Isolation of Phenolic Glycolipid I from Human Lepromatous Nodules

TO THE EDITOR:

Skin biopsy material, amounting to a total weight of 20 g, was obtained from highly bacteriologically positive lepromatous (polar lepromatous) leprosy patients at Acworth Leprosy Hospital, Bombay, India. The bacillary load in the tissues ranged from 5×10^8 to 1×10^9 acid-fast bacilli/g wet weight. The tissue was chopped into small pieces, incubated with 1% trypsin (Sigma Chemical Co., St. Louis, Missouri) for 30 min at 37°C, and extracted with $CHCl_3/CH_3OH/H_2O$ (8:4:3) at 50°C for 18 hr. The biphasic mixture was resolved by centrifugation, and the bottom phase dried. The lipids therein (1.2 g) were suspended in a minimum volume of chloroform and applied to a Florisil (100–200 mesh; Floridin Company, Pittsburgh, Pennsylvania) column (2.1 × 13 cm) and eluted first with 2 bed volumes (48 ml) each of chloroform [which removed 542 mg (45%) of the lipid], 1% methanol in chloroform [26.6 mg (2.2%)], 2% methanol [53.2 mg (4.4%)], 5% methanol [21.8 mg (1.8%)], 10% methanol [11.8 mg (1%)], 50% methanol [42.6 mg (3.6%)], and absolute methanol [57.2 mg (4.8%)], amounting to only about 63% recovery of total lipids but near full recovery of glycolipid. Eluates were monitored for the presence of phenolic glycolipid I (PGL-I) by chromatography in chloroform-methanol (95:5) and in comparison with the authentic armadillo-derived material.

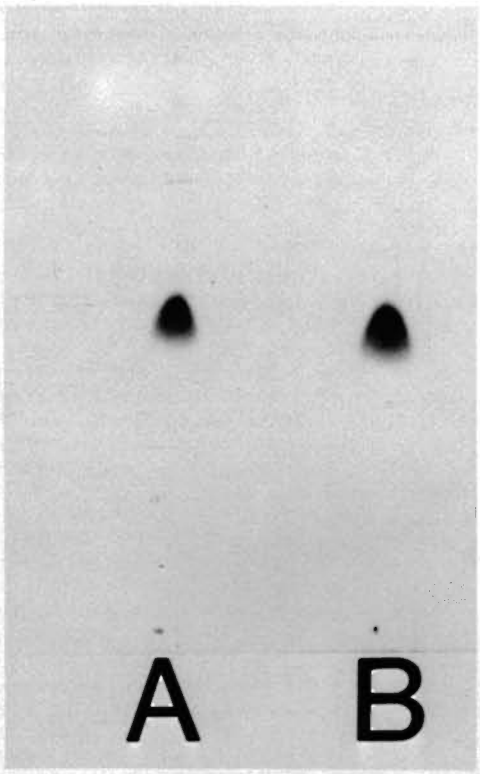

FIG. 1. TLC of pure PGL-I from human nodules (A) against PGL-I from infected armadillo liver (B). Solvent, $CHCl_3$-CH_3OH-H_2O (45:5:0.5); spray, 10% H_2SO_4, 110°C, 5 min.

The majority of the partially purified PGL-I was present in the 1% methanol in chloroform eluate with trace quantities in the 2% methanol eluate. Final purification was achieved by preparative thin-layer chromatography (TLC) (silica gel G, Redi Plates; Fisher Scientific Co., Pittsburgh) in $CHCl_3$-CH_3OH (95:5). This step served to remove a persistent, unidentified substance which comprised about 40% of the glycolipid preparation prior to preparative TLC. Analytical TLC in the same solvent or $CHCl_3$-CH_3OH-H_2O) (45:5:0.5) (Fig. 1) showed a highly purified product, R_f 0.45, with a yellow color in response to 10% H_2SO_4, a pink-mauve color with an α-naphthol-H_2SO_4 spray ([3]), and identical in these respects to PGL-I from infected armadillo organs. Exactly 8 mg of glycolipid was recovered from 20 g of nodular material representing 0.04% of its wet weight and 0.6% of the total nodular lipid, dry weight.

The infrared spectrum of the human-derived glycolipid was as expected, with minor absorption bands at 1500 cm^{-1} and 1600 cm^{-1}, due to the aromatic nucleus, major hydroxyl group absorption at 3600 cm^{-1}, and ester group absorption at 1730 cm^{-1}. Likewise, the ultraviolet absorption spectrum was as expected with two broad and strong peaks at 228 nm and 274 nm due to the phenyl substituent.

