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Demonstration of Antibodies Reacting with Different Determinants on *Mycobacterium leprae* Antigen 7[1]

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Infection with mycobacteria is usually associated with development of cell mediated immune reactions and formation of antibodies against various constituents of the infective organism. Very few data are available to indicate if the same or different components are involved in induction of cellular and humoral immunity and about the importance of interactions between these two types of immune responses, particularly whether formation of antibodies may suppress the development of cell mediated immune reactions that are of main importance for protective immunity.

Our approach to this problem has been to isolate antigenic components of *M. leprae* and other mycobacteria and to test the components for reactivity in assays for cell mediated immune reactions and with serum antibodies in individual patients.

A specific radioimmunoassay has been developed for demonstration and quantification of antibodies against BCG antigen 60 ([14, 16]) and the cross-reacting *M. leprae* antigen 7 ([23]). Antibodies against this component of *M. leprae* occur frequently in leprosy sera ([14, 15, 23, 31]), the concentration being higher in patients with lepromatous than tuberculoid leprosy ([31]). Subsequent experiments with a highly purified preparation of *M. leprae* antigen 7 have shown that this component is also involved in cell mediated immune reactions in leprosy; it is a potent stimulator in the lymphocyte stimulation test ([10]) and induces typical delayed type hypersensitivity skin reactions in many patients with tuberculoid leprosy ([26]). In the present work, the same preparation was labeled with ^{125}I for studies of its reactivity with serum antibodies in the same patients.

M. leprae antigen 7 is of macromolecular nature, being recovered in the void volume after gel filtration on Sephadex G200 columns ([23]). The cross-reacting BCG antigen 60 is precipitated by ammonium sulfate and trichloroacetic acid and contains polysaccharide moieties reacting with concanavalin A ([16]). Pilot experiments based on these

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observations revealed that antibodies reacting with *M. leprae* antigen 7 were partly neutralized by the addition of polysaccharides purified from *M. tuberculosis*.

The purpose of the present investigation was to extend the above mentioned initial observations to study whether antibodies occurring in leprosy patients react with different antigenic determinants on *M. leprae* antigen 7 and, if so, whether differences in antibody specificity are associated with variation in the clinical course of the disease.

MATERIALS AND METHODS

Patient sera. Sera were obtained at the Armauer Hansen Research Institute (AHRI) from patients attending the Addis Ababa Leprosy Hospital. Most of the sera were from patients whose lymphocytes were isolated and studied for reactivity in the lymphocyte stimulation test to the same purified *M. leprae* antigen 7, as described elsewhere [10]. The patients were classified clinically, and in most cases histologically, according to the extended Ripley-Jopling scale [24, 27, 28]. The group termed "lepromatous leprosy" in this paper consisted of patients with LL, LI, and BL leprosy. The group termed "tuberculoid leprosy" consisted of patients with BT, BT/TT, and TT leprosy. The sera were separated from venous blood, stored at −25°C, and transported in the frozen state to Oslo for subsequent testing in radioimmunoassay.

Mycobacteria and antigenic preparations thereof. *M. leprae* were provided by IMMLEP as freeze-dried bacilli (the AB23R preparation), purified from liver tissue of infected armadillos according to protocol 3 of the report of the 3rd IMMLEP Scientific Working Group which is procedure IV of reference 29. Preparation of *M. leprae* sonicates and immunization of rabbits with *M. leprae* sonicates to produce antisera reacting with various components of the bacillus are described elsewhere [15]. *M. leprae* antigen 7 was isolated by ultrasonication of a crude cell wall preparation of *M. leprae* as described elsewhere [10]. The preparation (MLS 21) was tested for purity by crossed immunoelectrophoresis (CIE). The total ultrasonic extract of *M. leprae* gave more than 15 distinct precipitate lines in CIE against anti-*M. leprae* antiserum [8]. The purified antigen 7 gave a single, strong precipitate line with form and position typical of *M. leprae* antigen 7 when tested in CIE with the same rabbit anti-*M. leprae* antiserum [10]. The antigen was labeled with ^{125}I by electrolytic iodination [13, 16]. For testing of this preparation, a mixture consisting of equal parts of unlabeled, total *M. leprae* sonicate and the labeled preparation was placed in the circular antigen well of a CIE plate with anti-*M. leprae* antibody in the top gel. The plate was run, washed, pressed and dried and then exposed to an X-ray film for autoradiography [16, 23]. The film showed intense labeling corresponding to the precipitate line of antigen 7. On prolonged exposure to obtain a more sensitive system for detection of labeled, contaminating components, faint labeling corresponding to two other precipitate lines was seen.

M. bovis (BCG) were obtained as 14 day cultures on Sauton medium from K. Bunch-Christensen, Statens Seruminstitut, Copenhagen, Denmark, and were identical to the vaccine strain distributed by that laboratory. The cross-reacting antigen, BCG antigen 60 [15], was purified from these bacteria as follows: A suspension of BCG bacilli (40 mg wet weight/ml in distilled water) was treated four times in a bacterial press (Yeda Scientific Instruments, Rehovot, Israel) applying a pressure of 100–150 kg/cm^2 to break the cells and remove cytoplasmic antigens. After each pressing, the pellet was ultrasonified for 30 seconds to ensure that the cytoplasmic contents were liberated from the cell wall as completely as possible. After washing, and final centrifugation, the sediment was suspended in distilled water and sonified for 2 × 25 min with the Branson B-12 sonifier at 80W effect (Branson Sonic Power Co., Danbury, Connecticut, U.S.A.). In CIE using anti-BCG antibody this material gave a strong precipitate line corresponding to antigen 60 with a few barely detectable precipitating components as contaminants. This preparation was labeled with ^{125}I by electrolytic iodination, and the labeled preparation checked for purity by CIE and autoradiography as for the labeled *M. leprae* antigen. The exposed films showed strong labeling corresponding to BCG antigen 60.

