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Class Specific Anti-*Mycobacterium leprae* Antibody Assay in Lepromatous Leprosy (BL-LL) Patients During the First Two to Four Years of DDS Treatment[1]

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A decrease in concentration of antimycobacterial antibodies during dapsone (DDS) treatment of patients with lepromatous leprosy was initially demonstrated by Ross ([21]) with a modified Middlebrook-Dubos hemagglutination test. Rees, *et al.* ([18]) found a similar gradual decrease in antibody activity during DDS treatment of lepromatous leprosy using a double diffusion test in gel with *Mycobacterium tuberculosis* as antigen.

We have previously ([16]) developed a sensitive radioimmunoassay (RIA) for quantification of antibodies against *M. leprae* antigen 7, a cell wall component of *M. leprae* ([2, 7]). This technique was used in the study of antibodies against *M. leprae* antigen 7 in 15 lepromatous (LL-BL) leprosy patients in serum samples taken prior to antileprosy treatment, and at different intervals for 10 to 16 months during DDS treatment. We demonstrated a small, but significant decrease in the activity of antibodies against *M. leprae* antigen 7 during this observation period in 14 of the 15 patients ([16]). Additional serum samples have been collected from these patients, and the observation period has now been extended to 2–4 years after the start of DDS treatment.

Solid-phase radioimmunoassays (sRIA) have later been developed for demonstration and quantification of IgA-, IgM-, and IgG-anti-*M. leprae* antibodies ([13, 14]). By the use of these assays we found low IgA-, IgM-, and IgG-anti-*M. leprae* antibody activity in sera from non-leprosy controls, higher activity in sera from tuberculoid leprosy patients, and the highest activity in sera from lepromatous leprosy patients. When a group of lepromatous leprosy patients treated with DDS for at least ten years and with no signs of active disease was compared with a group of newly diagnosed patients with lepromatous leprosy prior to treatment, there was a marked and significant decrease of IgG-anti-*M. leprae* antibody activity, less but significant decrease of IgA antibodies, and no decrease of IgM-anti-*M. leprae* antibody activity ([15]).

The purpose of the present investigation was to apply the sRIA on serum samples from the lepromatous leprosy patients

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through the extended observation period with three main intentions:

1) To see if the previous finding of a marked decrease in IgG-anti-*M. leprae* and less decrease in IgA-anti-*M. leprae* would be confirmed.
2) To see if the lack of decrease in IgM-anti-*M. leprae* antibody activity during DDS treatment would be confirmed in the second, independent series when groups of observations were compared and if a decrease in IgM-anti-*M. leprae* antibody activity might be demonstrated by comparing the antibody activity in consecutive serum samples from individual patients.
3) To compare the results of the immunoglobulin class specific anti-*M. leprae* antibody assays with the results of assays for anti-*M. leprae* antigen 7 antibodies in individual sera.

MATERIALS AND METHODS

Patients

Fifteen patients attending the Addis Ababa Leprosy Hospital were selected for the study. Fourteen of these patients were identical with the patients described previously ([16]). The patients were classified clinically and histologically according to the extended Ridley-Jopling scale ([17, 19, 20]): 6 patients having LL, 4 patients having LI, and 5 patients having BL leprosy. An additional patient (No. 11) included in Figures 1, 2, and 3 had a dual mycobacterial infection, lepromatous leprosy and tuberculosis, and is considered separately. All patients started treatment with DDS at the point of inclusion into the study, except one patient (No. 13) with a disease history of ten years. He had been treated with DDS for two years and then had had no treatment for eight years before inclusion into the study. The first Bacteriological Index (BI) was determined and the first serum sample was obtained immediately before the beginning of DDS treatment. The median observation period was 3 years, with a range of 1½ to 4 years.

Treatment consisted of DDS in a dose of 100 mg daily, except in patient No. 1, a 13-year-old boy at the start of treatment who initially received 50 mg DDS daily; the dosage was later increased to 100 mg daily. The patients were examined clinically, histologically, and bacteriologically at the beginning of the study. Subsequently the patients were seen regularly, and evaluated by clinical criteria for regression of nodules, decrease of infiltration of the skin, nerve size and tenderness, nerve conduction velocity, and BI. Special attention was paid to signs of reactions: either reversal reaction (RR), as indicated by inflammatory skin lesions and/or signs of active neuritis with changes in motor nerve conduction velocity (MCV), muscle strength and cutaneous sensation; or erythema nodosum leprosum (ENL), as indicated by symptoms of general malaise and the appearance of the typical red tender subcutaneous nodules and/or lymphadenitis and/or neuritis with changes in nerve size, tenderness, MCV, and loss of strength and sensation.

