation of protein A binding sites (data not shown).

Figure 2 shows the standard binding curves obtained in the direct assay, the indirect assay for IgG antibodies, and the indirect assay for IgM antibodies reacting with *M. leprae*. Background values measured as the amount of radioactivity bound when the incubation with human serum was omitted, reached 200 cpm in the direct assay and 1,500 cpm in the indirect assay. In the indirect assay for IgM antibodies, omission of the incubation with rabbit anti-human IgM antiserum served as a control for the effectiveness of the absorption procedure, and the resulting amount of bound radioactivity did not exceed the background values.

In each experiment, dilution series of the positive and negative reference serum pools were included. In 10 separate experiments, the coefficient of variation was 3.8% in the direct assay, using a 1:100 dilution of the positive reference serum pool, which indicated good reproducibility between experiments. A coefficient of variation of around 4.5% was calculated for the other radioimmunoassays.

Anti-*M. leprae* antibody profile in leprosy patients and normal controls. The antibody activity directed against *M. leprae* in sera from leprosy patients categorized according to their clinico-histopathological classification was compared with the antibody activity present in sera from normal individuals. The results obtained with the direct assay and expressed as percent cpm of the positive reference are shown in Fig. 3. In general, the method of evaluating the results,
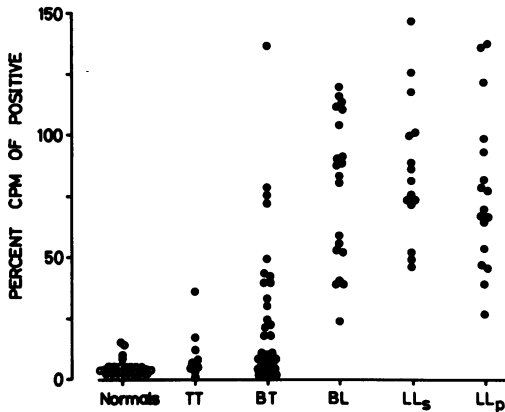
FIG. 3. IgG anti-*M. leprae* antibodies determined with the direct assay in sera from normal individuals and tuberculoid (TT and BT) and lepromatous (BL, LL$_S$, and LL$_P$) leprosy patients.
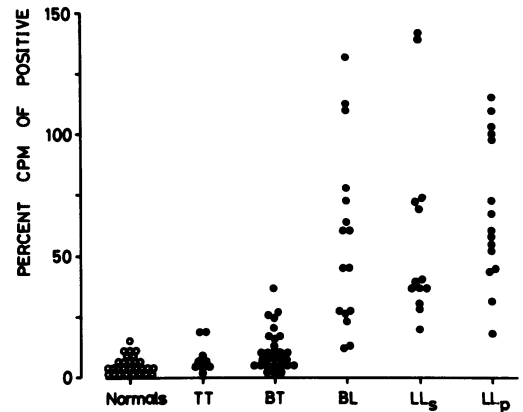

FIG. 4. IgM anti-*M. leprae* antibodies detected in sera from normal individuals and leprosy patients.

either as percent cpm or as percent activity of the positive reference, did not alter the antibody profile across the disease spectrum. However, as may be anticipated from the standard binding curves, the individual samples with a low or high percent cpm tended to give respectively lower or higher values when expressed as percent activity.

The sera from normal individuals contained little anti-*M. leprae* antibody activity (4.3 ± 3.8% cpm). By setting the minimum positive antibody activity at the mean value of the normal group plus two standard deviations of the mean, i.e., at 12.0% cpm, a positive antibody response could be assigned to 2 out of 31 normal individuals. Many patients classified toward the tuberculoid end of the spectrum showed an antibody response comparable to that of the normal controls. A positive response could be assigned to 3 out of 9 polar tuberculoid (TT) patients and to 16 out of 34 borderline tuberculoid (BT) leprosy patients. All of the patients classified towards the lepromatous end of the spectrum showed a positive antibody response directed against *M. leprae*.

The same sera tested in the direct assay at 1:100 and 1:1,000 serum dilutions were analyzed with the indirect assay for IgG antibodies at 1:1,000 and 1:10,000 serum dilutions. With the indirect assay, antibodies directed against *M. leprae* could be detected at a 50 to 100 times higher serum dilution than that used for the direct assay. The results with the indirect assay were similar to those obtained with the direct assay. An interassay correlation coefficient of r = 0.87 was found when the results were expressed as percent cpm of the positive reference, and r = 0.82 was found when expressed as percent activity of the positive reference.

