SERA FROM SYPHILIATIC PATIENTS CROSS REACT WITH SPECIFIC GLYCOPEPTIDOLIPID AND WHOLE LIPID EXTRACT OF *MYCOBACTERIUM AVIUM* COMPLEX IN AN ANTI-CARDIOLIPIN ANTIBODY TEST

Keywords: Cardiolipin, Glycopeptidolipid, Syphilis, Mycobacterium.

Antiphospholipid or anticardiolipin (aCL) antibodies comprise a family of immunoglobulins which have been associated with symptoms including strokes, venous thrombosis, thrombocytopenia, and recurrent abortion.[1,2] There are reports with regard to the occurrence of aCL antibodies in patients with septicemia and acute disease states such as mycoplasma infection,[3] malaria,[4] lyme disease[5] and a number of viral infections including HIV.[6]

Mycobacterium sp. contain a great deal of complex molecules in their cell wall, such as glycopeptidolipid (GPL), lipoarabinomann, lipooligosaccharide, and phenolic

Correspondence address:
Mohammed R. Pourshafie, Ph.D.,
Department of Microbiology,
Pasteur Institute of Iran,
Tehran, I.R. Iran
Fax : (98-21) 6465132
Tel: (98-21) 6469871-4 (ext. 252).

glycolipid. In this study the immuno-cross-reactivity between GPL, a major lipid component of *Mycobacterium avium* complex (MAC), and CL were examined.

GPL and whole lipid extract (WLE) were purified from MAC serovar 4 (TMC 1463).[8] Nine sera from syphiliatic patients as well as normal control volunteers were used. ELISA assay was performed as described by Harris et al.[2] The microtiter plates were coated with mycobacterial lipids or with 30 μL per well of 50 μg/mL CL in ethanol. Fifty microliters of patients' serum (primary antibody) followed by alkaline-phosphatase labeled, affinity-purified goat antihuman IgG was added. Enzyme substrate (P-nitrophenylphosphate-diethanolamine buffer) was then added. A Bio-rad computerized program (Bio-Rad Laboratories, Hercules, CA) enabled the construction of calibration curves and the determination of the values of the unknown samples from the curve. Log-logit equations were used to construct the calibration curves. All experiments were done in triplicates and significance was assessed by means of Student's t-test with α set *a priori* at $p<0.05$.

Figure 1 shows a comparison among CL, WLE and GPL-coated plates following incubation with sera from syphiliatic patients in an ELISA test. The binding of sera from syphiliatic patients to CL was more evident than WLE and GPL. The binding of anti-CL antibody standards on CL or WLE-coated plates was also studied.

The binding of anti-CL antibody was more evident in the presence of CL than WLE. The cross-reacting of anti-CL antibody was apparent in the presence of 10 or 1.5 μg of WLE (data not shown). To examine the degree of binding of anti-CL monoclonal antibody (mAb) to mycobacterial lipids, the plates were then incubated with anti-CL mAb as the primary antibody. Results indicated a comparable binding of the anti-CL mAb to CL, GPL and WLE (Fig. 2). Using a polyclonal antibody against CL, however, showed a significant difference in binding to CL as compared to GPL or WLE.

The presence of anti-CL antibody has been reported in various infectious diseases[3-7] and also in 14% of the general population.[9] With such a wide range of distribution, the binding of anti-CL antibody to WLE isolated from MAC may have occurred via any of the molecules present in the WLE. On the other hand, the GPL molecule has a well-defined structure. The 3,4-di-O-methyrhamose is attached to the alaninol on the terminal end of the peptide moiety and the 6-deoxyhexose is linked to the allo-theronine in the peptide moiety, a structure unlike CL. The incubation of anti-CL

