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Human serum amyloid P component (SAP) is a glycoprotein structurally belonging to the pentraxin family of proteins, which has a characteristic pentameric organization. Mice with a targeted deletion of the SAP gene develop antinuclear Abs, which was interpreted as evidence for a role of SAP in controlling the degradation of chromatin. However, in vitro SAP also can bind to phosphatidylethanolamine, a phospholipid which in normal cells is located mainly in the inner leaflet of the cell membrane, to be translocated to the outer leaflet of the cell membrane during a membrane flip-flop. We hypothesized that SAP, because of its specificity for phosphatidylethanolamine, may bind to apoptotic cells independent of its nuclear binding. Calcium-dependent binding of SAP to early, nonpermeable apoptotic Jurkat, SKW, and Raji cells was indeed observed. Experiments with flip-flopped erythrocytes confirmed that SAP bound to early apoptotic cells via exposed phosphatidylethanolamine. Binding of SAP was stronger to late, permeable apoptotic cells. Experiments with enucleated neutrophils, with DNase/RNase treatment of late apoptotic Jurkat cells, and competition experiments with histones suggested that binding of SAP to late apoptotic cells was largely independent of chromatin. Confocal laser microscopic studies indeed suggested that SAP bound to these apoptotic cells mainly via the blebs. Thus, this study shows that SAP binds to apoptotic cells already at an early stage, which raises the possibility that SAP is involved in dealing with apoptotic cells in vivo. The Journal of Immunology, 2001, 167: 647–654.

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Atoosa Familian,* Bas Zwart,* Han G. Huisman,* Irma Rensink,* Dorina Roem,* Peter L. Hordijk,* Lucien A. Aarden,* and C. Erik Hack*†

Human serum amyloid P component (SAP) is a glycoprotein consisting of 10 noncovalently associated identical subunits of 23,500 Da organized as two face-to-face pentagonal structures. SAP is structurally related to C-reactive protein (CRP), a major acute-phase reactant (APR) in humans. Both proteins are found in all mammals. Individuals unable to synthesize SAP or CRP have not been described, suggesting these proteins fulfill important biological functions (1–3).

SAP and CRP are members of the pentraxin family, proteins with a characteristic pentameric organization. They share 51% of amino acid identity and 59% nucleotide sequence identity. Their genes are closely linked and are located in band q2.1 of chromosome 1, and either protein can bind C1q and activate the complement system via the classical pathway. Although in humans circulating levels of CRP may increase up to 1000-fold during an acute phase response, plasma levels of SAP hardly change, being in a range of 30–40 μg/ml. However, in some other animal species, SAP rather than CRP reacts as APR (1, 3).

SAP does not exist only in plasma but also can be found in amyloid deposits (4) and in the normal renal glomerular basement membrane (5, 6), as well as in elastic fibers in the blood vessel walls (7). In the presence of calcium ions, SAP can bind to several ligands such as amyloid fibrils of any type (4), agarose (8), heparin and dermatan sulfate (9), C1q (10, 11), C4 binding protein (12, 13), laminin (14), type V collagen (15), and phosphoethanolamine-containing compounds such as phosphatidylethanolamine (16, 17), DNA (18), chromatin (19, 20), and histones (21, 22).