BCG sonicate for absorption experiments was made in the following way: BCG

bacilli were pelleted by centrifugation at 3000 × g for 30 min, and the pellet weighed and resuspended in 0.9% NaCl at a concentration of 60 mg/ml. The suspension was sonified for 20 minutes in a rosette cooling cell submerged in iced water with the Branson B-12 sonifier at 80W.

Mycobacterial polysaccharides. Crude arabinomannan (AM) and arabinogalactan (AG) were prepared from *M. tuberculosis* by extraction with NaOH followed by fractional precipitation with ethanol following the procedure of Azuma, *et al.* [5] except that the bacteria were given a preliminary extraction with acetone to remove triglycerides. AM was precipitated by 66% (v/v) ethanol but not by 50%, AG by 75% but not by 66%. The composition of the polysaccharides was determined qualitatively by thin layer chromatography of acid hydrolysates and quantitatively by gas liquid chromatography of trimethylsilyl derivatives of monosaccharides in hydrolysates. The analysis summarized in the Table showed that the AG preparation was pure since it contained about equal amounts of arabinose and galactose and very small amounts of mannose and glucose, whereas the AM preparation was slightly contaminated with AG. The total recovery value is lower than in an optimal analysis, probably being due to incomplete removal of water and partial denaturation of sugar moieties during storage after initial preparation of the polysaccharides.

Radioimmunoassay (RIA). The procedure previously described for assay of antibodies against BCG antigen 60 [14, 16] and *M. leprae* antigen 7 [23] was used. The technique is based on separation of antibody-bound labeled antigen 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 [20]. Briefly, each tube contained 100 μl of the appropriate serum dilution and 100 μl of labeled *M. leprae* antigen 7. The mixtures were incubated for 30 minutes at room temperature before addition of 2 ml 0.5% of formalinized staphylococci of the Cowan 1 strain (NCTC 85308). All dilutions of unlabeled "cold" and labeled proteins and of sera were made in immunoassay buffer of the following composition: 0.01 M phosphate buffer pH 7.4 in 0.14 M NaCl with 0.02 M NaN_3 and 0.2% bovine serum albumin (Sigma 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, and in experiments where this is appropriate, expressed as radioactivity bound to staphylococci in percent of maximum binding activity by reference sera containing strong anti-*M. leprae* antigen 7 activity. For this purpose a pool of 4 rabbit anti-*M. leprae* antisera and a pool of 4 lepromatous sera with particularly strong anti-*M. leprae* antigen 7 activity were used.

TABLE. *Composition of mycobacterial polysaccharide preparations.*

	Arabino-galactan		Arabino-mannan	
	μg	Ratio to galac-tose	μg	Ratio to man-nose
Arabinose	314	1.88	119	0.65
Mannose	12	0.06	183	—
Galactose	167	—	43	0.24
Glucose	14	0.08	8	0.04
Recovered	507		353	
Used	850		670	
Recovery[a]	60%		53%	

[a] Recovery was calculated as % of total used (w/w) after drying *in vacuo* overnight over P_2O_5.

Inhibition assays with the RIA technique. For inhibition assay, 100 μl of the selected dilution of patient serum were mixed with 100 μl of the solution to be tested for inhibiting activity. The contents were mixed and left at room temperature for 30 min. Then labeled antigen was added and the procedure continued as described above. Materials tested for inhibition are described in the Results section. In many instances, calculation of a ratio was found suitable for indicating the inhibitory activity of a particular substance. This ratio was calculated as follows:

$$R = \frac{\text{counts/400 sec after addition of inhibitor} - a}{\text{counts/400 sec without inhibitor} - a}$$

where a is "unspecific uptake," i.e., amount of radioactivity recovered in the

bacterial pellet in a control tube containing only labeled antigen and the suspension of staphylococci.

RESULTS

Properties of *M. leprae* antigen 7 and basic design of experiments. The physicochemical characteristics of *M. leprae* antigen 7 and the cross-reacting BCG antigen 60 have not been established in detail. Both components are however macromolecules, appearing in the void volume after gel filtration through Sephadex G200 ([16,23]), and they are precipitated by ammonium sulfate and trichloroacetic acid. The concentration of BCG antigen 60 may be assayed by the Folin-Ciocalteaux test, by the Biuret reaction, and by measuring optical density at 280 nm. The values obtained by these tests, using human serum albumin as a standard, correspond to only about 25% of the dry weight. This indicates that the molecule contains both peptide and other constituents. In pilot experiments with RIA, the reaction between BCG antigen 60 and corresponding rabbit and human antibodies was partially inhibited by polysaccharide preparations from *M. tuberculosis* containing AM and AG. BCG antigen 60 reacts with concanavalin A ([16]). The same is the case with *M. leprae* antigen 7 (Harboe and Closs, unpublished experiments), and these interactions are both inhibited by α-methylmannoside. RIA was used to investigate to what extent AG and AM could inhibit the reaction between antibodies in leprosy sera and labeled *M. leprae* antigen 7. Complete inhibition by AG and AM would indicate that all anti-*M. leprae* 7 antibodies in the sera are directed against AG- and AM-containing determinants, whereas no inhibition would indicate that the anti-*M. leprae* 7 antibodies are directed against other determinants on the antigen 7 molecules. To record the degree of inhibition, the ratio between binding activity after addition of polysaccharide and binding without addition of polysaccharide was calculated as described above. Initial experiments were performed with pools made from sera of patients with lepromatous and tuberculoid leprosy. Inhibition was obtained and was more pronounced in the tuberculoid serum pool than in the lepromatous serum pool, and the ratio decreased upon increasing dilution of the serum pools. It was therefore decided to test individual sera from patients with lepromatous and tuberculoid leprosy. To try to compensate for variation in amount of antibody in individual sera ([14,23,31]), the ratio was recorded in relation to the amount of antibody which was expressed as binding of *M. leprae* antigen 7 in percent of maximal binding by anti-*M. leprae*.

