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Preliminary Study of Cellular Immunity to *Mycobacterium leprae* Protein in Contacts and Leprosy Patients

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The peripheral blood mononuclear cell (PBMC) responses in leprosy show broad variation according to the disease spectrum. There is a good response at the tuberculoid pole characterized by an active response in cell-mediated immunity (CMI). In contrast, cells of patients from the lepromatous pole do not proliferate in the presence of specific antigen.

Mycobacterium leprae presents a complicated structure which has been studied for the last 20 years ([4, 6, 8, 12]), showing interesting results concerning the different components of *M. leprae*. Many protein constituents have been identified by immunoelectrophoretic and polyacrylamide gel electrophoretic techniques. Recently, the technique for converting bands cut from Western blots into antigen-bearing particles and testing them in lymphocyte transformation tests has been reported ([7, 10, 11, 13, 14]).

We have used this technique and have identified different molecular weight fractions and their behavior in the cellular response in family contacts and leprosy patients.

MATERIALS AND METHODS

Patients and contacts. Heparinized blood was obtained from patients with leprosy and their contacts. The disease was classified according to the criteria of Ridley and Jopling ([16]). All patients received treatment with sulfone, rifampin, and clofazimine.

Mycobacterial extracts. Soluble extract of *M. leprae* (MLSE) was prepared by the rupture of bacilli purified from the tissues of experimentally infected armadillos by the Draper protocol ([5]) with eight passes through a French pressure cell followed by centrifugation at 49,000 × *g* × 1 hr at 4°C, to eliminate bacillary debris, and filtration through a Millipore membrane (pore size 0.45 μm) ([3]).

MLSE fractions. Soluble extract (50 μg protein/lane) was electrophoresed on 10% polyacrylamide gels containing sodium dodecylsulfate (SDS), using a discontinuous SDS buffer system ([9]). The samples were diluted in a sample buffer, pH 6.8, containing 62 mM Tris-HCL, 2% SDS, 50 mM 2-mercaptoethanol and 10% glycerol, and boiled for 3 min before application on the gels. The gels were run at 25 mA (constant current) in the stacking gel and 35 mA in the separating gel until the bromophenol blue tracking dye reached the bottom of the gel. The gel was stained with 0.25% Coomassie brilliant blue. High molecular weight standard mixture SDS-6H (Sigma Chemical Co., St. Louis, Missouri, U.S.A.) was used to determine the molecular weights (MW) of the protein bands.

The proteins in unstained preparative gels (800 μg protein/11 cm) were electrophoretically transferred to nitrocellulose sheets (Sigma) 0.45 μm in a Trans-blot cell (Bio-Rad Laboratories, Richmond, California, U.S.A.) using a buffer pH 8.3 containing 25 mM Tris base, 192 mM glycine, and 20% methanol at a constant current of 100 mA ([17]) for 16 hr at room temperature. The nitrocellulose membranes were cut into 18 horizontal sections, selecting the strongest bands in stained vertical strips, corresponding to 120-kDa to 14-kDa MW fractions.

The strips were then solubilized as described previously ([1]) by incubation and intermittent mixing for 1 hr with 500 μl

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