

Antibodies to Sulfolipids in Human Leprosy Sera¹

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Sufficient evidence has accumulated in recent years confirming the presence of common antigens in *Mycobacterium tuberculosis* and *M. leprae*. Antibodies to different components, in particular to protein antigens of *M. tuberculosis*, have been shown in animals immunized with leprosy bacilli⁽¹⁶⁾ and in patients suffering from lepromatous leprosy⁽¹⁰⁾. Furthermore, cross reactivity between a polysaccharide fraction of *M. tuberculosis* and *M. leprae* has been shown⁽⁸⁾. Among the lipids, antibodies to phosphatides and in particular to mannophosphoinositides of mycobacteria have also been demonstrated in sera of lepromatous patients^(4,12).

Recently, we have demonstrated the presence of antibodies to sulfolipids in human tuberculous sera⁽¹⁵⁾. Therefore, it was of interest to determine the presence of antibodies to sulfolipids in leprosy patients. The results are presented in this communication.

MATERIALS AND METHODS

Large quantities of *M. tuberculosis* H37Rv originally obtained from NCTC London were grown in modified Youman's medium.

Isolation of the antigen. Cells were harvested after four weeks and total sulfolipids were isolated and purified according to the procedure developed in our laboratory by Prabhudesai, *et al.*⁽¹³⁾. The procedure is based on the sequential extraction of lipids from mycobacterial cells with cold methanol, hot methanol, and chloroform-methanol (2:1, v/v). The chloroform-methanol (2:1) fraction was absorbed on silica gel H and neutral lipids were eluted with chloroform.

Cord factor and sulfolipids were separated from this gel by dry column chromatography. The purity of the sulfolipids was checked on micro thin layer chromatography plates and they were found to be free from contamination with other lipids or with compounds containing amino groups.

Preparation of the sera. Blood from patients with leprosy was obtained by the dermatology department of this Institute. The sera were separated and merthiolated at a 1:10,000 level. Sera from normal subjects were processed in a similar manner.

Immunological analysis

Agglutination. All the serum samples were examined for the presence of agglutinating antibodies by the techniques of Takahashi⁽¹⁵⁾. The procedure involves sensitization of a standard kaolin particle suspension with sulfolipid antigen at a concentration of 15 µg lipid/ml, followed by its reaction with antisera. The reaction was interpreted as positive when coarse and solid agglutinates were present, and as negative when the kaolin powder rose in swirls after the tubes were disturbed, in the same way as in the control tube.

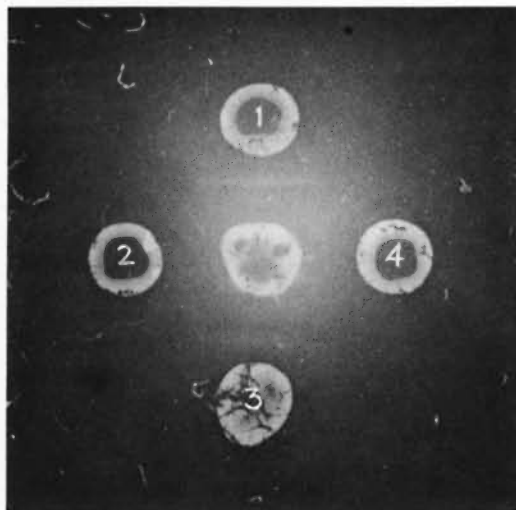
Agar gel diffusion. The precipitation reaction was studied by double diffusion in agar gel by the method of Ouchterlony⁽¹¹⁾ using sulfolipid as antigen.

RESULTS

Sera from 23 lepromatous leprosy patients, 5 tuberculoid leprosy patients, and 20 normal individuals were examined for agglutination and precipitation using the total sulfolipids of *M. tuberculosis* as antigen. Positive antibody responses were observed by both the precipitation and agglutination techniques in 22 of 23 lepromatous leprosy patients. None of the tuberculoid patients or normal subjects showed any reaction with total sulfolipids (The Figure).

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THE FIGURE. Precipitin reaction in agar gel of sera from lepromatous leprosy patients with total sulfolipids of *Mycobacterium tuberculosis*. The central well contains sulfolipid antigen. Wells 1 and 3 contain sera from lepromatous leprosy patients. Wells 2 and 4 contain normal human serum.

DISCUSSION

The occurrence of antimycobacterial antibodies in the low resistant form of leprosy is well documented. Antimycobacterial antibodies have been found in a high proportion of lepromatous patients but, as in the present study, they can be detected in few or no patients with tuberculoid disease (^{7, 10}). Reggiardo and Middlebrook (¹⁴) have isolated serologically active glycolipids from *M. bovis* BCG which were reactive with sera from patients with tuberculosis and leprosy. Recently, Brennan and Barrow (¹), using a partially purified lipid fraction from *M. leprae*, observed distinct lines of precipitation with antisera from lepromatous patients and from an infected armadillo. There was no reaction with sera from patients with tuberculosis or with *M. avium* infection. They suggested that the lipid antigens of *M. leprae* may be species specific and related to the A, B, C, or G mycosides. The presence of mycosides, cord factor, and mannosides in *M. leprae* has been suggested by various authors (^{2, 3, 4}).