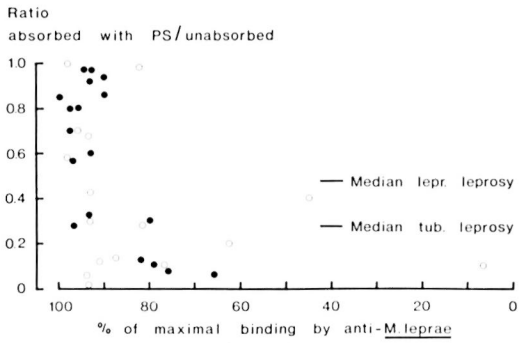

FIG. 1. Inhibition of antibodies against *M. leprae* antigen 7 by polysaccharides. Each point represents one patient, ● lepromatous leprosy, ○ tuberculoid leprosy. A ratio of 1.0 means no inhibition. A ratio of 0 means that binding of labeled *M. leprae* antigen 7 by 100 μl of serum diluted 10^{-3} was completely inhibited by 100 μl of a solution containing 50 μg arabinogalactan (AG) and 50 μg arabinomannan (AM). For further explanation see text.

Figure 1 shows the results of inhibition experiments in which 100 μl of a solution containing 50 μg AG and 50 μg AM (or 100 μl buffer as the control) were mixed with 100 μl of individual sera diluted 10^{-3}.

When sera with the highest antibody activity are compared, e.g., those where sera diluted 10^{-3} showed more than 90% of maximal binding by anti-*M. leprae*, it is evident that there is a marked variation in content of anti-polysaccharide antibodies in individual sera. In some sera the addition of polysaccharide inhibited the binding activity almost completely, indicating that almost all of the antibodies in these sera diluted 10^{-3} were directed against AG-AM determinants of the *M. leprae* antigen 7 molecule. Other sera showed a ratio close to 1.0; almost no inhibition was obtained with polysaccharide which indicates that most of the antibodies in these instances were directed against other determinants on the molecule. The median value of the

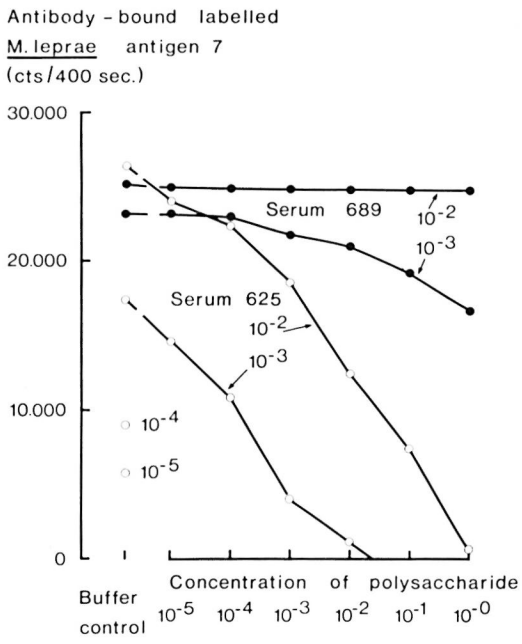

FIG. 2. Inhibition of antibodies against *M. leprae* antigen 7 in two selected sera by different dilutions of a solution containing 5 mg AG and 5 mg AM per ml.

ratio was distinctly higher in sera from patients with lepromatous leprosy (0.7) than in sera from patients with tuberculoid leprosy (0.3), indicating a tendency to more exclusive occurrence of anti-AG/AM antibodies in tuberculoid leprosy.

Figure 2 illustrates the marked difference in behavior between two sera selected on the basis of the experiments illustrated in Fig. 1. In Fig. 1, a single dilution of serum (10^{-3}) was tested with a single dilution of polysaccharide (10^{-1}), whereas in this figure two sera were examined for reactivity at different dilutions of serum and inhibiting polysaccharide. One serum showed a virtually flat curve at a dilution of 10^{-2}, indicating that at this dilution the polysaccharides had almost no inhibiting activity. At 10^{-3}, the curve was slowly declining with slight inhibition at high concentrations of polysaccharide. The other serum had higher binding activity in the control tube when diluted 10^{-2}, but polysaccharide had a marked inhibiting activity and neutralized all anti-*M. leprae* 7 activity in the serum even at this dilution.

To obtain more quantitative information, the data from the experiment illustrated in Fig. 2 were transformed to another form in Fig. 3. The activity is expressed here in "antibody units" remaining at each dilution of serum after addition of increasing amounts of polysaccharide. To the left in Fig. 2, the open circles show that serum 625/78 bound 26,500 counts at dilution 10^{-2}, and 17,400, 9000, and 5800 counts at the three subsequent tenfold dilutions. At dilution 10^{-2}, 26,500 counts represent 100 antibody units, 17,400 10 units, 9000 1 unit, and 5800 0.1 unit. At dilution 10^{-3}, 17,400 represent 100 units, 9000 10 units, etc. The number of antibody units corresponding to the number of counts recovered in the bacterial pellet at different dilutions of serum 625/78 with different amounts of polysaccharide were then read on graphs recording antibody units on the ordinate in log scale and counts on the abscissa in arithmetic scale, and then transferred to a second graph shown in Fig. 3. The same principle was used for serum 689/78, shown in Fig. 3. In Fig. 3 each curve starts at 100 antibody units. If polysaccharide inhibited to the same extent in two dilutions of the same serum, the curves should be superimposed. The curves bend and get lower at each dilution. This means that the antibodies in the next dilution of serum are more easily inhibited by polysaccharide. The curves of (lepromatous) serum 689/78 are strikingly different from those of (tuberculoid) serum 625/78, inhibition by polysaccharide being far more pronounced in the latter case. In serum 625/78 diluted 10^{-2}, 14 antibody units remained after addition of polysaccharide diluted 10^{-3}. The same point in serum 689/78 would correspond to serum diluted about $5 \cdot 10^{-4}$, i.e., serum diluted 500 times more. The titer of anti-*M. leprae* 7 in both sera was about 10^6. Thus, the observations cannot be explained by different amounts of anti-*M. leprae* 7 antibodies in the two sera, but by difference in specificity.