Recently, some insight in the function of SAP was obtained in mice that were made deficient by targeted disruption of the gene coding for SAP. The majority of these SAP-deficient mice appeared to develop antinuclear Abs, which was interpreted as evidence that SAP is controlling the degradation of chromatin in vivo (23). Moreover, it was postulated that SAP bound to apoptotic cells via chromatin fragments exposed on the blebs of these cells. The formation of chromatin-bearing blebs on apoptotic cells occurs in a late stage of apoptosis, i.e., when these cells become leaky. These cells often are referred to as late apoptotic or secondary necrotic cells. Earlier during apoptosis, when the integrity of the membrane is still intact, changes in membrane phospholipids occur (24–27). In normal cells, phospholipids are distributed asymmetrically between the inner and outer leaflet of the membrane. In those cells, most aminophospholipids, i.e., phosphatidylethanolamine and to a lesser extent, phosphatidylethanolamine, are located in the inner leaflet of the plasma membrane (28–31). One of the earliest events during apoptosis is the loss of this asymmetry, leading to the exposure of phosphatidylethanolamine in the outer leaflet (32), a phenomenon called “membrane flip-flop.” As SAP can bind to phosphatidylethanolamine, it can be hypothesized that this pentraxin may bind to apoptotic cells as soon as their membranes are flip-flopped. As there are no studies regarding the precise ligands for SAP on apoptotic cells, we studied binding of SAP to early and late apoptotic cells as well as to erythrocytes incubated with Ca-ionophore as a model for flip-flopped membranes. Our results indicate that SAP can bind to both early and late apoptotic cells independent of chromatin.
Materials and Methods

Materials
Phosphorylethanolamine (PE) and phosphorylcholine (PC) were obtained from Sigma (St. Louis, MO). Phycocerythrin-labeled streptavidin (streptavidin-phycocerythrin) was obtained from Becton Dickinson Immunocytometry System (Mountain View, CA). Propidium iodide (PI) was purchased from Calbiochem (La Jolla, CA).

Recalcified plasma
Blood was collected from healthy volunteers in siliconized tubes containing sodium citrate at a final concentration of 10 mM. Plasma was separated by centrifugation at 1300 × g for 10 min at 4°C, supplemented with CaCl2 to yield a final concentration of 10 mM, and incubated for 10 min at 37°C followed by 30 min at 4°C. Hereafter, a clot formed that was removed by centrifugation at 1300 × g for 10 min at 4°C. The supernatant (recalci- fied plasma) was stored in aliquots at −70°C until use.

Purification of SAP
Human SAP was purified from normal human plasma by affinity column chromatography as described by Ying et al. (33). In brief, recalciﬁed plasma was applied to a Bio-gel A 0.5-m column (Bio-Rad Laboratories, Hercules, CA) equilibrated with veronal-buffered saline, pH 7.4, containing 10 mM CaCl2, and 2 mM MgCl2 (VB + ). The column was washed extensively with the same buffer, and SAP was eluted with veronal-buffered saline containing 10 mM EDTA (VBEDTA). Purity of SAP was determined by SDS-PAGE (12.5%, w/v). The purified protein gave a single band of Mr ≈ 25,000 under reducing conditions. Human SAP also was purchased from Sigma. Under reducing conditions, purified SAP migrated as a single band comparable to commercial protein. In immunoblotting experiments with rabbit antiserum directed against human SAP, either preparation of SAP yielded a single band of Mr ≈ 25,000.

Antibodies
Rabbit antiserum against human SAP was made by repeated i.m. injection with SAP in rabbits. Polyclonal anti-human SAP Abs (PaSAP) were puriﬁed from the serum by immunoaffinity column chromatography; SAP was covalently coupled to cyanogen bromide-activated Sepharose 4B (Pharmacia Fine Chemicals, Uppsala, Sweden); rabbit serum was passed through the column and recycled three times; the column then was washed with PBS, pH 7.4, until A280 nm was <0.01. Finally anti-SAP Abs were eluted with 0.1 M glycine-HCl, pH 2.5, and dialyzed against PBS. Rabbit polyclonal anti-SAP also was purchased from Dako (Carpinteria, CA).

Mouse mAbs against human SAP were prepared by fusing spleen cells from mice immunized with recombinant SAP or SAP-depleted plasma containing varying amounts of plasma or purified SAP and incubated for 30 min at 37°C. Cells were washed four times with HEPES buffer, pH 7.4 containing 0.5% BSA. Positive clones were subcloned by repeated limiting dilutions. All mAbs were puriﬁed from culture supernatant by affinity chromatography on protein-A Sepharose (Pharmacia).