Immunological techniques

Solid-phase radioimmunoassay (sRIA). Solid phase radioimmunoassay (sRIA) was carried out as described in detail previously ([13, 14]). Briefly, sonified *M. leprae* were used for coating polystyrene tubes, and either LSP [lepromatous serum pool prepared from 40 newly diagnosed patients with lepromatous (LL-BL) leprosy] or patient serum was added to the tubes. The tubes were incubated for 24 hr at 4°C, washed and ^{125}I-labelled purified anti-human IgA, -IgM, or -IgG was added. The tubes were again incubated for 4 hr at 4°C, washed, and counted in a gammacounter. For each set of experiments, LSP and background controls were included. Background control activity, obtained either by omitting coating of the tubes with sonified *M. leprae* or adding of serum, was less than 1% of the counts obtained with sonified *M. leprae*-coated tubes and LSP diluted 1:10.

Antibody activity was calculated as described previously ([15]). A standard curve was made for each set of experiments from the counts obtained with LSP diluted 1×10^{-1}, 1×10^{-2}, 1×10^{-3}, and 1×10^{-4}. The steepest part of this standard curve providing the largest difference in counts between two neighboring dilutions of LSP was used. Each patient's serum was diluted 1×10^{-2}, 1×10^{-3}, and 1×10^{-4} for the IgM and IgG assays and 1×10^{-1}, 1×10^{-2}, and 1×10^{-3} for the IgA assay. The dilution of the pa-

tient's serum falling within the steepest part of the standard curve was used for calculation, and the other two dilutions were used as controls. The results were expressed as antibody activity in percentage of LSP and calculated in the following way: 100% of LSP means that the same number was obtained with 1×10^{-3} dilution of the patient serum as with 1×10^{-3} dilution of LSP; 10% of LSP means the same number of counts with 1×10^{-2} dilution of patient serum as with 1×10^{-3} dilution of LSP; and, finally, 1000% of LSP means that the same number of counts was obtained with 1×10^{-4} dilution of patient serum as with 1×10^{-3} dilution of LSP. The calculations were made on a semilogarithmic paper with the number of counts on the abscissa and the percentage on the ordinate.

The effect of rheumatoid factor (RF) was investigated using monoclonal RF in a model experiment. The amount of monoclonal RF needed to obtain a false-positive reaction was distinctly greater in our sRIA than the amount of RF occurring in LSP ([14]). Furthermore, sRIA has been shown to be particularly insensitive to RF.

Radioimmunoassay (RIA) for antibodies against *M. leprae* antigen 7. RIA for antibodies against *M. leprae* antigen 7 was carried out as described previously ([16]). The ^{125}I-labelled *M. leprae* antigen 7 preparation was made by electrolytic iodination. This RIA is based upon separation of antibody-bound labelled antigen from free labelled antigen by protein A-containing staphylococci (Cowan type 1). In brief, 100 μl of the serum samples (tested in dilutions 1×10^{-3} and 1×10^{-4}) was mixed with 100 μl of ^{125}I-labelled *M. leprae* antigen 7, incubated for 30 min at 20°C, and 2 ml 1% formalinized and heat treated staphylococci was added. After careful mixing of the reagents, the tubes were spun at 1500 *g* for 20 min, the supernatant was discharged, and the pellet counted in a gammacounter. At each set of experiments, 1×10^{-2}, 1×10^{-3}, and 1×10^{-4} dilutions of LSP were included to prepare a standard curve. Antibody activity was again expressed as percent of LSP and calculated as for sRIA.

Leprosy bacilli

Leprosy bacilli were obtained from Dr. R. J. W. Rees, London, through the WHO Immunology of Leprosy (IMMLEP) program. The bacilli were isolated from liver tissue of *M. leprae*-inoculated armadillos as described by Draper ([5]).