IgM anti-*M. leprae* antibody response in leprosy patients and normal controls. Sera from normal individuals and leprosy patients were adsorbed with *S. aureus* Cowan I and assayed for IgM anti-*M. leprae* antibodies. The results were expressed as percent cpm of the positive reference and are shown in Fig. 4. The normal individuals showed a marginal antibody response (mean value, 6 + 5% cpm of positive). The discrimination level between positive and negative IgM antibody responses, determined by the mean of the normal group plus two standard deviations of the mean, was set at 16% cpm. A positive response could be assigned to
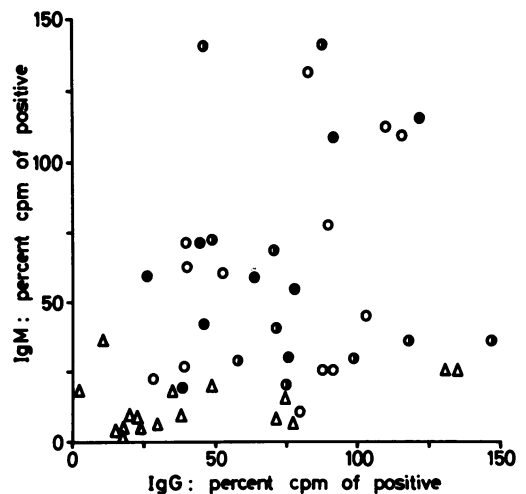

FIG. 5. Relation between the IgG anti-*M. leprae* antibody response determined with the indirect assay and the IgM anti-*M. leprae* antibody levels in tuberculoid (△), BL (○), LL$_S$ (◑), and LL$_P$ (●) leprosy patients.

TABLE 1. Relation between IgG anti-*M. leprae* antibody responses and clinical signs in tuberculoid leprosy patients

Clinical signs	No. of patients	% cpm[a] of positive reference		No. of positive responders	No. of negative responders
		Direct assay	Indirect assay		
Duration of disease (yr)					
<1	16	28 ± 37	47 ± 33	6	10
1–3	11	24 ± 23	57 ± 34[b]	7	4
>3	10	8 ± 11	25 ± 15	1	9
No. of skin patches					
≤4	21	13 ± 17	31 ± 21[b]	5	16
≥5	16	32 ± 37	63 ± 35	9	7
No. of affected nerves					
≤2	16	15 ± 33	35 ± 30[b]	3	13
≥3	20	34 ± 33	58 ± 35	12	8
State of disease					
Quiescent	20	7 ± 5	26 ± 15[b]	2	18
Active	17	46 ± 41	73 ± 32	13	4

[a] Mean ± standard deviation.
[b] $P < 0.05$ for the comparison between subgroups.

10 out of 38 tuberculoid patients and to nearly all lepromatous patients (42 out of 44).

Figure 5 illustrates the relationship between the IgG, as determined in the indirect assay, and the IgM anti-*M. leprae* antibody response in leprosy patients. Only the data from those patients who demonstrated a positive response for either IgG or IgM antibodies or both are shown. The results of the two assays were not related (r = 0.07). A difference in the relation of the IgG versus the IgM response in tuberculoid and lepromatous patients was observed. Some tuberculoid patients demonstrated an IgG response of the same magnitude as that of lepromatous patients, but their IgM anti-*M. leprae* response was always low compared with that of the lepromatous patients. Of interest are the two tuberculoid patients who showed a positive IgM response, whereas no elevated levels of IgG anti-*M. leprae* antibodies could be detected.

Relation between the anti-*M. leprae* antibody response and clinical signs of leprosy. Since within each group of leprosy patients a considerable variation in antibody levels could be demonstrated, an attempt was made to correlate the individual's antibody response with his clinical features. Within the paucibacillary tuberculoid leprosy group, 38 patients were classified by the duration of disease, the number of patches, the number of enlarged nerves, and the presence of signs of activity. Active tuberculoid leprosy was defined by the presence of erythematous raised patches, tender nerves, and signs of recent nerve damage. Patients were divided into groups of positive and negative antibody responders

according to the criteria described above (Table 1).