Biotinylation of anti-SAP and purified SAP
PaSAP either prepared in our laboratory or obtained commercially, mAb aSAP-14 or purified SAP were biotinylated with LC-biotin-cinimide ester (Pierce, Rockford, IL) according to instruction of manufacturer.

Depletion of SAP from plasma
SAP-depleted plasma was prepared by passage of recalciﬁed plasma over a Bio-gel A 0.5-m column equilibrated with VB + at 4°C. To ensure that trace amounts of SAP also were removed, this procedure was repeated once. By a quantitative ELISA performed with monoclonal anti-human SAP (aSAP-14) as a coating Ab and biotinylated PaSAP as a detecting Ab, it was established that this depleted plasma contained less than 1% of the normal plasma level of SAP.

Induction of membrane flip-flop in erythrocytes
Human erythrocytes were isolated from fresh heparinized whole blood by centrifugation at 1300 × g for 10 min, cleared from buffy coat, and washed carefully with sterile isotonic saline to remove plasma and contaminating white blood cells. Cells were suspended in IMDM at 37°C. After extensive washing with HEPES buffer, cells were incubated for 20 min at 37°C in the dark. Binding assays also were performed with cells incubated with 5 µg/ml biotinylated SAP in HEPES buffer following the same procedure described above.

Binding of SAP to cells
Vital and apoptotic cells were washed with IMDM and distributed in 96-wells microtiter plates (2 × 104 cells/well). The cells then were centrifuged at 1000 × g for 2 min, and the pellets were resuspended in 100 µl of IMDM containing varying amounts of plasma or purified SAP and incubated for 30 min at 37°C. Cells were washed four times with HEPES buffer, pH 7.4 containing 0.5% BSA. Binding was detected by a subsequent incubation with biotinylated PaSAP or mAb aSAP-14 (5 µg/ml in HEPES buffer, final volume 50 µl) for 30 min at 4°C. The cells were washed three times with HEPES buffer and stained with Annexin V-FITC (Boehringer Ingelheim, Ingelheim, Germany; final dilution 1/150) and streptavidin-phycocerythrin (final dilution 1/150) in the same buffer, final volume 50 µl, for 20 min at 4°C in the dark. Binding assays also were performed with cells incubated with 5 µg/ml biotinylated SAP in HEPES buffer following the same procedure described above.

Binding of SAP to neutrophils or cytoplasts was analyzed in similar experiments except that the Jurkat cells were replaced by the appropriate cells. In case cytoplasts were analyzed, permeability of the cells was assessed by uptake of fluorescein diacetate (FDA). To test the nature of the cytoplasmic protein as a marker for loss of membrane integrity followed by a FITC-labeled second Ab (as an alternative for PI). As this prevented the use of FITC-labeled annexin V, merocyanine 540 (final concentration 20 µg/ml; Sigma) was applied as a
marker for membrane flip-flop. Binding of SAP was determined with biotinylated mAb and allophycocyanin-conjugated streptavidin as described above.

To confirm calcium dependency of the binding of SAP, recalcified plasma at the same indicated dilutions was preincubated either with or without 10 mM EDTA for 30 min at 37°C before incubation with vital or apoptotic cells. The experiments were completed as described above.

As other tests for specificity, recalcified plasma was preincubated for 30 min at 37°C with varying concentrations of PE (0 – 40 mg/ml), PC (0 – 40 mg/ml), or histones (H1, H2a, H2b, H3, or H4; 0 – 40 mg/ml) before incubation with cells.

Binding of SAP to apoptotic cells deprived of nucleic acids was investigated by incubating late apoptotic cells for 60 min at 37°C with different concentrations of DNase I (0 – 250 μg/ml; Sigma), with or without RNase (0–500 μg/ml; Sigma), before incubation with recalcified plasma. Complete digestion of DNA and RNA was assessed by PI staining of the cells with either fluorescence microscopy or FACS analysis.

