# Interaction of Surfactant Protein A with the Intermediate Filaments Desmin and Vimentin[†]

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ABSTRACT: Surfactant protein A (SP-A), a member of the collectin family that modulates innate immunity, has recently been involved in the physiology of reproduction. Consistent with the activation of ERK-1/2 and COX-2 induced by SP-A in myometrial cells, we reported previously the presence of two major proteins recognized by SP-A in these cells. Here we identify by mass spectrometry one of these SP-A targets as the intermediate filament (IF) desmin. In myometrial preparations derived from desmin-deficient mice, the absence of binding of SP-A to any 50 kDa protein confirmed the identity of this SP-A-binding site as desmin. Our data based on partial chymotrypsin digestion of pure desmin suggested that SP-A recognizes especially its rod domain, which is known to play an important role during the assembly of desmin into filaments. In line with that, electron microscopy experiments showed that SP-A inhibits in vitro the polymerization of desmin filaments. SP-A also recognized in vitro polymerized filaments in a calcium-dependent manner at a physiological ionic strength but not the C1q receptor gC1qR. Furthermore, Texas Red-labeled SP-A colocalized with desmin filaments in myometrial cells. Interestingly, vimentin, the IF characteristic of leukocytes, is one of the major proteins recognized by SP-A in protein extracts of U937 cells after PMA-induced differentiation of this monocytic cell line. Interaction of SP-A with vimentin was further confirmed using recombinant vimentin in solid-phase binding assays. The ability of SP-A to interact with desmin and vimentin, and to prevent polymerization of desmin monomers, shed light on unexpected and wider biological roles of this collectin.

Surfactant protein A (SP-A)[1] is a member of the collectin family partially associated with surfactant layers in the lung. SP-A presents a globular C-terminal domain with calcium-dependent lectin activity and an N-terminal collagen-like domain (*1*). The globular domain of SP-A recognizes carbohydrates and lipids on the surface of pathogens (*2*). Several calcium binding sites are also present in this globular domain (*3*), and calcium induces conformational changes in SP-A (*4*) that influence further interactions of the protein with its different ligands.

SP-A regulates the function of both alveolar type II epithelial cells (AE-II) (the cells producing lung surfactant) and macrophages. In vitro, several studies have demonstrated that SP-A inhibits lipid secretion and promotes the uptake of lipids by AE-II, thus indicating a role of SP-A in surfactant homeostasis. However, SP-A-deficient mice presented normal phospholipid metabolism unless challenged (*5*). This apparent lack of phenotype in SP-A-deficient mice could be related to alternate mechanisms of surfactant metabolism existing in SP-A gene-targeted mice (*6*). Therefore, SP-A is necessary for the normal clearence of surfactant lipids from the alveolar space. With regard to macrophages, SP-A modulates phagocytosis of pathogens, cytokine production, respiratory burst, and chemotaxis mediated by this cell type (*2*). Some of these processes, such as phagocytosis or chemotaxis, involve cytoskeleton rearrangements necessary for monocyte and/or macrophages to move to areas of infection and efficiently exert pseudopod-based phagocytosis. Interestingly, SP-A stimulates the polymerization of actin in alveolar macrophages in a highly organized and directional manner (*7*). These results could explain some attributed functions of SP-A toward macrophages, especially in phagocytosis and chemotaxis processes. With the exception of different forms of

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[1] Abbreviations: AE-II, alveolar type II epithelial cells; HBSS, Hank's Balanced Salt Solution with calcium and magnesium; IF, intermediate filament; LC−MS, liquid chromatography−mass spectrometry; MEM, minimal essential medium eagle; PMA, phorbol myristate acetate; PVDF, polyvinylidene difluoride; RPMI, Roswell Park Memorial Institute (medium); SP-A, surfactant protein A; TEM, transmission electron microscopy; TR-SP-A, Texas Red-labeled SP-A; $VD_3$, 1α,25-dihydroxyvitamin $D_3$.