When the patients were classified by the duration of disease, the positive antibody responders were mainly found in the group with a history of less than 3 years. However, it should be mentioned that the analysis on duration of disease is based on the time the patient himself observed the first signs of the disease and may thus be biased. A significant increase in antibody activity was observed in tuberculoid patients with the more widespread forms of the disease, i.e., with more than four patches or more than two affected nerves. The analysis was especially valid when the state of disease was described as either quiescent or active. No significant differences in the IgM anti-*M. leprae* antibody response on the basis of clinical features could be demonstrated.

Patients classified toward the lepromatous end of the spectrum were classified by their bacterial load (bacterial and morphological index), extent of nerve damage, and type of skin infiltration. Also within this group, the individual's antibody responses varied considerably, but no indication for a correlation between either IgG or IgM anti-*M. leprae* antibody levels and clinical signs was observed (data not shown).

Anti-*M. leprae* antibody response during 1 year of chemotherapy in lepromatous leprosy patients. The effect of treatment with DDS (100 mg daily) on the humoral immune response was studied in 15 lepromatous leprosy patients. Four had been classified as borderline lepromatous (BL) (no. 1 to 4), five as subpolar lepromatous (LL_S) (no. 5
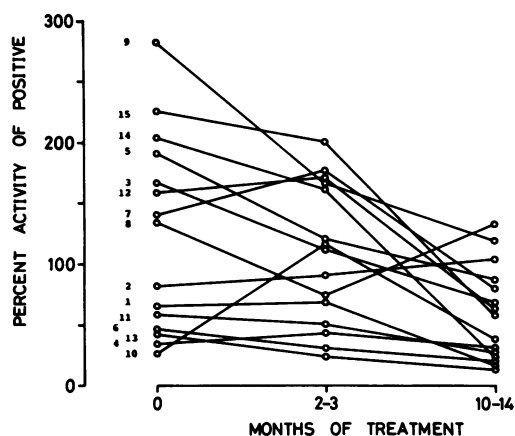
FIG. 6. Effect of DDS treatment on the IgG anti-*M. leprae* antibody levels determined with the direct assay.
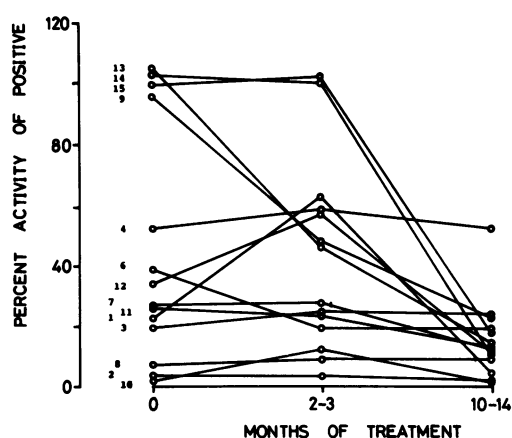

FIG. 7. IgM anti-*M. leprae* antibody response in lepromatous leprosy patients during DDS treatment.

to 9) and six as polar lepromatous (LL$_P$) (no. 10 to 15). In all patients, 1 year of chemotherapy resulted in a marked improvement as judged by clinical and bacteriological parameters. The results from the direct assay and the indirect assay for IgM antibodies, expressed as percent activity of the positive reference, are shown in Fig. 6 and 7.

In general, 1 year of chemotherapy resulted in a significant decrease in anti-*M. leprae* antibody levels ($P < 0.01$). As a group, the mean IgG antibody activity measured with the direct assay was 124% of positive at the start of treatment and decreased to 59% at the end of the observation period. The mean value for IgM antibodies decreased from 50% activity at the beginning to 18% at the end. In most patients, the decline in antibody levels occurred gradually. Abrupt changes, as indicated by the assessments at 2 to 3 months after the start of chemotherapy, were observed in some patients, whereby a change in antibody levels of one class did not necessarily coincide with a change in the other antibody class.