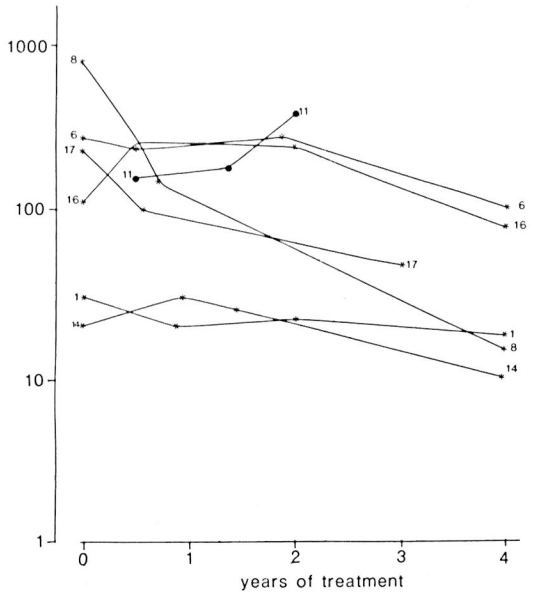

FIG. 1. IgA-anti-*M. leprae* antibody activity in serial serum samples obtained from six lepromatous leprosy patients and one patient with dual mycobacterial diseases (No. 11) during the first 2–4 years of DDS treatment. The activity is expressed as percentage of the IgA-anti-*M. leprae* activity in a lepromatous serum pool (LSP).

Statistical methods

Wilcoxon's ranking test was used for calculation of the statistical significance of differences between groups ([4]). The Kolmogorov-Smirnov-two-sample test was used for comparison of the results obtained by sRIA for IgA-, IgM- and IgG-anti-*M. leprae* antibody activity with the results obtained by radioimmunoassay for antibodies against *M. leprae* antigen 7 ([23]).

RESULTS

Table 1 shows the observation period, the IgA-anti-*M. leprae* antibody activity in the sera at the start and the end of the observation period, and the difference between these two in the 15 lepromatous leprosy patients. The observation period varied from 1½ to 4 years. The IgA-anti-*M. leprae* antibody activity at the start of the observation period varied from 25% to 2000% of

TABLE 1. *IgA anti-*M. leprae *antibody activity in 15 patients with lepromatous leprosy before and after 1½ to 4 years treatment with DDS.*[a]

Patient no.	Observation period (yr)	At the start of the study	At the end of the study	Difference	Activity at the end in % of the activity at the start
1	4	30	20	−10	66
2	2	150	30	−120	20
3	4	50	30	−20	60
6	4	300	100	−200	33
7	2	100	40	−60	40
8	4	800	10	−790	1.25
10	3	750	600	−150	80
12	1½	260	140	−120	54
13	1½	2000	400	−1600	20
14	4	25	10	−15	40
16	4	100	80	−20	80
17	3	230	50	−180	22
18	1½	300	100	−200	33
19	2	500	40	−460	8
20	3	400	50	−350	12
Median value	3	260	50	−150	33

[a] The values are expressed as percentage of the activity in a lepromatous serum pool (LSP) used for reference.

the activity in LSP, the median value being 260% of LSP. The variation at the end of the study was from 10% to 600% of LSP, with a median value of 50%. There was a decrease in IgA-anti-*M. leprae* antibody activity in all sera taken at the end of the observation period compared to the sera taken at the start of the study. The median IgA-anti-*M. leprae* activity at the end was 33% of the activity before the start of DDS treatment.

Figure 1 shows the IgA-anti-*M. leprae* antibody activity in sera taken at different intervals from the start to the end of the observation period in six of the patients (Nos. 1, 6, 8, 14, 16, and 17). The curves from these six patients are typical for the patterns observed in the whole group, and the same patients have been used in Figures 1, 2, and 3. In addition, patient No. 11 with a dual mycobacterial infection has been included in Figures 1, 2, and 3; he will be discussed later.

The curves showed a marked decrease (patients Nos. 8 and 17) or a moderate decrease in antibody activity from the start to the end of the study. There was a transient increase in IgA-anti-*M. leprae* antibody activity in some of the sera after initiation of DDS treatment, illustrated by patients Nos. 14 and 16 in Figure 1. Nine of the patients had one or several episodes of reactions during the observation period (RR in two patients and ENL in seven patients). There was an associated increase in IgA-anti-*M. leprae* antibody activity in 5 (1 with RR and 4 with ENL) of these 9 patients during or soon after these episodes of reactions.