Tests on selected sera are particularly useful to demonstrate differences in antibody specificity in individual sera. In pools of sera rarely occurring antibodies are diluted out, and the most frequently occurring antibodies will dominate the pattern. In experiments on pools made from sera of lepromatous and tuberculoid leprosy patients similar observations were made. The difference between the two was less marked, but again higher dilutions were

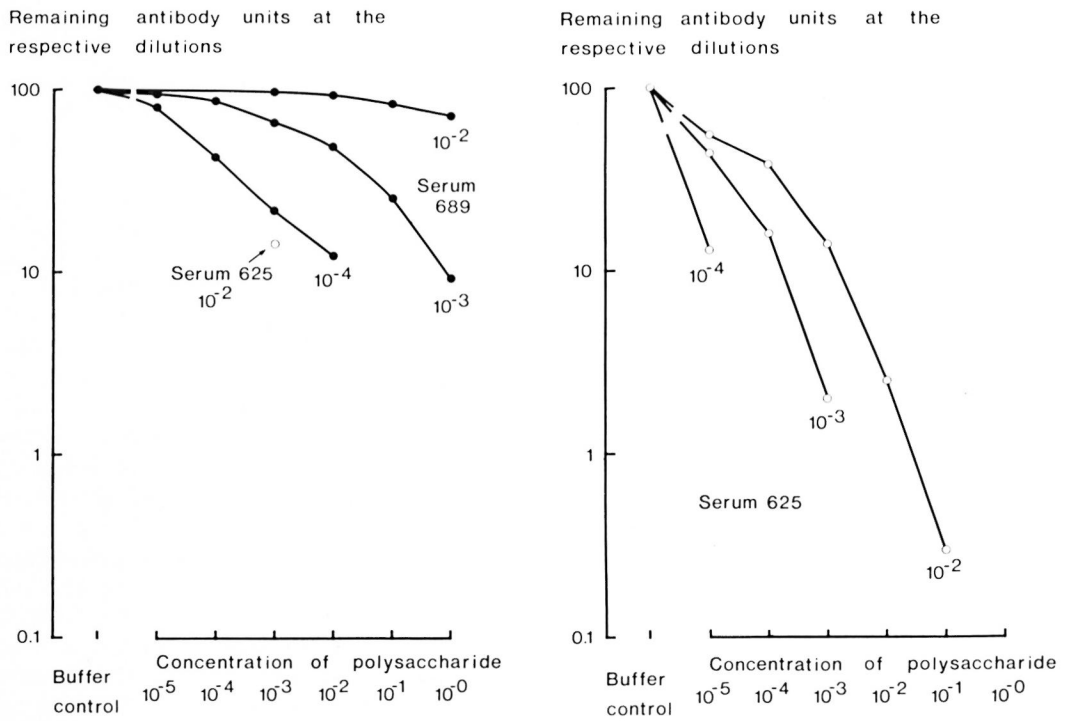

FIG. 3. Data from experiment shown in Fig. 2 expressed as "antibody units" remaining at the indicated dilutions after addition of increasing amounts of polysaccharide. At each dilution the curve starts at 100 units. Steeper curves at increasing dilution of serum means that the antibodies are more easily inhibited by AG and AM at higher dilutions.

more easily inhibited by polysaccharides and the curves of the tuberculoid pool were distinctly steeper than the curves of the lepromatous pool.

Specificity of anti-polysaccharide antibodies. Inhibition experiments were made with AG and AM to obtain additional information on the specificity of anti-polysaccharide antibodies that react with *M. leprae* antigen 7. Initially, four sera were selected and tested in a single dilution (10^{-3}) in inhibition experiments with different concentrations of the two polysaccharides. In three sera, the two polysaccharides had virtually equal inhibiting capacity whereas in one AM had definitely stronger inhibitory activity than AG. Figure 4 shows the findings with two sera to illustrate this difference in specificity. Figure 5 shows the findings with a series of individual sera tested in dilution 10^{-3} with AG or AM diluted 10^{-3}. In 12 of the 14 sera tested, AG and AM had similar inhibiting capacity, the ratio being close to 1.0. In sera with decreasing amounts of anti-*M. leprae* 7 antibodies there was a tendency to decreasing ratio. Note that two sera showed a marked deviation from this general behavior. In one of them (serum 735/78) AM had a markedly higher inhibiting capacity than AG; in the other (serum 725/78) the reverse was the case, demonstrating variation in anti-polysaccharide specificity in individual sera.