DISCUSSION

This study describes a convenient solid-phase radioimmunoassay for the determination of antibodies directed against surface antigens of *M. leprae*. The method used for the immobilization of cells on the wells of polyvinyl microtiter plates has been applied successfully in other radiometric immunoassays (14). During preliminary experiments, we observed a substantial difference between the antibody binding capacities of various *M. leprae* preparations. Human-derived *M. leprae*, isolated from nodules of lepromatous leprosy patients (7), also bound antibodies, but considerable batch-to-batch variations were observed. The *M. leprae* bacilli used

in this study (batch AB40) were isolated from infected armadillo tissue by a twofold extraction on a two-phase separation medium (World Health Organization Report of the Fourth IMM-LEP Scientific Working Group Meeting, 19–22 June, 1978). Another batch of armadillo-derived bacilli (batch AB21), isolated with the aid of proteolytic enzymes (World Health Organization Report of the Second IMMLEP Task Force Meeting, 1–5 December, 1975), showed at similar concentrations only minimal antibody binding activity (unpublished data).

The ability of protein A to react with immunoglobulins and its application in radioimmunoassays have been well documented (6, 9, 13, 25). Antibody binding studies have shown that protein A reacts preferentially with the F$_c$ part of human IgG1, IgG2, and IgG4 (16). It has also been shown to react with monoclonal IgM1 and IgA1 (12, 17, 24), but its binding to polyclonal IgM and IgA may be minimal (18, 25). However, due to its predominance, the antibody binding sites on immobilized antigen are mainly occupied by IgG-type antibodies. To detect specific IgM (or IgA) antibodies quantitatively, the insertion of an adsorption step, removing IgG antibodies, is necessary (2, 26; L. Mackinlay and S. Chantler, Abstr. no. 19.6.19, 4th Internatl. Congr. Immunol., Paris, 1980). Therefore, the sera were first adsorped with *S. aureus* Cowan I, a bacterial strain characterized by a high density of protein A on its surface. As a consequence, the indirect assay for IgM antibodies directed against *M. leprae* only measures IgM2 antibodies, but we presume that the results are representative of the total IgM response directed against *M. leprae*.

With the solid-phase radioimmunoassay, the levels of IgG antibodies directed against *M. leprae* were determined in leprosy patients and normal controls. In general, patients classified

towards the lepromatous end of the spectrum showed significantly higher antibody responses than those classified towards the tuberculoid end of the disease spectrum. Since high levels of antimycobacterial antibodies have been found mainly in the multibacillary form of the disease, a clear correlation between bacterial load and humoral immune response has been suggested (20). Indeed, reducing the bacterial load by chemotherapy seems to affect the amount of circulating antibodies in the majority of lepromatous leprosy patients (19, 21). Similar observations have been made in tuberculoid leprosy patients (33). However, the antibody levels vary greatly even within the classified groups, and, in patients classified towards the lepromatous end of the spectrum, the level of circulating antibodies directed against *M. leprae* could not be related with the bacterial load assessed by skin smears or other clinical signs. In these highly disseminated infections, the mechanisms regulating the antibody response may be very complex because of the continuous presence of antigenic material. In addition, the specificity of the antibody response may be questioned. *M. tuberculosis* and its purified protein derivative have demonstrated the ability to induce polyclonal antibody production in vitro (4, 8). Since *M. leprae* and *M. tuberculosis* are closely related (31), similar in vivo mechanisms may contribute to the observed variations in antibody responses in the multibacillary infection.

In tuberculoid leprosy patients, the IgG anti-*M. leprae* antibody levels appeared to be associated with the extent and activity of the disease. Clearly, the more widely disseminated tuberculoid infections have, or have had, a larger number of bacilli. In these patients, the stimulation of antibody synthesis, for example, by circulating antigens triggering antibody production in the central lymphon compartment (28), is more likely to occur.

The association of high antibody levels with the state of disease activity in tuberculoid leprosy was even more highly significant than the association with the degree of dissemination. The clinical phenomena which accompany the active phase may be attributed to delayed-type hypersensitivity reactions (7), and the increased antibody levels may be explained by a simultaneous increased level of helper T-cell activity (5). On the other hand, the disease activity may be associated with the first phases of dissemination when the disease changes from a localized to a systemic infection. The concomitant change in antigen presentation to the lymphoid system may contribute to the appearance of delayed-type hypersensitivity reactions.