Table 2 shows the results from the IgM-anti-*M. leprae* antibody assay. The variations in IgM-antibody activity at the start of the study was from 16% to 560% of LSP, with a median value of 230%. The variation at the end of the study was between 20% and 280% of LSP with a median value of 100%. A decrease in IgM-anti-*M. leprae* activity after three years (median duration) of DDS treatment could be demonstrated in 12 of the 15 patients. Median IgM-anti-*M. leprae* activity at the end was 53% of the activity before the start of DDS treatment.

When the group of sera taken before the start of DDS treatment was compared with the group of sera taken at the end of the observation period, the difference was not statistically significant ($0.1 > p > 0.05$). Similar statistical analysis showed a significant decrease in antibody activity in the other three assays (sRIA for IgA- and IgG-anti-*M. leprae* antibody and RIA for antibodies against *M. leprae* antigen 7) with $p < 0.005$ when group observations at the

TABLE 2. *IgM anti-*M. leprae *antibody activity in 15 patients with lepromatous leprosy before and after 1½ to 4 years treatment with DDS.*[a]

Patient no.	Observation period (yr)	At the start of the study	At the end of the study	Difference	Activity at the end in % of the activity at the start
1	4	20	20	0	100
2	2	450	280	−170	62
3	4	170	50	−120	29
6	4	370	200	−170	54
7	2	160	70	−90	44
8	4	560	160	−400	28
10	3	340	180	−160	53
12	1½	80	60	−20	75
13	1½	230	30	−200	13
14	4	16	22	+6	138
16	4	50	80	+30	160
17	3	300	130	−170	43
18	1½	140	100	−40	71
19	2	240	100	−140	42
20	3	400	100	−300	25
Median value	3	230	100	−140	53

[a] The values are expressed as percentage of the activity in a lepromatous serum pool (LSP) used for reference.

start were compared with the group observations at the end of the study.

Figure 2 illustrates the findings in the IgM assay. The decline in antibody activity was less, with more horizontal curves than in Figures 1 and 3. Figure 2 also shows a transient increase in antibody activity in four of the patients (Nos. 6, 14, 16, 17) after the start of DDS treatment. Again, such an increase could be correlated with reactions in 4 (1 with RR and 3 with ENL) of the 9 patients. We saw a transient increase of IgM-anti-*M. leprae* antibody activity in one patient (No. 15) without any clinical sign of reaction recorded during the observation period.

Table 3 and Figure 3 show the results in the IgG assay. The IgG-anti-*M. leprae* antibody activity varied from 30% to 4000% of the activity in LSP at the start of the study, with a median value of 320%, and from 10% to 450% of LSP at the end of the study, with a median value of 100%. Figure 3 illustrates the marked decrease in IgG-anti-*M. leprae* antibody activity and a transient increase in IgG activity in three of the patients (Nos. 6, 14 and 16) after the start of DDS treatment. This could be related to reactions in 4 (1 with RR and 3 with ENL) of the 9 patients with reactions during the observation period.

We could demonstrate a transient increase in both IgA-, IgM- and IgG-anti-*M. leprae* antibody activity in 3 patients in association with reactions (1 with RR and 2 with ENL).

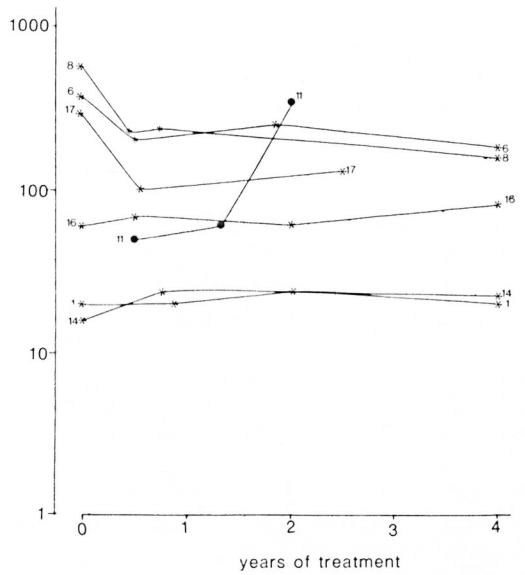

FIG. 2. IgM-anti-*M. leprae* antibody activity in six lepromatous leprosy patients and one patient with dual mycobacterial diseases (No. 11) during DDS treatment; otherwise as in Figure 1.

