INTERNATIONAL JOURNAL OF LEPROSY

Volume 51, Number 3
Printed in the U.S.A.
(ISSN 0148-916X)

INTERNATIONAL JOURNAL OF LEPROSY
And Other Mycobacterial Diseases

VOLUME 51, NUMBER 3 SEPTEMBER 1983

Relation Between Anti-*Mycobacterium leprae* Antibody Activity and Clinical Features in Borderline Tuberculoid (BT) Leprosy[1]

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

For several decades, the main view has been that antibodies against mycobacterial antigens occur frequently and in high amounts in lepromatous leprosy and rarely and in low concentrations in tuberculoid leprosy. This view was based on studies employing various immunological methods, such as complement fixation tests ([4,11]), passive hemagglutination tests ([1]), and double diffusion precipitation reactions in gel ([16,18,20,24]).

The introduction of new, sensitive radioimmunoassays for the demonstration and quantification of antibodies led to a revision of this view. When groups of patients were studied, it was demonstrated that the median antibody concentration decreased gradually from the lepromatous to the tuberculoid end of the spectrum. It became apparent, however, that there was a striking variation in antibody activity in individual sera from patients with similar clinical and histological classification, e.g., in borderline tuberculoid (BT) leprosy. This type of variation in antibody activity has been demonstrated in radioimmunoassay (RIA) for the quantification of antibodies against BCG antigen 60 ([8]) and its crossreacting antigen *Mycobacterium leprae* antigen 7 ([15,26]). Similar findings were also made in solid-phase radioimmunoassay (sRIA) for IgG, IgA and IgM anti-*M. leprae* antibodies ([13]). The reason for this variation in antibody activity has not been established.

The purpose of the present investigation was to study groups of patients with borderline tuberculoid (BT) leprosy to obtain additional information on the relationship between activity and clinical features in this form of leprosy.

MATERIALS AND METHODS

Patient sera. Sera were obtained from two groups of patients with BT leprosy at the All-Africa Leprosy and Rehabilitation Training Centre (ALERT), Addis Ababa,

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

[2] E. J. M. Touw Langendijk, M.D.; T. Warndorff van Diepen, 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).

Ethiopia. The cases were classified clinically, and in most cases histologically, according to the extended Ridley-Jopling scale ([17,21,22]). One group comprised the first 40 patients with newly diagnosed BT leprosy who, according to the available information, had not been treated previously and were entered into the TUBA trial. The second group comprised the first 40 patients with BT leprosy entered into the SUS trial. They were suspected from their case histories to have dapsone (DDS) resistant disease, appearing with active skin lesions or new skin lesions despite information on previous DDS treatment of long duration. They were put on DDS treatment under supervision with regular clinical examinations to establish if they were clinically resistant to DDS. Studies on antibody activity during continuous treatment with DDS, including cases with clinical DDS resistance following change of drug therapy, are reported in the accompanying paper ([6]).

The sera used in the present study were obtained from the first venous blood sample taken at entry into the study. 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 RIA. Due to damage during storage and transportation, three sera from the SUS group were not included in the study.

Mycobacteria and antibody assays. *M. leprae* was provided by Dr. R. J. W. Rees from the IMMLEP bank as freeze-dried bacilli purified from liver tissue of infected armadillos. Two radioimmunoassays were used.

Antibodies against *M. leprae* antigen 7 were determined by RIA using protein A containing staphylococci to separate antibody-bound labeled antigen from free antigen ([15]). Labeling and testing of the labeled antigen and performance of the assay are described in the accompanying paper ([6]). Antibody activity is expressed in percent of the activity in a lepromatous serum pool (LSP) used for reference, and the calculations were made as described previously ([13,14]).

A solid phase RIA was used for the quantification of IgG antibodies against various antigenic components in *M. leprae* sonicate as described previously ([12,13]) and in the accompanying paper ([6]).

