Serological Activity of Purified Glycolipid from *Mycobacterium leprae*

To the Editor:

A readily available and chemically characterized leprosy-specific antigen would be a valuable diagnostic and taxonomic tool. Brennan and Barrow [1] found serological activity in lipid fractions from *Mycobacterium leprae*. Hunter and Brennan [3] subsequently identified a major component of the active fractions as a glycolipid resembling mycoside A from *Mycobacterium kansasii* but possessing an unique trisaccharide. Unfortunately the pure lipid had no serological activity, as measured by immunodiffusion experiments using sonicated suspensions of glycolipid.

To show immunological activity in lipids is technically difficult since they are effectively insoluble in aqueous systems. Sonicated suspensions consist of aggregates of lipid molecules, not necessarily having their antigenic determinants exposed (though hydrophilic groups would be expected to be outermost in lipids sonicated in aqueous media). In the case of the unique glycolipid derived from *M. leprae*, the antigenic determinant is presumably the carbohydrate moiety.

We have incorporated purified glycolipid from *M. leprae* into liposomes following the technique of Six, *et al.* [4] and have shown in a limited series of experiments that these liposomes are aggregated by, and form precipitates in agarose gels with, sera from leprosy patients.

Liposomes were prepared with sphingomyelin-cholesterol-dicetyl phosphate-glycolipid (2:1.5:0.2:0.1 in molar ratios) and swollen in Tris-buffered saline (0.15 M NaCl, 20 mM Tris adjusted to pH 8 with HCl). They were sonicated to produce vesicles small enough to diffuse in agarose. Agarose was 0.8% w/v in Tris-buffered saline. Control liposomes were prepared in the same way but without glycolipid. Two glycolipid preparations were used, one of about 95% purity and the other without detectable contaminants (assessed by thin-layer chromatography).

Both were obtained from supernatants of homogenates of livers and spleens of experimentally infected nine-banded armadillos [6] and were purified by column- and thin-layer chromatography. The glycolipid had identical physical and chemical properties to the material described by Hunter and Brennan [3].

Control liposomes produced no precipitation lines with any of the sera used. Liposomes with glycolipid produced precipitates with undiluted sera from three patients with active lepromatous (LL) leprosy, a pool of serum from several lepromatous patients, and serum from an experimentally infected armadillo. No precipitates were formed with 2 sera from patients with tuberculoid leprosy (BT and TT), sera from 2 mice heavily infected with *M. lepraemurium* or with sera from 2 patients with active pulmonary tuberculosis. The LL serum showing the strongest precipitation line produced a visible line when diluted 1:8 with Tris-buffered saline, with an amount of liposomes containing 9 μg of pure glycolipid. Nearly pure and pure glycolipid produced precipitates of equal intensity.

In a single experiment one serum from a

patient with lepromatous leprosy aggregated liposomes containing 9 μg of glycolipid at dilutions of 1:10, 1:20 and 1:40 but another such serum failed to aggregate the liposomes. Neither of two sera from *M. lepraemurium*-infected mice aggregated the liposomes.

Liposomes are simple models of biological membrane vesicles, consisting of a double layer of lipid whose hydrophilic ends are outermost in aqueous media. They have been used as carriers for immunological studies on natural ([2]) and synthetic ([5]) glycolipids. It seems that in the case of the leprosy-specific glycolipid they also allow expression of the antigenic determinant in an aqueous system. The leprosy-specific glycolipid is an abundant product of the bacteria ([3, 7]). Our demonstration that it is serologically active even when highly purified suggests many possible experimental uses for this material.

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