**FACS analysis**

Binding of Annexin V-FITC or SAP was measured by flow cytometry. Leakage of the cells was assessed by staining with PI. After appropriate incubations, the cells were finally washed, and relative fluorescence intensity was measured with a flow cytometer (FACScan; Becton Dickinson). Experiments including streptavidin-allophycocyanin intensity were performed with a FACSCalibur (Becton Dickinson).

**Confocal laser scanning microscopy**

Early, late, or mixed apoptotic Jurkat cells were incubated with 50% v/v recalcified plasma in IMDM for 30 min at 37°C. After washing with washing buffer (HEPES buffer containing 2% BSA), the cells were incubated with 5 μg/ml mAb aSAP-14 in washing buffer for 30 min at 4°C, washed, and stained with annexin V coupled to FITC (diluted 1/1500) and 20 μg/ml Alexa568-conjugated goat anti-mouse Ab (Molecular Probes, Eugene, Oregon) in the same buffer for 20 min at 4°C in the dark. At the end of incubation, the cells were transferred to poly-L-lysine-coated microscope slides (Sigma) and fixed with 1% paraformaldehyde for 10 min. Images were recorded with a Zeiss LSM 510 confocal laser scanning microscope (Zeiss, Oberkochen, Germany) using appropriate filter settings.

**Results**

**Binding of SAP to apoptotic cells**

Based on binding of annexin V-FITC and PI, cells can be divided into three subpopulations, i.e., vital and early and late apoptotic cells (Fig. 1A). To analyze binding of SAP to vital, early apoptotic, or late apoptotic cells within one experiment, populations of these cells were combined and incubated with recalcified plasma. Bound SAP was detected by incubation with biotinylated mAb aSAP-14 and streptavidin coupled to allophycocyanin. Based on annexin V and PI staining, the cells were divided into three different populations (A). Histograms show binding of SAP to each population in presence (B) or absence (C) of recalcified plasma.

**FIGURE 1.** Binding of SAP to vital, early, and late apoptotic Jurkat cells. Early and late apoptotic Jurkat cells were prepared as described in Material and Methods, mixed, and incubated in IMDM with or without 50% (v/v) recalcified plasma and subsequently with biotinylated mAb aSAP-14. Thereafter, cells were stained with Annexin V-FITC, PI, and streptavidin-allophycocyanin. Based on annexin V and PI staining, the cells were divided into three different populations (A). Histograms show binding of SAP to each population in presence (B) or absence (C) of recalcified plasma.

**FIGURE 2.** Dose-response curves of binding of SAP to cells. The combination of early and late apoptotic Jurkat cells was incubated with increasing amount of purified SAP followed by biotinylated mAb aSAP-14. Top, Binding to early apoptotic cells. Bottom, Binding to late apoptotic population. Binding to vital cells is indicated in each panel, and curves represent MFI (n = 4).
Binding of SAP to other cells

To show specificity of binding for apoptotic cells, we also tested other cell lines, i.e., SKW and Raji cells (both Burkitt’s lymphoma B cell lines) as well as isolated neutrophils. Apoptosis was induced by incubation with etoposide or cyclohexamide for cell lines and 24 h culture for neutrophils as described in Materials and Methods. Binding of SAP to early and late apoptotic SKW, Raji, and neutrophils was observed, similar to Jurkat cells (data not shown).

Specificity of the binding of SAP to apoptotic cells

Specificity of the binding of SAP to apoptotic cells (Jurkat as well as SKW and Raji) was demonstrated in several ways.

Binding of purified SAP. Binding of SAP was tested by incubating apoptotic or vital cells with the purified protein instead of recalcified plasma. A dose-dependent binding of purified SAP was observed only to the apoptotic cells (Fig. 2). The same results were observed when biotinylated SAP was used instead of purified SAP and biotinylated Abs (data are not shown).

