INTERNATIONAL JOURNAL OF LEPROSY

Volume 51, Number 3
Printed in the U.S.A.

Effect of Treatment on Antibody Activity Against *Mycobacterium leprae* Antigen 7 in Tuberculoid Leprosy[1]

John Sverre Dahle, Titia Warndorff van Diepen,
Els J. M. Touw Langendijk, Morten Harboe, and Ayele Belehu[2]

A radioimmunoassay (RIA) for the demonstration and quantification of antibodies against *Mycobacterium leprae* antigen 7 was developed by Melsom, *et al.* ([11]) who demonstrated a decline in antibody activity in sera from lepromatous patients during the first year of dapsone (DDS) treatment. They noted that the fall in antibody activity was small and attributed this to the large amount of mycobacterial antigen present in these lepromatous patients.

By extending the observation period to three years, Melsom, *et al.* ([10]) demonstrated a marked decrease of IgG and IgA anti-*M. leprae* antibody activity measured by solid phase radioimmunoassays (sRIA) and of anti-*M. leprae* 7 activity in the same lepromatous patients.

Yoder, *et al.* ([16]) determined the antibody activity against *M. leprae* antigen 7 by RIA in groups throughout the spectrum and observed that the median value in four groups of patients decreased gradually from the lepromatous to the tuberculoid end of the spectrum, but there was a striking variation in antibody activity of individual sera in each group. By studying groups of patients treated for various lengths of time, they showed that DDS treatment led to a marked decrease in antibody activity in borderline tuberculoid leprosy and that relapse was associated with renewed synthesis and increased antibody activity.

The purpose of the present study was to determine the antibody activity in individual patients with borderline tuberculoid (BT) leprosy during long-term treatment.

MATERIALS AND METHDS

Patient sera. From a group of 80 consecutive patients with tuberculoid leprosy at the All-Africa Leprosy and Rehabilitation Training Centre (ALERT), Addis Ababa, Ethiopia, 21 patients fulfilled the criteria for inclusion in this study: at least four serum samples should be available, taken at different times during a minimum observation time of 16 months. The patients were classified clinically and in most cases histologically according to the extended Ridley-Jopling scale ([12,13,14]) as having borderline tuberculoid (BT) leprosy.

The patients were divided into two groups: One, called SUS, contained 12 patients suspected to be harboring DDS resistant bacilli. They still had active lesions or developed new quiescent or active lesions despite long-term DDS treatment. The other group, called TUBA, consisted of nine new patients who according to the available information had not been treated previously. All patients received a standard supervised treatment with DDS, 100 mg daily. Three patients (TUBA Nos. 1, 14 and 19) received in addition rifampin, 600 mg daily, for three weeks initially, and one patient (TUBA No. 9) received TB450 (isoniazid 300 mg + thioacetazone, 150 mg, daily) for one year initially together with standard DDS treatment. During treatment, the patients were clinically examined at regular intervals and sera were collected.

Six patients in the SUS groups were clinically proven to have DDS resistant leprosy

[1] Received for publication on 30 November 1982; accepted for publication in revised form on 15 February 1983.

[2] J. S. Dahle, M.D., University of Oslo, Department of Dermatology, Ullevaal Hospital, Oslo 1, Norway. T. Warndorff van Diepen, M.D.; E. J. M. Touw Langendijk, M.D., All-Africa Leprosy and Rehabilitation Training Centre (ALERT), P.O. Box 165, Addis Ababa, Ethiopia. M. Harboe, M.D., Ph.D., University of Oslo, Institute for Experimental Medical Research, Ullevaal Hospital, Oslo 1, Norway. A. Belehu, Ph.D., Armauer Hansen Research Institute (AHRI), P.O. Box 1005, Addis Ababa, Ethiopia (deceased 17 May 1983).

since they continued to show active lesions or developed new quiescent or active lesions despite supervised daily administration of 100 mg DDS after entering this study. In these six patients, the treatment was changed from DDS to clofazimine (Lamprene®), 100 mg daily, initially supplemented with rifampin, 600 mg daily, for three weeks in five of them.

In nine of the TUBA patients a lepromin test was carried out at the first consultation upon entering the study. The median observation period was 29 months with a range from 16–39 months. The sera were separated from venous blood, stored at −25°C in Addis Ababa, and subsequently transported in a frozen state to Oslo, Norway, for testing by radioimmunoassay.

Mycobacteria and antigenic preparations thereof. *M. leprae* were provided by Dr. R. J. W. Rees from the IMMLEP bank as freeze-dried bacilli purified from liver tissue of infected armadillos according to protocol 3 of the report of the Third IMMLEP Scientific Working Group, World Health Organization (WHO), which is procedure IV of reference 15. The disruption of *M. leprae* by ultrasonification for subsequent immunization of rabbits to produce antisera reacting with various components of the bacillus is described elsewhere ([5]).