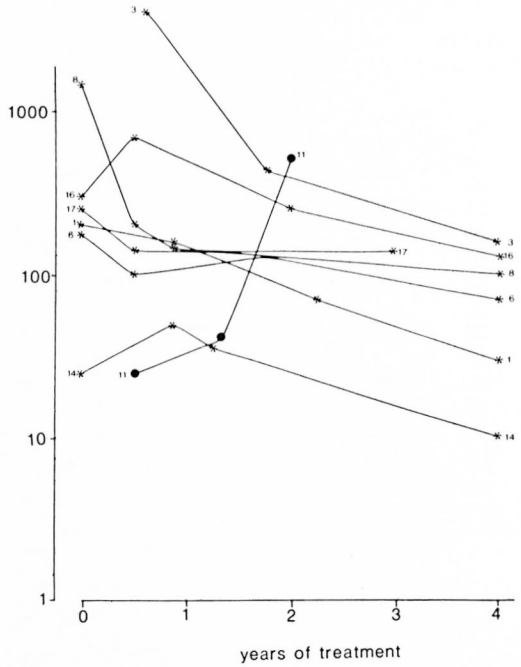

FIG. 3. IgG-anti-*M. leprae* antibody activity in seven lepromatous leprosy patients and one patient with dual mycobacterial disease (No. 11) during DDS treatment; otherwise as in Figure 1.

Patient No. 11 (included in Figs. 1, 2, and 3) is the only one in whom IgA-, IgM- and IgG-anti-*M. leprae* and anti-*M. leprae* 7 antibody activity increased markedly throughout the observation period. He entered the study in May, 1975, with a diagnosis of BB/BL leprosy and developed a typical reversal reaction during DDS treatment. In August, 1975, treatment with prednisolone had to be started, and he was given 30 mg daily for one week, 20 mg daily for two weeks and 10 mg daily for seven months. In October, 1975, he developed clinical and x-ray features of active pulmonary tuberculosis. Since he suffered from a dual mycobacterial infection, he was excluded from the calculation of the decrease in antibody activity in Tables 1 through 4. The first serum sample taken from this patient prior to treatment was no longer available for study, and the first sample recorded in the figures was obtained four months after the start of treatment. In the previous study ([16]) of the activity of antibodies against *M. leprae* antigen 7, it was shown that antibodies against *M. leprae* antigen 7 for this patient were markedly lower at the start of the study than in this sample obtained four months after the start of DDS treatment.

TABLE 3. *IgG anti-*M. leprae *antibody activity in 15 patients with lepromatous leprosy before and after 1½ to 4 years treatment with DDS.*[a]

Patient no.	Observation period (yr)	At the start of the study	At the end of the study	Difference	Activity at the end in % of the activity at the start
1	4	200	30	−170	15
2	2	500	40	−460	8
3	4	3500	170	−3300	5
6	4	180	70	−110	39
7	2	1500	100	−1400	7
8	4	1500	100	−1400	7
10	3	90	90	0	100
12	1½	4000	450	−3550	11
13	1½	70	40	−30	58
14	4	30	10	−20	33
16	4	320	140	−180	44
17	3	250	140	−110	56
18	1½	150	70	−80	47
19	2	450	260	−190	88
20	3	700	150	−550	21
Median value	3	320	100	−180	21

[a] The values are expressed as percentage of the activity in a lepromatous serum pool (LSP) used for reference.

TABLE 4. *Antibodies against* M. leprae *antigen 7 in 15 patients with lepromatous leprosy before and after 1½ to 4 years treatment with DDS.*[a]

Patient no.	Observation period (yr)	At the start of the study	At the end of the study	Difference	Activity at the end in % of the activity at the start
1	4	250	50	−200	20
2	2	100	60	−40	60
3	4	320	60	−260	19
6	4	500	120	−380	24
7	2	220	22	−198	10
8	4	350	60	−290	17
10	3	250	200	−50	80
12	1½	600	300	−300	50
13	1½	700	140	−560	20
14	4	300	100	−200	33
16	4	500	200	−300	40
17	3	250	70	−180	28
18	1½	300	100	−200	33
19	2	800	450	−350	56
20	3	900	130	−770	14
Median value	3	320	100	−260	28
11	2	11	170	+159	

[a] The values are expressed as percentage of the activity in a lepromatous serum pool (LSP) used for reference.