Inhibition by BCG sonicate. *M. leprae* antigen 7 cross-reacts extensively with a similar antigen in many other species of mycobacteria ([15, 18]). From its macromolecular nature it would be expected to contain a series of different antigenic determinants on its surface. Direct evidence for this view has been presented above in the demonstration of antibodies reacting with AG-AM and other determinants on the molecule. Some of the determinants cross-react with other mycobacterial species. Other determinants may be relatively or completely specific for *M. leprae*, as has been shown with regard to other compo-

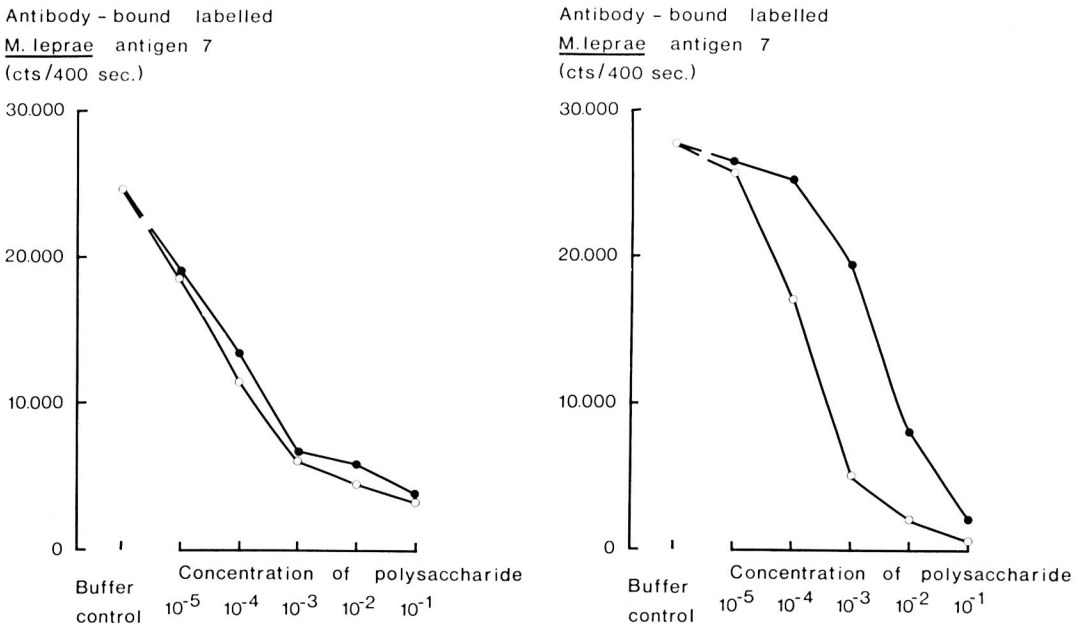

FIG. 4. Inhibition of antibodies against *M. leprae* antigen 7 by different dilutions of a solution containing 5 mg AG (●) or AM (○) per ml. The left part shows serum 684/78 where AG and AM had similar inhibitory activity; the right part, serum 735/78, where AM inhibited the antibodies more effectively than AG.

nents of the leprosy bacillus ([22]). To explore this question, similar inhibition experiments were made using BCG sonicate to inhibit binding of labeled *M. leprae* antigen 7 by antibodies in lepromatous and tuberculoid leprosy sera; 100 μl of each serum diluted 10^{-2} were absorbed with 100 μl of BCG sonicate. The results are shown in Fig. 6. Once again variation in antibody specificity in individual sera was observed; the binding activity of some sera was completely inhibited by addition of BCG sonicate, whereas other sera were inhibited to a much smaller extent. Again, there was some relationship to the clinical features, in that sera from patients with tuberculoid leprosy were more completely inhibited by BCG sonicate than lepromatous leprosy sera. In Fig. 1, the ratio "absorbed with polysaccharide/unabsorbed" is recorded; in Fig. 6 the similar ratio with BCG sonicate. The two ratios were closely correlated ($r = 0.77$, $p < 0.001$). Sera which tend to show specificity for *M. leprae*, i.e., they are not completely absorbed by BCG sonicate, are those where the anti-*M. leprae* 7 antibodies are poorly inhibited by AG and AM.

To investigate if absorption with BCG sonicate may be used to demonstrate a sub-

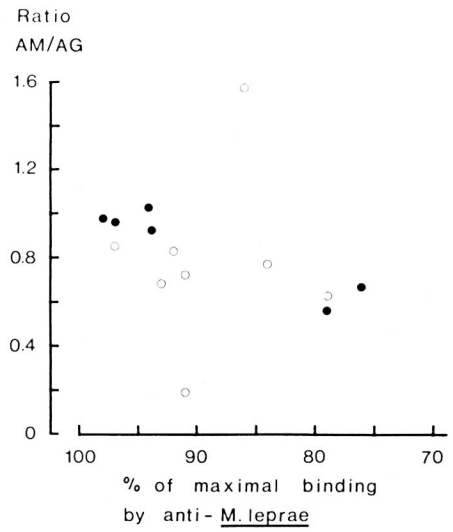

FIG. 5. Comparison of inhibitory activity of AM and AG on anti-*M. leprae* 7 antibodies in 6 lepromatous leprosy sera (●) and 8 tuberculoid leprosy sera (○). In most sera, AM and AG had similar inhibitory capacity, the ratio being close to 1.0. Serum 725/78 showed a high and serum 735/78 a very low ratio, illustrating variation in anti-polysaccharide specificity in individual sera.

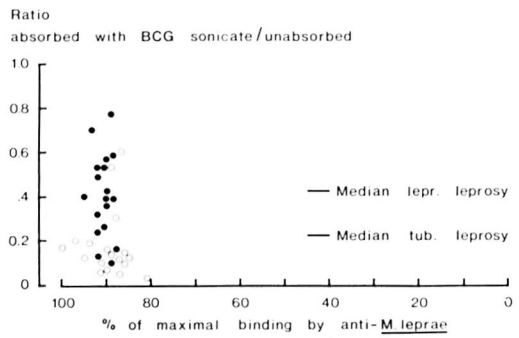

FIG. 6. Inhibition of antibodies against *M. leprae* antigen 7 by BCG sonicate. Recording as in Fig. 1. Variation in inhibitory capacity was observed in individual sera, median inhibition being higher in tuberculoid (○) than in lepromatous leprosy (●).