Aliquots of sonified *M. leprae* were centrifuged at 20,000 *g* for 20 min to remove insoluble material ([2]). Aliquots of the supernatant were labeled with ^{125}I by electrolytic iodination ([4,8]). To test each labeled preparation, a mixture consisting of equal parts of unlabeled *M. leprae* sonicate and the labeled preparation was placed in the circular antigen well of a crossed immunoelectrophoresis (CIE) plate with rabbit anti-*M. leprae* antibody in the top gel. The plate was run, washed, pressed, dried and then exposed to an x-ray film for autoradiography ([1,6,11]). The film showed intense labeling corresponding to the precipitate line of antigen 7 where most of the radioactivity was localized. This preparation was therefore used directly for the assay of anti-*M. leprae* antigen 7.

Radioimmunoassay (RIA). For the assay of antibodies against *M. leprae* antigen 7, the procedure previously described for the assay of antibodies against BCG antigen 60 ([4,6]) and *M. leprae* antigen 7 ([11]) was used.

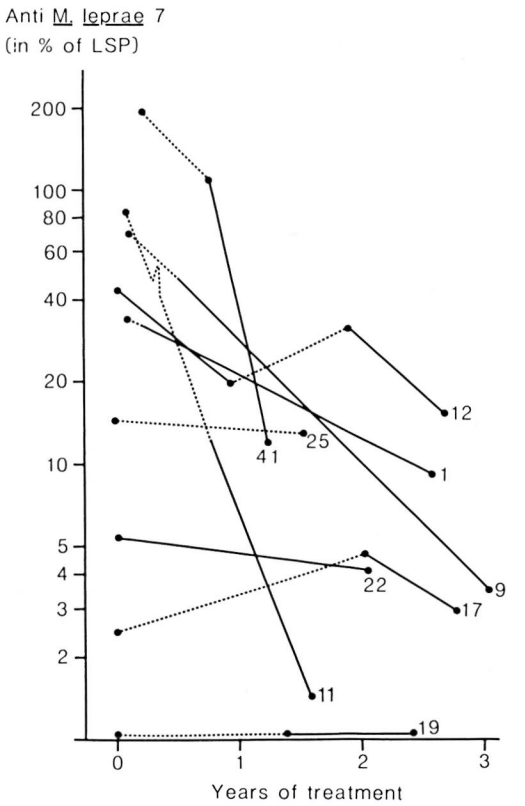

FIG. 1. Effect of prolonged DDS treatment on anti-*M. leprae* antigen 7 antibody activity in newly diagnosed borderline tuberculoid leprosy. The activity is expressed in percent of the activity in a lepromatous serum pool (LSP) used for reference. The solid lines indicate periods of clinical improvement; whereas stippled lines indicate periods with signs of active inflammation in skin lesions.

The technique is based on the separation of antibody-bound labeled *M. leprae* antigen 7 from free antigen by the use of protein A-containing staphylococci which serve as a solid phase and have a marked capacity to bind IgG antibodies ([8]). Briefly, each tube contained 100 μl of the appropriate serum dilution and 100 μl of labeled *M. leprae* sonicate. The mixtures were incubated for 30 min at room temperature before adding 2 ml of 0.5% formalinized staphylococci of the Cowan 1 strain (NCTC 85308). All dilutions of unlabeled, "cold" and labeled proteins and of sera were made in an immunoassay buffer of the following composition: 0.01 M phosphate buffer, pH 7.4, in 0.14 M NaCl with 0.02% w/v NaN_3 and 0.2% w/v bovine serum albumin (Sigma

