Inhibitory effects of anticardiolipin antibodies on lymphocyte proliferation and neutrophil phagocytosis

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Abstract

Anticardiolipin antibodies purified from serum from patients with systemic lupus erythematosus (SLE) by cardiolipin micelles were studied for their effects on lymphocytes and neutrophils. At a concentration of 160 µg/ml they markedly suppressed the [³H]thymidine incorporation of mononuclear cells stimulated by phytohaemagglutinin (4.9 (SEM 1.9%) of the control) and pokeweed mitogen (26.7 (10.5%) of the control). In addition, anticardiolipin antibodies changed the cell cycle of phytohaemagglutinin stimulated lymphocytes such that the S and G2+M phases were significantly diminished (G0/G1=64.62%, S=20.59%, G2+M=14.78% in the presence of normal human IgG v G0/G1=86.07%, S=10.32%, G2+M=3.59% in the presence of anticardiolipin antibodies). The suppression of lymphocyte proliferation by anticardiolipin antibodies was shown not to be caused by an alteration of T cell subpopulations. However, the interleukin 2 receptors on the cell surface and the soluble interleukin 2 receptors in the supernatant of phytohaemagglutinin stimulated mononuclear cells were decreased in the presence of anticardiolipin antibodies. On the other hand, the phagocytic activity of neutrophils was 40% inhibited at a higher concentration of anticardiolipin antibodies (300 µg/ml) through suppression of C3b/C4b and Fc receptors on polymorphonuclear leucocytes. These results suggest that anticardiolipin antibodies exert inhibitory effects on both lymphocytes and phagocytes in addition to the coagulation cascade. These newly found activities of anticardiolipin antibodies were mediated by the non-specific membranotropic property of the antibodies.

Anticardiolipin antibodies have been reported to be associated with a broad spectrum of disease, such as deep vein thrombosis, coronary artery occlusion, pulmonary hypertension, neurological disorders, and habitual abortion in patients with systemic lupus erythematosus (SLE).¹⁻⁶ As these antibodies can bind to negatively charged phospholipids in the coagulation cascade and plasma membrane they may cause paradoxical hypercoagulability,⁷ thrombocytopenia,⁸ and haemolytic anaemia.⁹ Khamashta *et al* showed that purified anticardiolipin antibodies could stick to freeze-thawed platelets and red cells but not to untreated cells.¹⁰ Misra *et al* further showed that anticardiolipin antibodies purified by

cardiolipin-cholesterol liposome from the serum of a patient with infectious mononucleosis reacted with the membrane of lectin activated lymphocytes and certain cell lines.¹¹ These results suggest that activation of membrane is mandatory for the expression of binding epitopes with anticardiolipin antibodies. Thus, possibly, anticardiolipin antibodies may exert certain biological effects on the activated cells through non-specific binding to the cell membrane as long as the epitopes are exposed. However, the biological activity of anticardiolipin antibodies, other than anticoagulation, has never been reported. In this study anticardiolipin antibodies were partially purified from SLE serum by cardiolipin micelles and their effects on the functions of normal human lymphocytes and phagocytes were investigated.

Patients and methods

PATIENTS' SERUM SAMPLES

Serum samples from patients fulfilling four or more of the 1982 revised American Rheumatism Association criteria for the classification of SLE¹² were screened for the presence of anticardiolipin antibodies by enzyme linked immunosorbent assay (ELISA). Only those samples with an absorbance at 410 nm higher than 0.6 (normal serum <0.3) were purified further.

PURIFICATION OF ANTICARDIOLIPIN ANTIBODIES WITH CARDIOLIPIN MICELLES

We used the method described by Harris *et al*,¹³ with some modifications. Briefly, cardiolipin was dissolved in ethanol (Sigma Chemicals, St Louis, MO, USA) and evaporated in a vacuum oven to dryness. Cardiolipin micelles were created by adding phosphate buffered saline solution, pH 7.2, to dried cardiolipin with vigorous agitation in vortex. Serum (10 ml) containing anticardiolipin antibodies was then mixed with 5 ml of micelles (3 mg/ml) and incubated at 37°C for one hour and at 4°C overnight with constant shaking. After incubation the mixture was spun at 27 000 g for 15 minutes. The pellet was suspended in 30 ml phosphate buffered saline, pH 7.2, and centrifuged again at 27 000 g for 15 minutes. Washing was repeated three times to remove the non-bound proteins. Finally, the pellet was suspended in 2 ml of 1 M NaI and the suspension incubated at room temperature for 60 minutes to interrupt the linkage between antibodies and micelles. The suspension was again centrifuged at 27 000 g for 15 minutes. On this occasion the

