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# Surface blebs on apoptotic cells are sites of enhanced procoagulant activity: Implications for coagulation events and antigenic spread in systemic lupus erythematosus

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**ABSTRACT** **The restriction of phosphatidylserine (PtdSer) to the inner surface of the plasma membrane bilayer is lost early during apoptosis. Since PtdSer is a potent surface procoagulant, and since there is an increased incidence of coagulation events in patients with systemic lupus erythematosus (SLE) who have anti-phospholipid antibodies, we addressed whether apoptotic cells are procoagulant and whether anti-phospholipid antibodies influence this. Apoptotic HeLa cells, human endothelial cells, and a murine pre-B-cell line were markedly procoagulant in a modified Russell viper venom assay. This procoagulant effect was entirely abolished by addition of the PtdSer-binding protein, annexin V, confirming that it was PtdSer-dependent. The procoagulant effect was also abolished by addition of IgG purified from the plasma of three patients with anti-phospholipid antibody syndrome, but not IgG from normal controls. Confocal microscopy of apoptotic cells stained with fluorescein-isothiocyanate-conjugated-annexin V demonstrated $Ca^{2+}$-dependent binding to the surface of membrane blebs on apoptotic cells, but not to intracellular membranes. Recent data indicate that the surface blebs of apoptotic cells constitute an important immunogenic particle in SLE. We propose that the PtdSer exposed on the outside of these blebs can induce the production of anti-phospholipid antibodies, which might also enhance the immunogenicity of the bleb contents. When apoptosis occurs in a microenvironment in direct contact with circulating plasma, the unique procoagulant consequences of the apoptotic surface may additionally be expressed. This might explain the increased incidence of pathological intravascular coagulation events that occur in some lupus patients who have anti-phospholipid antibodies.**

In the plasma membrane, phosphatidylserine (PtdSer), a potent surface procoagulant, is usually restricted to the inner surface of the bilayer (1). At this site, PtdSer is shielded from binding to circulating PtdSer-binding proteins and, hence, from exerting its procoagulant effect. However, the procoagulant effect of PtdSer may be revealed in a number of physiologic and pathologic conditions, including activated platelets and the irreversibly sickled erythrocytes of sickle cell anemia (2–4). Small surface microparticles on activated platelets (5), rich in essential phospholipid cofactors, appear to act as the catalytic surface for assembly of the prothrombinase complex (6). Similar surface microparticles or "spicules" have been observed to shed from the surface of sickled erythrocytes (3, 7). These spicules are identical in size to those structures seen on activated platelets (5), are PtdSer-rich (8), are similarly procoagulant (3, 8), and are devoid of spectrin (7). It has been proposed that uncoupling of the membrane skeleton from the lipid bilayer induces the formation of these surface structures (8). The normal restriction of PtdSer to the inner surface of the plasma membrane bilayer is also perturbed in apoptotic cells (9). Interestingly, the prominent small surface blebs on these cells resemble the microparticles and spicules observed in platelets and sickled erythrocytes (10). It is also striking that fodrin (nonerythroid spectrin) is cleaved during apoptosis (11), and it has been postulated that the consequent disruption of the membrane skeleton may be responsible for the observed membrane blebbing (11, 12).

The observation that nonbilayer phospholipid structures are potentially immunogenic (13) has led to the suggestion that alteration of lipid phase in the course of membrane remodeling *in vivo* may lead to the production of anti-phospholipid antibodies. The importance of the binding and altered conformation of circulating phospholipid-binding proteins like $\beta_2$-glycoprotein 1 and annexin V in the induction of this immune response has been underscored (25). The *in vivo* settings in which nonbilayer phospholipid structure arises are therefore likely marked by the elaboration of anti-phospholipid antibodies [as is the case for sickle cell disease (14)]. Anti-phospholipid antibodies are also found in patients with systemic lupus erythematosus (SLE), a disease in which apoptotic cell surface blebs appear to be a major immunogenic particle (15). Paradoxically, a subgroup of lupus patients who develop anti-phospholipid antibodies (which have anticoagulant effects in *in vitro* phospholipid-poor coagulation assays), suffer from pathological coagulation events in the venous and arterial circulations (16). We demonstrate here that anti-phospholipid antibodies and the PtdSer-binding protein annexin V bind to the surface of apoptotic cells, and abolish their procoagulant effect. Our data suggest that the procoagulant events and anti-phospholipid antibodies observed in patients might be consequences of the same pathophysiologic event *in vivo*: that is, the exposure of anionic phospholipids in nonbilayer arrangements at the surface of the apoptotic cell.