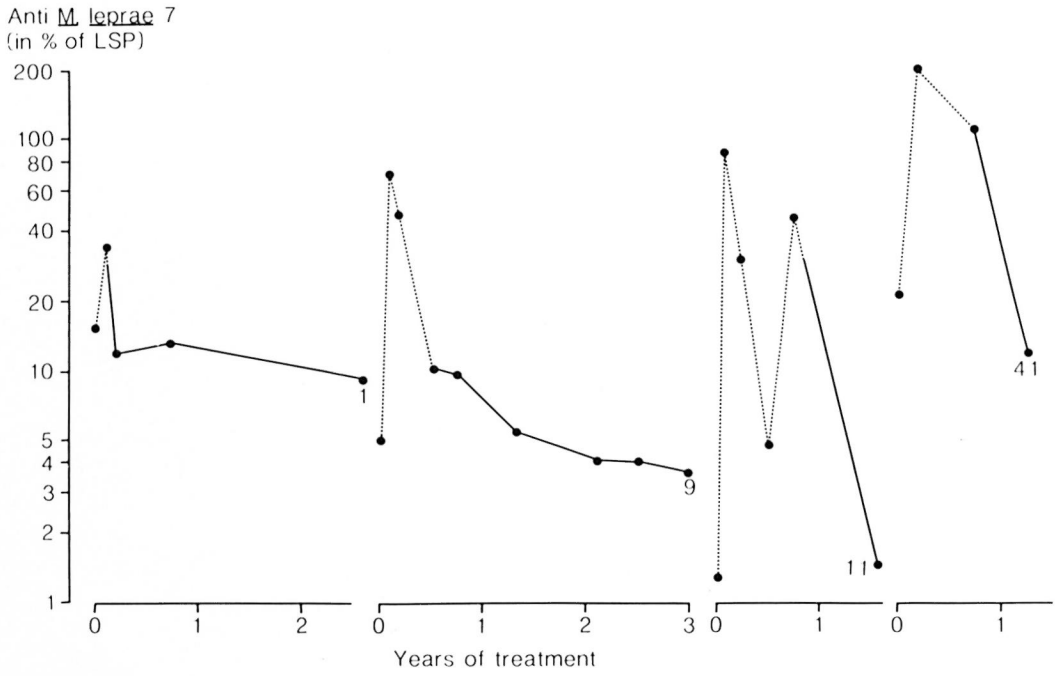

FIG. 2. Four newly diagnosed patients with borderline tuberculoid leprosy with a rapid rise in anti-*M. leprae* antigen 7 activity following initiation of treatment. Recording of antibody and clinical activity as in Figure 1.

Chemical Co., St. Louis, Missouri, U.S.A.). After the reagents had been mixed, the tubes were spun at 2300 × *g* for 20 min, the supernatant was carefully aspirated and the radioactivity determined in the bacterial pellet. All values are given as mean values of duplicate tests. The antibody activity, expressed in percent of the activity of a lepromatous serum pool (LSP) used for reference, was calculated as described previously ([10]).

RESULTS

Figure 1 shows the effect of prolonged DDS treatment on antibody activity against *M. leprae* antigen 7 in nine previously untreated patients with borderline tuberculoid (BT) leprosy. To facilitate the illustration of the main points, Figure 1 shows the anti-*M. leprae* antigen 7 activity at the beginning and at the end of the observation period for each patient and, if relevant, at additional points of marked changes in clinical appearance.

Seven of the nine newly diagnosed patients responded clinically to the treatment. In five of these (TUBA Nos. 1, 9, 11, 12 and 41) there was a marked decrease in antibody activity; whereas Case No. 17 showed an unusual pattern with active skin lesions for two years during DDS treatment which was associated with an increase in antibody activity. When after the delivery of a child the lesions subsided, the anti-*M. leprae* 7 activity decreased.

One case (TUBA No. 25), who did not respond clinically to the treatment and showed no significant reduction in antibody activity, was suspected to have primary DDS resistant disease. Patient TUBA No. 19 showed no antibody activity at all during the treatment period of 2½ years. In this patient, the clinical features of hypopigmentation without loss of cutaneous sensibility and inconclusive histology led to a revision of the diagnosis, and the treatment was stopped.

Five of the clinically responding patients (TUBA Nos. 1, 9, 11, 17 and 41) showed signs of inflammation in the skin lesions ("active" skin lesions) during the first part of treatment, indicated with stippled lines in Figure 1, which were associated with increased antibody activity. In four of them the rise in antibody activity occurred rapidly, as shown in Figure 2, with the highest

TABLE 1. *Anti-*M. leprae *antigen 7 antibody activity as % of LSP in newly diagnosed BT leprosy patients during DDS treatment.*[a]

Patient no.	Observation period (months)	Antibody activity		
		Start[b]/ top[c] value	At end of study	Difference
TUBA 1	31	14/35	9	−26
9	36	5/70	3.5	−66.5
11	19	0/80	0.5	−79.5
12	32	43/	16	−27
17	34	2.5/	2.9	+0.4
22	27	5/	4	−1
25	18	14/	13	−1
41	16	21/200	12	−188
Total 8	213	449.5	60.9	−388.6

[a] Mean reduction in antibody activity per patient per year in this group was 22% of LSP.

[b] Start value is the anti-*M. leprae* antigen 7 antibody activity in the first serum sample.

