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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

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SUMMARY

IgA, IgM and IgG anti-*M. leprae* antibody activity was quantitated by solid phase radioimmunoassay in groups of untreated leprosy patients throughout the spectrum, in lepromatous leprosy patients treated for more than 10 years, in an indeterminate leprosy group, and in a non-leprosy control group. IgA, IgM and IgG anti-*M. leprae* antibody activity increased from the group of healthy individuals exposed to *M. leprae* but without clinical signs of leprosy to tuberculoid (BT and BT/TT) and further to lepromatous (BL to LL) leprosy. There was a considerable overlap in IgA antibody activity, while the overlap between controls and tuberculoid and lepromatous leprosy was less than 20% in the IgM and IgG assays. After more than 10 years of treatment, the IgG anti-*M. leprae* activity had decreased markedly, whereas there was less effect in the IgA assay and no significant change in the IgM assay. In contrast to earlier findings, the group of 'strictly indeterminate leprosy' showed signs of an active humoral immune response against *M. leprae*. The IgM anti-*M. leprae* activity was higher in indeterminate leprosy than in the control group with virtually no overlap. IgA anti-*M. leprae* was higher in indeterminate leprosy, but with considerable overlap with the controls. No difference between these two groups was found in the IgG assay. The results are discussed in relation to the value of the various immunoglobulin specific anti-*M. leprae* assays for different purposes, including development of techniques for sero-diagnosis of leprosy.

INTRODUCTION

A solid phase radioimmunoassay (sRIA) was developed for demonstration and quantification of IgA and IgM anti-*M. leprae* antibodies in cord sera from babies of leprous mothers. IgM anti-*M. leprae* antibodies were demonstrated in 50%, and both IgA and IgM antibodies in 30% of cord sera from babies of mothers with active lepromatous leprosy. IgA anti-*M. leprae* could not be demonstrated in 23 cord sera of mothers with inactive lepromatous leprosy, tuberculoid leprosy and no clinical signs of leprosy, while IgM anti-*M. leprae* antibodies could be demonstrated in low concentration in three of these sera (Melsom *et al.*, 1981b). At delivery, the lepromatous leprosy mothers had a markedly higher concentration of both IgM and IgA anti-*M. leprae* antibodies than the tuberculoid and non-leprosy mothers. These findings indicated that immunoglobulin class-specific anti-*M. leprae* assays might be particularly valuable for sero-diagnosis in leprosy.

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Various antibody assays and assays for cell-mediated immune reactions are currently being studied for their value in diagnosis of subclinical infection in leprosy. Antibody assays appear to be particularly valuable in individuals prone to develop lepromatous forms of the disease with negative lymphocyte transformation and skin tests. Due to its long incubation time (Newell, 1966) and the high infectivity in diffuse lepromatous leprosy with few clinical signs and symptoms, it would be particularly valuable if patients with lepromatous leprosy could be identified before development of clinical symptoms.

The purpose of the present work was first, to apply our class specific assays for IgA, IgM and IgG anti-*M. leprae* antibodies in sera from healthy individuals exposed to *M. leprae* but without clinical signs of the disease, and from patients with tuberculoid (BT and BT/TT) and lepromatous (LL to BL) leprosy, second, to study the effect of prolonged dapsone (DDS) treatment in lepromatous leprosy, and third, to study a group of patients with 'strictly indeterminate leprosy', previously studied with regard to cellular immune reactivity and precipitating anti mycobacterial antibodies (Myrvang *et al.*, 1973a) with the more sensitive radioimmunoassays for anti-*M. leprae* antibodies.

MATERIALS AND METHODS

Patients and controls

Group 1 consisted of 21 non-leprosy controls of whom 11 had worked from one to seven years and the remaining 10 from one week to one year at the Addis Ababa Leprosy Hospital as gate clinic attendants, ward nurses or laboratory assistants. They had all been heavily exposed to *M. leprae* bacilli shed from the large number of untreated lepromatous leprosy patients attending the outpatient clinic at the hospital, but they had no clinical signs of leprosy (Myrvang, 1974). The non-leprosy controls came from a somewhat better socio-economic background than the leprosy patients.

