

## 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.

—Fukumi Furukawa, M.D.

Department of Dermatology

—Gakuji Ohshio, M.D.

—Yoshihiro Hamashima, M.D.

Department of Pathology

Faculty of Medicine

Kyoto University

Shogoin-Kawara-cho

Sakyo, Kyoto 606, Japan

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