[c] In the patients with more than twofold increase in antibody activity during the first six weeks the highest value was used as reference point for calculation of decrease in antibody activity.

activity being recorded 1–4 weeks after the beginning of treatment. This rapidly increasing antibody activity was associated with inflamed skin lesions in all four cases, two of them having overt reversal reactions. In these cases, the highest value was used as the first point in Figure 1 and as the reference point for calculating the decrease in antibody activity in Table 1. Mean reduction in antibody activity per patient per year in this group was 22% of the activity in the LSP. The whole group was used for the calculation with the exception of case TUBA No. 19 whose diagnosis of leprosy was revised.

TABLE 2. *Anti-*M. leprae *antigen 7 antibody activity as % of LSP in six patients, suspected from their case histories to have DDS resistant disease but who improved on supervised DDS treatment.*[a]

Patient no.	Observation period (months)	Antibody activity		
		Start value	At end of study	Difference
SUS 2	20	41	43	+2
4	19	7	4.5	−2.5
12	32	80	18	−62
15	16	42	23	−19
22	29	23	17	−6
33	24	58	39	−19
Total 6	140	251	144.5	−106.5

[a] Mean reduction in antibody activity per patient per year in this group was 9% of LSP.

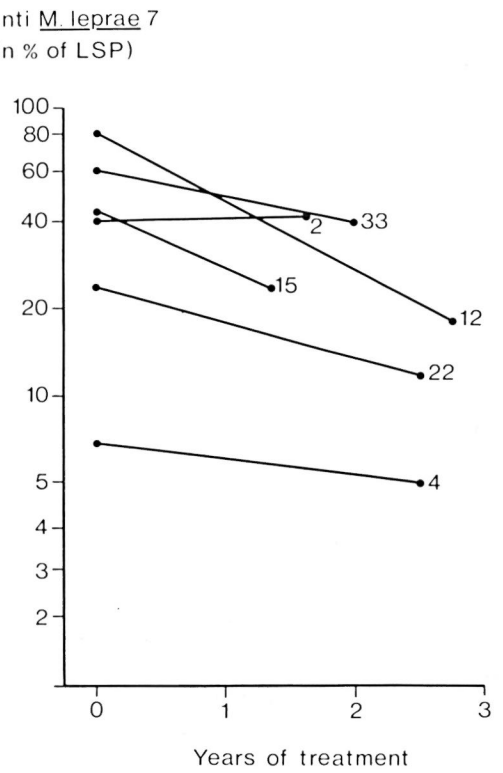

FIG. 3. Effect of prolonged supervised DDS treatment on anti-*M. leprae* antigen 7 antibody activity in six borderline tuberculoid leprosy patients, who were suspected from their case histories to have DDS resistant leprosy. All improved on supervised DDS treatment alone. Recording of antibody and clinical activity as in Figure 1.

TABLE 3. *Anti-*M. leprae *antigen 7 antibody activity as % of LSP in five patients who were clinically resistant to supervised DDS treatment.*[a]

Patient No.	Observation period (months)	Antibody activity		
		Start value	At end of study	Difference
SUS 3	14	19	37	+18
7	3	28	17	−11
14	27	43	32	−11
16	30	14	11	−3
24	21	57	40	−17
Total 5	95	161	137	−24

[a] Mean reduction in antibody activity per patient per year in this group was 3% of LSP.

