Antibodies to Mannophosphoinositides in Leprosy Patients

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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. (4) 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. lepra 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. lepra.

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 antmycobacterial 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 (11) as the mannophosphoinositides of the bacilli. Weber et al. (15) 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 Younan'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 lepromatous 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-
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 technique 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 mannoses were homogenized in saline at a concentration of 100 \( \mu \text{g} \) 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 mannose antigens. The antigen used was a saline homogenate of the lipid emulsified at a concentration of 100 \( \mu \text{g} \) 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 mannoses in the agar gel. Further, sera from lepromatous leprosy and tuberculoid leprosy cases did not fix complement significantly in the presence of total mannoses.

DISCUSSION

In general, investigations on the presence of common antigens in \( M. \text{tuberculosis} \) and \( M. \text{lepra} \) 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 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 \( M. \text{lepra} \). The present investigation suggests the presence of mannophosphoinositides in \( M. \text{lepra} \) as in \( M. \text{tuberculosis} \).

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 mannofosfoinositidos en muestras de suero obtenidas de pacientes con lepra lepromatosa.

RÉSUMÉ

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

REFERENCES