Effect of *Mycobacterium leprae* on Peripheral Nerve Protein Phosphorylation; a Preliminary Study

TO THE EDITOR:

Protein phosphorylation plays an important role in the regulation of cellular metabolism ([1]). An important question that needs to be answered in leprosy is how *Mycobacterium leprae* interacts with the host cell signal transduction pathways ([5]). We report here the *in vitro* effect of *M. leprae* on rat and human peripheral nerve phosphorylation.

Tris hydroxymethylaminomethane (Tris), phenyl methyl sulfonyl fluoride, benzamidine hydrochloride, Triton X-100, beta mercaptoethanol and trypsin were obtained from Sigma Chemical Company, St. Louis, Missouri, U.S.A. Other reagents used in the study were of analytical grade. Gamma ^{32}P ATP was obtained from Bhabha Atomic Research Centre, Bombay, India. Normal human peripheral nerves were obtained from amputated limbs, the conditions being osteogenic carcinoma or soft tissue sarcomas. Tibial nerves were traced and collected immediately after surgery, and they were immediately frozen at –20°C until their use (usually within a month). Rats were sacrificed by cervical dislocation and the peripheral nerves (sciatic, tibial and sural) were dissected and processed immediately at 4°C. The human and rat nerve samples were cleared of the connective tissue and minced. The minced tissue was homogenized in a glass teflon homogenizer in 10 volumes of Tris-HCl buffer, pH 7.6 (0.02 M) containing phenyl methyl sulfonyl fluoride (0.002 M), benzamidine hydrochloride (1 mg/ml) and 0.1% (v/v) Triton X-100 (10 ml/g wet tissue). The homogenate was centrifuged at 1000 × *g* for 20 min. The supernatant was used for the study of protein phosphorylation. Protein was estimated according to Lowry, *et al.* ([4]).

***M. leprae* isolation.** *M. leprae* were isolated from skin biopsies of leprosy patients and mouse foot pads infected with *M. leprae*. The tissues were minced with scissors and homogenized in a glass homogenizer in sterile 0.05 M phosphate buffer (pH 7.0). After coarse tissue debris was removed by low speed centrifugation (100 × *g* for 3 min), sterile 0.5% w/v trypsin solution was added to the bacillary suspension at a final concentration of 0.05%. This suspension was incubated at 37°C for 45 min and then centrifuged (1500 × *g* for 15 min). The sediment was suspended in 0.05 M phosphate buffer (pH 7.0) and treated with NaOH at a final concentration of 0.25 N for 10 min at 37°C. The bacilli suspension was then neutralized with 0.1 N HCl and centrifuged. The pellet bacilli were suspended in 0.01 M Tris-HCl buffer (pH 7.2) containing 0.15 M NaCl, 0.001 M $MgCl_2$ and 0.1% v/v Triton X-100. The suspension was again centrifuged and the bacillary pellet was finally suspended in the buffer used for nerve tissue homogenization. After purification the bacilli were acid fast and *o*-diphenol oxidase positive (World Health Organization. Laboratory techniques for leprosy and Appendix 2. Chapter 7. Identification of *M. leprae*. Geneva: World Health Organization, 1987, pp. 107–136).

Protein phosphorylation. Unless otherwise indicated, the reaction mixture for protein phosphorylation consisted of 100 μg protein of the nerve homogenate 1000 × *g* supernatant, 0.02 M Tris-HCl buffer, pH 7.5, 0.02 M $MgCl_2$ and 1.3 nM gamma ^{32}P ATP (2×10^6 DPM) in a total volume of 150 μl. The reaction was initiated by the addition of ^{32}P ATP and incubation was carried out at 37°C for 5 min. The reaction was terminated by the addition of sodium dodecyl sulfate (SDS)-dissociation buffer, heated at 100°C for 3 min and subjected to SDS-gel electrophoresis on 10% or 12.5% gels according to Laemmli ([3]). The stained gel was destained, dried on a gel dryer, and kept in contact with X-ray film at –70°C for 7 to 10 days for autoradiography ([6]). Molecular weight markers used were myosin (205 kDa), beta-galactosidase (116 kDa), phosphorylase B (97 kDa), bovine serum albumin (66 kDa), egg albumin (45 kDa) and carbonic anhydrase (29 kDa).

To investigate the effect of *M. leprae* on

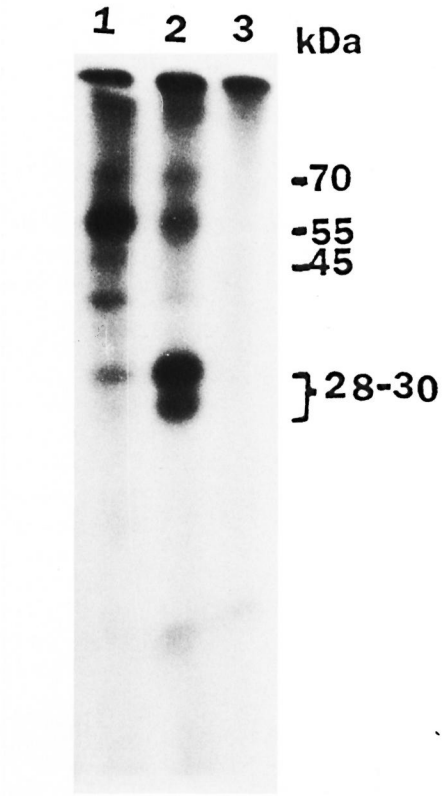

FIG. 1. Autoradiogram showing effect of *M. leprae* on phosphorylation of rat peripheral nerve proteins. Assay was carried out as described in text and SDS-gel electrophoresis was done on 12.5% gel. Lane 1 = reaction mixture for phosphorylation with added *M. leprae*; lane 2 = reaction mixture; lane 3 = *M. leprae* alone without peripheral nerve extract in reaction mixture.