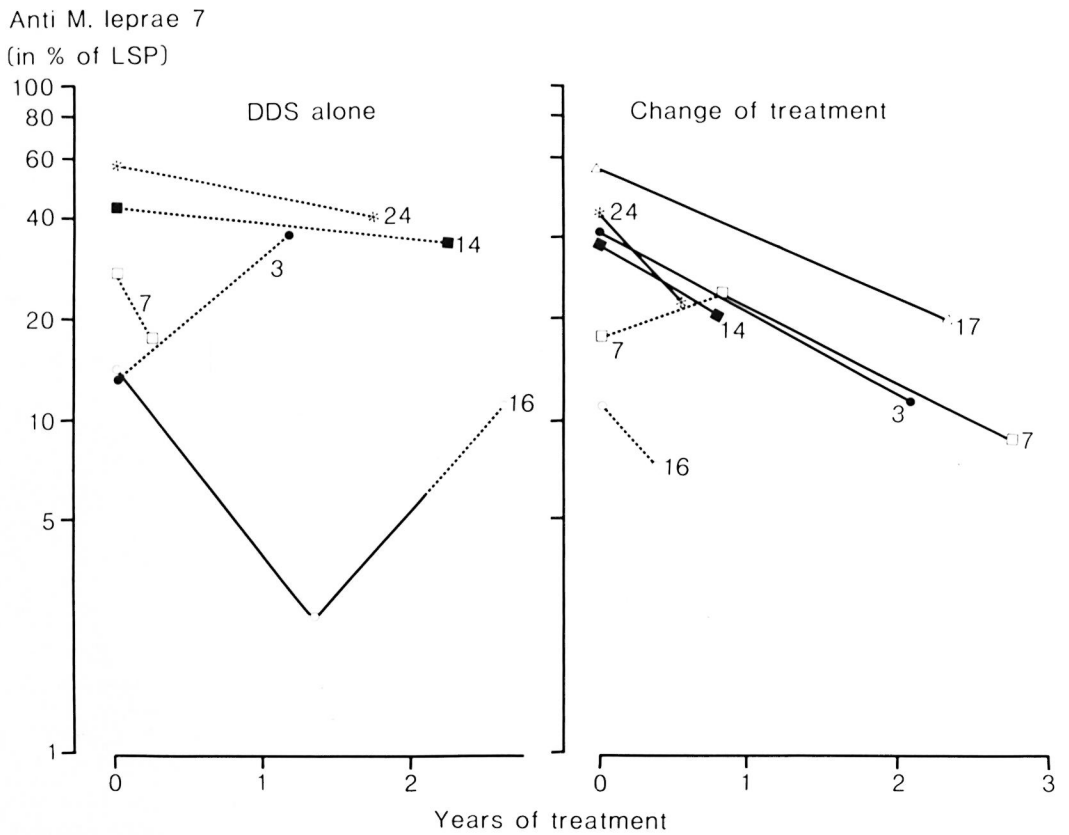

FIG. 4. Effect of prolonged treatment on anti-*M. leprae* antigen 7 antibody in clinically DDS resistant patients with borderline tuberculoid leprosy before and after change of therapy. Recording of antibody and clinical activity as in Figure 1.

In the SUS group of 12 patients suspected of having DDS resistant disease from their clinical history, there was a marked variation in response to continued DDS treatment under supervision.

Six patients responded clinically to DDS treatment. Five of these showed a decrease in anti-*M. leprae* 7 activity, as illustrated in Figure 3, but the slope of the curves was less steep than in the TUBA group with newly diagnosed patients. The mean reduction per patient per year was 9% of LSP (Table 2).

The results obtained in five of the six patients who did not respond clinically to continued DDS treatment are shown in the left part of Figure 4. The pattern is variable. Patient SUS No. 7 received DDS alone for only three months since new lesions appeared, but the second sample showed lower antibody activity than the first one. Case 16 showed an initial decrease in antibody activity and clinical improvement for two years, followed by clinical deterioration associated with an increase in anti-*M. leprae* 7 activity. Case SUS No. 3 showed a marked increase; Cases 24 and 14 showed barely decreasing antibody activity. Table 3 shows that the mean reduction per person per year was 3% of LSP in this group.

After change of treatment, clinical improvement was obtained in five cases, and this was associated with a marked decrease in antibody activity in all cases. The mean reduction in antibody activity in this group was 12% of LSP per patient per year, as shown in Table 4. This marked decrease was seen in all but one case after a few months on effective treatment, as illustrated in the right part of Figure 4 and in more detail in Figure 5. One case (SUS No. 7) showed an increase in antibody activity for nearly one

TABLE 4. *Anti-*M. leprae *antigen 7 antibody activity as % of LSP in six patients with clinically DDS resistant disease after change of treatment.*[a]

Patient no.	Observation period (months)	Antibody activity		
		At start of Lamprene® treatment	At end of study	Difference
SUS 3	25	37	11	−26
7	33	17	8	−9
14	9	32	20	−12
17	28	58	20	−38
16	5	11	7	−4
24	7	40	22	−18
Total 6	107	195	88	−107

[a] Mean reduction in antibody activity per patient per year in this group was 12% of LSP.

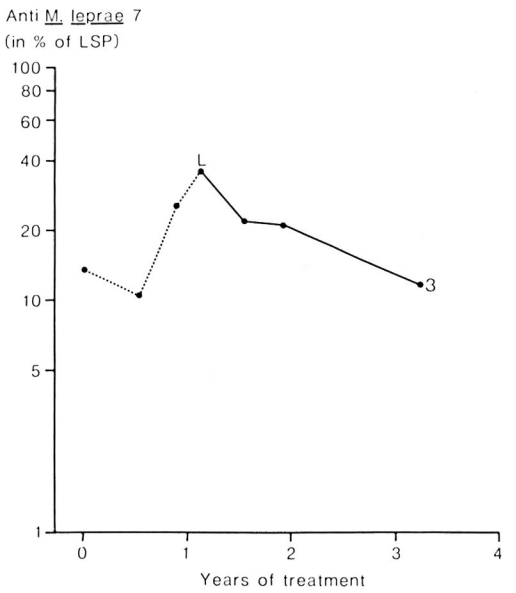

FIG. 5. Effect of prolonged treatment on anti-*M. leprae* antigen 7 antibody activity in a patient with DDS resistant disease (SUS No. 3) before and after change from DDS to Lamprene® (marked L). Recording of antibody and clinical activity as in Figure 1.

year after change of therapy while her lesions remained active. Then clinical improvement was observed in association with decreasing antibody activity.