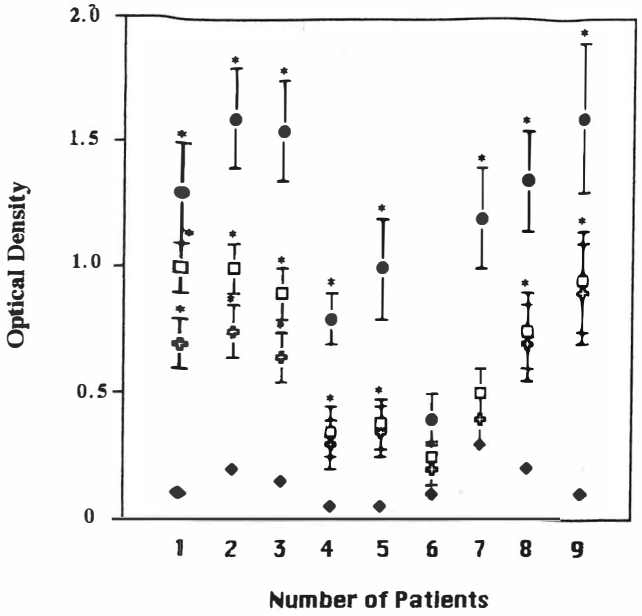

Fig. 1. Nine sera samples from syphiliatic patients were collected and then added on the ELISA plates coated with 1.5 μg of CL ●, WLE □ and GPL + . Nine samples of normal sera were also plated on the CL-coated wells ◆. The samples were done in triplicates and the mean values reported. An asterisk (*) indicates a significant difference between syphiliatic and normal control sera. Arrows indicate standard deviation.

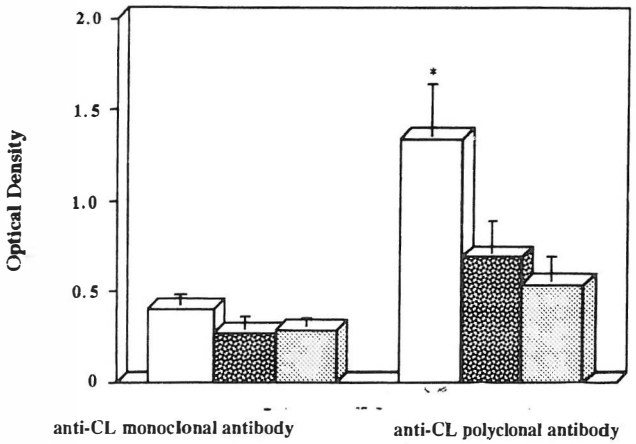

Fig. 2. Plates were coated with 1.5 μg per well of CL □ , WLE ▨, and GPL ▭ . An asterisk (*) indicates significance.

monoclonal antibody with CL, GPL, and WLE indicated a similar binding pattern, suggesting the presence of a similar recognition site on all three lipids. There is an inconsistency with regard to binding of the antibodies to phospholipids from syphiliatic patients. Some suggest that it may bind to CL[10] or phosphatidylcholine.[11]

The information presented here may suggest the presence of multivalent antibodies against CL which bind to several antigenic sites of different structural integrity.

MOHAMMED R. POURSHAFIE, Ph.D.
HOSSAIN MOTIEIAN NAJAR, Ph.D.*

*From the Department of Microbiology, Pasteur Institute of Iran, Tehran, and the *Cell Bank Department, Pasteur Institute of Iran, Tehran, I.R. Iran.*

REFERENCES

1. Reyes H, Dearings L, Shoenfeld Y, Peter J: Antiphospholipid antibodies: a critique of their heterogeneity and hegemony. Sem Thromb Hemost 20: 89-100, 1994.
2. Harris E, Gharavi A, Boey M, Patel B, Mackworth C, Loizou S, Hughes G: Anticardiolipin antibodies: detection by radioimmunoassay and association with thrombosis in systemic lupus erythematosus. Lancet 2: 1211-1214, 1983.
3. Vaarala O, Vaara M, Palosuo T: Effective inhibition of cardiolipin binding antibodies in Gram-negative infection by bacteria lipopolysaccharide. Scand J Immunol 28: 607-612, 1988.
4. Colaco CB, Male DK: Anti-phospholipid antibodies in syphilis and a thrombotic subset of SLE: distinct profiles of epitope specificity. Clin Exp Immunol 59: 449-456, 1985.
5. Mackworth-Young CG, Harris EN, Steare AC: Anticardiolipin antibodies in Lyme disease. Arthritis Rheum 31: 1052-1056, 1988.
6. Canoso RT, Zon LI, Groopman JE: Anticardiolipin antibodies associated with HTLV-III infection. Br J Haematol 65: 495-498, 1987.
7. Pourshafie M, Ayub Q, Barrow WW: Comparative effects of *Mycobacterium avium* glycopeptidolipid and lipopeptide fragment on functions and ultrastructure of mononuclear cells. Clin Exp Immunol 93: 72-80, 1993.
8. Sammaritano L, Gharavi A: Antiphospholipid antibodies syndrome. Clin Lab Med 12: 41-59, 1992.
9. Weiss M, Sodock V, Sigal L, Phillips M, Merryman P, Abranson S: False positive seroreactivity to *B. burgdorferi* in SLE: the value of immunoblot analysis. Lupus 4: 131-7, 1995.
10. Kataoka T, Nojima S: Immunochemical studies of phospholipids. VI. Haptenic activity of phosphatidylinositol and the role of lecithin as an auxiliary lipid. J Immunol 105: 503-509, 1970.