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myosin (*8, 9*) and annexin IV (*10*), interactions between SP-A and cytosolic proteins have not been described so far. Therefore, the capacity of SP-A to modulate alveolar cell functions might arise from already observed interactions of SP-A with cell membrane-expressed receptors.

Several receptors have been proposed for SP-A, some of them remaining incompletely characterized. Different reports indicate that SP-A exhibits both pro-inflammatory and anti-inflammatory activities. Recently, Gardai and co-workers proposed a mechanism to explain these dual effects: binding of SP-A globular heads to signal inhibitory receptors (SIRPα) will block stimulatory signaling pathways, whereas binding of the SP-A collagenous tail to the calreticulin−CD91 complex will induce the activation of p38 mitogen-activated protein (MAP) kinase and the production of pro-inflammatory mediators (*11*). One of the most accepted specific SP-A receptors is SPR210, first described by Chroneos and co-workers (*12*). SPR210 mediates SP-A-enhanced uptake by macrophages (*13*) and SP-A-induced inhibition of phospholipid secretion by AE-II (*12*). Recently, SPR210 has been successfully identified as a cell surface form of myosin 18A (*9*). In vitro, direct interactions between SP-A and CD14 have been reported by Sano and co-workers. SP-A−CD14 interaction could explain the regulatory effects of SP-A in LPS-elicited responses in macrophages (*14*). However, SP-A is able to diminish LPS-induced production of TNF-α in alveolar macrophages of CD14-null mice (*15*), suggesting that SP-A can also inhibit LPS-induced cytokine production through interactions with other receptors like TLR-4 (*16*).

Other SP-A receptors are less characterized. Strayer and associates found a 32 kDa protein associated with the regulation of surfactant metabolism. Antibodies against this 32 kDa protein block the inhibitory effect of SP-A in secretagogue-stimulated surfactant secretion from AE-II cells (*17*). Stevens and co-workers described a 170−200 kDa protein under nonreducing conditions and a 55 kDa protein under reducing conditions present in AE-II cell membranes, related to the SP-A-mediated lipid uptake by AE-II (*18*). Additional molecular or structural characterization of this receptor has not been provided.

Although SP-A was first described in alveolar epithelium, it has also been found in extrapulmonary tissues. Indeed, SP-A can be locally produced by fetal (*19, 20*) or reproductive tissues, including the uterus (*21*). This suggests a new role of SP-A in the physiology of reproduction, as recently demonstrated by Condon and associates (*22*). In this context, we have previously detected two major SP-A binding sites in myometrial cell extracts, with molecular masses of 200 and 50 kDa (*23*). Our observation that Texas Red-labeled SP-A (TR-SP-A) specifically binds to the surface of myometrial cells suggested for the first time the presence of SP-A binding sites in myometrial cells. These interacting sites might account for the activation of ERK-1/2 and COX-2 induced by SP-A in rat myometrial cells (*23*).

In this study, we have used different biochemical approaches to identify the ~50 kDa binding site recognized by SP-A in myometrial cells and in protein extracts of U937 cells after PMA-induced differentiation as desmin and vimentin, respectively. Our data thus demonstrate for the first time a direct interaction between SP-A and intermediate filaments.

## EXPERIMENTAL PROCEDURES

*Materials.* Human recombinant desmin and vimentin were purchased from Fitzgerald (Concord, MA). Recombinant gC1qR/p33 was a gift from B. Ghebrehiwet (Stony Brook, NY). 1α,25-Dihydroxyvitamin $D_3$ ($VD_3$) was obtained from BioMol (Plymouth Meeting, PA). Phorbol myristate acetate (PMA), bovine serum albumin (BSA), α-chymotrypsin, 1,3,4,6-tetrachloro-3α,6β-diphenylglycouril (iodogen), Triton X-100, monoclonal mouse anti-desmin antibody (DE-U-10), monoclonal anti-vimentin antibody (LN-6), FITC-conjugated anti-mouse antibody, and horseradish peroxidase (HRP)-conjugated anti-mouse IgM were obtained from Sigma Chemical Co. (St. Louis, MO). C1q from human serum was purchased from Merck-Calbiochem (Nottingham, U.K.). $Na^{125}I$ (0.78 MBq/μL) was purchased from ICN Biomedical Inc. (Irvine, CA). Fetal calf serum (FCS) was from BioMedia (Boussens, France). All other cell culture reagents, as well as mouse IgG1 isotype control, Texas Red succinimidyl ester, and Prolog Fade, were from Invitrogen (Cergy Pontoise, France).