DISCUSSION

In previous studies ([16]) groups of BT leprosy patients treated for various periods of time were investigated. They showed that DDS treatment led to a marked decrease in antibody activity in BT leprosy and that relapse was associated with renewed synthesis and increased antibody activity. In each group there was a striking variation in the antibody activity of individual sera. Therefore, the need for investigation of individual patients with BT leprosy during long-term treatment was obvious.

The present study confirms and extends our main earlier observations. The majority of the patients responding clinically to DDS treatment showed a decrease in antibody activity. The decline in antibody activity was more pronounced in the newly diagnosed (TUBA) patients than in the DDS sensitive SUS patients with long-standing leprosy (Table 1 compared with Table 2) in whom the basic stimulation of the immune system has been going on for years and is probably more profound.

Patients who were suspected from their case histories to have DDS resistant disease (the SUS group) were included in the present investigation to get an extra opportunity to evaluate the correlation between clinical effect during treatment and alteration in antibody activity. This group was heterogeneous. Six of these patients responded clinically to supervised DDS treatment which was associated with a definite decrease in antibody activity in five cases. The decline in antibody activity was, however, somewhat less than in the TUBA group, as mentioned above. In the remaining six SUS patients who were clinically not responding to supervised DDS treatment, the antibody activity was variable, and a definite decrease in antibody activity was first obtained after change to effective treatment.

The main observation is, therefore, that there is a strong correlation between clinical findings and antibody activity when groups of patients are investigated and that in individual patients the curves of clinical and antibody activity run mostly parallel. However, some patients deviate from this main pattern in different ways. The reasons for this deviation remain to be elucidated, but factors such as genetic predisposition, variation in antigen load, exposure to other crossreacting mycobacteria, reactions, and

pregnancy are probably involved. Awareness of these deviations is important. Interpretation of the findings in individual serum samples may indeed be difficult and should always be made in relation to the clinical condition.

During this long-term study of individual patients, a new and interesting observation was made. Four of eight patients (50%) in the TUBA group reacted with a rapid and marked increase in antibody activity shortly after initiation of treatment. This pattern was only observed in TUBA patients responding clinically to therapy and is probably due to increased antigen liberation from the bacteria which stimulates the immune system.

A similar pattern was observed by Kaplan and Chase ([9]), who measured anti-mycobacterial antibody activity before and after treatment in patients with tuberculosis. They showed that before treatment most patients with tuberculosis had only weak antibodies against native antigens in mycobacterial culture filtrates, even with advanced disease or prolonged symptoms. Only 46% of newly diagnosed patients showed antibody activity in their precipitation tests. Sera from patients with relapsed tuberculosis obtained before antimicrobial therapy contained precipitating antibodies more frequently (in 66% of the cases) and reacted with more antigens in culture fluids. Chemotherapy resulted in increased antibody activity in patients with both newly diagnosed and relapsed tuberculosis. They observed two patterns of antibody response after the initiation of therapy. Antibody negative patients required at least 6–8 weeks to seroconvert; whereas patients with preexisting antibody showed a more rapidly detectable increased antibody synthesis. In both groups, an early increase in antibody response after the initiation of treatment was always associated with clinical improvement.

All four patients in Figure 2 were seropositive before the initiation of therapy, but since the next sera were obtained after 4–10 weeks and then had reached maximum activity, it is likely that increased antibody synthesis is induced in a few weeks.

During the period of increased antibody synthesis, all four patients showed signs of inflammation in skin lesions and two had overt reversal reactions. But after 2–8 months all improved, accompanied with a definite decrease in antibody activity. Therefore, in BT patients an early and strong increase in antibody activity after the initiation of treatment seems to be a favorable sign, even if increased inflammatory activity and a reversal reaction may occur initially.

The four TUBA patients with rapidly increasing antibody activity obtained different forms of treatment. Case No. 1 received rifampin for three weeks initially together with DDS, and Case No. 9 received TB450 (isoniazid, 300 mg + thioacetazone, 150 mg, daily) for one year initially together with the standard dose of DDS. However, the same pattern of response was observed in Case Nos. 11 and 41 who received DDS alone. Therefore, this particular pattern of response to treatment does not appear to depend on the kind of treatment given but on the institution of effective chemotherapy.