## EXPERIMENTAL PROCEDURES

**Materials.** Leupeptin, pepstatin, and aprotinin were purchased from Calbiochem. Human umbilical vein endothelial cells (HUVECs) and their culture media were obtained from Clonetics (San Diego). Phenylmethylsulfonyl fluoride (PMSF), rabbit brain cephalin, Russell viper venom (RVV) and DEAE-Sepharose CL6B were from Sigma, and fluorescein isothiocyanate (FITC) was from Pierce. The human placental cDNA library in Lambda Zap II and *Pfu* DNA polymerase were obtained from Stratagene. The prokaryotic expression vector pET22b and the $Ni^{2+}$-charged His-Bind columns were obtained from Novagen. Protein assay reagents were from Bio-Rad. All other reagents and compounds were analytical grade.

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Abbreviations: HUVEC, human umbilical vein endothelial cells; PMSF, phenylmethylsulfonyl fluoride; PtdSer, phosphatidylserine; PPP, platelet-poor plasma; RVV, Russell viper venom; SLE, systemic lupus erythematosus.

**Methods.** *Cell culture.* HeLa cells were passaged in 10% (vol/vol) heat-inactivated calf serum using standard tissue culture procedures. HUVECs were cultured and trypsinized according to the manufacturer's directions and were used at the third or fourth passage. The 103 cell line (gift of N. Rosenberg, Tufts University) was generated by transformation of murine bone marrow pre-B-cells with a temperature-sensitive Abelson murine leukemia virus (17). These cells were grown in suspension culture in 5% $CO_2$/95% air at 33°C in RPMI 1640 medium supplemented with 10% heat-inactivated fetal calf serum, 50 μM 2-mercaptoethanol, penicillin, and streptomycin.

*Preparation of apoptotic cells and fragments.* Apoptosis was induced in HUVECs by irradiation with ultraviolet B (15), followed by incubation at 37°C for 9–12 hr or by nutrient deprivation of HeLa cultures (incubation at 37°C in Krebs–Ringer buffer for 16 hr). Incubation under both of these conditions produced many floating, blebbed cells and dispersed blebs (18). Mixed populations of detached, apoptotic blebbed cells and fragments were prepared by aspiration of the medium immediately preceding harvesting, followed by centrifugation of this medium at 16,000 × *g*. In the case of the HUVEC bleb prep, an extra phosphate-buffered saline (PBS) washing step followed by 16,000 × *g* centrifugation was included to remove all serum. Typically, yields ranged from 40 to 200 μg of bleb protein per 10-cm dish of apoptotic cells. Apoptosis of 103 cells was induced by shifting cells to 39°C for 4–14 hr. In all cell types, cell death by apoptosis was confirmed by visualization of internucleosomal DNA cleavage on agarose gels and by classic apoptotic morphology (data not shown).

*Cloning and expression of human annexin V.* Annexin V was cloned by PCR performed on DNA purified from a commercially available human placental cDNA library in Lambda ZapII (Stratagene no. 936203). One hundred microliters of the amplified library was diluted with an equal volume of SET/SDS (100 mM NaCl/20 mM EDTA/50 mM Tris, pH 8.0/1% SDS) and extracted twice with phenol. Glycogen (10 μg) and NaOAc (20 μl) were added prior to ethanol precipitation of phage DNA. The DNA pellet was washed in 70% ethanol and resuspended in 10 μl of TE (10 mM Tris, pH 8.0/0.2 mM EDTA). Two microliters of this DNA was used as template in a PCR with *Pfu* DNA polymerase, with the oligonucleotides annex-5′ (5′-AGTAGTCGCCATGGCACAGG-3′) and annex 3′ (5′-CGCAAGCTTGTCATCTTCTCCACAGAG-3′). These sequences were derived from the published human annexin V cDNA sequence (19) with alterations to aid in subsequent cloning. The PCR-generated cDNA fragment, containing the entire annexin V open reading frame, was cloned into the prokaryotic expression vector pET22b, generating a his6-tagged fusion gene. Recombinant protein was purified from *Escherichia coli* BL21 induced for 2 hr at 30°C with 1 mM isopropyl β-D-thiogalactoside (IPTG). Bacteria were lysed by sonication on ice in binding buffer (5 mM imidazole/0.5 M NaCl/40 mM Tris, pH 7.9). Triton X-100 was added to 0.5%, and the lysate was centrifuged at 15,000 × *g* for 20 min at 4°C. The supernatant was passed over a 1-ml $Ni^{2+}$-charged His-Bind column, washed with 10 ml of wash buffer (60 mM imidazole/0.5 M NaCl/40 mM Tris, pH 7.9), and eluted with 2 ml of elution buffer (1 M imidazole/0.5 M NaCl/20 mM Tris, pH 7.9). Protease inhibitors (0.5 mM PMSF, 10 units of aprotinin, 2 μM leupeptin, and 2 μM pepstatin A) were added, and the eluate was dialyzed extensively against PBS containing 10% (vol/vol) glycerol and 0.5 mM PMSF. Purity of the preparation was ≈90% when assessed by SDS/PAGE and Coomassie blue staining (data not shown).