Table 4 shows the results of the assay for antibodies against *M. leprae* antigen 7. The variation at the start of the study was from 100% to 900% of LSP, with a median value of 320%, and from 22% to 450%, with a median value of 170%, of the activity found in LSP at the end of the study. All 15 patients showed a decrease in anti-*M. leprae* activity when the results at the start of the study were compared with the results at the end. Median *M. leprae* 7 antibody activity at the end was 28% of the activity before the start of DDS treatment.

When the Kolmogorov-Smirnov-two-sample test was used to compare the results obtained for IgA-, IgM-, and IgG-anti-*M. leprae* antibody activity with the results obtained for antibodies against *M. leprae* antigen 7 in each serum sample, there was no significant correlation between the results of the sRIA assays and the RIA results. The correlation coefficient was 0.5 between IgA-anti-*M. leprae* antibodies and antibodies against *M. leprae* antigen 7, 0.3 between IgM-anti-*M. leprae* antibodies and antibodies against *M. leprae* antigen 7 and, finally, 0.3 between IgG-anti-*M. leprae* antibodies and antibodies against *M. leprae* antigen 7. The best correlation was therefore seen between IgA-anti-*M. leprae* antibodies and antibodies against *M. leprae* antigen 7 ([23]).

DISCUSSION

Quantification of IgA-, IgM- and IgG-anti-*M. leprae* antibody activity and antibodies against *M. leprae* antigen 7 in serial serum samples taken prior to antileprosy treatment and at different intervals until 1½ to 4 years (median duration 3 years) after the start of DDS treatment showed a marked decline in several kinds of antibody activity during the observation period. The assays for IgG- and IgA-anti-*M. leprae* and antibodies against *M. leprae* antigen 7 showed a similar decrease of antibody activity; the median antibody activity at the end of the observation period was about one third of the activity before the start of DDS treatment in these three assays. These findings support and extend the findings in an earlier study of two groups of lepromatous leprosy patients, demonstrating a marked decrease in IgG-anti-*M. leprae* antibody activity after ten years of DDS treatment ([15]).

In the previous study, we did not find a significant decrease in IgM-anti-*M. leprae* antibody activity after ten years of DDS

treatment ([15]) although in the present investigation we have demonstrated a decline in IgM-anti-*M. leprae* antibody activity after three years of DDS treatment. The variation of IgM-anti-*M. leprae* antibody activity in individual patients with untreated lepromatous leprosy is large, from 10% to 540% in the present investigation and from 10% to 1000% of the activity in LSP in the previous investigation. Therefore, due to the large variation of the individual results, the decrease of IgM-anti-*M. leprae* antibody activity during DDS treatment does not seem to be sufficient to cause a significant decrease when group observations are compared. Indeed, when we calculated the results in the present investigation using Wilcoxon's ranking test as before ([4]) for calculation of the statistical significance of differences between groups, we found significant differences in the IgA- and IgG-anti-*M. leprae* assays and in the assay for antibodies against *M. leprae* antigen 7 from the start compared to the end of the study ($p < 0.005$ for all three assays), while the difference in the IgM assay was not significant ($0.1 > p > 0.05$). In analogy with the previous studies, the present investigation showed that the IgM-anti-*M. leprae* assay is less sensitive than the others in demonstrating a decrease in antibody activity during DDS treatment. The basis for this difference is of considerable interest but, at present, unknown.

As previously ([16]), patient No. 11 was excluded from the main series because of the appearance of active pulmonary tuberculosis during the observation period. He was the only patient who showed a prolonged increase of IgA-, IgM-, and IgG-anti-*M. leprae* and antibodies against *M. leprae* antigen 7 during the two-year observation period. Antibodies against *M. leprae* antigen 7 and most of the anti-M. leprae antibodies detected by the immunoglobulin class specific assays crossreact with antigens in other mycobacteria such as *M. tuberculosis* ([6, 7, 11]), and the increased antibody activity is probably a response to increased antigenic load during the dual mycobacterial infection caused by progressing tuberculosis.