Group 2 consisted of 17 patients with BT and BT/TT leprosy, clinically and histologically classified according to the extended Ridly–Jopling scale (Ridley & Jopling, 1966; Ridley & Waters, 1969; Myrvang *et al.*, 1973b). They were treated with DDS, 50 to 100 mg daily, and the serum samples were taken at the start of DDS treatment.

Group 3 consisted of 17 patients with LL-BL leprosy, clinically and histologically classified according to the extended Ridley–Jopling scale. The serum samples were taken at the start of DDS treatment.

Group 4 consisted of 16 patients with LL-BL leprosy, who had been treated with DDS (50 to 100 mg daily) for at least 10 years, and whose skin smears had been negative for at least five years.

Group 5 consisted of those nine patients where frozen serum samples were available from the group of patients classified both clinically and histologically as indeterminate leprosy ('strictly indeterminate leprosy') by Myrvang *et al.* (1973a). The clinical diagnosis was based on clinical examination and bacterial indices from skin smears. A skin biopsy was taken from all patients, and histological classification was done blindly without clinical information. The patients were newly diagnosed when the serum samples were obtained.

Indeterminate leprosy was clinically defined as a condition with one or a few hypopigmented macules, usually with some sensory loss, but with normal peripheral nerves. The histological picture was considered to be indeterminate when there was no granuloma present but one or more of the following features were seen (1) infiltration of lymphocytes and histiocytes around skin appendages, peripheral nerves and vessels with or without proliferation of spindle-shaped cells in the superficial dermis; (2) proliferation of Schwann cells; or (3) acid fast bacilli in nerve, arrector pili muscles or sub-epidermal zone (Ridley, 1971).

The lepromatous serum pool (LSP) is identical with LSP used in previous investigations (Melsom *et al.*, 1981a,b). It consisted of sera from 40 lepromatous (LL-BL) leprosy patients taken either at the time of diagnosis or within 1/2 year after the start of DDS treatment. All the patients attended the Addis Ababa Leprosy Hospital.

Solid phase radioimmunoassay (sRIA) was carried out as described previously (Melsom *et al.*,

1981a,b). In short, Nunc polystyrol test tubes were coated with sonicated *M. leprae* bacilli obtained from Dr R. J. W. Rees, London, through the Immunology of Leprosy (IMMLEP) component of the UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases and prepared by Draper's method from liver of *M. leprae* infected armadillos (Draper, 1976). LSP or patient serum was added to the coated test tubes, which were incubated for 24 hr and washed. Purified ^{125}I labelled rabbit anti-human IgA, IgM or IgG antibodies were added. The tubes were incubated for 24 hr, washed and counted. ^{125}I labelled rabbit anti-human IgA, IgM and IgG preparations were purified and controlled as previously described (Melsom *et al.*, 1981a,b).

In each set of experiments, controls were included where either LSP or coating of the test tubes with sonicated *M. leprae* was omitted. The counts in these controls were less than 1% of the counts obtained with sonicated *M. leprae* coated tubes and LSP diluted 1:10 (Melsom *et al.*, 1981b).

Calculation of the results. The patient's sera were tested at dilutions of 1×10^{-1}, 1×10^{-2} and 1×10^{-3} for the IgA anti-*M. leprae* antibody assay and 1×10^{-2}, 1×10^{-3} and 1×10^{-4} for the IgM and IgG anti-*M. leprae* assays. The LSP was run in parallel at dilutions of 1×10^{-1}, 1×10^{-2}, 1×10^{-3} and 1×10^{-4}, and a standard curve was made on semi-logarithmic paper with the dilution of LSP on the ordinate and the number of counts on the abscissa recording the results obtained with LSP in each set of experiments. IgM anti-*M. leprae* antibody activity was then calculated by the following method: antibody activity in patient's serum corresponding to '100% of LSP' means that the same number of counts was obtained with the patient's serum diluted 1×10^{-3} as in LSP diluted 1×10^{-3}, 10% of LSP gave the same number of counts with the patient's serum diluted 1×10^{-2} as with LSP diluted 1×10^{-3}, and finally 1,000% of LSP means that the same number of counts was obtained with the patient's serum diluted 1×10^{-4} as with LSP diluted 1×10^{-3}. The steepest part of the standard curve (the largest number of counts between two next dilutions of LSP) was usually between 1×10^{-2} and 1×10^{-3} dilution of LSP. The dilution of patient's serum lying on this part of the standard curve was used for calculation of the percentage of IgM anti-*M. leprae* antibody activity. The two other dilutions were used as controls. The IgA and IgG anti-*M. leprae* antibody activity was calculated by a similar method, but the patient's serum was diluted 1×10^{-1}, 1×10^{-2} and 1×10^{-3} for the IgA assay.