*Fluorescein isothiocyanate (FITC)-labeling of recombinant human annexin V.* Annexin V (0.35 mg) was conjugated to 0.1 mg of FITC as recommended by the manufacturer.

*Staining of cells with recombinant FITC-annexin V for flow cytometry.* The 103 cells grown at 33°C or at 39°C for 14 hr after temperature shift were pelleted by centrifugation at 300 × *g* and resuspended in 1 ml of PBS containing 3% calf serum. Each sample was divided into two equal aliquots and incubated on ice for 30 min with 0.6 μg of recombinant FITC-annexin V per ml in the presence of either 2 mM $CaCl_2$ or 2 mM EGTA. Cells were recovered by centrifugation and washed three times with PBS containing 3% calf serum and either 2 mM $CaCl_2$ or 2 mM EGTA. Cells were analyzed by flow cytometry on a FACScan instrument (Becton Dickinson).

*Immunofluorescence and confocal microscopy.* Immunofluorescence microscopy was performed on adherent HeLa cells grown on coverslips as described (15). Control or apoptotic cells were washed twice with ice-cold wash buffer (20 mM Tris, pH 7.4/150 mM NaCl) prior to incubation (20 min, 4°C) with FITC-annexin (diluted in wash buffer to 5 μg/ml) in the presence of either 1 mM $CaCl_2$ or 1 mM EDTA. Coverslips were then washed by dipping in wash buffer supplemented with either $CaCl_2$ or EDTA prior to fixation with 4% paraformaldehyde (5 min, 4°C), permeabilization with acetone (30 sec, 4°C), and subsequent staining with 5 μg of propidium iodide per ml. Coverslips were mounted on glass slides with Permount before viewing on a Leica confocal microscopy system.

*Purification of IgG fractions from human plasma.* Platelet-poor plasma (PPP) was prepared by centrifugation of citrated human venous blood obtained from patients with primary anti-phospholipid antibody syndrome or from healthy volunteers. After diluting 1:1 with 40 mM Tris (pH 8.5), the PPP was incubated (20 min, room temperature) with DEAE-Sepharose CL6B slurry. The beads were then removed by gentle centrifugation, and the IgG-containing supernatant was dialyzed against buffer containing 2.5 mM Hepes (pH 7.4) and 37.5 mM NaCl. The samples were concentrated 4-fold by Speedvac (to yield a final buffer concentration of 10 mM Hepes (pH 7.4) and 150 mM NaCl). Preparations were routinely analyzed by SDS/PAGE and Coomassie blue staining to confirm >95% purity (data not shown).

*Modified Russell viper venom (RVV) assay.* Aliquots (75 μl) of PPP were warmed (1 min) in a 37°C waterbath immediately before adding one of the following: (*i*) 105 μl of assay buffer (10 mM Hepes, pH 7.4/150 mM NaCl), (*ii*) 75 μl of apoptotic cells/fragments and 30 μl of assay buffer, (*iii*) 75 μl of apoptotic cells/fragments and 30 μl of annexin V or purified IgG, (*iv*) 75 μl of cephalin and 30 μl of assay buffer, or (*v*) 75 μl of cephalin and 30 μl of annexin V. After warming for another 1 min at 37°C, 12.5 ng of RVV was added to each reaction, followed by $CaCl_2$ (final concentration, 6.25 mM) to initiate clotting. Clot formation was detected by visual inspection, and all assays were performed at least in triplicate. To avoid bias in measuring the clotting times, clot formation was judged by two separate researchers, one of whom did not know the components of the reaction mix.

*Miscellaneous.* The protein content of all samples was determined using the Bio-Rad DC protein assay with γ-globulin as a standard. Statistical analysis was performed with the SIGMASTAT program (Jandel Scientific, Corte Madera, CA).

*Patients and controls.* IgG was purified from plasma obtained from three healthy volunteers or from three female patients (two with primary and one with secondary anti-phospholipid antibody syndrome) who had prolonged clotting times in the RVV test (a sensitive assay for the lupus anticoagulant), and had a history of a clinically significant arterial thrombotic event.

