

Antibodies to Mannophosphoinositides in Leprosy Patients¹

G. K. Khuller and D. Subrahmanyam²

Evidence has accumulated in recent years for the presence of common antigens in *Mycobacterium tuberculosis* and *Mycobacterium leprae*. Thus, Pepys *et al.* (⁹), using precipitin tests found three antigens common to the various PPD preparations and lepromin. Tuma and Silva (¹⁶) observed positive precipitins when antigens isolated from *M. tuberculosis* and BCG reacted with sera from rabbits immunized with leprosy bacilli. Navalkar *et al.* (⁶) found humoral antibodies in six serum samples from bacillary positive leprosy patients against several mycobacterial antigens while such antibodies could not be detected in six other sera from bacillary negative patients.

Estrada-Parra and his coworkers (²) isolated a group-specific polysaccharide from tissues infected with *M. leprae* which reacted in agar gel with sera from active tuberculous and lepromatous leprosy patients giving precipitin bands. Salazar-Mallen *et al.* (¹⁰) obtained similar evidence with a polysaccharide present in *M. tuberculosis* and *M. leprae*.

Goihman-Yahr *et al.* (³) observed cross reactivity of lepromin with other mycobacterial antigens by intradermal tests.

Using soluble protein antigens from different mycobacteria, including *M. tuberculosis* and BCG, Ulrich *et al.* (¹⁷) concluded that the frequency of circulating antimycobacterial antibodies was high in persons with lepromatous leprosy.

Recently, extensive studies in several laboratories (^{8, 14, 15}) have established the presence of antibodies in tuberculous patients to the phospholipids of the tubercle

bacilli. These antigens have been identified (¹⁴) as the mannophosphoinositides of the bacilli. Weber *et al.* (¹⁸) reported positive agglutination reactions in 67 per cent of the leprosy patients with Takahashi antigen. This antigen was, however, a crude preparation contaminated with nonphosphatide components of the tubercle bacilli. Takahashi (¹⁵) observed positive agglutination reaction in some tuberculin-positive but not tuberculin-negative leprosy patients with the phosphatide antigen and concluded that this might be due to concomitant tuberculosis infection in the patients but not due to a common phospholipid antigen between the species. An investigation was undertaken to examine whether such antibodies exist in leprosy patients using purified phospholipid antigens and the results are reported in this communication.

MATERIALS AND METHODS

Large quantities of *M. tuberculosis* (H₃₇Rv and H₃₇Ra) originally obtained from NCTC, London, were grown in a modified Youman's medium (¹³). At the end of a six weeks growth period, the bacilli were harvested and the lipids were extracted and purified as detailed elsewhere (^{12, 13}). The mannophosphoinositides were separated from the other phospholipids by preparative thin layer chromatography and purity of the lipids was checked by the established analytic methods followed in a previous investigation (⁴).

Preparation of sera. Blood from leprous inpatients of the Leprosy Home, Delhi, was collected in sterile centrifuge tubes. Tuberculosis infection was ruled out in these patients by clinical and radiologic examination. The serum was separated within two hours after collection of the blood sample and merthiolated at 1:10,000 level and was immediately used in the serologic reac-

¹ Received for publication 1 July 1970.

² G. K. Khuller, M.Sc., Research Assistant, National Institute of Communicable Diseases, Delhi, India; D. Subrahmanyam, M.Sc., Ph.D., Professor of Biochemistry, Postgraduate Institute of Medical Education & Research, Chandigarh, India

tions. Sera from normal persons were processed in a similar manner.

Immunologic analysis. Agglutination. The serum samples were examined for the presence of antibodies by the agglutination technic of Takahashi (15) using purified total mannophosphoinositides as antigen.

Agar gel diffusion. The precipitin reaction was studied by double diffusion in agar gel by the method of Ouchterlony (7). For this purpose the mannosides were homogenized in saline at a concentration of 100 μg lipid-phosphorus/ml. and the fine suspension was used as the antigen. Normal serum and saline controls were included in the plates.