Statistical calculations. For calculation of statistical significance of difference between the groups, Wilcoxon's modified ranking test was used (Diem, 1962). The test was used as a one tailed test, since previous experiments indicated that the anti-*M. leprae* antibody increased from non-leprosy controls to BT-BT/TT and finally BL-LL leprosy patients.

RESULTS

Fig. 1 shows the IgA anti-*M. leprae* antibody activity, Fig. 2 the IgM-, and Fig. 3 the IgG anti-*M. leprae* antibody activity. Each point represents one individual, and the activity is expressed as a percentage of the activity in the respective immunoglobulin class in LSP.

The median antibody activity increased from healthy, exposed controls (group 1) to tuberculoid leprosy (group 2) and further, to lepromatous leprosy (group 3) in all immunoglobulin classes. The overlap between controls and tuberculoid leprosy was considerable in the IgA assay and less in the IgG and IgM assays. In tuberculoid and lepromatous leprosy there was a wide variation in antibody content in individual sera from patients with similar clinical classification in all immunoglobulin classes.

The median values of IgA anti-*M. leprae* antibody activity increased from 18% in group 1, to 50% in group 2 and, finally, to 115% of LSP in group 3. The overlap between controls and groups 2 and 3 was considerable. The difference between groups 1 and 2, and 1 and 3 was statistically significant ($P < 0{\cdot}005$). There was no significant difference between groups 2 and 3.

The overlap between groups 2 and 3 and controls was small (three of 17 sera in both group 2 and 3) in the IgM assay. The median value increased from 12% in group 1, to 45% in group 2, and 110% of IgM anti-*M. leprae* antibody activity in LSP in group 3. The difference was statistically significant between all three groups (group 1 to 2 $P < 0{\cdot}005$, and group 2 to 3, $P = 0{\cdot}05$), even though the overlap between groups 2 and 3 was considerable.

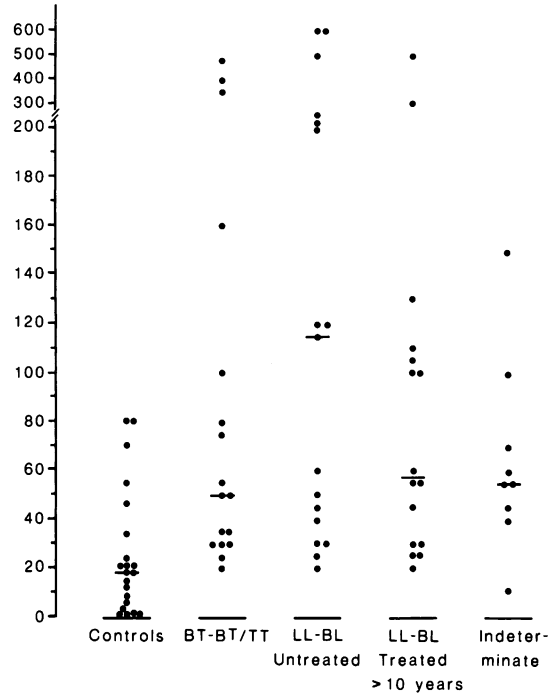

Fig. 1. IgA anti-*M. leprae* antibody activity in controls. BT-BT/TT leprosy patients, untreated LL-BL leprosy patients, LL-BL leprosy patients treated for more than 10 years, and 'strictly indeterminate leprosy' patients. Each point represents one individual, and the activity is expressed as percentage of the IgA anti-*M. leprae* activity in a lepromatous serum pool (LSP). The horizontal bars represent the median values.