## RESULTS AND DISCUSSION

**Apoptotic Cells Are Procoagulant in the RVV Assay.** The physiologic coagulation of plasma requires the regulated exposure of a negatively charged phospholipid-rich surface to initiate and propagate the catalytic cascade (5, 20). This has been observed with activated platelets, in which surface mi-

croparticles appear to provide the catalytic membrane surface for optimal thrombin generation (5). Unregulated generation of a similar procoagulant surface occurs in irreversibly sickled erythrocytes and has been postulated to contribute to the hypercoagulable state in this disease (3). By using a modified RVV test, it has been demonstrated (9) that apoptotic thymocytes are procoagulant, a phenomenon that has been postulated to be due to the exposure of PtdSer at the apoptotic cell surface. We addressed whether this procoagulant effect was also a feature of other apoptotic cells, and whether procoagulant activity resided in apoptotic fragments. The pre-B-cell line 103 was generated by transformation of murine bone marrow pre-B-cells with a temperature-sensitive Abelson murine leukemia virus (17). Since these cells are nonadherent in culture, the effects of control cells in the RVV assay can be easily assessed. After the 103 cells were shifted to the nonpermissive temperature (39°C), they became apoptotic within 4 hr, and >50% of cells were apoptotic by 14 hr (data not shown; also see Fig. 3). Apoptosis in this population was confirmed by demonstrating the characteristic DNA ladder on an agarose gel (Fig. 1*A*). Equal numbers ($6 \times 10^6$ cells) of nonapoptotic and apoptotic 103 cells were subsequently tested in the modified RVV assay. The control clotting time obtained in the absence of added cells was unaltered by the addition of nonapoptotic cells; in contrast, apoptotic cells were procoagulant in this assay, decreasing the clotting time from 75 to 55 sec ($P < 0.001$) (Fig. 1*B*). Rabbit brain cephalin, another exogenous source of phospholipid, was also markedly procoagulant in this assay (see Fig. 4).

In previous studies, we have examined apoptosis in adherent cell populations by separating apoptotic cells from their nonapoptotic counterparts (18). This was accomplished by using gentle washing to detach the loosely adherent apoptotic cells from the substratum. When these detached apoptotic cells were tested in the RVV assay, they were also markedly procoagulant, decreasing the clotting time to 53% or 58% of control values (HeLa cells and HUVECs, respectively) (Fig. 2 *A* and *B*; $P = 0.002$ and $P = 0.01$, respectively). This effect was dose-dependent (data not shown), reaching a maximum at 22 μg of apoptotic cell protein added per assay. We have therefore observed this procoagulant effect in all three of the different apoptotic cell types examined to date (HeLa, HUVEC, pre-B-cells). Furthermore, since apoptosis was induced by a variety of different protocols (nutrient deprivation, ultraviolet B irradiation, temperature shift), it is likely that the procoagulant effect is a general feature of apoptotic cells.

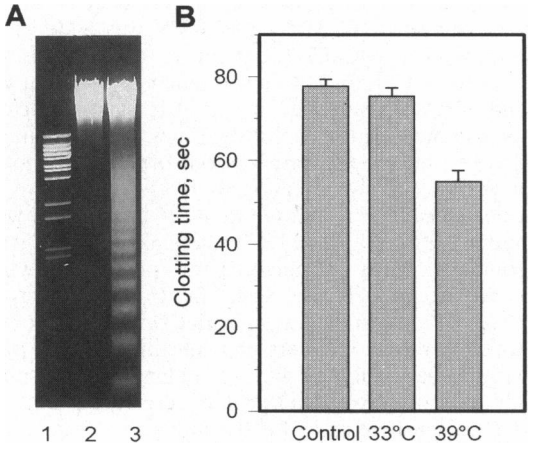


FIG. 1. Apoptotic B cells (line 103) are procoagulant in the RVV test. (*A*) DNA obtained from cells cultured at 33°C (lane 2) or 39°C (lane 3) for 14 hr was electrophoresed on agarose gels and visualized with ethidium bromide. The characteristic ladder pattern of internucleosomal DNA cleavage is seen in the cells cultured at 39°C. The migration of marker DNA is shown in lane 1. (*B*) Cells ($6 \times 10^6$) cultured at 33°C (nonapoptotic) or 39°C for 14 hr (apoptotic) were added to the modified RVV assay. Mean ± SD of triplicate clotting time determinations are presented. The experiment was repeated on three separate occasions with similar results.