Although more than 20 years have passed since Middlebrook, *et al.* (⁹) described an anionic sulfur-containing lipid in a virulent strain of *M. tuberculosis*, the existence of

sulfolipids in *M. leprae* has not yet been demonstrated. In this report the presence of anti-sulfolipid antibodies in lepromatous patients indirectly suggests the presence of sulfolipids in *M. leprae*. This has been documented in *M. tuberculosis* (¹³). Recently, antibodies to sulfolipids-methylated bovine serum albumin complexes have been observed to afford protection against tuberculosis in guinea pigs (⁶). Therefore, sulfolipid antigens could be tried as an antileprosy vaccine, and work in this direction is warranted.

SUMMARY

Antibodies to sulfolipids of *Mycobacterium tuberculosis* H37Rv have been demonstrated in patients with lepromatous leprosy by precipitin reaction in the agar gel and kaolin agglutination techniques.

RESUMEN

Por medio de una reacción de precipitación en gel de agar y por aglutinación, usando kaolin como soporte, se demostró la presencia de anticuerpos que reaccionan con sulfolípidos del *Mycobacterium tuberculosis* H37Rv, en pacientes con lepra lepromatosa.

RÉSUMÉ

Chez des malades atteints de lèpre lépromateuse, on a pu démontrer la présence d'anticorps aux sulfolipides de *Mycobacterium tuberculosis* H37Rv, en ayant recours à des techniques de réaction de précipitation en agar-gel et d'agglutination sur kaolin.

REFERENCES

1. BRENNAN, P. J. and BARROW, W. M. Evidence for species-specific lipid antigens in *Mycobacterium leprae*. *Int. J. Lepr.* **48** (1980) 382-387.
2. DRAPER, P. The walls of *Mycobacterium lepraemurium*; chemistry and ultrastructure. *J. Gen. Microbiol.* **69** (1971) 313-324.
3. GOREN, M. B., BROKL, O. and ROLLER, P. Cord factor (trehalose-6-6'-dimycolate) of in vivo-derived *Mycobacterium lepraemurium*. *Biochim. Biophys. Acta* **574** (1979) 70-78.
4. KHULLER, G. K. and SUBRAHMANYAM, D. Antibodies to mannophosphoinositides in leprosy patients. *Int. J. Lepr.* **38** (1970) 365-368.
5. KHULLER, G. K., MALIK, U. and NALINI, P. Antibodies to mycobacterial sulfolipids in human tuberculous sera. *IRCS Medical Science* **9** (1981) 492.
6. KHULLER, G. K., MALIK, U. and SUBRAHMANYAM, D. Immunobiological properties of sulfolipids of mycobacteria. *Tubercule* **63** (1982) 107-111.
7. KRONVALL, G., BJUME, B., STANFORD, J., MAZEL, S. and SAMUEL, D. Mycobacterial antigens

- in antibody responses of leprosy patients. *Int. J. Lepr.* **43** (1975) 299-306.
8. MALLEN, M. S., CHAVARRIA, E. A. and GUTIÉRREZ, A. E. Estudios de inmunoprecipitación en la lepra. *Rev. Invest. Salud. Publica.* **27** (1967) 3-14.
 9. MIDDLEBROOK, G., COLEMAN, C. M. and SCHAEFER, W. B. Sulfolipid from virulent tubercle bacilli. *Proc. Natl. Acad. Sci. USA* **45** (1959) 1801-1804.
 10. NAVALKAR, R. G., NORLIN, M. and OUCHTERLONY, O. Characterization of leprosy sera with various mycobacterial antigens using double diffusion-in-gel analysis. A preliminary report. *Int. Arch. Allerg.* **25** (1964) 105-113.
 11. OUCHTERLONY, O. Antigen-antibody reactions in gels. *Acta Path. et Microbiol. Scandinav.* **26** (1949) 507-515.
 12. PINTO, M. R. M. and ASSECULERATNE, S. N. An investigation of Takahashi's antitubercle phosphate kaolin agglutination test (KAT) in leprosy. *Int. J. Lepr.* **42** (1974) 48-51.
 13. PRABHUDESAI, A. V., MALIK, U., SUBRAHMANYAM, D. and KHULLER, G. K. Isolation and purification of sulfolipids of *Mycobacterium tuberculosis* H37Rv. *Indian J. Biochem. Biophys.* **18** (1981) 71-73.
 14. REGGIARDO, Z. and MIDDLEBROOK, G. Serologically active glycolipid families from *Mycobacterium bovis* BCG. I. Extraction, purification and immunologic studies. *Am. J. Epidemiol.* **100** (1974) 469-476.
 15. TAKAHASHI, Y. Serum agglutination of kaolin particles sensitized with tubercle phosphatide and its clinical evaluation as a sero-diagnostic test for tuberculosis. *Am. Rev. Respir. Dis.* **85** (1962) 708-719.
 16. TUMA, M. and SILVA, C. Antigenic relationship between the Hansen bacillus and other mycobacteria. *Int. J. Lepr.* **30** (1962) 71-76.