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micelles floated on the top and the supernatant was removed. The supernatant fraction was then intensively dialysed against phosphate buffered saline, pH 7.2, at 4°C for 24 hours. Finally, the dialysate was changed to RPMI-1640. The protein concentration of the antibodies was measured by a Bio-Rad protein assay kit (Bio-Rad Laboratories, Richmond, CA, USA) and the purity by 10% sodium dodecyl sulphate-polyacrylamide gel electrophoresis. The anticardiolipin activity of the antibodies was estimated by an ELISA.¹⁴

ISOLATION OF MONONUCLEAR CELLS AND POLYMORPHONUCLEAR LEUCOCYTES FROM PERIPHERAL BLOOD

Heparinised venous blood from normal subjects was mixed with a quarter volume of 2% dextran solution (mol wt 500 000) and incubated at 37°C for 20 minutes. Supernatant rich in leucocytes was collected and diluted with the same volume of Hanks's balanced salt solution. After centrifugation at 300 g for 30 minutes over a Ficoll-Hypaque cushion (specific gravity 1.077) the mononuclear cells were aspirated from the interphase while polymorphonuclear leucocytes were obtained from the bottom. The purity of the polymorphonuclear leucocytes was greater than 95% as confirmed by Wright's stain.

MITOGEN INDUCED [³H]THYMIDINE INCORPORATION IN MONONUCLEAR CELLS

Mononuclear cells (100 µl; 1×10^6 /ml) were placed in flat bottomed microwells in triplicate. Phytohaemagglutinin 20 µl; 20 µg/ml) and 0.08 ml of anticardiolipin antibodies (400 µg/ml) were added to the wells. In the control wells 0.08 ml of RPMI-1640 or purified human IgG (400 µg/ml) was added instead of anticardiolipin antibodies. Stimulation of [³H]thymidine by pokeweed mitogen was carried out as for the phytohaemagglutinin assay except that the concentration of pokeweed mitogen was 100 µg/ml. The mixture was incubated at 37°C in 5% CO₂ – 95% air for 68 hours and then pulsed with 18.5 kBq [methyl-³H]thymidine/well (specific activity 248 GBq/mmol; NEN, Boston, USA) at 37°C for four hours. The cells were harvested and the radioactivity measured by a beta counter.

CELL CYCLE ANALYSIS OF MITOGEN STIMULATED MONONUCLEAR CELLS

The mixture of 0.4 ml mononuclear cells (2.25×10^6 /ml), 0.5 ml anticardiolipin antibodies (400 µg/ml) or human IgG (400 µg/ml), and 0.1 ml mitogen (phytohaemagglutinin 20 µg/ml or pokeweed mitogen 100 µg/ml) in a sterile plastic tube was incubated at 37°C in 5% CO₂ – 95% air for three days. The nuclear DNA was stained with 50 µg/ml of propidium iodide in 0.1% sodium citrate and 0.2% Triton X-100. The cell cycle was analysed by EPICS C flow cytometry (Coulter Electronics, Hialeah, Miami, FL, USA). The percentage of each phase in the cell cycle was calculated with the PARA-1 program provided by Coulter Electronics.

DETERMINATION OF T CELL SUBPOPULATIONS AND INTERLEUKIN 2 RECEPTORS

Fluorescent conjugated monoclonal antibodies against CD3, CD4, CD8, and interleukin 2 receptor (Coulter Immunology) were used to stain the surface markers of mononuclear cells stimulated with phytohaemagglutinin in the presence of anticardiolipin antibodies or human IgG by flow cytometry.

In addition, the culture supernatants were collected after three days for measurement of soluble interleukin 2 receptors.

The concentration of soluble interleukin 2 receptor in various supernatants was measured by a commercial test kit (AMAC, Westbrook, ME, USA). The sensitivity of the assay was 0.2 fmol. The coefficients of variation of intra and interassays were 5.5% and 11.3% respectively.

