Lipid Composition of Human Leprous Tissue

TO THE EDITOR:

Mycobacteria contain a high amount of lipid, mostly phospholipids and glycolipids ([1]). Analysis of skin biopsies of human and armadillo leprous tissue showed the presence of high lipid content and also certain characteristic lipids of mycobacteria, such as mycolic acids, a 6-deoxyhexose containing lipid (glycolipid-1) and a wax ester ([6, 8]). This has led to speculation that mycobacteria may greatly contribute to the lipid and its metabolism in human lepromas. However, not many studies are available which have studied detailed lipid constituents in leprous tissue of patients in the whole spectrum of leprosy. Therefore, in the present study lipid constituents in human leprous tissue in the leprosy spectrum have been investigated, and an attempt has been made to explain the origin of the stored lipids in lepra cells.

Skin biopsies from active lesions were taken from 33 patients ranging in age from 25–45 years. The duration of the disease was more than 2 years in all cases. All were untreated patients taken from the leprosy clinic of the Postgraduate Institute of Medical Education and Research, Chandigarh, India. These patients had no known systemic disease, including diabetes mellitus, and hypertension and had normal electrocardiograms. All had the same degree of subcutaneous fat, exhibited the same level of nutrition, and were matched for age and sex for all groups. Of the 33 patients classified according to Ridley and Jopling ([5]), 11 were lepromatous (LL), 9 borderline lepromatous (BL), and 13 had tuberculoid (TT) leprosy. Eleven healthy age- and sex-matched controls were included in the study. The bacterial index (BI) of the LL and BL patients ranged from 2+ to 6+ and in TT disease no bacilli could be detected in slit-skin smears. None of the patients was in reaction.

Lipids from the biopsy material were extracted with chloroform : methanol, 2:1 (v/v) partitioned with aqueous KCl, and an aliquot of the extracted total lipids was quantitated by gravimetry. Individual fractions of neutral lipids and phospholipids were separated by thin-layer chromatography with the solvent systems, petroleum ether : diethyl ether : acetic acid (90:10:1 v/v) and chloroform : methanol : aqueous ammonia 14 N (65:35:4 v/v), respectively. Using arithmetic reference standards and identifying the lipid spots by exposing to I_2 vapor,

TABLE 1. *Total lipids and their fractions in tissues of various types of leprosy (mean ± S.D.).*

Lipids (mg/g tissue)	Controls (N = 11)	LL[a] (N = 11)	BL[b] (N = 9)	TT[c] (N = 13)
Total lipid	2.53 ± 0.21	3.08 ± 0.24	3.05 ± 0.32	2.61 ± 0.24
Phospholipids	1.16 ± 0.21	2.27 ± 0.23	2.24 ± 0.31	1.19 ± 0.21
Triglycerides	0.26 ± 0.05	1.74 ± 0.12	1.72 ± 0.12	0.39 ± 0.12
Free fatty acids	0.06 ± 0.02	0.08 ± 0.03	0.09 ± 0.04	0.05 ± 0.02
Cholesterol	0.93 ± 0.17	0.97 ± 0.21	0.98 ± 0.22	0.96 ± 0.13

[a] LL = lepromatous leprosy.
[b] BL = borderline lepromatous leprosy.
[c] TT = tuberculoid leprosy.

they were scraped from the glass plates and eluted with suitable solvents. Cholesterol was estimated colorimetrically by reacting with $FeCl_3$–galacial acetic acid reagent while triglycerides as its glycerol moiety. Phospholipid phosphorus was estimated colorimetrically after digesting with $HClO_4$. Free fatty acid was quantitated by titrating with alcoholic NaOH. These methods have been described in detail in a earlier publication ([4]).