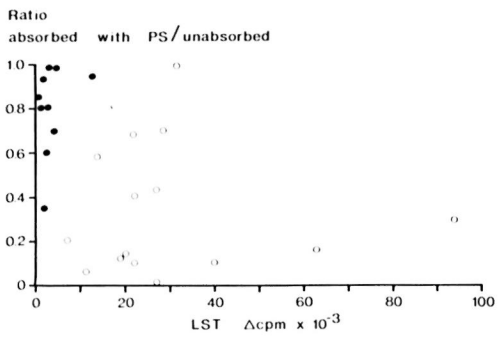

FIG. 7. Correlation plot between antibodies against *M. leprae* antigen 7 and the lymphocyte response to stimulation with the same preparation in 10 patients with lepromatous leprosy (●) and 15 patients with tuberculoid leprosy (○). For further explanation see text.

population of *M. leprae* specific antibodies among those reacting with labeled *M. leprae* antigen 7, absorption experiments were made in a corresponding system using labeled BCG antigen 60 and individual leprosy sera. In the latter system, all antibodies reacting with labeled BCG antigen 60 should by definition be inhibitable by BCG sonicate. The two sera with the highest ratios in Fig. 6 were first selected for absorption. It was found that the amount of BCG sonicate used for absorption in the experiment shown in Fig. 6 was insufficient to absorb the anti-BCG activity completely. When larger amounts were used (200 and 300 μl of sonicate), binding of both BCG antigen 60 and *M. leprae* antigen 7 decreased further, producing virtually parallel curves. RIA with labeled *M. leprae* antigen 7 thus appears to be less promising for demonstration of *M. leprae* specific antibodies than RIA with other labeled preparations of *M. leprae* ([17]) and the fluorescent leprosy antibody absorption test ([1]). Experiments to evaluate to what extent *M. leprae* specific reactivity can be demonstrated in tests for cell mediated immune reactions with the same preparation of *M. leprae* antigen 7 are described elsewhere ([10]).

Relation between antibody formation and cell mediated immune reactions against *M. leprae* antigen 7 in individual patients. The same preparation of *M. leprae* antigen 7 was tested for antibody content in sera by RIA and for reactivity in the lymphocyte stimulation test (LST) in 10 patients with lepromatous leprosy and 15 patients with tuberculoid leprosy. No correlation was found in either of the groups between anti-*M. leprae* 7 antibody content in serum and response in the LST to stimulation with 0.1 μg antigen/ml culture fluid.

Figure 7 shows the data obtained with the ratio "absorbed with polysaccharide/unabsorbed" was plotted against response in the LST. Again, no correlation was found in the two groups. The tuberculoid leprosy group is particularly interesting in the analysis since it contains patients with wide variation in the LST response. The three patients with the highest LST response all had ratios below 0.3.

DISCUSSION

The nature of antigenic determinants on macromolecules has been elucidated particularly through detailed studies of sperm whale myoglobin ([2]) and lysozyme ([3]). In these molecules they constitute a small number of sites on the surface of the molecules with distinct borders. The different sites have different immunological specificity and react with different antibody molecules in antisera produced against the parent molecule. Current evidence indicates that repetitive occurrence of antigenic sites on protein molecules is rare except in proteins consisting of identical subunits and in glycoproteins with many identical immunogenic side chains of a carbohydrate nature.

M. leprae antigen 7 is of particular inter-

est in immunological studies of mycobacterial infections. Antibodies against this component occur frequently in leprosy ([15, 23, 31]); thus it appears to be useful to investigate if the same component of the leprosy bacillus may be involved both in induction of a humoral and a cell mediated immune response. For this purpose, a particular preparation in which only antigen 7 could be demonstrated by crossed immunoelectrophoresis was studied for reactivity with serum antibodies and in various tests for cell mediated immune reactions. Results of the latter studies are reported in detail elsewhere ([10]). The antigen cross-reacts extensively with other mycobacteria. In BCG, the corresponding component is BCG antigen 60 ([15]), and this component corresponds to the major precipitating antigen in purified protein derivative (PPD) of *M. tuberculosis* ([9]).

RIA inhibition experiments with purified polysaccharides would be expected to be sensitive to detect variation in specificity in individual sera and to demonstrate antibodies reacting with polysaccharide- and other determinants on the *M. leprae* antigen 7 molecule. The purity of the materials used for inhibition is essential for evaluation of the results. In Figs. 2 and 3, the slight inhibition of serum 689/78 by high concentrations of the polysaccharide preparation might be explained by contamination with tuberculin-like peptide in very small amounts. Azuma, *et al.* ([5]) found that crude polysaccharides prepared by ethanol precipitation contained protein that could only be removed by extensive chromatography. Their crude polysaccharides gave skin reactions in tuberculin-positive humans, while the purified polysaccharides, which were free from protein, did not. Contamination with small amounts of peptides could not, however, explain the marked inhibition of the antibodies in serum 625/78 and the variation in inhibiting capacity towards individual sera. The polysaccharide solution used for the inhibition experiments contained AM and AG. The observations are easily explained by the occurrence of different antigenic determinants of *M. leprae* antigen 7 of AG-AM and non-AG-AM nature with different relative amounts of antibodies against these types of determinants in individual sera.

In crossed immunoelectrophoresis of concentrated BCG culture fluid against anti-BCG, component 89 forms a very distinct precipitate with characteristic form and position. It has been identified as a polysaccharide antigen ([9]). When AG or AM were incorporated in the intermediate gel, both preparations lifted the BCG 89 line upwards leaving all other lines unaffected. The AG and AM preparations form a precipitate in crossed immunoelectrophoresis with slightly different electrophoretic mobility. When mixed, a single precipitate is formed, very similar to the BCG 89 precipitate. These observations indicate that a major part of the antibodies is directed against arabinose determinants. Further studies on individual sera are needed to delineate variation in fine anti-polysaccharide specificity as indicated by the inhibition experiments with AG and AM in Figs. 4 and 5.