*Animals and Tissue Processing.* Female desmin-deficient mice were obtained by introducing a null mutation into the desmin gene of mouse strain C57BL/6J (*24*). These mice, their corresponding wild type, and Wistar female rats (Janvier, France) were treated by intraperitoneal injection of 30 μg of estradiol for the last 2 days before being euthanized at the age of 30 days by standard carbon dioxide asphyxiation. All treatments were performed in accordance with the principles and procedures outlined in the European guidelines for the care and use of experimental animals. The uterus was removed, and the myometrium was separated from the endometrium by stripping, as previously described (*25, 26*).

*Cell Culture.* Primary cultures of myometrial cells were prepared from rat myometrium by collagenase type II digestion, as previously described (*27*). Myometrial cells were then cultured at a plating density of $15 \times 10^3$ cells/cm$^2$ in MEM supplemented with 10% fetal calf serum at 37 °C in an atmosphere of 5% $CO_2$ and 95% humidified air. The human promonocytic U937 cell line used was obtained from the European Collection of Cell Cultures (ECCAC, Salisbury, U.K.) and was cultured in RPMI supplemented with 10% heat-inactivated and endotoxin-free FCS. U937 cells were differentiated after incubation of the cells with PMA (1 nM) for 24 h or $VD_3$ (10 nM) for 72 h in culture medium.

*Isolation of SP-A and Protein Labeling.* Human SP-A was purified from the bronchoalveolar lavage of patients with alveolar proteinosis following sequential extractions with butanol and octyl glucoside (*28*). The purity of SP-A was checked by SDS−PAGE in a 12% polyacrylamide gel under reducing conditions. The endotoxin content was 0.12 pg/μg of protein (*Limulus* Amebocyte Lysate assay, BioWhittaker). Fluorescent Texas Red-labeled SP-A derivative was prepared as previously described (*23*). The covalent coupling of the fluorochrome was assessed by SDS−PAGE with viewing of the gel under UV light. The capacity of this fluorescent derivative of SP-A to interact with DPPC vesicles was found to be identical to that of the native SP-A. Radioiodinated SP-A and C1q were prepared by the iodogen method of Greenwood et al. (*29*) as previously described (*30*). The specific activities of the $^{125}I$-labeled SP-A and C1q were 2.25

$\times 10^6$ and $2.56 \times 10^6$ cpm/$\mu$g, respectively. The purities of the preparations were assessed by SDS−PAGE and autoradiography. As previously shown (*23*), labeled SP-A retained its capacity to bind DPPC and Re-LPS, two well-known ligands of SP-A.