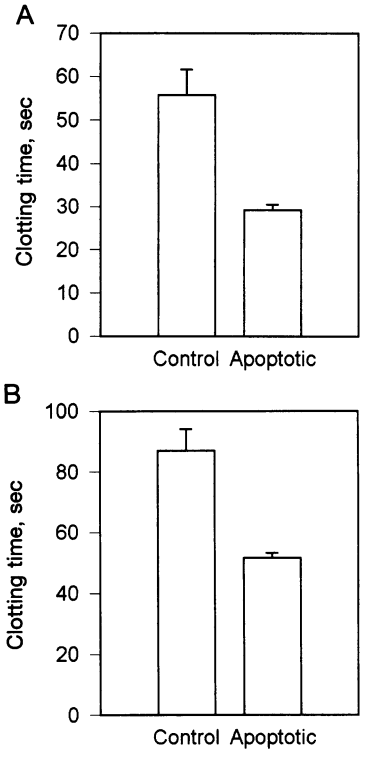


FIG. 2. Detached apoptotic HeLa cells (*A*) and endothelial cells (*B*) are procoagulant in the RVV assay. Apoptosis was induced by nutrient deprivation of HeLa cells (*A*) or by irradiation of HUVECs (*B*). Mixed populations of apoptotic cells and detached apoptotic bodies were prepared and added to the modified RVV assay. Results are means ± SD of triplicate clotting time determinations. Experiments were repeated on five (HeLa) or two (HUVEC) separate occasions with similar results.

**Procoagulant Activity of Apoptotic Cells Is PtdSer-Dependent.** The restriction of PtdSer to the inner leaflet of the plasma membrane ensures that PtdSer-binding proteins bind poorly to healthy cells. This topological restriction is perturbed in apoptotic cells, and as a consequence, the PtdSer-binding protein annexin V is able to bind to the surface of apoptotic cells (21). To directly address the sites of PtdSer exposure, recombinant human annexin V was purified, and fluorescein-labeled. Fluorescence-activated cell sorter (FACS) analysis of 103 cells with FITC-annexin V confirmed the $Ca^{2+}$-dependent binding of FITC-annexin V specifically to apoptotic, but not control 103 cells (Fig. 3 *A* and *B*). Unlike 103 cells, apoptotic HeLa cells are ideally suited for morphologic analysis. Therefore, we used FITC-annexin to identify the sites of PtdSer exposure in HeLa cells by immunofluorescence confocal microscopy. No staining of control HeLa cells was observed, either in the presence or absence of $Ca^{2+}$ (data not shown). When apoptotic cells were incubated with FITC-annexin before permeabilization, $Ca^{2+}$-dependent binding of FITC-annexin to the surface of apoptotic HeLa cells was observed (Fig. 3*C*). There was diffuse staining of the plasma membrane (absent in nonapoptotic cells) as well as strong staining of the surfaces of both small blebs and apoptotic bodies. Annexin V staining was absent from the internal membranes in nonpermeabilized cells, confirming that annexin V binding precedes the loss of membrane integrity in apoptotic cells. Annexin V is therefore a sensitive tool for the detection of early apoptotic