The specificity of the anti-*M. leprae* 7 antibodies that are not inhibited by AG-AM remains to be elucidated. At present we prefer to use the term "other determinants" to indicate that their nature is unknown and to indicate the need for additional structural studies of *M. leprae* antigen 7 to obtain information on this point. The "other determinants" may consist of other polysaccharide residues, e.g., glucose, peptide moieties, and may even be of lipid nature. The structure of the cross-reacting BCG antigen 60 is currently being studied in our laboratory as the first step in this field.

It is widely recognized that the mean concentration of anti-mycobacterial antibodies is higher in lepromatous than in tuberculoid leprosy ([4, 11, 12, 14, 21, 25, 30, 31]). Differences in antibody specificity have obviously been looked for by various investigators, but data showing this have to the best of our knowledge not been presented. Extensive experiments in our laboratory with patient sera and concentrated immunoglobulin preparations in the intermediate gel of crossed immunoelectrophoresis plates have confirmed that the mean antibody concentration is higher in lepromatous leprosy, but they have not provided evidence of different antibody specificity in lepromatous and tuberculoid leprosy (Closs, unpublished observations). The present

data are apparently the first to indicate that there is a difference in the fine specificity of anti-*M. leprae* 7 antibodies in lepromatous and tuberculoid leprosy. This is particularly interesting in view of the possibility that the "other determinants" may be of a peptide nature, and that antibodies against such determinants may interfere with cell mediated immune reactions against *M. leprae* antigen 7.

The relationship between antibody formation and the development of cell mediated immunity is of great interest in leprosy. For a number of years it has been a main view that lepromatous leprosy is associated with the formation of anti-mycobacterial antibodies and lack of cell mediated immune reactivity against *M. leprae,* whereas the reverse is the case in tuberculoid leprosy ([7, 12, 25, 29]). Various observations indicate that this view needs to be reevaluated ([14, 19]): The median anti-*M. leprae* 7 antibody concentration decreases gradually from the lepromatous to the tuberculoid end of the spectrum. But in each group, e.g., in LL and BT leprosy, there is a striking variation in the antibody content of individual sera ([14, 31]). Similar observations have been made in studies of cell mediated immunity. Myrvang, *et al.* ([24]) found that the mean response in the lymphocyte transformation test decreased gradually from the tuberculoid to the lepromatous end of the spectrum, but in the tuberculoid leprosy group there was a marked variation in the lymphocyte transformation test response in patients with a similar clinical classification. This observation has later been confirmed and extended ([6]).

For evaluation of the relationship between humoral and cellular immune responses against *M. leprae,* work on isolated components is required since identical results may be obtained even if different components in an *M. leprae* sonicate act as the stimulating agent in the LST. In the present studies with a purified preparation of *M. leprae* antigen 7, an inverse relationship between cell mediated immune reactivity and antibodies was not found. In the analysis in Fig. 7, antibody reactivity with AG-AM and non-AG-AM determinants was also taken into account; still no apparent correlation. In further investigations, particular attention should probably be given to patients with tuberculoid leprosy who have a strong response to *M. leprae* antigen 7 in the LST. Dissection of the component and analysis of its various constituents should be further pursued to establish if occurrence of antibodies against certain constituents of the molecule are incompatible with the development and/or presence of cell mediated immune reactions against this prominent component of the leprosy bacillus.

SUMMARY

A purified preparation of *M. leprae* antigen 7 was used to investigate the specificity of anti-*M. leprae* 7 antibodies in leprosy sera in a radioimmunoassay. A solution containing arabinogalactan (AG) and arabinomannan (AM) inhibited the antibodies in some sera to a great extent, whereas the antibody activity was virtually unchanged in other sera under the same conditions. These findings indicate that the antibodies are directed against different determinants on *M. leprae* antigen 7. Antibodies against determinants other than AG and AM occurred particularly in lepromatous leprosy sera. In 12 out of 14 sera, AG and AM had similar inhibiting capacity. In one serum, AG had markedly higher inhibiting capacity than AM; in the last serum, the reverse was the case, demonstrating variation in anti-polysaccharide specificity in individual sera.

RESUMEN

Se usó una preparación del antígeno 7 purificada del *M. leprae* para investigar, por radioinmunoensayo, la especificidad de los anticuerpos anti-*M. leprae* 7 presentes en los sueros de pacientes con lepra. Se encontró que una solución conteniendo arabinogalactana (AG) y arabinomanana (AM) inhibió, en forma importante, a los anticuerpos en algunos sueros mientras que la actividad de anticuerpo permaneció intacta en otros sueros trabajados bajo las mismas condiciones. Estos hallazgos, indican que los anticuerpos están dirigidos contra diferentes determinantes del antígeno 7 del *M. leprae*. Otros anticuerpos contra determinantes distintos a AG y AM existieron particularmente en los sueros de pacientes con lepra lepromatosa. En 12 de 14 sueros, la AG y la AM tuvieron una capacidad inhibitoria similar. En un suero, la AG tuvo una capacidad inhibitoria marcadamente mayor que la AM; en un último suero, el caso fue el inverso, demostrando variación en la especificidad anti-polisacárido de los sueros individuales.

RÉSUMÉ

Une préparation purifiée de l'antigène 7 de *M. leprae* a été utilisée pour investiguer la spécificité des anticorps 7 anti-*M. leprae*, dans des échantillons de sérum de malades atteints de lèpre, par le biais d'une technique d'épreuve immunologique. Une solution contenant de l'arabinogalactan (AG) et de l'arabinomannan (AM) a inhibé les anticorps, dans certains échantillons de sérum, de manière très prononcée, alors que l'activité en anticorps était pratiquement inchangée dans d'autres échantillons de sérum, étudiés dans les mêmes conditions. Ces observations montrent que les anticorps sont dirigés contre différents facteurs de l'antigène 7 de *M. leprae*. Des anticorps contre les facteurs autres que AG et AM sont decelés plus particulièrement dans les échantillons de lèpre lépromateuse. Dans 12 des 14 échantillons de sérum, AG et AM présentaient une capacité d'inhibition similaire. Dans un échantillon de sérum, AG a témoigné d'un pouvoir d'inhibition nettement supérieur à celui présenté par AM. Dans le dernier échantillon de sérum, c'est l'inverse que l'on a observé, ce qui démontre une variation dans la spécificité anti-polysaccharidique des échantillons individuels de sérums.