The median values in the assays for IgA-, IgM-, and IgG-anti-*M. leprae* antibodies and antibodies against *M. leprae* antigen 7 were well above 100% of the activity in LSP in the pretreatment sera. In view of the marked effect of treatment on antibody activity, this may be due to some of the sera in the LSP being obtained from patients after initiation of antileprosy treatment, and partly due to loss of activity during transport and storage of the sera, since the pool was collected in 1974 to 1975. The activity of the reference LSP was very stable during the last three years and the period of these studies. The recording of the level of antibody activity might be different if the sera were compared with another reference LSP. However, this would not influence the conclusions with regard to demonstration of decreasing antibody activity in individual patients where all samples from each patient were tested simultaneously in each assay.

When the results of the IgA-, IgM-, and IgG-anti-*M. leprae* assays were compared with the assay for antibodies against *M. leprae* antigen 7 at the start and the end of the observation period, there were no significant correlations. The RIA for anti-*M. leprae* 7 antibodies is based on binding of antibody-bound labelled antigen to protein A-containing staphylococci, an assay which reacts with about 90% of the IgG ([12]), 30% of the IgM ([10]), and 10% of the IgA molecules in normal sera ([22]). Antibodies of these three immunoglobulin classes may therefore be demonstrated by the assay, although it is expected to be less sensitive for IgM and IgA than for IgG antibodies. In the immunoglobulin class specific sRIA, sonified *M. leprae* bacilli are used for the coating of polystyrol test tubes. By crossed immunoelectrophoresis (CIE) more than 20 distinct antigenic components have been demonstrated in concentrated *M. leprae* sonicates ([1]). The relative amount and the identity of the antigenic components bound to the wall of the test tube have not been determined so far. By the use of monospecific ([8, 9]) and oligospecific antisera against mycobacterial components, it has been demonstrated that *M. leprae* antigen 7 is bound to the polystyrol test tubes and that the sRIA demonstrates antibodies against other components of *M. leprae* in addition to anti-*M. leprae* 7 antibodies (Harboe, unpublished observations). By CIE with patient serum in the intermediate gel, it has been shown that the specificity of the hu-

moral immune response varies markedly in individual patients with lepromatous leprosy ([7]). The sRIA assay thus appears to detect so many antibodies in addition to anti-*M. leprae* 7 that no significant correlation was found between the two types of assays.

The decrease in anti-*M. leprae* 7 activity during DDS treatment demonstrated in the present study is more marked than in a previous study of the same patient group ([16]). This difference can be partly explained by the extended observation period. Another difference between the two investigations is the change of reference. Previously, we used a rabbit anti-*M. leprae* antiserum as reference, recording the activity of a patient serum at a given dilution as "percentage of maximal binding by rabbit anti-*M. leprae*." Later findings indicated that reference to a standard LSP gave more consistent results and better opportunities for calculation of wide variations in antibody activity by referring to its activity on a log scale. Batch variations of ^{125}I-labelled *M. leprae* antigen 7 have also been observed; the batch presently used provided an assay with calculable results over a wide range of antibody activity.

The present results indicate that the relationship between a transient increase in antibody activity and the occurrence of reactions needs further study. Similar observations have been made previously, *e.g.*, Cruickshank and Ellis ([3]). Using an agglutination test with killed *M. tuberculosis* H37Rv in a patient with lepromatous leprosy, a marked rise in agglutination titer was observed during ENL with a subsequent decrease in antibody activity after disappearance of the reaction.

Although the effect varied in the different assays, it is evident that DDS treatment of lepromatous leprosy which results in clinical improvement is associated with a decrease in anti-*M. leprae* antibody activity. The median antibody activity at the end of an observation period with a median duration of three years was about one third of the activity before the start of treatment. This difference corresponds in magnitude to the difference between untreated lepromatous and untreated borderline tuberculoid (BT) leprosy ([15]). Additional information about the basic mechanisms involved, *e.g.*, change in affinity or avidity or fine specificity of the antibodies, is needed to obtain a better understanding of the nature and the significance of the humoral immune response in leprosy.