Statistical calculations. Wilcoxon's rank sum test for unpaired samples was used to test differences between groups ([25]). The statistical significance of association between high and low antibody activity, as defined in the Results section, and various clinical features were evaluated by the χ^2-test for small numbers calculated from 2×2 contingency tables ([19]). Probability (p) values larger than 0.05 were regarded as not significant (n.s.).

RESULTS

The Figure shows the findings in the two antibody assays. Each point corresponds to one patient, filled circles representing the SUS group of previously treated suspected DDS resistant cases, open circles the TUBA group of new cases. Anti-*M. leprae* 7 antibody activity varied from 550% to 1% of the activity in the LSP used for reference, the median value for the whole group being 19% of the LSP. The activity in the SUS group was higher than in the TUBA group, with median values of 40% and 11.5%, respectively. The difference between the two groups was close to the significance level, $p \approx 0.05$.

The findings in the IgG anti-*M. leprae* assay were similar with a marked variation in activity between individual sera. The median value for the whole group was 22% of the activity in the LSP. Again, the SUS group showed higher activity (median value 30% of the LSP) than the TUBA group of new cases (median value 8% of the LSP). The difference between these two groups was statistically significant, $p < 0.01$.

These findings strongly indicated that the basic differences in clinical features which led to the division of the borderline tuberculoid patients into these two groups was associated with differences in antibody activity. To obtain further information on this point, two groups of sera were defined, those with high antibody activity and those with low antibody activity. These groups are shown in The Figure. High antibody activity was defined as being more than three times the median activity, meaning above 57% of the activity in the LSP in the anti-*M. leprae* 7 assay and above 66% of the LSP

TABLE 1. *Relationship between treatment status and antibody activity in BT leprosy.*

	Anti-*M. leprae* 7			IgG anti-*M. leprae*		
	High[a]	Low[b]		High[a]	Low[b]	
Previously treated, still active cases (SUS group)	16	2	18	11	5	16
New cases (TUBA group)	8	15	23	7	17	24
	24	17	41	18	22	40
	$\chi^2 = 12.18$ 1 d.f. $p < 0.001$			$\chi^2 = 6.08$ 1 d.f. $p < 0.02$		

[a] Higher than three times median value of the whole group of 77 sera.
[b] Lower than one third of the median value of the whole group.

in the IgG anti-*M. leprae* assay. Similarly, low antibody activity was defined as activity below one third of the median value of the whole group, meaning less than 6.3% of the activity in the LSP in the anti-*M. leprae* 7 assay and less than 7.3% of the LSP in the IgG anti-*M. leprae* assay.

Table 1 shows the relationship between antibody activity and treatment status in BT leprosy. Eighteen of the previously treated but still active cases in the SUS group had antibody activity above three times or below one third of the median value of the whole group in the anti-*M. leprae* 7 assay, with 16 cases in the former and only two in the latter group. In the group of new cases, the reverse was found with eight patients having high and 15 low antibody activity. This difference was statistically significant with a p value <0.001 in the χ^2-test. In the IgG anti-*M. leprae* assay the difference between these two groups was also statistically significant, $p < 0.02$.

Table 2 shows various comparisons made to see if differences between the two groups were significantly correlated with antibody activity.

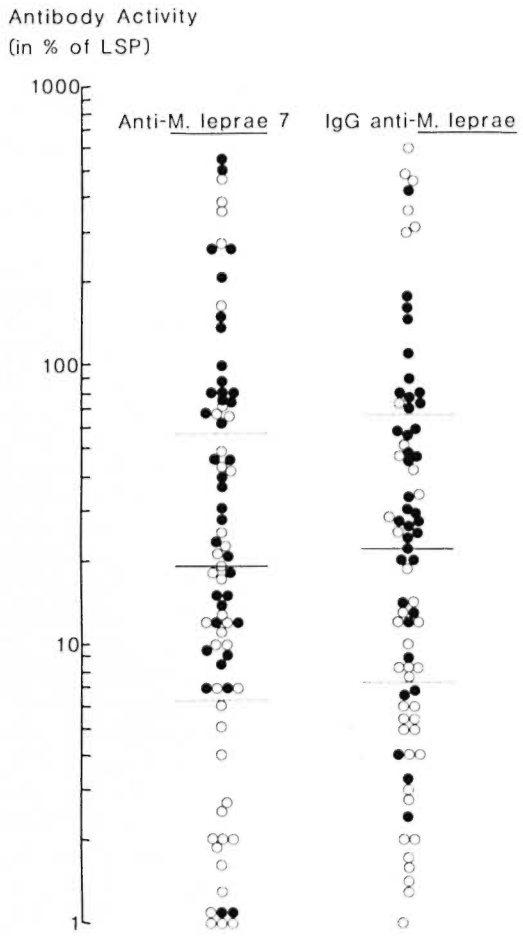
In the newly diagnosed cases, the appearance of "active lesions" with signs of inflammation was not significantly correlated with high antibody activity.