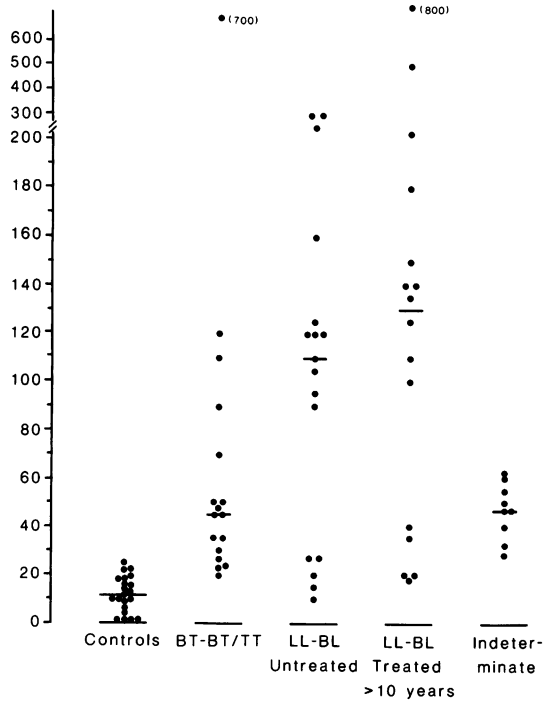

Fig. 2. IgM anti-*M. leprae* antibody activity, otherwise as for Fig. 1.

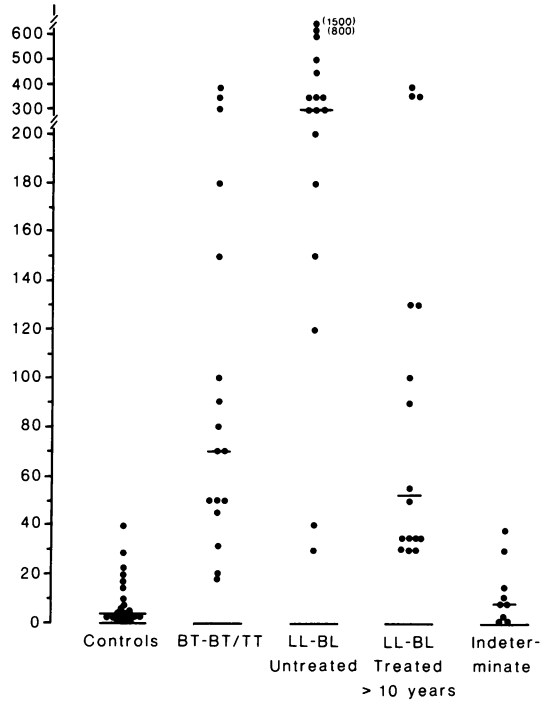

Fig. 3. IgG anti-*M. leprae* antibody activity, otherwise as for Fig. 1.

The overlap between groups 1 and 2, and 1 and 3 was also small in the IgG assay (three of 17 sera in group 2, and two of 17 sera in group 3), and the difference was highly significant statistically between groups 1 and 2 ($P < 0{\cdot}005$). The median concentration increased from 5% in group 1, to 70% in group 2, and finally, 300% of IgG anti-*M. leprae* antibody activity in LSP in group 3. The increase in antibody activity from tuberculoid to lepromatous leprosy was therefore highest in the IgG assay, and the difference between these two groups was statistically highly significant ($P < 0{\cdot}005$).

The effect of treatment on antibody activity also varied between the immunoglobulin classes. In the IgA assay there was a moderate decrease in median anti-*M. leprae* activity from 115 to 55%. In the IgM assay, the median values were similar in the untreated and the treated lepromatous leprosy patients (110 and 130% of LSP). The difference between these two groups was not significant with regard to both IgA and IgM anti-*M. leprae* antibody activity. The IgG anti-*M. leprae* activity showed a marked decrease upon treatment, the median value in untreated lepromatous leprosy being 300% of LSP and, after treatment, 53% of LSP. The difference between these two groups was highly significant ($P < 0{\cdot}005$).