*Autoradiography of SP-A Binding Proteins, Using [$^{125}$I]SP-A.* Detection of [$^{125}$I]SP-A binding proteins present in blotted membranes was performed as previously described (*23*) with modifications. Briefly, myometrial tissues from rats or mice were homogenized in lysis buffer consisting of 1% CHAPS in 50 mM Tris (pH 7.5) and 300 mM NaCl, supplemented with a cocktail of protease inhibitors (10 $\mu$g/mL aprotinin, 1 mM PMSF, and 2 $\mu$g/mL pepstatin and leupeptin), 100 $\mu$M orthovanadate, and 2 mM iodoacetamide. The extracts were centrifuged at 12000*g* for 20 min at 4 °C. Extractions were dialyzed against 5 mM Tris and 150 mM NaCl (pH 7.4) at 4 °C, and total proteins were precipitated in 65% saturation ammonium sulfate (12000*g* for 30 min at 4 °C). The pellet was dialyzed again before SDS−PAGE. Detergent-extracted proteins were analyzed by SDS−PAGE in 8% polyacrylamide gels using big gels (160 mm length, 100 mm width, 1 mm thickness) in the case of mass spectrometry identification or small gels adapted to a Mini-Protean-II system (Bio-Rad). Proteins were transferred onto PVDF (0.45 $\mu$m) at 100 mA for small gels (1 h) or 150 mA for big gels (1 h). Membranes were blocked by incubation (overnight, 4 °C) with 2% BSA in 5 mM Tris and 150 mM NaCl (pH 7.4) with 0.1% Tween 20 (washing buffer). Blots were washed and incubated (overnight at 4 °C or for 2 h at room temperature) with 0.2% BSA in washing buffer supplemented with [$^{125}$I]SP-A ($0.5-2 \times 10^6$ cpm) and 2 mM $CaCl_2$. Blots were thoroughly washed with washing buffer containing 2 mM $CaCl_2$, dried, and applied against X-ray films for autoradiography (Hyperfilm, Amersham Biosciences). Finally, blotted proteins were stained with Coomassie Blue following the manufacturer's instructions. Differentiated or nondifferentiated U937 cells were washed in cold PBS and resuspended lysis buffer. After incubation for 1 h at 4 °C under continuous vortexing, cell debris was precipitated (12000*g* for 20 min at 4 °C) and proteins in clarified supernatants or ammonium sulfate-precipitated fractions were separated in 8% SDS−PAGE gels. After being blotted, membranes were hybridized with [$^{125}$I]SP-A as described above or with anti-vimentin antibodies. Anti-vimentin antibody was developed using HRP-conjugated anti-mouse IgM as the secondary antibody and an ECL system (Amershan Biosciences).

*LC−MS/MS Analysis.* In-gel digestion of the cut band was performed with the Progest system (Genomic Solution) according to a standard trypsin protocol. Gel pieces were washed twice by successive separate baths of 10% acetic acid, 40% ethanol, and acetonitrile (ACN). They were then washed twice with successive baths of 25 mM $(NH_4)_2CO_3$ and ACN. Digestion was subsequently performed for 6 h at 37 °C with 125 ng of modified trypsin (Promega) dissolved in 20% methanol and 20 mM $(NH_4)_2CO_3$. The peptides were extracted successively with 2% trifluoroacetic acid (TFA) and 50% ACN and then with ACN. Peptide extracts were dried in a vacuum centrifuge and suspended in 20 $\mu$L of 0.05% TFA, 0.05% HCOOH, and 2% ACN. HPLC was performed on an Ultimate LC system combined with a Famos autosampler and a Switchos II microcolumn switch system (Dionex). A 4 $\mu$L sample was loaded at 5 $\mu$L/min on a precolumn cartridge (stationary phase, C18 PepMap 100, 5 $\mu$m; column, 300 $\mu$m inside diameter, 5 mm; Dionex) and desalted with 0.05% TFA, 0.05% HCOOH, and 2% ACN. After 2.5 min, the precolumn cartridge was connected to the separating PepMap C18 column (stationary phase, C18 PepMap 100, 3 $\mu$m; column, 75 $\mu$m inside diameter, 150 mm; Dionex). Buffers consisted of 0.1% HCOOH and 3% ACN (A) or 0.1% HCOOH and 95% ACN (B). The peptide separation was achieved with a linear gradient from 5 to 30% B for 25 min at a rate of 200 nL/min. If the regeneration step at 100% B and the equilibration step at 100% A were included, one run took 45 min. Eluted peptides were analyzed online with a LCQ Deca XP$^+$ ion trap (Thermo Electron) using a nanoelectrospray interface. Ionization (ionization potential of 1.3−1.5 kV) was performed with liquid junction and a noncoated capillary probe (10 mm inside diameter; New Objective). Peptide ions were analyzed using Xcalibur 1.4 with the following data-dependent acquisition steps: (1) full MS scan [mass-to-charge ratio (*m*/*z*) of 400−1900, centroid mode], (2) ZoomScan on a selected precursor (scan at high resolution in profile mode on a *m*/*z* window of 4), and (3) MS/MS (qz = 0.22, activation time = 50 ms, and collision energy = 40%; centroid mode). Steps 2 and 3 were repeated for the two major ions detected in step 1. Dynamic exclusion was set to 30 s. A database search was performed with Bioworks 3.2 (Thermo Electron). Trypsin digestion, Cys carboxyamidomethylation, and Met oxidation were set to enzymatic cleavage, static, and possible modifications, respectively. Precursor mass tolerance and fragment mass tolerance were 1.4 and 1, respectively. The UniprotKB database [combination of Swiss-Prot (version 52.5) and TrEMBL (version 35.5) databases; 4629251 entries on May 15, 2007] was used. Identified tryptic peptides were filtered according to (i) their cross-correlation score (Xcorr), superior to 1.7, 2.2, and 3.3 for mono-, di-, and tricharged peptides, respectively, and (ii) their probability inferior to 0.05. A minimum of two different peptides was required. In the case of identification with only two or three MS/MS spectra, similarity between the experimental and theoretical MS/MS spectra was visually confirmed.