In the SUS group further subdivision was

TABLE 2. *Relationship between antibody activity and clinical features in BT leprosy.*

Group	Anti-*M. leprae* 7		Ratio	Significance[b]	IgG anti-*M. leprae*		Ratio	Significance[b]
	High[a]	Low[a]			High[a]	Low[a]		
Total material	24	17	1.4		18	22	0.82	
Males	11	8	1.4	n.s.	10	10	1.0	n.s.
Females	13	9	1.4		8	12	0.67	
New cases (TUBA)	8	15	0.53	$p < 0.001$	7	17	0.41	$p < 0.02$
Previously treated cases (SUS)	16	2	8.0		11	5	2.20	
TUBA, active lesions	3	9	0.33	n.s.	3	10	0.30	n.s.
TUBA, inactive lesions	5	6	0.83		4	7	0.57	
SUS ≥ 5 yrs treatment	8	0	∞	n.s.	6	0	∞	$p < 0.05$
SUS < 5 yrs treatment	8	2	4.0		5	5	1.0	
SUS, active lesions	13	0	∞	$p < 0.02$	7	0	∞	n.s.
SUS, quiescent lesions	3	2	1.5		4	2	2.0	
SUS, neuritis	8	0	∞	n.s.	6	0	∞	$p < 0.05$
SUS, no neuritis	8	2	4.0		5	5	1.0	

[a] Defined as in Table 1.
[b] Calculated by χ^2 test as in Table 1.



THE FIGURE. Antibody activity in borderline tuberculoid (BT) leprosy. The activity in the assay for antibodies against *M. leprae* antigen 7 and IgG antibodies against various antigens of *M. leprae* is recorded in percent of the activity in a lepromatous serum pool (LSP) used for reference. Each point represents one patient; ● = previously treated but still active cases (the SUS group); ○ = newly diagnosed cases (the TUBA group).

correlated to a significant extent to variation in antibody activity. The relationship to length of treatment is shown in Table 3. In both assays, high antibody activity was correlated with longer duration of disease and treatment. The difference was not statistically significant in the anti-*M. leprae* 7 assay due to the presence of only two SUS cases in the low activity group in this assay; whereas it was statistically significant in the IgG anti-*M. leprae* assay.

Signs of inflammatory activity in the lesions were correlated to antibody activity, in cases with active skin lesions to activity in the anti-*M. leprae* 7 assay and in cases with neuritis to the activity in the IgG anti-*M. leprae* assay.

TABLE 3. *Relationship between antibody activity and length of treatment in the SUS group of BT leprosy.*

	Duration of treatment in years			
	High activity[a]		Low activity[a]	
	Median	Mean	Median	Mean
Anti-*M. leprae* 7	4.0	5.7	2.5	2.5
IgG anti-*M. leprae*	6.0	6.4	2.0	2.2

[a] Defined as in Table 1.