MEASUREMENT OF POLYMORPHONUCLEAR LEUCOCYTE PHAGOCYTOSIS

Fluoresbrite carboxylate microspheres (0.75 µm; Polysciences, Warrington, PA, USA) were first opsonised with fresh normal human serum at 37°C for 45 minutes. Polymorphonuclear leucocytes (50 µl; 1.2×10^7 /ml) were incubated with 150 µl of anticardiolipin antibodies (0.4 mg/ml) or human IgG (0.4 mg/ml) at 37°C for one hour. Opsonised fluorescent beads (10 µl; 2×10^9 particles/ml) were then added to the mixture and incubated for another hour. The beads which had not undergone phagocytosis were removed after three washes at 300 g for 10 minutes. The percentage and fluorescence intensity of the polymorphonuclear leucocytes which had taken part in phagocytosis of the beads were determined by EPICS C flow cytometry.¹⁵

DETERMINATION OF C3b RECEPTORS AND IgG Fc RECEPTORS ON POLYMORPHONUCLEAR LEUCOCYTES

An indirect immunofluorescence antibody method was used to determine the C3b and IgG Fc receptors on polymorphonuclear leucocytes. Monoclonal mouse antihuman C3b receptors (Dako, Kakopatts, Denmark) and fluorescein isothiocyanate labelled rabbit antimouse IgG2a antibodies were the primary and secondary antibodies for staining the C3b receptors on the polymorphonuclear leucocytes. Heat aggregated (63°C, 45 minutes) human IgG (250 µg/ml) and fluorescein isothiocyanate labelled goat antihuman IgG were the ligand and antibodies for staining the Fc receptors on the polymorphonuclear leucocytes. The percentage and fluorescence intensity of these receptors on the polymorphonuclear leucocytes were analysed by flow cytometry at 488 nm.

STATISTICAL ANALYSIS

Results are expressed as the mean (SEM) for the whole study. Statistical significance was assessed by non-parametric Wilcoxon signed ranks test.

Figure 1 Electrophoretic analysis of molecules bound and unbound with cardiolipin micelles by 10% sodium dodecyl sulphate-polyacrylamide gel electrophoresis. Lane 1 = calibration proteins; lane 2 = human IgG (40 µg); lane 3 = supernatant containing unbound molecules from a normal human serum; lane 4 = supernatant containing bound molecules from an SLE serum; lane 5 = bound molecules from a normal serum; lane 6 = affinity purified anti-dsDNA antibodies; lane 7 = human IgG (20 µg).

Results

CARDIOLIPIN BINDING ACTIVITY OF PARTIALLY PURIFIED ANTICARDIOLIPIN ANTIBODIES

Anticardiolipin antibodies purified from SLE sera by cardiolipin micelles contained IgG and albumin as shown by sodium dodecyl sulphate-polyacrylamide gel electrophoresis (fig 1). The cardiolipin binding activity of these antibodies was measured by ELISA. Anticardiolipin antibodies at a protein concentration of 400 µg/ml were equivalent to an absorption of 0.5 at 280 nm. Most of the experiments were carried out at this protein concentration.

Figure 2 Effects of anticardiolipin antibodies (aCL) on the phytohaemagglutinin (PHA) and pokeweed mitogen (PWM) induced $[^3\text{H}]$ thymidine incorporation in normal human mononuclear cells in three day cultures. The percentage response represents the counts per minute (cpm) of mitogen stimulated mononuclear cells in the presence of 160 µg/ml of human IgG (HIgG) or anticardiolipin antibodies divided by the cpm of mitogen stimulated mononuclear cells (control).

INHIBITION OF $[^3\text{H}]$ THYMIDINE INCORPORATION IN MONONUCLEAR CELLS

Anticardiolipin antibodies, at a final concentration of 160 µg/ml, markedly suppressed phytohaemagglutinin and pokeweed mitogen induced $[^3\text{H}]$ thymidine incorporation in normal mononuclear cells (fig 2). This suppression by anticardiolipin antibodies was dependent on dose (fig 3). The antibodies did not change the cell viability even when the concentration was increased to 480 µg/ml, as confirmed by a trypan blue dye exclusion test. In an additional experiment the cardiolipin micelles were incubated with 1 M sodium iodide at room temperature for one hour and then treated by the same procedures as in the preparation of antibodies. The supernatant obtained did not change the mitogen response of the mononuclear cells (data not shown). Furthermore, sodium iodide at concentrations ranging from 10^{-2} to 10^{-6} mol/l did not suppress the mitogen response of mononuclear cells (table 1). In addition to the normal mononuclear cells, anticardiolipin antibodies also suppressed the proliferation of certain cell lines such as WI-L2 (B lymphoma cell) and Jurkat cells (T lymphoma cell) (table 2).