Sera from the group of 'strictly indeterminate leprosy' gave particularly interesting findings, again pointing to the importance of immunoglobulin class specific assay. The findings in the IgG assay were unrewarding, the median values and the scatter being virtually the same in healthy, exposed controls and in patients with indeterminate leprosy. In the IgA assay, the median value was higher in the indeterminate group (55% of LSP) than in the control group (18% of LSP). The scatter resulted in a marked overlap between the two groups, but the difference between them was statistically significant ($P < 0{\cdot}005$). The findings in the initial IgM assay showed an increase in median value from 11% of LSP in healthy contacts, to 50% of LSP in indeterminate leprosy with no overlap between the groups (variation from 1 to 20% of LSP in healthy contacts, and from 25 to 63% of LSP in indeterminate leprosy). The 30 sera in these two groups were tested simultaneously in the

IgM assay a second time, coding the sera so that the origin of the sera was unknown during the assay and calculation of the results. The results were similar, the median values on repeated testing being 12·5 and 48% of LSP respectively. One serum overlapped between the two groups in that three sera in the control group showed slightly higher values than the serum with the lowest activity in the indeterminate group, the variation now being from 0·5 to 30% of LSP in the exposed controls and from 25 to 60% of LSP in the indeterminate group. The values shown in Fig. 2 in the control and indeterminate groups are the mean values of the initial and repeated results of the IgM anti-*M. leprae* antibody assay.

DISCUSSION

The present sRIA is a sensitive assay for the demonstration and quantification of IgA, IgM and IgG anti-*M. leprae* antibodies which are immunoglobulin specific (Melsom *et al.*, 1981a,b), but whose specificity with regard to reactive components in the *M. leprae* sonicate (Harboe *et al.*, 1977a; Closs, Mshara & Harboe, 1979) remains to be determined.

Prior exposure to cross-reacting antigens is an important modulating factor in development of the immune response to antigens under experimental or during subsequent infections (Barbana *et al.*, 1973; Shneerson & Robbins, 1975; Fazekas de St Groth & Webster, 1966). Tests such as the present ones, employing antigens of *M. leprae* that cross-react with corresponding antigens in other species of mycobacteria, may therefore be more sensitive for demonstration of increased antibody activity during subclinical or overt, early infection with leprosy than tests for *M. leprae* specific antibodies. Such assays, based upon cross-reacting *M. leprae* antigens, may also detect antibodies in serum from non-leprosy controls who have been exposed to other mycobacteria and/or *M. leprae*.

The difference between tuberculoid leprosy and non-leprosy controls was greater in the IgM and IgG assays than in the IgA assay. The overlap between controls and patients with tuberculoid leprosy was relatively small both in the IgM and IgG assays (17·5%). Therefore, both assays for IgM and IgG anti-*M. leprae* antibody activity separate leprosy patients from controls and may be used for further development of methods for serological diagnosis of leprosy.

The assay of IgA, IgM and IgG anti-*M. leprae* antibodies in patients with tuberculoid leprosy and lepromatous leprosy confirms and extends our previous findings in other radioimmunoassays of sera from patients with various forms of leprosy (Harboe *et al.*, 1977b; Melsom *et al.*, 1978; Yoder *et al.*, 1979; Melsom *et al.*, 1981b). The median antibody concentration was higher in lepromatous than in tuberculoid leprosy, but with a marked variation between individual patients with similar clinical classification. This applies to antibodies of all three immunoglobulin classes investigated, but the difference between lepromatous and tuberculoid leprosy was greater in the IgG and IgM assays than in the IgA assay. The difference between groups 1, 2 and 3 in the present IgM and IgG assays is also greater than the difference between corresponding groups in a previous investigation with cross-reacting BCG antigen 60 using protein A containing staphylococci to a separate antibody-bound ^{125}I labelled antigen from free antigen (Harboe *et al.*, 1977a).