DISCUSSION

The present investigation confirmed the previous demonstration of marked variation in antibody activity in individual patients with BT leprosy ([13,26]) both in the assay for antibodies against *M. leprae* antigen 7 and in the polyvalent solid RIA for IgG antibodies against various antigenic components present in *M. leprae* sonicates. Yoder, *et al.* ([26]) studied groups of BT leprosy patients treated for various periods of time and found that DDS treatment led to a marked decrease in antibody activity and that suspected or proven relapse was associated with renewed antibody synthesis and increased anti-*M. leprae* 7 antibody activity in the serum. These observations are probably reflected in the difference observed between the SUS and TUBA groups in the present investigation. The SUS group consisted of cases with a continued presence of active lesions or cases who developed new lesions despite DDS treatment for several years. As shown in the accompanying paper ([6]) this group was heterogeneous. Although the patients were suspected to have DDS resistant disease on the basis of their clinical history, some of them responded to supervised DDS treatment. In these cases, the presence of active lesions or the appearance of new lesions was probably related to irregular or insufficient administration of DDS, and these cases correspond to "relapse cases" in the study of Yoder, *et al.* ([26]). In other cases, supervised treatment with DDS did not lead to clinical improvement, and these patients were then clinically di-

agnosed as having DDS resistant disease. In both groups, active multiplication of bacilli is expected to occur, resulting in an increased antigenic load in the organism and probably in an increased liberation of antigen from bacilli-containing cells which imply a potent stimulus to the immune system, resulting in increased antibody synthesis.

This is probably the main reason for the increased antibody activity in the SUS group compared with the newly diagnosed cases.

Skin lesions with signs of inflammation and the development of acute neuritis have been demonstrated to be associated with increased activity in lymphocyte transformation tests ([2, 3, 7]), that is, with increased delayed-type hypersensitivity against antigens of *M. leprae*. The main current view is that the development of active inflammation in skin lesions or nerves is not only associated with increased cell-mediated immune reactions, but directly mediated by the cell-mediated hypersensitivity to mycobacterial antigens liberated in the skin lesions or peripheral nerves. The correlation between signs of inflammation of lesions and high antibody activity observed in the present work indicates that the underlying processes are associated with the stimulation of both humoral and cellular immune responses. It has often been claimed that there is an inverse relationship between cell-mediated and humoral immune responses to *M. leprae* throughout the leprosy spectrum ([5,16]). Previous studies by various radioimmunoassays have shown that this view cannot be upheld since there is a wide variation in antibody activity in individual patients with similar clinical classification ([8,13,26]), and no strict inverse relationship between cell-mediated and humoral immune responses was observed when assayed against one defined antigenic component of *M. leprae* ([9]). The present study adds a new facet to this discussion by showing that increased inflammatory activity in skin lesions and nerves is associated with high antibody levels in patients with persisting BT leprosy, and there is an obvious need for combined studies of humoral and immune reactions during reactions in leprosy.

In newly diagnosed cases of BT leprosy there was no significant correlation between the presence of active skin lesions and high antibody activity. In such cases the basic stimulation of the immune system is probably less profound, and the stimulation by antigens of actively inflamed lesions is not strong enough to "break through" to induce a marked increase in antibody activity. The cause of the wide variation in antibody activity in individual cases with newly diagnosed BT leprosy has not been established. Further studies should be made in this group which appears to be particularly useful as an indicator of other factors influencing the immune response during the development of paucibacillary leprosy. In early cases of tuberculoid leprosy, it is expected that several other factors, such as genetic predisposition, extent and kind of previous exposure to environmental mycobacteria, concurrent infection and malnutrition, may strongly influence the antibody activity as measured in the current assays. In these cases it would be particularly important to correlate antibody activity with various indicators of antigenic load. This load represents mycobacterial antigen not only in skin lesions but also elsewhere in the body, since several authors have provided evidence that leprosy is a generalized disease, not only in the lepromatous forms but also in the apparent paucibacillary forms of tuberculoid leprosy ([10,23]).

SUMMARY

Antibody activity against *Mycobacterium leprae* antigen 7 was determined by radioimmunoassay and IgG antibodies against various antigens present in an *M. leprae* sonicate by a solid phase radioimmunoassay in 77 patients with borderline tuberculoid (BT) leprosy.