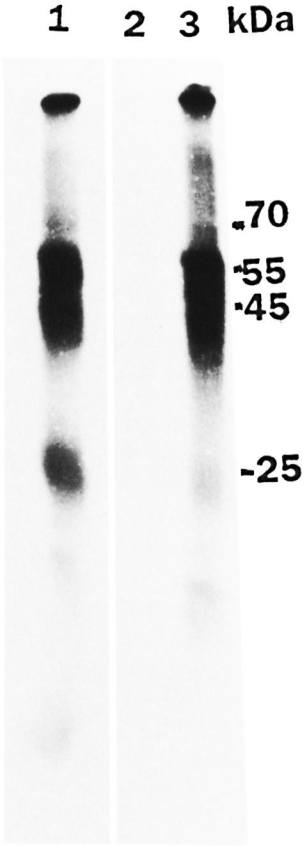

FIG. 2. Autoradiogram showing effect of *M. leprae* on human peripheral nerve protein phosphorylation. Assay was carried out as described in text and SDS-gel electrophoresis was done on 10% gel. Lane 1 = reaction mixture for phosphorylation; lane 2 = *M. leprae* alone without peripheral nerve extract in reaction mixture; lane 3 = reaction mixture with added *M. leprae*.

peripheral nerve phosphorylation, 50 μl containing 10^5 bacilli were added to the reaction mixture prior to the initiation of the reaction by gamma ^{32}P-labeled ATP.

Autoradiograms of the reaction mixture in which rat peripheral nerve extract was incubated with gamma ^{32}P ATP under normal assay conditions showed various phosphorylated bands (Fig. 1, lane 2). The major phosphorylated band was at 28–30 kDa. When *M. leprae* were added to the phosphorylation mixture, there was a significant decrease in phosphorylation of 28–30 kDa (Fig. 1, lane 1). *M. leprae* alone in the reaction mixture showed no phosphorylated bands (Fig. 1, lane 3). Essentially similar results were obtained for human peripheral nerve. A protein band at 25 kDa that was phosphorylated in the human peripheral nerve (Fig. 2, lane 1) was significantly de-

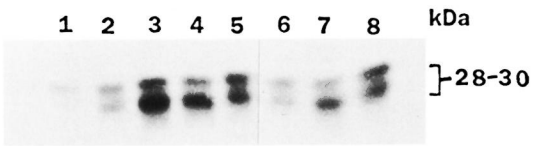

FIG. 3. Autoradiogram showing phosphorylation of 28–30 kDa protein band following incubation of gamma ^{32}P ATP with rat peripheral nerve (different ages) 1000 × *g* supernatant. Assay and SDS-gel electrophoresis on 12.5% gel were conducted as described in text. Lanes 1 to 5 had reaction mixture for phosphorylation and lanes 6 to 8 had added *M. leprae* to the reaction mixture. The age of rat used was 1 week (lane 1), 2 weeks (lane 2), 4 weeks (lanes 3 and 6), 10 weeks (lanes 4 and 7) and 30 weeks (lanes 5 and 8). An equal amount of protein (100 μg) was used in all reaction mixtures.

creased in the presence of added *M. leprae* (Fig. 2, lane 3).

Phosphorylation using peripheral nerves from rats of different ages (from 1 to 30 weeks) showed the presence of the ^{32}P-phosphorylated 28–30 kDa protein, the maximal phosphorylation being in rats at age 4 weeks and above (Fig. 3, lanes 1 to 5). with added *M. leprae,* phosphorylation of the 28–30 kDa protein was significantly reduced in all of the age groups at or above 4 weeks (Fig. 3, lanes 6 to 8).

Phosphorylation of rat peripheral nerve 28–30 kDa protein has been well characterized ([2, 7, 8]). Studies in our laboratory have identified the 25-kDa protein of human peripheral nerve and the 28–30 kDa protein of rat peripheral nerve to be glycoproteins which could be phosphorylated (unpublished data). The molecular weights of these proteins appeared similar to the Po protein ([2, 7]). In the present study, the addition of *M. leprae* caused a decrease in phosphorylation of the 28–30-kDa and 25-kDa proteins in rat and human peripheral nerves, respectively. This could be due to any of the following reasons: a) *M. leprae* alters the protein kinases; b) *M. leprae* alters protein phosphatase; c) *M. leprae* could interact or bind to specific nerve proteins blocking substrate sites. This *in vitro* observation might have possible implications in *M. leprae*-host interactions. The significance of this finding is currently being investigated.

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