Figure 3 Dose dependent effect of anticardiolipin antibodies (aCL) on phytohaemagglutinin induced $[^3\text{H}]$ -thymidine $[^3\text{H-TdR}]$ incorporation in human mononuclear cells derived from three different subjects in three day old cultures. The percentage of control was calculated by the counts per minute (cpm) of phytohaemagglutinin stimulated mononuclear cells in the presence of anticardiolipin antibodies divided by the cpm without addition of anticardiolipin antibodies.

Table 1 Effects of sodium iodide on phytohaemagglutinin and pokeweed mitogen induced $[^3\text{H}]$ thymidine incorporation in mononuclear cells in three day old cultures

Concentration of sodium iodide (mol/l)	$[^3\text{H}]$ Thymidine incorporation (cpm)	
	Phytohaemagglutinin (2 µg/ml)	Pokeweed mitogen (10 µg/ml)
0	61 634	7818
10^{-6}	64 064	7773
10^{-4}	62 644	7598
10^{-2}	66 358	8561

Table 2 Effects of anticardiolipin antibodies (aCL) on the proliferation of WI-L2 and Jurkat cell lines in three day old cultures

	[³ H]Thymidine incorporation (cpm)	
	WI-L2 (5×10 ⁴ cells/well)	Jurkat (5×10 ⁴ cells/well)
Experiment 1		
Medium	298 603	5383
Human IgG (160 µg/ml)	284 787	4613
aCL (160 µg/ml)	174	1687
Experiment 2		
Medium	334 584	6884
Human IgG (160 µg/ml)	341 351	6415
aCL (160 µg/ml)	2876	997

EFFECT OF ANTICARDIOLIPIN ANTIBODIES ON THE CELL CYCLE OF MONONUCLEAR CELLS

Compared with human IgG, anticardiolipin antibodies at a concentration of 160 µg/ml markedly suppressed the synthetic (S), gap 2 (G2), and mitotic (M) phases of the phytohaemagglutinin induced cell cycle of mononuclear cells. Figure 4 shows a typical case.

CHANGES OF LYMPHOCYTE SURFACE MARKERS BY ANTICARDIOLIPIN ANTIBODIES

To determine whether decreased mitogen stimulated [³H]thymidine incorporation in mononuclear cells in the presence of anticardio-

Table 3 Effects of anticardiolipin antibodies (aCL) on the percentage of surface markers of phytohaemagglutinin (PHA) stimulated mononuclear cells in three day old cultures

	% Of mononuclear cells				
	CD3	CD4	CD8	IL-2R†	CD4/ IL-2R
Experiment 1					
PHA	74.0	29.4	59.7	51.0	0.49
PHA+human IgG*	76.7	27.0	58.6	74.5	0.46
PHA+aCL*	56.7	12.5	15.6	28.3	0.80
Experiment 2					
PHA	69.3	34.3	38.2	45.8	0.90
PHA+human IgG	60.4	38.2	22.6	35.1	1.69
PHA+aCL	47.2	10.1	10.8	25.8	0.94
Experiment 3					
PHA	79.5	50.6	25.1	19.7	2.02
PHA+human IgG	77.3	50.3	27.1	19.2	1.86
PHA+aCL	72.6	43.4	21.8	14.2	1.99

*The concentration of human IgG and of anticardiolipin antibodies was 160 µg/ml.
†IL-2R=interleukin 2 receptor.

Figure 4 Effect of anticardiolipin antibodies (160 µg/ml) on the cell cycle of phytohaemagglutinin stimulated human mononuclear cells; MNC=mononuclear cells; PHA=phytohaemagglutinin; HlgG=human IgG; aCL=anticardiolipin antibodies.

lipin antibodies changes the T cell subpopulations or not, the percentages of CD3, CD4, and CD8 positive lymphocytes in mononuclear cells were measured by flow cytometry. Although the percentages of these three subpopulations were all decreased by anticardiolipin antibodies, the CD4/CD8 ratio was not changed. In addition, the percentage of interleukin 2 receptor positive cells in phytohaemagglutinin stimulated mononuclear cells was markedly decreased in the presence of anticardiolipin antibodies (table 3). This suggests that anticardiolipin antibodies non-selectively suppress the surface marker expression on mitogen stimulated lymphocytes.