In both assays there was a wide variation in antibody activity in individual patients although all were diagnosed as having BT leprosy. The median antibody activity was lower in newly diagnosed cases than in patients appearing with active skin lesions or new skin lesions despite dapsone (DDS) treatment of long duration. Further comparison of patients with high and low antibody activity revealed that high antibody activity was significantly correlated statistically with active skin lesions, new skin lesions and neuritis despite DDS treatment of long duration. The reason for variation in antibody activity in newly diagnosed BT

leprosy remains unclear, and this patient group is of particular interest for further characterization of the basis for variation in antibody activity in tuberculoid leprosy.

RESUMEN

Se determinó la actividad de anticuerpo contra el antígeno 7 de *Mycobacterium leprae* (por radioinmunoensayo) y la actividad de anticuerpo IgG contra los antígenos presentes en un sonicado de *M. leprae* (por radioinmunoensayo en fase sólida), en 77 pacientes con lepra tuberculoide intermedia, BT.

En ambos ensayos se presentó una amplia variación en la actividad de anticuerpo en los pacientes individuales, aunque todos ellos se diagnosticaron como BT. La mediana de la actividad de anticuerpo fue más baja en los casos de reciente diagnóstico que en los pacientes con lesiones activas en la piel (nuevas o crónicas), independientemente del tratamiento con dapsona (DDS). La comparación adicional de los pacientes con alta y baja actividad de anticuerpo reveló que la alta actividad de anticuerpo correlacionaba estadísticamente con la presencia de lesiones activas en la piel, de lesiones nuevas en la piel y de neuritis, siendo esto independiente del tratamiento prolongado con DDS. La razón de la variación en la actividad de anticuerpo en pacientes BT de reciente diagnóstico permanece obscura pero este grupo de pacientes es de interés particular porque su estudio detallado nos puede ayudar a entender las causas de la variación en la actividad de anticuerpo observada en lepra tuberculoide.

RÉSUMÉ

On a procédé au dosage radioimmunologique de l'activité en anticorps contre l'antigène 7 de *Mycobacterium leprae*, ainsi qu'au dosage radiobiologique en phase solide des anticorps IgG contre divers antigènes présents dans un sonicat de *M. leprae*, chez 77 malades atteints de lèpre tuberculoïde dimorphe (BT).

Pour l'un et l'autre de ces dosages, on a constaté une grande variation dans l'activité en anticorps chez des malades individuels, quoique tous aient été diagnostiqués comme atteints de lèpre BT. L'activité moyenne en anticorps était plus faible chez des cas récemment diagnostiqués que chez des malades présentant des lésions cutanées actives, ou de nouvelles lésions cutanées, malgré un traitement prolongé à la dapsone (DDS). La comparaison des malades présentant respectivement des activités élevées ou faibles en anticorps a révélé qu'une activité élevée en anticorps présentait une corrélation statistiquement significative avec les lésions cutanées actives, les nouvelles lésions cutanées, et la névrite, et ceci malgré un traitement prolongé par la DDS. La raison de cette variation dans l'activité en anticorps chez des malades BT récemment diagnostiqués, n'est pas claire. Ce groupe de malades présente un intérêt particulier pour une étude ultérieure des facteurs qui expliquerait les variations dans l'activité en anticorps dans la lèpre tuberculoïde.

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 and Helén Bergsvik for their excellent technical assistance. Armauer Hansen Research Institute is sponsored by the Norwegian and Swedish Save the Children Organizations.