At the start of this study, a lepromin test was carried out in the TUBA patients. However, it is unlikely that this would affect the results of the antibody assay in a significant way. To our knowledge, there are no published data in humans indicating so. Further, inoculation of armadillos with living *M. leprae* leads to increased anti-*M. leprae* antigen 7 antibody activity, but only after six months in connection with sustained bacillary multiplication ([3]). In analogy, BCG vaccination leads to a moderate and short lasting increase in anti-*M. leprae* antigen 7 activity probably related to a limited multiplication of these bacteria *in vivo* (Harboe, unpublished observations).

Any early indicator that could help to determine the course of the disease and the response to therapy would be of significance, particularly in cases of BT leprosy where few criteria are available to evaluate the course early after initiation of treatment due to the low number of bacilli in the skin lesions and the slow course. This particular pattern with early and marked increase in antibody activity seems to be such an indicator to confirm the diagnosis and indicate that the therapy is effective. The strong correlation between signs of inflammation and reactions and a rapid and marked increase in antibody activity observed in these four TUBA patients (Nos. 1, 9, 11 and 41) also indicates that the underlying processes

are associated with the stimulation of both humoral and cellular immune responses. This is an additional indicator that the previously claimed strict inverse relationship between cell-mediated and humoral immune responses throughout the leprosy spectrum cannot be upheld.

Further studies should be made on the development of antibody activity in individual BT patients during treatment—and particularly during the first six months with the new combined-drug regimens recommended by WHO.

SUMMARY

Anti-*Mycobacterium leprae* antigen 7 antibody activity was determined by radioimmunoassay during treatment in a longtime study of individual patients with newly diagnosed borderline tuberculoid (BT) leprosy and in BT leprosy patients who were suspected from their case histories to have dapsone (DDS) resistant leprosy. There was a strong correlation between clinical and antibody activity, and clinical improvement following treatment led to a marked decrease in antibody activity in most cases.

A characteristic pattern of rapid and marked increase in antibody activity shortly after the initiation of treatment was observed in patients with newly diagnosed BT leprosy. This pattern may become of practical importance in the evaluation of patients with BT leprosy as an indicator that the therapy is effective, even though this pattern was associated with a transient increase in inflammatory activity in the skin lesions. The association of inflammatory activity with increased antibody activity strongly indicates that the underlying processes are associated with the stimulation of both humoral and cellular immune responses.

RESUMEN

Se usó un radioinmunoensayo para determinar la actividad de anticuerpo contra el antígeno 7 del *Mycobacterium leprae* en el suero de pacientes con lepra tuberculoide intermedia (BT) de reciente diagnóstico y de pacientes con lepra BT que, según sus historias clínicas, parecían haber desarrollado resistencia a la dapsona (DDS). El estudio se hizo a largo plazo, durante el tratamiento de los pacientes. Se encontró que hubo una gran correlación entre la actividad clínica y la presencia de anticuerpos y que la mejoría clínica que siguió al tratamiento condujo a una marcada disminución en la actividad de anticuerpo en la mayoría de los casos.

En los pacientes con lepra BT de reciente diagnóstico se observó, de manera característica, un rápido y marcado incremento en la actividad de anticuerpo poco después de iniciado el tratamiento. Este patrón de la respuesta en anticuerpo puede llegar a ser de importancia práctica en la evaluación de los pacientes con lepra BT como un indicador de que la terapia es efectiva, aún cuando dicho patrón estuvo asociado con un incremento transitorio en la actividad inflamatoria de las lesiones de la piel. La asociación de la actividad inflamatoria con el incremento en la actividad de anticuerpo indica que los fenomenos subyacentes están asociados con la estimulación tanto de la inmunidad humoral como de la celular.

RÉSUMÉ

On a procédé à une détermination radioimmunologique de l'activité en anticorps contre l'antigène 7 de *Mycobacterium leprae,* au cours du traitement, dans le cadre d'une étude à long terme des malades individuels présentant une lèpre tuberculoïde dimorphe (BT) récemment diagnostiquée, et de malades atteints de lèpre BT dont on soupçonnait d'après leur anamnèse qu'ils pourraient présenter une lèpre résistante à la dapsone. On a noté une corrélation prononcée entre les signes cliniques et l'activité en anticorps; l'amélioration clinique à la suite du traitement a mené à une diminution marquée de l'activité en anticorps dans la plupart des cas.