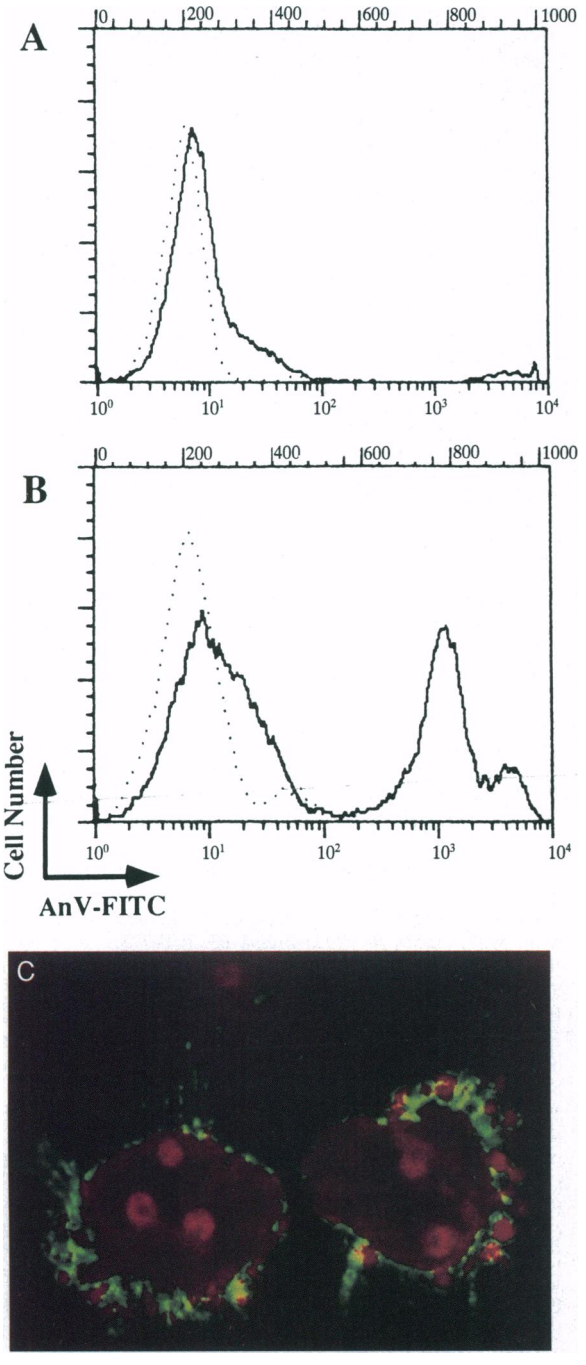


FIG. 3. Recombinant annexin V binds to the surface of apoptotic cells. (*A* and *B*) FITC-annexin V staining of pre-B-cells cultured at 33°C (*A*) or 39°C (*B*) for 14 hr. The solid line represents staining in the presence of $Ca^{2+}$, and the dotted line represents staining in the presence of EGTA as assessed by flow cytometry. (*C*) Confocal micrograph of apoptotic HeLa cells stained with FITC-annexin V in the presence of $Ca^{2+}$, prior to fixation, permeabilization with acetone, and staining with propidium iodide.

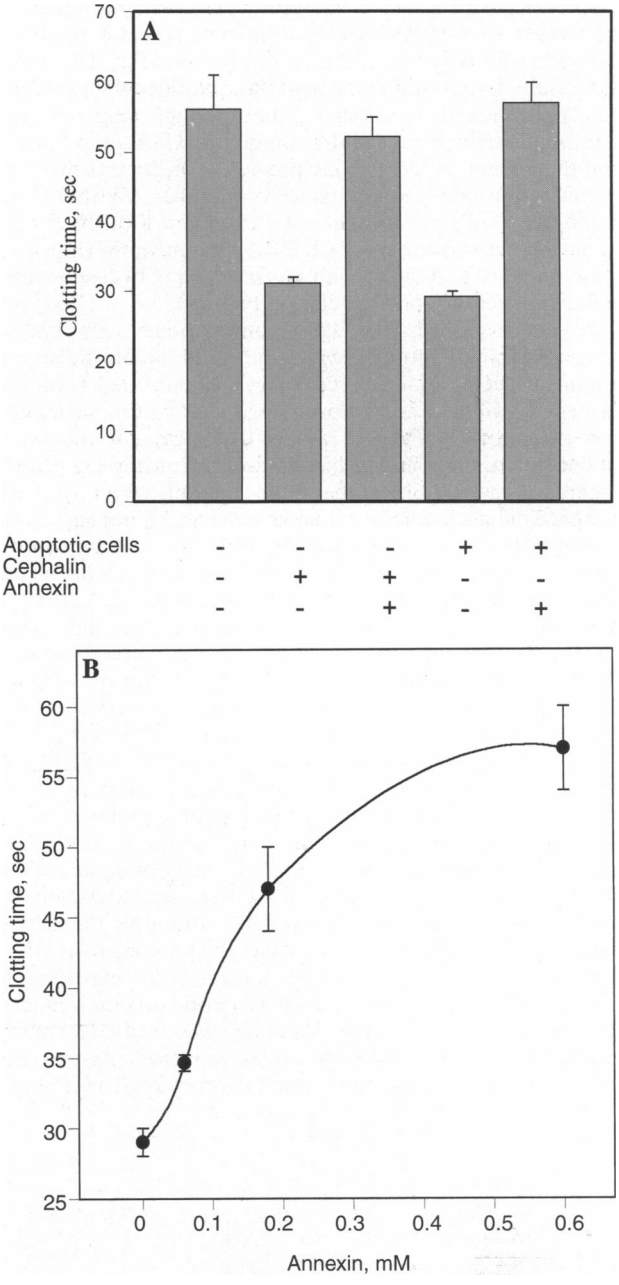


FIG. 4. Recombinant annexin V reverses the procoagulant effect of apoptotic HeLa cells and cephalin. (*A*) The indicated combinations of apoptotic HeLa cells, cephalin, and annexin V were used in the modified RVV assay. (*B*) Increasing amounts of annexin were added to a modified RVV assay performed in the presence of a constant amount of apoptotic HeLa cells (0.13 μg of cell protein per μl). Half-maximal reversal of the procoagulant effect was obtained with 150 μM annexin V. The results are presented as means ± SD of triplicate clotting time determinations. The experiment was repeated on three separate occasions with two different preparations of recombinant annexin V.

cells and might have utility for studying the early membrane phospholipid rearrangements that are a feature of the apoptotic process. There is accumulating evidence to suggest that the targeting of negatively charged phospholipids by the immune system in SLE occurs as a phospholipid–protein complex, and that the "anti-phospholipid antibodies" are directed against complexes of phospholipid-binding proteins like $\beta_2$-glycoprotein 1 or annexin V with phospholipid, rather than phospholipid alone (reviewed in ref. 22). The demonstration of $Ca^{2+}$-dependent annexin-binding to the surface blebs of apoptotic cells, a site at which most of the lupus autoantigens are clustered (15), is therefore of great significance. Since binding of autoantibody to one component of a multicomponent complex can influence the subsequent processing and presentation of other antigens in the complex (23), it is also possible that anti-phospholipid antibody coating of apoptotic blebs might enhance the subsequent immunogenicity of these autoantigen clusters.