The lipid compositions of tissue from leprosy patients and controls are given in Table 1. An increase in the total lipids was observed in LL and BL patients compared to normal subjects and TT patients. The increase in total lipids is also reflected in an increase in phospholipid and triglyceride content. Cholesterol and free fatty acid values were unaltered in all types of leprosy patients. Among the individual phospholipid classes (Table 2) phosphatidylcholine (PC) and sphingomyelin (SPM) were increased and phosphotidylethanolamine (PE) decreased in LL disease. The BL patients registered an increase in both SPM and PE as well. An elevation in cardiolipin content was observed in TT patients only. Lyso PC and lyso PE were unaltered in all types of disease.

The present study indicates a substantial elevation of the phospholipid and triglyceride levels in leprous tissue from bacillary-positive (BL and LL) disease, while the cholesterol and fatty acid content remain unaltered. This confirms the earlier observations of Khandke, *et al.* ([3]). Phospholipids and neutral fat have been suggested as the major components of lepra cell lipids. ([2, 6]). It is possible that these lipids are derived from bacilli phagocytized by the macrophages ([2]). They may also originate from fatty degeneration (lipophanerotis) of the lepra cells ([8]). Although most people believe that the bacilli and lipophanerosis are responsible for stored lipids in leprous tissue, alternatively, the increased lipid levels may be due to reduced levels of tissue lipase since

TABLE 2. *Phospholipid fractions in tissues of various types of leprosy (values are percentage of total phospholipids mean ± S.D.).*

Phospholipid fractions	Controls (N = 11)	LL[a] (N = 11)	BL[b] (N = 9)	TT[c] (N = 13)
Lysophosphatidylethanolamine (LPE)	1.17 ± 0.51	1.23 ± 0.51	1.24 ± 0.59	1.27 ± 0.38
Lysophosphatidylcholine (LPC)	1.26 ± 0.46	1.31 ± 0.51	1.33 ± 0.39	1.27 ± 0.41
Phosphatidylcholine (PC)	48.96 ± 3.3	52.7 ± 3.4	46.07 ± 4.8	47.3 ± 5.7
Sphingomyelin (SPM)	20.35 ± 1.42	25.31 ± 1.73	23.84 ± 1.96	20.08 ± 1.34
Phosphatidylethanolamine (PE)	17.70 ± 1.78	14.16 ± 0.93	22.23 ± 1.51	19.01 ± 1.34
Phosphatidylinositol (PI)	1.83 ± 0.84	1.67 ± 0.39	1.89 ± 0.51	1.92 ± 0.72
Cardiolipin (CL)	2.01 ± 0.71	2.11 ± 0.79	2.32 ± 0.56	3.81 ± 0.66

[a] LL = lepromatous leprosy.
[b] BL = borderline lepromatous leprosy.
[c] TT = tuberculoid leprosy.

in active LL disease serum lipase activity has been shown to be inhibited ([7]). However, in the present study there is no change in values of lipids in the bacillary-negative TT patients but very high levels of phospholipids and triglycerides in BL and LL patients. This suggests that the origin of the leprous tissue lipids is partly bacterial and partly might be due to fatty degeneration of macrophage cytoplasm since the rise in lipids is out of proportion to that contributed by *Mycobacterium leprae* alone.

M. leprae are rich in phospholipids ([6]) and so are likely to be responsible for the increased phospholipids found in the leprous tissues. This is supported by the unchanged phospholipid values in the bacilli-free TT cases where only the cardiolipin fraction was elevated but none of the other phospholipid fractions was altered. In the bacillary-positive BL and LL patients, however, the individual phospholipid fractions varied, but not according to any predictable pattern. Reduction in the levels of PE in LL cases may be due to its more marked conversion to PC by the methyl transferase reaction for some unknown reason. An increase in the SPM level in the bacillary-positive cases (BL and LL) is difficult to explain since this fraction is not present in any significant quantity in either the leprosy bacilli or macrophages. Although *M. leprae* contain sufficient quantities of cardiolipin in their cell wall, the near total absence of bacilli and the insignificant amount of this phospholipid present in macrophages, epithelioid, and giant cells of tuberculoid granuloma does not explain increased levels of cardiolipin in tuberculoid tissue.

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