The suggested distinction between mechanisms regulating the antibody response in tuberculoid and lepromatous leprosy is further confirmed when the IgM anti-*M. leprae* antibody responses are analyzed. Some tuberculoid antibody patients mounted a strong IgG anti-*M. leprae* antibody response, but their IgM anti-*M. leprae* responses were always low compared with those of lepromatous leprosy patients. Two tuberculoid leprosy patients showed a positive IgM and a negative IgG anti-*M. leprae* antibody response. Presumably, these patients were assessed during the first stages of antibody production, whereas in the other tuberculoid leprosy patients, the proportion of the antibody responses of both classes resembles a normal antibody synthesis pattern.

Nearly all lepromatous leprosy patients were positive IgM anti-*M. leprae* antibody responders, which may be interpreted as a sign of a persistent infection, analogous for example, to the presence of specific IgM antibodies in measles during latent infection of the brain (30). Accordingly, even after 1 year of chemotherapy, when most bacilli are still present (although most of them should have been killed), we could still detect a strong but decreased IgM anti-*M. leprae* antibody response in lepromatous leprosy patients.

For leprosy control programs, the analysis of antibody responses may be particularly useful in tuberculoid leprosy patients, in which group the majority of leprosy patients can be classified. Serial antibody assessments may provide information on the response to treatment and on the possibility of relapse (33). In addition, it might prove useful to classify newly diagnosed tuberculoid patients as high or low antibody responders, on which basis different treatment regimens may be designed. The observed relation among antibody responses, the extent of nerve damage, and the presence of signs of activity may provide a method to define a group of tuberculoid leprosy patients who are at high risk to develop physical handicaps.

LITERATURE CITED

1. **Abe, M., S. Izumu, T. Saito, and S. K. Mathur.** 1976. Early serodiagnosis of leprosy by indirect immunofluorescence. Lepr. India **48:**272–276.
2. **Ankerst, J., P. Christensen, L. Kjellen, and G. Kronvall.** 1974. A routine diagnostic test for IgA and IgM antibodies to rubella virus: adsorption of IgG with *Staphylococcus aureus.* J. Infect. Dis. **130:**268–273.
3. **Axelsen, N. H., M. Harboe, O. Closs, and T. Godal.** 1974. BCG antibody profiles in tuberculoid and lepromatous leprosy. Infect. Immun. **9:**952–958.

4. Banck, G., and A. Forsgren. 1978. Many bacterial species are mitogenic for human blood B lymphocytes. Scand. J. Immunol. 8:347–354.
5. Barnetson, R. S., A. Barnetson, J. M. H. Pearson, and G. Kronvall. 1976. Does nonspecific T-lymphocyte stimulation of B-lymphocytes occur during reversal reaction in borderline leprosy? Scand. J. Immunol. 5:287–291.
6. Biberfeld, P., V. Ghetie, and J. Sjoquist. 1974. Demonstration and assaying of IgG antibodies in tissues and on cells by labeled staphylococcal protein A. J. Immunol. Methods 6:249–259.
7. Bjune, G., R. S. Barnetson, D. S. Ridley, and G. Kronvall. 1976. Lymphocyte transformation test in leprosy; correlation of the response with inflammation of lesions. Clin. Exp. Immunol. 25:85–94.
8. Fauci, A. S., and K. R. Pratt. 1976. Activation of human B lymphocytes. I. Direct plaque-forming cell assay for the manifestation of human B lymphocytes. J. Exp. Med. 144:674–684.
9. Goding, J. W. 1978. Use of staphylococcal protein A as an immunological reagent. J. Immunol. Methods 20:241–253.
10. Greenwood, F. C., W. M. Hunter, and J. S. Blover. 1963. The preparation of I-131 labeled human growth hormone of high specific radioactivity. Biochem. J. 89:114–123.
11. Harboe, M., O. Closs, G. Bjune, G. Kronvall, and N. H. Axelsen. 1978. Mycobacterium leprae specific antibodies detected by radioimmunoassay. Scand. J. Immunol. 7:111–120.
12. Harboe, M., and I. Fölling. 1974. Recognition of two distinct groups of human IgM and IgA based on different binding to staphylococci. Scand. J. Immunol. 3:471–482.
13. Jonsson, S., and G. Kronvall. 1972. Protein A containing Staphylococcus aureus as anti-gamma globulin reagent in radioimmunoassays. Scand. J. Immunol. 1:414–415.
14. Klinman, N. 1979. Detection of monoclonal antibodies using radiolabeled anti-immunoglobulin reagents, p. 101–104. In Hybridoma technology with special reference to parasitic diseases. UNDP/World Bank/WHO, Geneva.
15. Kronvall, G. 1973. A rapid slide agglutination method for typing pneumococci by means of specific antibody adsorbed to protein A-containing staphylococci. J. Med. Microbiol. 6:187–190.
16. Kronvall, G., and R. C. Williams. 1969. Differences in anti-protein A activity among IgG subgroups. J. Immunol. 103:828–833.
17. Lind, I., M. Harboe, and I. Fölling. 1975. Protein A reactivity of two distinct groups of human monoclonal IgM. Scand. J. Immunol. 4:843–848.
18. McDowell, G., A. Grov, and P. Oeding. 1971. IgA and IgM reacting with staphylococcal Protein A. Acta Pathol. Microbiol. Scand. Sect. A 79:801–805.
19. Melsom, R., B. Naafs, M. Harboe, and O. Closs. 1978. Antibody activity against Mycobacterium leprae antigen 7