The reason for the marked variation in antibody content in sera from individuals with similar classification, previously demonstrated in anti-*M. leprae* 7 assays (Melsom *et al.*, 1978; Yoder *et al.*, 1979) and shown presently in IgG, IgA and IgM anti-*M. leprae* in groups 2 and 3, remains unknown. This variation is currently being studied from several points of view (Harboe *et al.*, in preparation). Of particular interest are the patients with either very high or low antibody concentration at diagnosis and the patients with high IgG anti-*M. leprae* antibody concentration after prolonged therapy, as seen in Fig. 3. In both groups 3 and 4 of lepromatous leprosy there were several patients with particularly low IgM anti-*M. leprae* antibody activity, as seen in Fig. 2. Sixty per cent of these patients also showed low IgA and IgM anti-*M. leprae* antibody activity. The significance of this poor *M. leprae* antibody activity among some lepromatous leprosy patients remains unknown.

A decrease in antibody concentration during DDS treatment of lepromatous leprosy patients (Rees *et al.*, 1965; Melsom *et al.*, 1978) and of tuberculoid leprosy patients (Yoder *et al.*, 1979) has been demonstrated previously. The present data confirm these earlier observations. The decrease in

antibody activity was only significant in the IgG anti-*M. leprae* antibody assay, and was more marked than previously demonstrated by Yoder *et al.* (1979) by *M. leprae* antigen 7 antibody activity in identical patients. Therefore, IgG antibodies appear to be the most sensitive indicator of effect of treatment. The lack of decrease in IgM anti-*M. leprae* antibodies after 10 years of treatment of lepromatous leprosy patients might indicate that slow release of cell wall antigens from dead mycobacteria is a sufficient stimulus to maintain production of IgM anti-*M. leprae* antibodies.

The significance of IgA antibodies in leprosy needs to be further studied, particularly in view of the data indicating that mucous membranes may be an important site of entry of leprosy bacilli into the body following exposure by droplet infection (Huang, 1980; Davey, 1978). Local IgA synthesis may represent the initial immune response against *M. leprae* infection and even be the first line of defence against *M. leprae* infection. Such local IgA synthesis, with occurrence of IgA antibodies in blood and external secretions, depends on several factors, among these antigen presentation at mucous membranes. The effect of exposure of mycobacterial antigens from mycobacteria in water and soil remains unknown. Such exposure will probably lead to local and perhaps systemic production of IgA antibodies.

Indeterminate leprosy often presents diagnostic problems, and there is a definite need for improved methods for diagnosis of this condition in individual patients. This would be of importance both to assess the incidence of indeterminate leprosy with increased occurrence, and to obtain better information on how frequently it develops either towards tuberculoid, borderline or lepromatous leprosy. The patients classified, both clinically and histologically, as 'strictly indeterminate leprosy' by Myrvang *et al.* (1973a) appeared to be quite homogenous in their immunological reaction to *M. leprae*. They showed either no response or a very poor response in *in vitro* tests for cellular immune reactions like the lymphocyte transformation test and the leukocyte migration inhibition test, and *in vivo* in the early lepromin reaction. These findings indicate that 'strictly indeterminate leprosy' had not triggered off cellular immune reactivity, or at least hypersensitivity, in these leprosy patients. Precipitating antibodies against disintegrated BCG and *Mycobacterium duvalii* bacilli were not demonstrated, but Myrvang (1975) pointed out that 'the question whether *M. leprae* has stimulated antibody formation in indeterminate leprosy patients should be left open until examined by more sensitive techniques'. We have demonstrated a significant *M. leprae* antibody production of the IgM class in patients with 'strictly indeterminate leprosy', where all but one of the nine patients tested had a higher concentration of IgM antibodies than the control group, as seen in Fig. 2. The median concentration of IgA anti-*M. leprae* antibodies was higher in the indeterminate group compared to the control group; the difference was significant ($P < 0{\cdot}005$) even though the overlap between these two groups was considerable. There was no difference in IgG anti-*M. leprae* antibody activity between these two groups.