The ^{1}H-NMR spectrum showed greater

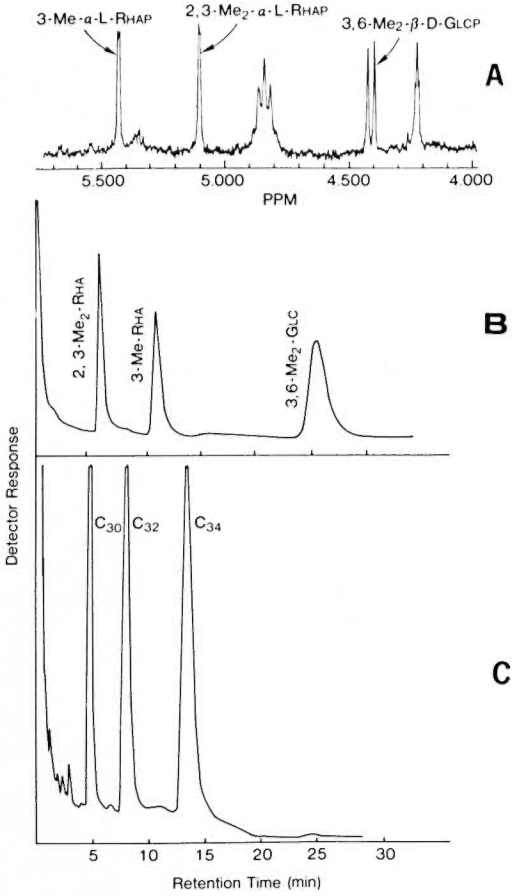

FIG. 2. (A) ^{1}H-NMR of the pure PGL-I from human leprosy nodules. NMR was conducted on an NT-360 spectrometer at a concentration of 6 mg/ml $CDCl_3$. (B) GLC of the alditol acetates derived from human PGL-I. GLC conditions: 1.8 m column of 3% SP-2340 on 100/120 Supelcoport, 180°C, isothermal. (C) GLC of the acyl methyl esters from PGL-I of the human source. Saponification conditions, 10% NaOH in benzene-methanol (1:2), 100°C for 18 hr. Fatty acids were methylated with diazomethane. GLC conditions: 1.8 m column of 3% OV-1 on 80/100 Supelcoport, temperature gradient 240–300°C at 4°C/min.

Me = *O*-methyl; Rhap = rhamnopyranose; Glcp = glucopyranose; C_{30} = 2,4,6,8-tetramethylhexacosanoate; C_{32} = 2,4,6,8-tetramethyloctacosanoate; C_{34} = 2,4,6,8-tetramethyltriacontanoate.

resolution than that obtained for the armadillo-derived product (2), but with the same qualitative detail. There were the two doublets at 6.90 ppm and 7.14 ppm of the phenyl nucleus, and the three anomeric signals at 4.42, 5.10, and 5.45 ppm (Fig. 2A) attributable to the H_1 of 3,6-di-*O*-Me-β-D-glucopyranoside, 2,3-di-*O*-methyl-α-L-rhamnopyranoside, and 3-*O*-methyl-α-L-rhamnopyranoside, respectively (2).

The sugar residues of the glycolipid were examined by gas chromatography (GC) of the alditol acetates as described (2) (Fig. 2B). These were identical in chromatographic properties and in the mass fragmentation pattern to the derivatives of the three sugars from the armadillo-derived glycolipid, and thus were recognized as 3,6-di-*O*-methyl-glucose, 2,3-di-*O*-methyl-rhamnose, and 3-*O*-methyl-rhamnose (2). Likewise, the fatty acids of the diacylated phenolic phthiocerol were recognized as C_{30}, C_{32}, C_{36} mycocerosic acids (Fig. 2C), identical in retention time and mass fragmentation pattern to the products from the armadillo-derived glycolipid (2). Thus, in all aspects of its structure, PGL-I from human lepromatous leprosy tissue is identical to the product from armadillo-derived tissue.

Further proof of structural concordance was provided by immunological means. In ELISA (1) at 100 ng/ml, the pure human-derived PGL-I reacted vigorously with serum from a lepromatous leprosy patient (A_{488} 0.516). Likewise, it reacted readily with a monoclonal antibody (A_{488} 0.617) directed against the terminal sugar epitope (4).

Thus, the issue of the degree of correspondence between PGL-I from human and infected-armadillo sources can be put to rest—they are chemically and immunologically identical, and a procedure for its isolation from human leprosy nodules is now described.

—Nalini Vemuri, Ph.D.
—Lakshmi Khandke, Ph.D.
—P. R. Mahadevan, Ph.D.

The Foundation for Medical Research
Bombay 400018, India

—Shirley W. Hunter, Ph.D.
—Patrick J. Brennan, Ph.D.

Department of Microbiology
Colorado State University
Fort Collins, Colorado 80523, U.S.A.

Correspondence to Dr. N. Vemuri; current address: Department of Microbiology, Colorado State University, Fort Collins, Colorado 80523, U.S.A.

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