during the first year of DDS treatment in lepromatous (BL-LL) leprosy. Lepr. Rev. 49:17–29.
20. Myrvang, B., C. M. Feek, and T. Godal. 1974. Antimycobacterial antibodies in sera from patients throughout the clinico-pathological disease spectrum of leprosy. Acta Pathol. Microbiol. Scand. Sect. B 82:701–706.
21. Rees, R. J. W., K. R. Chatterjee, J. Pepys, and R. D. Tee. 1965. Some immunologic aspects of leprosy. Am. Rev. Respir. Dis. 92:139–149.
22. Ridley, D. S., and W. H. Jopling. 1966. Classification of leprosy according to immunity. A five-group system. Int. J. Lepr. 34:255–273.
23. Ridley, D. S., and M. F. Waters. 1969. Significance of variations within the lepromatous group. Lepr. Rev. 40:143–152.
24. Saltvedt, E., and M. Harboe. 1976. Binding of IgA to protein A containing staphylococci: relationship to subclasses. Scand. J. Immunol. 5:1103–1108.
25. Sjoquist, J., A. Forsgren, G. T. Gustafson, and G. Stalenheim. 1967. Interaction between gammaglobulins and protein A from Staphylococcus aureus. Nobel Symp. 3:341–347.
26. Skang, K., and E. Tjotta. 1974. Diagnosis of recent herpes simplex infections. A modified immunofluorescent test for the detection of specific herpes simplex IgM antibodies after staphylococcal adsorption of IgG. Acta Pathol. Microbiol. Scand. Sect. B 82:323–328.
27. Snedecor, G. W., and W. G. Cochran. 1967. Statistical methods, 6th ed. The Iowa State University Press, Ames.
28. Stoner, G. L. 1979. Importance of the neural predilection of Mycobacterium leprae in leprosy. Lancet ii:994–996.
29. Stoner, G. L., J. Touw, and A. Belehu. 1980. An assay for antibodies in leprosy sera reacting with ribonucleoprotein (RNP), a ribosomal antigen, using crossed immunoelectrophoresis with intermediate gel. Int. J. Lepr. 48:247–253.
30. Thomson, D., J. H. Connolly, B. O. Underwood, and F. Brown. 1975. A study of immunoglobulin M antibody to measles, canine distemper and rinderpest viruses in sera from patients with subacute sclerosing panencephalitis. J. Clin. Pathol. 28:543–546.
31. Wideback, K., G. Kronall, B. Bjorvatn, O. Closs, and M. Harboe. 1980. Comparative studies of antigen 21 in Mycobacterium and Nocardia species: possible taxonomic relationships with Mycobacterium leprae. Infect. Immun. 30:413–420.
32. World Health Organization. 1973. Cell-mediated immunity and resistance to infection. WHO Tech. Rep. Ser. no. 519.
33. Yoder, L., B. Naafs, M. Harboe, and G. Bjune. 1979. Antibody activity against Mycobacterium leprae antigen 7 in leprosy: studies on variation in antibody content throughout the spectrum and on the effect of DDS treatment and relapse in BT leprosy. Lepr. Rev. 50:113–121.