SUMMARY

Previously, a slight decrease in antibodies against *M. leprae* antigen 7 was demonstrated after one year of dapsone (DDS) treatment in 14 of 15 patients with lepromatous (BL-LL) leprosy. The same patients have now received DDS from 1½ to 4 years (median 3 years) and sera taken at the start, during, and at the end of the observation period have been retested for antibodies against *M. leprae* antigen 7 by radioimmunoassay and tested for IgA-, IgM-, and IgG-anti-*M. leprae* antibody activity by solid phase radioimmunoassay.

Both IgA- and IgG-anti-*M. leprae* antibody activity and the activity of antibodies against *M. leprae* antigen 7 showed a decrease in activity after three years of DDS treatment to a median value of about one third of the activity in the sera taken at the start of the study. A smaller but significant decrease in IgM-anti-*M. leprae* antibody activity could be demonstrated. A transient increase in antibody activity (both measured by RIA and sRIA) could be demonstrated and related to reactions (reversal reaction and ENL) in five patients. No significant correlation could be found when IgA-, IgM-, and IgG-anti-*M. leprae* antibody activity was compared with antibodies against *M. leprae* antigen 7 in individual sera.

RESUMEN

En un estudio anterior demostramos una ligera disminución en los anticuerpos contra el antígeno 7 de *M. leprae* en 14 de 15 pacientes con lepra lepromatosa (BL-LL) después de 1 año de tratamiento con dapsona (DDS). Estos mismos pacientes se han vuelto a estudiar, ahora después de 1.5 a 4 años de tratamiento (mediana 3 años) con DDS. Sus sueros, tomados al inicio, durante, y al final del periodo de observación, se han usado para buscar anticuerpos contra el antígeno 7 de *M. leprae* por radioinmunoensayo y anticuerpos IgG, IgM e IgA anti-*M. leprae*, por un radioinmunoensayo en fase sólida.

Tanto los anticuerpos IgA e IgM anti-*M. leprae*, como los anticuerpos contra el antígeno 7 de *M. leprae*, mostraron una disminución en su actividad después de 3 años de tratamiento con DDS hasta ⅓ de la actividad en los sueros tomados al inicio del estudio.

También se pudo demostrar una disminución pequeña pero significante en la actividad del anticuerpo IgM anti-*M. leprae*. Además se observó un aumento transitorio en la actividad de anticuerpo (medido tanto por RIA como por RIAs) que pudo relacionarse con estados reaccionales (reaccion reversa y ENL) en 5 pacientes. No se encontró ninguna correlación significante cuando la actividad de los anticuerpos IgA, IgM o IgG anti-*M. leprae* se comparó con los anticuerpos contra el antígeno 7 del *M. leprae* en los sueros individuales.

RÉSUMÉ

On a antérieurement mis en évidence une légère diminution des anticorps contre l'antigène 7 de *M. leprae* après une année de traitement à la dapsone (DDS), chez 14 malades sur 15 qui étaient atteints de lèpre lépromateuse (BL-LL). Les mêmes malades ont aujourd'hui reçu de la dapsone pour une durée de 1½ à 4 années (médiane 3 ans). Les échantillons de sérum prélevés au début, pendant, et à la fin de la période d'observation ont été réétudiés par une méthode de dosage radio immunologique en vue de mettre en évidence les anticorps contre l'antigène 7 de *M. leprae*; ces échantillons ont été également étudiés par une technique de dosage radio-immunologique en phase solide, pour leur activité en anticorps IgA, IgM, et IgG contre *M. leprae*.

L'activité des anticorps IgA et IgG contre *M. leprae*, de même que l'activité des anticorps contre l'antigène 7 de *M. leprae*, se sont révélées diminuées après 3 années de traitement par la DDS; l'activité notée avait une valeur médiane se situant environ à un tiers des activités mesurées dans les échantillons prélevés au début de l'étude. On a pu également démontrer une diminution plus faible, encore que significative, de l'anticorps IgM contre *M. leprae*. Une augmentation transitoire de l'activité en anticorps, mesurée à la fois par RIA et sRIA, a pu être démontrée, en relation avec les réactions (réaction inverse et ENL) chez 5 malades. Aucune corrélation significative n'a pu être mise en évidence lorsque l'activité en anticorps IgA, IgM, et IgG contre *M. leprae* était comparée à l'activité des anticorps contre l'antigène de *M. leprae*, dans des échantillons individuels de sérum.

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