Since annexin V has known anticoagulant properties (19), we addressed whether recombinant annexin V could reverse

the procoagulant effect of apoptotic cells. When annexin V was added to the RVV assay, the procoagulant effect of apoptotic cells was reversed to control levels (Fig. 4*A*). Identical results were obtained using rabbit brain cephalin, rather than apoptotic cells, as a source of phospholipid (Fig. 4*A*). The saturating inhibitory concentration of annexin V on phospholipid-dependent processes has previously been shown to be dependent on phospholipid concentration (24). With 0.13 $\mu$g of apoptotic cells per $\mu$l, half-maximal reversal of the procoagulant effect was observed at 150 $\mu$M annexin V (Fig. 4*B*). These data are consistent with annexin acting by a substrate-depletion effect in the coagulation pathway.

We addressed whether IgG from patients with known anti-phospholipid antibody syndrome could inhibit the procoagulant effect of apoptotic cells. IgG was purified from the plasma of patients who had prolonged clotting times (defined by a prolonged RVV test, a sensitive assay for the lupus anticoagulant) and had a history of a clinically significant thrombotic event. Since the anticoagulant effect of anti-phospholipid antibodies (as well as annexin V) appears to be via substrate depletion, we predicted that their ability to fully reverse the procoagulant effect would be dependent on the concentration of phospholipid used in the assay. Therefore, we chose a dilution of apoptotic cells within the linear range of the procoagulant effect. This concentration was lower than that used in the assays involving annexin V by a factor of 6. Under these conditions, IgG from these patients fully reversed the procoagulant effect of apoptotic cells in the RVV test (Fig. 5 *A–E*). In contrast, equivalent amounts of IgG purified from healthy controls had no effect on the clotting time in this assay (Fig. 5*F*). Patient IgG was less effective (on a molar basis) at inhibiting the procoagulant effect of apoptotic cells than annexin V, probably due to the heterogeneity of lupus patient IgG, different relative avidities for PtdSer, and steric differences between IgG and annexin V. Although the actual specificity(ies) of the IgG that reverses the procoagulant effect of apoptotic cells is (are) not yet known, these experiments demonstrate that the procoagulant apoptotic surface is indeed a target of lupus patient IgG. When IgG was purified from the serum of one of these patients (rather than from plasma), the purified IgG was unable to reverse the procoagulant effect of added cephalin or apoptotic cells (data not shown). Since activated platelets expose a similar procoagulant surface, it is likely that the relevant anticoagulant IgG molecules were consumed during the natural platelet-induced clotting process *in vitro*.

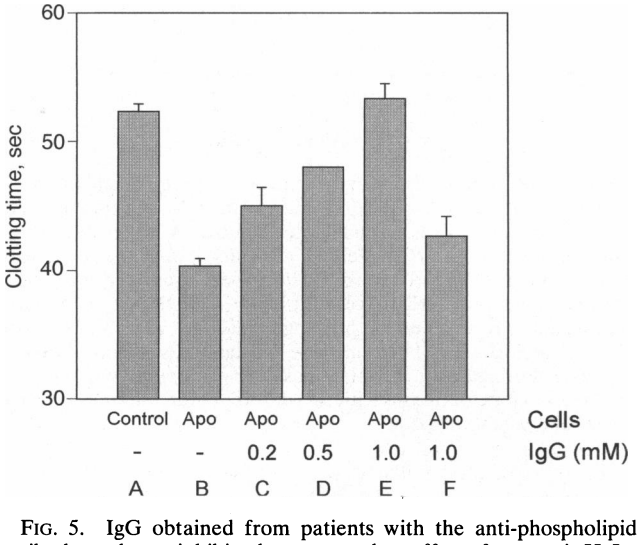


FIG. 5. IgG obtained from patients with the anti-phospholipid antibody syndrome inhibits the procoagulant effect of apoptotic HeLa cells. Modified RVV assays were performed in the absence of added cells or IgG ("control") or in the presence of a constant amount of apoptotic cells with the indicated amounts of IgG added. The results are presented as means ± SD of triplicate clotting time determinations. Three different patients and three different controls were examined, and all gave similar results. A representative example of each is presented.

