

## Double-stranded DNA Inhibits Cardiolipin-binding Activity in Lepromatous Leprosy Patients' Sera

TO THE EDITOR:

Cardiolipin (phosphatidyl glycerol) is one of the structural components of *Mycobacterium leprae* and has immunogenicity (1). Antibodies to cardiolipin are frequently observed in sera of patients with lepromatous leprosy by a flocculation test (7). Previously, we reported on the presence of anti-single stranded (ss) DNA antibodies and the absence of anti-double stranded (ds) DNA antibodies in lepromatous leprosy (2,3). Recent findings by Lafer, *et al.* (7) have indicated that cardiolipin can compete with the DNA-binding activity of monoclonal anti-DNA antibodies that react with a wide range of polynucleotides.

Sera were obtained from 45 patients with lepromatous leprosy and 30 age-matched, normal controls. Anti-cardiolipin antibodies were measured according to the method of Harris, *et al.* (4) with minor modification. Briefly, purified cardiolipin (Sigma, 10 µg/ml) was coated onto the surface of polyvinyl microtiter wells (Flow Laboratories). Serum samples diluted 1:20 in 1% bovine serum albumin (BSA)-phosphate buffered saline (PBS) were added to the plates. IgG-anti-cardiolipin antibodies were measured by using <sup>125</sup>I-protein A. When the mean level plus 2 standard deviations of normal controls was used as the cut-off point, 20% (9/45) of the patients with lepromatous leprosy were positive. Anti-ssDNA antibodies and anti-dsDNA antibodies were measured by the Koike, *et al.* (5) with minor modification (2). Anti-ssDNA antibodies were found in 5 out of 45 patients, but anti-dsDNA antibodies were not found in any sample examined. There were no significantly quantitative associations among the levels of anti-cardiolipin antibodies, anti-ssDNA and anti-dsDNA antibodies by statistical analysis using Spearman's rank correlation test.

We selected 7 samples with high titers of anti-cardiolipin antibodies and negligible titers of anti-ssDNA and anti-dsDNA antibodies. These samples were prepared for the inhibition study. A mixture of 100 µl of each of the inhibitors (cardiolipin, ssDNA, and dsDNA) at varying dilutions and each

THE TABLE. *Inhibitory effects of dsDNA and ssDNA on the cardiolipin-binding activity of lepromatous leprosy sera.*

Cases	% Inhibition					
	Cardiolipin (µg/ml)		dsDNA (µg/ml)		ssDNA (µg/ml)	
	200	100	200	100	200	100
1	57	30	100	100	0	12
2	52	48	77	61	18	17
3	31	35	100	97	8	10
4	34	33	76	75	18	20
5	46	37	84	83	22	15
6	48	45	95	80	15	10
7	52	38	85	79	19	13

of the samples to be tested was incubated for 1 hr at 37°C. This mixture was then utilized for the radioimmunoassay described above. The results of inhibition were expressed as percent inhibition of cardiolipin-binding activity, calculated as follows:

% inhibition =

$$\left[ 1 - \frac{\text{cpm in the presence of inhibitor} - \text{background}}{\text{cpm in the absence of inhibitor} - \text{background}} \right] \times 100$$

When the concentration of each inhibitor was 200 µg/ml, the mean ratios of percent inhibition of cardiolipin, dsDNA, and ssDNA were 45.7%, 88.1%, and 14.3%, respectively (The Table).

This result clearly demonstrates that polyspecific IgG-anti-cardiolipin antibodies in sera from lepromatous leprosy patients have the ability to crossreact with dsDNA. These findings may contribute to clarifying the biological significance of antinuclear antibodies in lepromatous leprosy.

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