Incubation with SAP-depleted plasma. Cells were incubated with 50% v/v recalcified plasma either depleted or not for SAP, as described in Materials and Methods. The results showed that early and late apoptotic cells did not bind SAP on incubation with SAP-depleted plasma. Supplementing the SAP-depleted plasma with purified SAP again resulted in fixation of this protein to apoptotic cells (Fig. 3). These results indicated that the observed SAP binding was not due to aspecific binding of the anti-SAP Abs to the cells.

Binding in presence of EDTA. Binding of SAP to various ligands is calcium-dependent (3, 16). Incubation of apoptotic cells with recalcified plasma in the presence of 10 mM EDTA resulted in a decreased binding of SAP to apoptotic cells, i.e., from a median fluorescence intensity (MFI) of 4530–1950 for late apoptotic cells
indicated histones. The experiments in A was incubated with plasma preincubated with or without increas-
SAP on apoptotic cells, the mixture of early and late apoptotic cells was inhibited by EDTA or PE.
revealed that the binding of SAP to the flip-flopped erythrocytes that observed with early apoptotic cells. Additional experiments less than that observed with late apoptotic cells and comparable to erythrocytes (Fig. 4). The intensity of staining was considerably attributable to interaction with flip-flopped membrane or with other (intracellular) structures, we did several experiments. First, we studied binding of SAP to erythrocytes that had been induced to undergo a membrane flip-flop by incubation with ionomycin. We presumed that binding to these cells would occur via the membrane only, because these cells lack a nucleus. Indeed, SAP, either in plasma or purified, appeared to bind to annexin V-positive erythrocytes (Fig. 4). The intensity of staining was considerably less than that observed with late apoptotic cells and comparable to that observed with early apoptotic cells. Additional experiments revealed that the binding of SAP to the flip-flopped erythrocytes was inhibited by EDTA or PE.
To expand the role of phosphatidylethanolamine as a ligand for SAP on apoptotic cells, the mixture of early and late apoptotic cells was incubated with plasma preincubated with or without increas-
ing amounts of PE or PC, as described in Materials and Methods. Complete inhibition to early apoptotic and significant inhibition to late apoptotic cells was observed only after preincubation with PE and not with PC (Fig. 5).
The difference in binding between late and early apoptotic cells may be because of binding of SAP to different intracellular structures. To evaluate the possibility of chromatin as the intracellular binding site for SAP in late apoptotic cells, late apoptotic Jurkat cells were incubated with increasing amounts of DNase and RNase before incubation with plasma. After treatment with DNase/ RNase, late apoptotic cells showed remarkably reduced binding of PI, whereas the binding of SAP was not affected by this treatment (Fig. 6A). In addition, preincubation of recalified plasma with different amounts of histones (H1, H2a, H2b, H3, or H4) as other possible ligands for SAP resulted in no inhibitory effect on binding to early and late apoptotic cells (Fig. 6B).
Neutrophil cytoplasts are enucleated cells consisting of a plasma membrane, cytoplasm, and some endoplasmic reticulum. Enucleation is achieved by separation of karyoplasts from neutrophils via specific gravity centrifugation (35). The composition of surface Ags of neutrophil cytoplasts is the same as intact neutrophils. Because cytoplasts have a similar cell membrane as nucleated neutrophils but are devoid of nuclear material, we examined binding of SAP to cytoplasts to establish the contribution of chromatin to the observed binding of SAP to apoptotic cells. Staining of the cytoplasts with merocyanin 540 and anti-p67 to assess membrane flip-flop and leakiness revealed three populations of cells, i.e., non-

![FIGURE 6](image1.png)

The experiments with purified histones and DNase/RNase treatment, as well as the experiments with neutrophilic cytoplasts did not show inhibition of binding of SAP to apoptotic cells, whereas the binding to merocyanin 540-positive cytoplasts was observed only after preincubation with PE (Fig. 6B).