REFERENCES

1. Almeida, J. O. Serology in leprosy. Bull. WHO **42** (1970) 673–702.
2. Barnetson, R. St.C., Bjune, G., Pearson, J. M. H. and Kronvall, G. Antigenic heterogeneity in patients with reactions in borderline leprosy. Br. Med. J. **4** (1975) 435–437.
3. Bjune, G., Barnetson, R. St.C., Ridley, D. S. and Kronvall, G. Lymphocyte transformation test in leprosy; correlation of the response with inflammation of lesions. Clin. Exp. Immunol. **25** (1976) 85–94.
4. Brants, J. Komplementbindungsreaktion mit dem Tuberkulose-Antigen von Witebsky, Klingenstein und Kuhn bei Lepra. Dermatol. Wochenschr. **95** (1932) 1688–1691.
5. Bullock, W. E. Leprosy: A model of immunological perturbation in chronic infection. J. Infect. Dis. **137** (1978) 341–354.
6. Dahle, J. S., Warndorff van Diepen, T., Touw Langendijk, E. J. M., Harboe, M. and Belehu, A. Effect of treatment on antibody activity against *Mycobacterium leprae* antigen 7 in tuberculoid leprosy. Int. J. Lepr. **51** (1983) 312–320.
7. Godal, T., Myrvang, B., Samuel, D. R., Ross, W. F. and Löfgren, M. Mechanism of "reactions" in borderline tuberculoid (BT) leprosy. Acta Pathol. Microbiol. Scand. [A] **236** (1973) 45–53.
8. Harboe, M., Closs, O., Bjorvatn, B. and Bjune, G. Antibodies against BCG antigen 60 in mycobacterial infection. Br. Med. J. **2** (1977) 430–433.
9. Harboe, M., Closs, O., Reitan, L. J. and Draper, P. Demonstration of antibodies reacting with different determinants on *Mycobacterium leprae* antigen 7. Int. J. Lepr. **49** (1981) 147–158.
10. Karat, A. B. A., Job, C. K. and Rao, P. S. S. Liver in leprosy: Histological and biochemical findings. Br. Med. J. **1** (1971) 307–310.
11. Lewis, P. A. and Aronsen, J. D. The complement fixation reaction as applied to leprosy. J. Exp. Med. **38** (1923) 219–232.
12. Melsom, R., Harboe, M., Duncan, M. E. and Bergsvik, H. IgA and IgM antibodies towards *M. leprae* in cord sera and in patients with leprosy: An indicator of intrauterine infection in leprosy. Scand. J. Immunol. **14** (1981) 343–352.
13. Melsom, R., Harboe, M., Myrvang, B., Godal,

T. and BELEHU, A. Immunoglobulin class specific antibodies to *M. leprae* in leprosy patients, including the indeterminate group and healthy contacts as a step in the development of methods for sero-diagnosis of leprosy. Clin. Exp. Immunol. **47** (1982) 225–233.

14. 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.
15. 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.
16. MYRVANG, B., FEEK, C. M. and GODAL, T. Antimycobacterial antibodies in sera from patients throughout the clinico-pathological disease spectrum of leprosy. Acta Pathol. Microbiol. Scand. [B] **82** (1974) 701–706.
17. MYRVANG, B., GODAL, T., RIDLEY, D. S., FRÖLAND, S. 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.
18. NORLIN, M., NAVALKAR, R. G., OUCHTERLONY, Ø. and LIND, A. Characterization of leprosy sera with various mycobacterial antigens using double diffusion-in-gel analysis-III. Acta Pathol. Microbiol. Scand. **67** (1966) 555–562.
19. RACE, R. R. and SANGER, R. *Blood Groups in Man.* 6th ed. Oxford: Blackwell, 1975.
20. REES, R. J. W., CHATTERJEE, K. R., PEPYS, J. and TEE, R. D. Some immunologic aspects of leprosy. Am. Rev. Resp. Dis. **92** Suppl. (1965) 139–149.
21. RIDLEY, D. S. and JOPLING, W. H. Classification of leprosy according to immunity. A five-group system. Int. J. Lepr. **34** (1966) 255–273.
22. RIDLEY, D. S. and WATERS, M. F. R. Significance of variations within the lepromatous group. Lepr. Rev. **40** (1969) 143–152.
23. SINGH, R. and KORANNE, R. V. Systemic involvement in tuberculoid leprosy—pathogenesis of leprosy. Lepr. India **51** (1979) 451–458.
24. ULRICH, M., PINARDI, M. E. and CONVIT, J. A study of antibody response in leprosy. Int. J. Lepr. **37** (1969) 22–27.
25. WHITE, C. The use of ranks in a test of significance for comparing two treatments. Biometrics **8** (1952) 33–41.
26. 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.