Un profil caractéristique d'augmentation rapide et prononcée dans l'activité en anticorps très peu de temps après l'instauration du traitement a été observé chez les malades souffrant d'une lèpre BT récemment diagnostiquée. Ce profil pourrait revêtir une importante pratique pour l'évaluation des malades atteints de lèpre BT, comme indicateur d'une thérapeutique efficace, même si ce profil est associé avec une augmentation transitoire de l'activité inflammatoire dans les lésions cutanées. L'association d'une activité inflammatoire avec une élévation de l'activité en anticorps, montre clairement que les mécanismes sous-jacents sont associés avec la stimulation des réponses immunitaires, tant humorales que cellulaires.

Acknowledgments. The work was supported by grants from Anders Jahre's Fund for the Promotion of Science, The Norwegian Council for Science and the Humanities, and by the Immunology of Leprosy (IMMLEP) component of the UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases. We thank Tadelle Gebretsion, Helén Bergsvik, and Brit Sundsten for their excellent technical assistance.

Armauer Hansen Research Institute is sponsored by the Norwegian and Swedish Save the Children Organizations.

REFERENCES

1. Closs, O., Mshana, R. N. and Harboe, M. Antigenic analysis of *Mycobacterium leprae.* Scand. J. Immunol. **9** (1979) 297–302.
2. Closs, O., Reitan, L. J., Negassi, K., Harboe, M. and Belehu, A. *In vitro* stimulation of lymphocytes in leprosy patients, healthy contacts of leprosy patients, and subjects not exposed to leprosy. Comparison of an antigen fraction prepared from *Mycobacterium leprae* and tuberculin purified protein derivative. Scand. J. Immunol. **16** (1982) 103–115.
3. Harboe, M. Radioimmunoassay and other serologic tests and their application in epidemiological work. Symposium on the Epidemiology of Leprosy, Geilo, Norway, 1981. Lepr. Rev. **52** Suppl. (1981) 275–288.
4. Harboe, M., Closs, O., Bjorvatn, B. and Bjune, G. Antibodies against BCG antigen 60 in mycobacterial infection. Br. Med. J. **2** (1977) 430–433.
5. Harboe, M., Closs, O., Bjorvatn, B., Kronvall, G. and Axelsen, N. H. Antibody response in rabbits to immunization with *Mycobacterium leprae.* Infect. Immun. **18** (1977) 792–805.
6. Harboe, M., Closs, O., Svindahl, K. and Deverill, J. Production and assay of antibodies against one antigenic component of *Mycobacterium bovis* BCG. Infect. Immun. **16** (1977) 662–672.
7. Harboe, M. and Fölling, I. Recognition of two distinct groups of human IgM and IgA based on different binding to staphylococci. Scand. J. Immunol. **3** (1974) 471–482.
8. Jonsson, S. and Kronvall, G. The use of protein A-containing *Staphylococcus aureus* as a solid phase anti-IgG reagent in radioimmunoassays as exemplified in the quantification of alpha-fetoprotein in normal human adult serum. Eur. J. Immunol. **4** (1974) 29–33.
9. Kaplan, M. H. and Chase, M. W. Antibodies to mycobacteria in human tuberculosis. I. Development of antibodies before and after antimicrobial therapy. J. Infect. Dis. **142** (1980) 825–834.
10. Melsom, R., Harboe, M. and Naafs, B. Class specific anti-*Mycobacterium leprae* antibody assay in lepromatous (BL-LL) leprosy patients during the first two to four years of DDS treatment. Int. J. Lepr. **50** (1982) 271–281.
11. Melsom, R., Naafs, B., Harboe, M. and Closs, O. Antibody activity against *Mycobacterium leprae* antigen 7 during the first year of DDS treatment in lepromatous (BL-LL) leprosy. Lepr. Rev. **49** (1978) 17–29.
12. Myrvang, G., Godal, T., Ridley, D. S. and Song, Y. K. Immune responsiveness to *Mycobacterium leprae* and other mycobacterial antigens throughout the clinical and histopathological spectrum of leprosy. Clin. Exp. Immunol. **14** (1973) 541–553.
13. Ridley, D. S. and Jopling, W. H. Classification of leprosy according to immunity. A five-group system. Int. J. Lepr. **34** (1966) 255–273.
14. Ridley, D. S. and Waters, M. F. R. Significance of variations within the lepromatous group. Lepr. Rev. **40** (1969) 143–152.
15. Shepard, C. C., Draper, P., Rees, R. J. W. and Lowe, C. Effect of purification steps on the immunogenicity of *Mycobacterium leprae.* Br. J. Exp. Pathol. **61** (1980) 376–379.
16. Yoder, L., Naafs, B., Harboe, M. and Bjune, G. 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** (1979) 113–121.