Individuals in both the control and indeterminate groups might have been exposed to saprophytic and other pathogenic mycobacteria with cross-reacting antigens to *M. leprae*. The present assays are not based upon *M. leprae* specific antibodies, and the increase of IgM (and IgA) anti-*M. leprae* antibody activity may be due to increased production of IgM (and IgA) antibodies against cross-reacting mycobacterial antigens and/or against *M. leprae* specific antigens. If the assays mostly demonstrate antibodies to cross-reacting mycobacterial antigens, previous exposure to saprophytic and/or other pathogenic mycobacteria may increase the reactivity to *M. leprae* antigens. This reactivity can be different between the two groups and theoretically explain some of the difference in IgM anti-*M. leprae* antibody activity between the two groups. The present assay does not solve this problem. We know, however, that the individuals in the control group have been heavily exposed to *M. leprae* bacilli.

We have previously demonstrated IgM anti-*M. leprae* antibodies in cord sera from almost 50% of babies born of mothers with lepromatous leprosy, and a significantly higher concentration of such antibodies in sera taken between 3 and 6 months from babies of lepromatous leprosy mothers compared to babies of tuberculoid leprosy and non-leprosy mothers. We could only detect IgM anti-*M. leprae* antibodies in one of 15 cord sera from babies of tuberculoid leprosy and non-leprosy mothers.

Therefore, the early production of IgM antibodies and their diagnostic value may not only be a feature of acute infectious disease, but it may also occur regularly in a very slowly developing

infection with an obligate intracellular parasite such as *M. leprae*. This has again been demonstrated in the present investigation by the increased IgM anti-*M. leprae* antibody activity in the indeterminate leprosy patients. The difference in IgM anti-*M. leprae* antibody activity between controls (group 1) and lepromatous and tuberculoid leprosy patients (groups 2 and 3) indicates that assays of IgM antibodies against *M. leprae* can be developed and used as sero-diagnosis for subclinical and early leprosy. On the other hand, the low concentration of IgG anti-*M. leprae* antibody activity in the indeterminate group, and previously demonstrated within the first 2 years of life in babies exposed to and possible infected with leprosy (Melsom *et al.*, 1981a), shows that IgG antibodies are poor indicators for subclinical and early leprosy infection. Simultaneous assays of IgM and IgG anti-*M. leprae* antibodies in serial samples from inoculated armadillos should be performed to establish the sequence in formation of IgG and IgM antibodies during an experimentally controlled development of systemic *M. leprae* infection.

Lately, there has been increased interest in the study of antibody formation in leprosy. Different assays for demonstration and quantification of such antibodies have been studied. Several basic methods, e.g. various radioimmunoassays, fluorescent antibody tests and ELISA techniques, have been used, and the specificity has varied from the demonstration of antibodies to cross-reacting mycobacterial antigens to *M. leprae* specific determinants. These assays have been evaluated in diagnosis of subclinical leprosy, to obtain additional information on humoral immune reactions related to classification and other clinical features, and to study the effect of treatment. This investigation is based upon another aspect, immunoglobulin class specific assays of IgA, IgM and IgG anti-*M. leprae* antibody activity. They need to be pursued further since assays that demonstrate antibodies of the three major immunoglobulin classes simultaneously may conceal information obtainable by assays of antibodies of only one immunoglobulin class, e.g. the demonstration of IgM anti-*M. leprae* antibody activity in indeterminate leprosy (Fig. 2). The class of antibody assayed (IgA, IgM or IgG anti-*M. leprae* antibody activity) should be carefully assessed. One might obtain different results depending upon the type of *M. leprae* antibody assay used. Therefore, this should be carefully considered in relation to the purpose of antibody studies in leprosy. The present findings indicate that antibodies of various immunoglobulin classes may be of different significance in different situations. IgG anti-*M. leprae* antibody activity can be used as an indicator of the effectiveness of anti-leprosy treatment and IgM anti-*M. leprae* antibody activity in the study of indeterminate leprosy and in development of methods for sero-diagnosis of leprosy. The importance of IgA anti-*M. leprae* antibodies needs to be further evaluated.

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