Complement fixation. The method of Levine (5) was followed for the complement-fixation reaction with the mannoside antigens. The antigen used was a saline homogenate of the lipid emulsified at a concentration of 100 μg lipid-P per ml. Controls with the addition of only antigen, or antibody or complement were run simultaneously.

RESULTS

Sera from 14 lepromatous leprosy, five tuberculoid leprosy and 20 normal cases were examined by agglutination, precipitin and complement fixation tests using total mannophosphoinositides as antigen. None of these cases gave positive agglutination reaction. However eight of the lepromatous leprosy cases gave a precipitin band in Ouchterlony gel plates as shown in Figure 1. None of the tuberculoid leprosy cases or normal controls reacted with the mannosides in the agar gel. Further, sera from lepromatous leprosy and tuberculoid leprosy cases did not fix complement significantly in the presence of total mannosides.

DISCUSSION

In general, investigations on the presence of common antigens in *M. tuberculosis* and *M. leprae* were conducted by assessing the serologic cross-reactions of the antisera with the antigens. Direct evidence for chemical identity of the antigens of the two mycobacterial species is at present fraught with difficulty in getting amounts of leprosy bacilli adequate for analysis. In the absence of suitable methods of cultivation of the

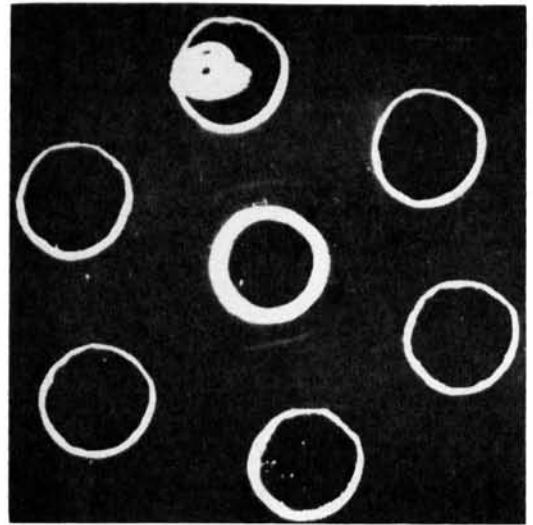


FIG. 1. Precipitin reaction in agar gel of sera from lepromatous leprosy patients with mannophosphoinositides of *Mycobacterium tuberculosis*. Center well has mannophosphoinositide antigen and each of the peripheral wells contains serum from a lepromatous leprosy case.

organisms *in vitro*, bacilli must be obtained from biopsy samples which yield too small an amount for rigorous chemical analysis.

Dharmendra (1) demonstrated the presence of phospholipid in *M. leprae*. The present investigation suggests the presence of mannophosphoinositides in *M. leprae* as in *M. tuberculosis*.

In general, it has been found (17) that the frequency of circulating antimycobacterial antibodies was high in persons with lepromatous leprosy in contrast to low frequency found in sera from patients with tuberculoid and indeterminate leprosy. Skinsnes (11) while analyzing the immunopathologic polarity in leprosy stated that humoral antibodies were low or poorly demonstrable in the tuberculoid-type of leprosy. In lepromatous leprosy the invasion by the leprosy bacilli of the host and abundant macrophage response with efficient phagocytosis seem to result in constant exposure of the antigen to the antibody-forming cells which may elicit humoral antibody response.

The results of the present investigation warrant further work in this direction covering larger number of samples.

SUMMARY

Antibodies to mannophosphoinositides have been demonstrated in serum samples from lepromatous leprosy patients by precipitin reaction in agar gels.

RESUMEN

Utilizando reacciones de precipitación en gel de agar, se han demostrado anticuerpos contra manofosfoinositidos en muestras de suero obtenidas de pacientes con lepra lepromatosa.