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REFERENCES

1. Abe, M., Yoshino, Y., Saikawa, K. and Saito, T. Fluorescent leprosy antibody absorption (FLA-ABS) test for detecting subclinical infection of *Mycobacterium leprae*. Int. J. Lepr. **48** (1980) 109–119.
2. Atassi, M. Z. Antigenic structure of myoglobin: The complete immunochemical anatomy of a protein and conclusions relating to antigenic structures of proteins. Immunochemistry **12** (1975) 423–438.
3. Atassi, M. Z. and Lee, C. L. The precise and entire antigenic structure of native lysozyme. Biochem. J. **171** (1978) 429–434.
4. Axelsen, N. H., Harboe, M., Closs, O. and Godal, T. BCG antibody profiles in tuberculoid and lepromatous leprosy. Infect. Immun. **9** (1974) 952–958.
5. Azuma, I., Kimura, H., Niinaka, T., Aoki, T. and Yamamura, Y. Chemical and immunological studies on mycobacterial polysaccharides. I. Purification and properties of polysaccharides from human tubercle bacilli. J. Bacteriol. **95** (1968) 263–271.
6. 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.
7. Bullock, W. E. Leprosy: A model of immunological perturbation in chronic infection. J. Infect. Dis. **137** (1978) 341–354.
8. Closs, O., Mshana, R. N. and Harboe, M. Antigenic analysis of *Mycobacterium leprae*. Scand. J. Immunol. **9** (1979) 297–302.
9. Closs, O., Harboe, M., Axelsen, N. H., Bunch-Christensen, K. and Magnusson, M. The antigens of *Mycobacterium bovis*, strain BCG, studied by crossed immunoelectrophoresis: a reference system. Scand. J. Immunol. **12** (1980) 249–263.
10. Closs, O., Reitan, L. J., Negassi, K., Harboe, M. and Belehu, A. *In vitro* stimulation of lymphocytes in leprosy patients and BCG vaccinated subjects not exposed to leprosy. Comparison of a purified antigen prepared from *Mycobacterium leprae* and tuberculin purified protein derivative. Scand. J. Immunol. Submitted for publication.
11. Godal, T. Immunological aspects of leprosy—present status. Prog. Allergy **25** (1978) 211–242.
12. Godal, T., Myrvang, B., Stanford, J. L. and Samuel, D. R. Recent advances in the immunology of leprosy with special reference to new approaches in immunoprophylaxis. Bull. Inst. Pasteur **72** (1974) 273–310.
13. 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.
14. Harboe, M., Closs, O., Bjorvatn, B. and Bjune, G. Antibodies against BCG antigen 60 in mycobacterial infection. Br. Med. J. **2** (1977) 430–433.
15. 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.
16. 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.
17. Harboe, M., Closs, O., Bjune, G., Kronvall, G. and Axelsen, N. H. *Mycobacterium leprae* specific antibodies detected by radioimmunoassay. Scand. J. Immunol. **7** (1978) 111–120.
18. Harboe, M., Mshana, R. N., Closs, O., Kronvall, G. and Axelsen, N. H. Cross-reactions between mycobacteria. II. Crossed immunoelectrophoretic analysis of soluble antigens of BCG and comparison with other mycobacteria. Scand. J. Immunol. **9** (1979) 115–124.

19. HARBOE, M. and CLOSS, O. Immunological aspects of leprosy. In: *Immunology 80,* Progress in Immunology IV. Fougereau, M. and Dausset, J., eds. New York: Academic Press, 1980, pp. 1231–1243.
20. 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 quantitation of α-fetoprotein in normal human adult serum. Eur. J. Immunol. **4** (1974) 29–33.
21. KRONVALL, G., BJUNE, G., STANFORD, J., MENZEL, S. and SAMUEL, D. Mycobacterial antigens in antibody responses of leprosy patients. Int. J. Lepr. **43** (1975) 299–306.
22. KRONVALL, G., STANFORD, J. L. and WALSH, G. P. Studies of mycobacterial antigens, with special reference to *Mycobacterium leprae*. Infect. Immun. **13** (1976) 1132–1138.
23. 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.
24. 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.
25. MYRVANG, B., FEEK, C. M. and GODAL, T. Antimycobacterial antibodies in sera from patients throughout the clinico-pathological disease spectrum. Acta Path. Microbiol. Scand. (Sect. B) **82** (1974) 701–706.
26. REITAN, L. J., TOUW LANGENDIJK, E., CLOSS, O., NEGASSI, K. and BELEHU, A. Comparison of a purified preparation of antigen ML7 with other *Mycobacterium leprae* antigens and PPD: Skin test activity, heat stability and effect on lymphocytes *in vitro*. In preparation.
27. RIDLEY, D. S. and JOPLING, W. H. Classification of leprosy according to immunity. A five-group system. Int. J. Lepr. **34** (1966) 255–273.
28. RIDLEY, D. S. and WATERS, M. F. R. Significance of variations within the lepromatous group. Lepr. Rev. **40** (1969) 143–152.
29. SHEPARD, C. C., DRAPER, P., REES, R. J. W. and LOWE, C. Effect of purification steps on the immunogenicity of *Mycobacterium leprae*. Brit. J. Exp. Path. **61** (1980) 376–379.
30. TURK, J. L. and BRYCESON, A. D. M. Immunological phenomena in leprosy and related diseases. Adv. Immunol. **13** (1971) 209–266.
31. 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.