The surface blebs of apoptotic cells are a major immunogenic particle in patients with SLE (15). These studies demonstrate that plasma membrane phospholipid distribution is altered in these surface structures, enabling the binding of annexin V and generating a procoagulant surface. When this process involves cells that are normally exposed to circulating plasma (e.g., vascular endothelial cells), this surface might manifest its procoagulant and immunogenic properties, resulting both in pathologic coagulation events and the generation of antiphospholipid antibodies. A similar situation may exist in sickle cell disease, where the procoagulant and immunogenic surface is found on irreversibly sickled erythrocytes (3, 14). The prevention of apoptosis-induced coagulation by administration of phospholipid-binding proteins like annexin V might have therapeutic potential in a number of pathologic coagulation states.

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1. Cullis, P. R. & De Kruijff, B. (1979) *Biochim. Biophys. Acta* **559,** 399–420.
2. Choe, H., Schlegel, R. A., Rubin, E., Williamson, P. & Westerman, M. P. (1986) *Br. J. Haematol.* **63,** 761–773.
3. Westerman, M. P., Cole, E. R. & Wu, K. (1984) *Br. J. Haematol.* **56,** 557–562.
4. Bevers, E. M., Comfurius, P., van Rijn, J. L. M. L., Hemker, H. C. & Zwaal, R. F. A. (1982) *Eur. J. Biochem.* **122,** 429–436.
5. Sims, P. J., Wiedmer, T., Esmon, C. T., Weiss, H. J. & Shattil, S. J. (1989) *J. Biol. Chem.* **264,** 17049–17057.
6. Sims, P. J., Faioni, E. M., Wiedmer, T. & Shattil, S. J. (1988) *J. Biol. Chem.* **263,** 18205–18212.
7. Allan, D., Limbrick, A. R., Thomas, P. & Westerman, M. P. (1982) *Nature (London)* **295,** 612–613.
8. Franck, P. F. H., Bevers, E. M., Lubin, B. H., Comfurius, P., Chiu, D. T.-Y., Op den Kamp, J. A. F., Zwaal, R. F. A., van Deenen, L. L. M. & Roelofsen, B. (1985) *J. Clin. Invest.* **75,** 183–190.
9. Fadok, V. A., Voelker, D. R., Campbell, P. A., Cohen, J. J., Bratton, D. L. & Henson, P. M. (1992) *J. Immunol.* **148,** 2207–2216.
10. Arends, M. J. & Wyllie, A. H. (1991) *Int. Rev. Exp. Pathol.* **32,** 223–254.
11. Martin, S. J., O'Brien, G. A., Nishioka, W. K., McGahon, A. J., Mahboubi, A., Saido, T. C. & Green, D. R. (1995) *J. Biol. Chem.* **270,** 6425–6428.
12. Martin, S. J. & Green, D. R. (1995) *Cell* **82,** 349–352.
13. Rauch, J. & Janoff, A. S. (1990) *Proc. Natl. Acad. Sci. USA* **87,** 4112–4114.
14. Kucuk, O., Gilman-Sachs, A., Beaman, K., Lis, L. J. & Westerman, M. P. (1993) *Am. J. Hematol.* **42,** 380–383.
15. Casciola-Rosen, L. A., Anhalt, G. & Rosen, A. (1994) *J. Exp. Med.* **179,** 1317–1330.
16. Petri, M. (1994) *Rheum. Dis. Clin. North Am.* **20,** 443–469.
17. Chen, Y. Y., Wang, L. C., Huang, M. S. & Rosenberg, N. (1994) *Genes Dev.* **8,** 688–697.
18. Casciola-Rosen, L. A., Miller, D. K., Anhalt, G. J. & Rosen, A. (1994) *J. Biol. Chem.* **269,** 30757–30760.
19. Maurer-Fogy, I., Reutelingsperger, C. P. M., Pieters, J., Bodo, G., Stratowa, C. & Hauptmann, R. (1988) *Eur. J. Biochem.* **174,** 585–592.

20. Chang, C. P., Zhao, J., Wiedmer, T. & Sims, P. J. (1993) *J. Biol. Chem.* **268,** 7171–7178.
21. Koopman, G., Reutelingsperger, C. P., Kuitjen, G. A., Keehnen, R. M., Pals, S. T. & van Oers, M. H. (1994) *Blood* **84,** 1415–1420.
22. Triplett, D. A. (1995) *Thromb. Res.* **78,** 1–31.
23. Lanzavecchia, A. (1995) *J. Exp. Med.* **181,** 1945–1948.
24. Ravanat, C., Archipoff, G., Beretz, A., Freund, G., Cazenave, J. P. & Freyssinet, J. M. (1992) *Biochem. J.* **282,** 7–13.
25. McNeil, H. P., Simpson, R. J., Chesterman, C. N. & Krilis, S. A. (1990) *Proc. Natl. Acad. Sci. USA* **87,** 4120–4124.