![FIGURE 7](image2.png)

and from 140 to 28 for early apoptotic cells (MFI for vital cells was 27; n = 3). Furthermore, SAP bound to apoptotic cells could be dissociated by incubation with EDTA (results not shown).

**Ligands for SAP on apoptotic cells**

Because of its specificity for phosphoethanolamine, SAP may bind to the flip-flopped membrane of apoptotic cells, but because of its specificity for nuclear materials, such as histones and DNA, it also may bind intracellularly to nucleosomes and other chromatin material. To determine whether SAP binding to apoptotic cells was attributable to interaction with flip-flopped membrane or with other (intracellular) structures, we did several experiments. First, we studied binding of SAP to erythrocytes that had been induced to undergo a membrane flip-flop by incubation with ionomycin. We presumed that binding to these cells would occur via the membrane only, because these cells lack a nucleus. Indeed, SAP, either in plasma or purified, appeared to bind to annexin V-positive erythrocytes (Fig. 4). The intensity of staining was considerably less than that observed with late apoptotic cells and comparable to that observed with early apoptotic cells. Additional experiments revealed that the binding of SAP to the flip-flopped erythrocytes was inhibited by EDTA or PE.
To expand the role of phosphatidylethanolamine as a ligand for SAP on apoptotic cells, the mixture of early and late apoptotic cells was incubated with plasma preincubated with or without increas-

![FIGURE 7](image3.png)
not point to chromatin as the main ligand for SAP in late apoptotic cells. To further examine this, binding of SAP to apoptotic cells also was studied with a confocal laser microscope. A combination of the cells incubated with etoposide for 5 or 20 h were incubated with 50% recalcified plasma followed by incubation with monoclonal anti-SAP Abs. The cells were stained with Annexin VFITC and Alexa568-conjugated streptavidin. In control experiments, cells incubated without plasma were found to be negative for red staining (Fig. 8A). Subsequent to incubation with plasma, the majority of the annexin V-positive cells appeared to bind SAP (Fig. 8B). Each population was tested separately as well. Cells incubated for 5 h with etoposide predominantly showed a patchy binding of annexin V and SAP (Fig. 8, C–E). Fluorescent intensity predominantly occurred in clusters on the surface of the cells, some clusters being positive for both reagents. Cells incubated with etoposide for 20 h showed nuclear staining for SAP as well as membrane staining (Fig. 8, F–H). Vital cells showed neither staining for annexin VFITC nor SAP (results not shown).

Discussion

The function of human SAP, a member of the pentraxin family of proteins, is unknown. In the present study, we provide evidence that SAP, either in recalcified plasma or purified, binds to apoptotic, but not to normal, Jurkat, Raji, or SKW cells. Moreover, binding of SAP was more pronounced to late than to early apoptotic cells. Experiments with purified SAP confirmed that binding of SAP in plasma to apoptotic cells did not occur via other plasma components.

A recent study has demonstrated binding of CRP, a homologue of SAP, to apoptotic tumor cell lines (36). Binding of CRP to apoptotic nontumor cells was not mentioned in that study. Here we demonstrate binding of SAP to flip-flopped erythrocytes as well as to flip-flopped cytoplasts (see Figs. 4 and 7). In addition, we have observed binding of SAP to flip-flopped neutrophils. Hence, these data rule out the possibility that SAP binds exclusively to tumor cell lines and not to normal cells.
To establish that SAP indeed binds to apoptotic cells via phosphatidylethanolamine exposed in the flip-flopped membrane, we also studied binding of SAP to erythrocytes. A membrane flip-flop was induced in these cells by increasing the intracellular calcium concentration (34). Indeed most erythrocytes incubated with calcium-ionophore stained positively for annexin V, a well-established marker for membrane flip-flop (32, 37). SAP was found to bind to flip-flopped, but not to normal, erythrocytes. This binding was specific, as it was observed with different SAP preparations, dependent on the presence of calcium, and inhibited by PE. The binding of SAP to flip-flopped erythrocytes much resembled that to early apoptotic cells. Altogether, these data led us to conclude that SAP binds to early apoptotic cells via phosphatidylethanolamine exposed in the outer leaflet of the membrane.