The inhibitory effect of anticardiolipin antibodies on phytohaemagglutinin stimulated mononuclear cell proliferation was further supported by the decreased generation of soluble interleukin 2 receptor in the supernatants (table 4).

SUPPRESSION OF POLYMORPHONUCLEAR LEUCOCYTE PHAGOCYTOSIS BY ANTICARDIOLIPIN ANTIBODIES

Anticardiolipin antibodies, at a concentration greater than 300 µg/ml, suppressed the phago-

Table 4 Effects of anticardiolipin antibodies (aCL) on the generation of soluble interleukin 2 receptors (sIL-2R) in phytohaemagglutinin (PHA) stimulated mononuclear cell supernatants in three day old cultures

	[³ H]Thymidine incorporation (cpm)	sIL-2R ⁺ (ng/ml)
Experiment 1		
Medium	319	<0.50
PHA	121 780	3.44
PHA+human IgG*	114 770	7.60
PHA+aCL*	1774	<0.50
Experiment 2		
Medium	196	<0.50
PHA	34 456	1.26
PHA+human IgG	36 549	1.89
PHA+aCL	3359	<0.50
Experiment 3		
Medium	204	<0.50
PHA	57 347	2.14
PHA+human IgG	56 943	2.08
PHA+aCL	987	<0.50

*The concentration of human IgG and of anticardiolipin antibodies was 160 µg/ml.

†The concentration of sIL-2R in the supernatants was measured by an ELISA test kit.

Table 5 Effects of anticardiolipin antibodies (aCL) on phagocytosis and on C3b and Fc receptor expressions on neutrophils*

	% Of neutrophils		
	Phagocytosis	C3b receptor	Fc receptor
Experiment 1			
Medium	35.9	73.6	53.9
Human IgG†	41.0	53.7	50.7
aCL†	18.5	39.4	29.4
Experiment 2			
Medium	49.8	64.6	66.7
Human IgG	53.7	62.9	57.4
aCL	24.6	43.6	42.3
Experiment 3			
Medium	48.4	53.8	63.2
Human IgG	44.6	54.7	52.5
aCL	38.2	39.4	31.4

*Neutrophils (2×10⁶/ml) were preincubated with medium, human IgG, or anticardiolipin antibodies for one and a half hours before the measurement of phagocytosis and C3b and Fc receptor expression by flow cytometry.

†The concentration of human IgG and of anticardiolipin antibodies was 300 µg/ml.

cytic activity of polymorphonuclear leucocytes. The suppression was due to inhibition of C3b/C4b and Fc receptor expression on the neutrophils (table 5).

Discussion

Anticardiolipin antibodies were found in 23–54% of the patients with SLE. Patients with other collagen vascular diseases, such as rheumatoid arthritis, Sjögren's syndrome, dermatomyositis/polymyositis,¹⁶ acute and chronic infections,^{17, 18} AIDS,¹⁹ and lymphoproliferative disorders,²⁰ contain such antibodies in the blood; they are also found in healthy elderly subjects.²¹ Patients with anticardiolipin antibodies may experience thrombosis in the venous, arterial, placental, or microvascular circulation.²² In addition to their effect on the blood coagulation system, there is some evidence that these autoantibodies can bind to certain cells and derange their functions. De Castellarnau *et al*,²³ Freyssinet *et al*,²⁴ Carreras *et al*,¹ and Coade²⁵ showed that in the presence of lupus anticoagulant the secretion of prostacyclins from vascular endothelial cells or smooth muscle cells was substantially decreased. Furthermore, Khamashta *et al* showed that purified anticardiolipin antibodies could bind to phosphatidylserine and phosphatidylinositol epitopes expressed on the surface of freeze-thawed platelets or red blood cells.¹⁰ Schorer *et al* found that liposome purified anticardiolipin antibodies selectively inhibited prostacyclin release from thrombin activated endothelial cells, but not from non-activated cells.²⁶ The most interesting finding, however, was that of Misra *et al*,¹¹ who showed that affinity purified anticardiolipin antibodies from the serum of a patient with infectious mononucleosis adhered to several cell types, including HEp-2, Molt 4, and phytohaemagglutinin activated peripheral lymphocytes, but did not adhere to the non-stimulated lymphocytes. Apparently, cell activation is necessary for the expression of binding epitopes with anticardiolipin antibodies. The negatively charged phospholipids, such as phosphatidylserine, phosphatidylinositol, or phosphatidylethanolamine, are located primarily in the inner lipid bilayer of the plasma membrane. Once the cells are activated, these phospholipids are expressed on the cell surface and facilitate binding of anticardiolipin antibodies to the membrane.^{10, 27, 28}