RÉSUMÉ

En utilisant la réaction aux précipitines dans des gels d'agar, on a démontré la présence d'anticorps aux mannophosphoinositides dans des échantillons de sérum provenant de malades atteints de lèpre lépromateuse.

REFERENCES

1. DHARMENDRA. The immunologic skin test in leprosy. Part 1. The isolation of a protein antigen of *Mycobacterium leprae*. Indian J. Med. Res. **30** (1942) 1-7.
2. ESTRADA-PARRA, S., CALDERON-MANES, S., SALAZAR-MALLÉN, M. and AMEZCUA, M. E. Isolation of a group-specific polysaccharides from tissue infected with *Mycobacterium leprae*. Internat. J. Leprosy **34** (1966) 294-297.
3. GOHMAN-YAHR, M., RAFFEL, S. and FERRESE, R. Cross reactivity of lepromin with other mycobacterial antigens. Internat. J. Leprosy **36** (1968) 129-143.
4. KHULLER, G. K. and SUBRAHMANYAM, D. On the mannophosphoinositides of *Mycobacterium* 607. *Experientia* **24** (1968) 851-852.
5. LEVINE, L. Microcomplement fixation. In: Handbook of Immunochemistry Ed. D. M. Weir, 1967, p. 707-712.
6. NAVALKAR, R. G. NORLIN, M. and OUCHTERLONY, Ö., Characterisation of leprosy sera with various mycobacterial antigens using double diffusion-in-gel analysis. A preliminary report. Internat. Arch. Allergy **25** (1964) 105-113.
7. OUCHTERLONY, Ö., Antigen-antibody reactions in gels. Ark. Kemi Min. Geol. **B26** (1949) 1.
8. PANGBORN, M. G. and MCKINNEY, J. A. Purification of serologically active phosphoinositides of *Mycobacterium tuberculosis*. J. Lipid Res. **7** (1966) 627-633.
9. PEPYS, J., AUGUSTIN, R. and PATTERSON, A. B. Common antigenic components of mycobacterial extracts. Tubercle (London) **40** (1959) 163-172.
10. SALAZAR-MALLÉN, M., AMEZCHUA CHARARRIA, E. and ESCOBAR GUTIERREZ, A. Estudios de inmunoprecipitación en la lepra. Rev. Invest. Salud. Publ. **27** (1967) 3-14 (Abstract in Internat. J. Leprosy **36** (1968) 368).
11. SKINSNES, O. K., Comparative pathogenesis of mycobacterioses. Ann. New York Acad. Sci. **154** (1968) 19-31.
12. SUBRAHMANYAM, D. Further studies on ethanolamine-containing phosphatides of mycobacteria. Indian J. Biochem. **1** (1964) 34-35.
13. SUBRAHMANYAM, D. Studies in the polyglycerolphosphatide of *Mycobacterium tuberculosis*. Canadian J. Biochem. **42** (1964) 1195-1201.
14. SUBRAHMANYAM, D. and SINGHVI, D. R. Phosphatide antigens of mycobacteria. Proc. Soc. Exper. Biol. & Med. **120** (1965) 102-105.
15. TAKAHASHI, Y. Specific serum agglutination of kaolin particles sensitized with tubercle phosphatide and its clinical evaluation as a serodiagnostic test for tuberculosis. American Rev. Resp. Dis. **85** (1962) 708-719.
16. TUMA, M. and SILVA, C. Antigenic relationship between the Hansen bacillus and other mycobacteria. Internat. J. Leprosy **30** (1962) 71-76.
17. ULRICH, M., PINARDI, M. E. and CONVIT, J. A study of antibody response in leprosy. Internat. J. Leprosy **37** (1969) 22-27.
18. WEBER, D., HAAS, H., ROZANSKY, R. and ZIFRONI, A. Evaluation of the tubercle phosphatide-kaolin agglutination test in tuberculosis. Acta tuberc. Scandinav. **45** (1964) 118-122.