We consistently found that SAP bound to late apoptotic cells with a higher affinity compared with early apoptotic cells or flip-flopped erythrocytes. The difference in binding affinity between late and early apoptotic cells may be attributable to presence of additional ligands on late apoptotic cells like chromatin, or alternately an enhanced exposure of phosphatidylethanolamine on these cells. On double-staining of late apoptotic cells with PI and SAP, it appeared that most cells that had taken up PI, indicating that these cells were leaky, strongly bound SAP. Considering the specificity of SAP for DNA, histones, and chromatin (18–22), it hence can be speculated that SAP bound to nucleosomes disposed to membranes of apoptotic cells or pass through the cells and bind to nucleus. To investigate this, we used confocal laser scanning microscopy. This approach revealed that SAP predominantly bound to membranes of bleb-like structures on the apoptotic cells, although in some apoptotic cells nuclear staining was observed next to membrane clusters. Treatment of the late apoptotic cells with DNase and RNase to digest DNA and RNA or preincubation of plasma before incubating those cells with various histones did not decrease binding of SAP to late apoptotic cells, whereas preincubation with PE showed complete inhibition of the binding to early and a significant inhibition of that to late apoptotic cells. Experiments with neutrophil cytoplasts, which are devoid of nuclear material (35), also showed restricted binding of SAP to flip-flopped cytoplasts and strongly to cytoplasts staining with an Ab against the cytoplasmic protein P67, which reflects loss of membrane integrity. Hence, although we cannot exclude the possibility that SAP binds to nucleosomes as well, we suggest that the stronger binding of SAP to late apoptotic cells was, at least in part, attributable to binding to the flip-flopped membranes of apoptotic blebs. Notably, strong binding of CRP also has been postulated to occur mainly to blebs (36).

Previously, we have hypothesized that another pentraxin, CRP, binds to flip-flopped membranes in particular when these membranes contain phospholipids hydrolyzed by secretory phospholipase A2. The reason for this requirement for secretory phospholipase A2 likely is that to be able to bind CRP, the tight package of lipase A2 likely is that to be able to bind CRP, the tight package of phospholipids needs to be loosened to allow CRP to get access to the lipase A2. The reason for this requirement for secretory phospholipids of proteins interacting specifically with phosphorylcholine and/or phosphatidylethanolamine may be more easily accessible in the flip-flopped membranes of these structures than in the planar membranes of early apoptotic cells.

Several mechanisms have been suggested for the clearance of apoptotic cells by phagocytes including direct recognition via specific PS receptors, lectin-like receptors, and thrombospondin-dependent vitronectin receptors (39–42). Bound to a ligand, SAP, as well as its homologue CRP, can bind to C1q and activate the classical complement pathway (3, 10). Therefore, our results are consistent with a scenario that apoptotic cells either via direct interaction of the blebs with C1q (43) or indirectly via fixation of SAP and CRP can activate complement. Hence, we suggest that in addition to the mechanisms just mentioned, apoptotic cells may also be cleared via opsonization with complement. Recent studies in mice made deficient for SAP or C1q by targeted gene disruption (23, 44) support this idea: these mice have a high risk for developing systemic lupus erythematosus, a disease which likely results from impaired clearance of apoptotic cells and subsequent exposure of the immune system to nucleosomes (45). Currently, we are investigating whether SAP bound to apoptotic cells indeed can activate complement.

In conclusion, our study demonstrates that SAP can bind to cells in the early stage of apoptosis, presumably via phosphatidylethanolamine exposed in flip-flopped membranes. This data suggest that SAP may be involved in the clearance of these cells in vivo.

References