In our study we clearly showed that anticardiolipin antibodies from SLE serum samples purified by cardiolipin micelles exerted a suppressive effect on lymphocyte and phagocyte functions in a manner dependent on dose. The anticardiolipin antibodies purified from pooled normal serum samples displayed similar activity also (data not shown). As far as we know this observation has not previously been reported. The inhibitory effect of anticardiolipin antibodies on mitogen induced [³H]thymidine incorporation of lymphocytes was not due to Fc dependent, IgG mediated, non-specific suppression of the immune response²⁹ because human IgG (160 µg/ml) had negligible effect. In

addition, the F(ab')₂ fragment of anticardiolipin antibodies after pepsin digestion retained the complete activity of the intact molecule (data not shown). This inhibition is quite significant because T cell hypofunction, such as hypo-reactivity to delayed hypersensitivity skin test³⁰ and phytohaemagglutinin hyporesponsiveness of mononuclear cells,³¹ are commonly found in active SLE. Whether anticardiolipin antibodies can cause T cell hypofunction in vivo is not clear at present because a number of auto-antibodies are present in patients with SLE, including antibodies to lymphocytes, dsDNA, or β₂ microglobulin. These antibodies have also been found to suppress the T cell function.^{15, 32} In addition, cross reactivity of anticardiolipin antibodies with ssDNA, dsDNA, and cytoskeletal proteins through a common epitope or phosphodiester group has been reported.³³ Although the anticardiolipin antibodies purified by cardiolipin micelles were contaminated with albumin and other serum proteins (fig 1), as reported by Schorer *et al*,²⁶ inhibition by these proteins was unlikely as larger quantities of similar proteins were already present in the 10% fetal bovine serum in RPMI-1640 medium. How anticardiolipin antibodies suppress the mitogen induced lymphocyte proliferation is still not clear. Nevertheless, the interleukin 2 receptors on lymphocytes (table 3) and the soluble interleukin 2 receptors (table 4) in the culture supernatant were remarkably diminished in the presence of anticardiolipin antibodies. It is conceivable that these changes are the result of diminished interleukin 2 production by type 1 helper T lymphocytes.

Another interesting observation in this study was that anticardiolipin antibodies suppressed C3b and Fc receptor expressions on polymorphonuclear leucocytes which might be responsible for defective phagocytosis of the cells (table 5). In our previous study¹⁵ we found that the phagocytosis of polymorphonuclear leucocytes was defective in patients with active SLE. Possibly, the release of many inflammatory mediators is diminished in SLE as a consequence of decreased interleukin 2 production in the presence of anticardiolipin antibodies. Tumour necrosis factor α is a potent stimulant for polymorphonuclear leucocytes and can also stimulate vascular endothelial cells to promote transendothelial neutrophil passage.^{34, 35} Once tumour necrosis factor α production is decreased owing to interleukin 2 deficiency, abnormal polymorphonuclear leucocyte functions can be noted.^{36, 37} Another possibility is that the binding of anticardiolipin antibodies to polymorphonuclear leucocyte membrane interferes with C3b and IgG Fc receptor expressions and subsequently perturbs the membrane phagocytic activity. This perturbation may occur through the phosphatidylinositol-protein kinase C system because this system plays an important part in the signal transduction of cell activation and proliferation.^{38, 39} Whether the defective membrane receptor expression after binding of anticardiolipin antibodies is caused by contracted intracellular receptor pool or impaired translocation of intracellular receptors to cell surface is not clear. Measurement of creatine kinase,

intracellular $[Ca^{2+}]_i$, phospholipid metabolites, and intracellular cAMP and cGMP is necessary to confirm this possibility. In conclusion, our results suggest that anticardiolipin antibodies can exert inhibitory effects on the functions of lymphocytes and neutrophils by non-specific binding to the cell membrane. The significance of this newly found biological activity of anticardiolipin antibodies in patients with SLE needs further investigation.

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