*Polymerization of Desmin Filaments.* Recombinant desmin was reconstituted at 1 mg/mL (18.8 $\mu$M; $M$ = 53 kDa) in 9 M urea buffer and aliquoted at −20 °C. From this stock, desmin was diluted 1/10 at 4 °C in 10 mM Tris-HCl (pH 8.5) or 5 mM Tris-HCl, 150 mM NaCl, and 2 mM $CaCl_2$ (pH 7.4) (final concentration of desmin of 1.8 $\mu$M) and incubated at 37 °C for 1 h. In selected experiments, SP-A was added to polymerizing buffer at different final concentrations (from 25 to 2.8 nM; $M$ = 640 kDa) before the addition of desmin monomers. One aliquot of each sample was analyzed by transmission electron microscopy.

*Transmission Electron Microscopy (TEM).* A drop of the sample was laid on a glow-discharged Formvar-coated copper grid (400 mesh) stabilized with evaporated carbon film. After 5 min, each grid was stained with 1% uranyl acetate (1 min) and then examined at 80 kV with a Philips EM208 electron microscope. Digital images were acquired using a camera (Advantage HR3 AMT-Hamamatsu), and diameters and lengths of all recognizable particles that intersected a grid line, including protofilaments, protofibrils,

and filaments, were measured with a Bioquant Image Analysis System (R&M Biometrics, Nashville, TN).

*Protease Digestion of Desmin.* Chymotryptic cleavage of desmin was carried out according to the method of Geisler et al. (*31*). Recombinant desmin was diluted in 10 mM Tris-HCl (pH 8.5). α-Chymotrypsin was added at a concentration equal to 1/400 of that of desmin (molar ratios), and the digestion was allowed to proceed for 0−60 min at 23 °C. Reaction was then stopped by addition of Laemmli sample buffer and heating. Afterward, desmin fragments were loaded in 15% SDS−PAGE gels in duplicate. One replicate was blotted on a PVDF membrane (pore size of 0.2 μm), and the other one was stained with Coomassie Blue. Afterward, membranes were incubated with [$^{125}$I]SP-A as described above to visualize SP-A binding peptides.

*Solid-Phase Binding Assays.* Nunc MaxiSorb plates were coated with 100 μL of polymerized desmin or vimentin filaments (1 μg/well) or equivalent amounts of desmin preincubated at 37 °C in 9 M urea (monomers) or 5 mM Tris-HCl buffer (pH 8.5) (protofilaments). Alternatively, some wells were coated with gC1qR/p33 (1 μg/well) in carbonate/bicarbonate buffer. Control wells were coated with the same volume of buffer without protein. After incubation of the plates at 37 °C for 2 h, wells were washed with 5 mM Tris, 150 mM NaCl, and 2 mM $CaCl_2$ (assay buffer). Plates were then saturated with 200 μL of 5% nonfat milk (1 h at 37 °C), washed, and incubated for 2 h at 37 °C with 100 μL of radiolabeled SP-A or C1q in assay buffer supplemented with 0.05% nonfat milk. Afterward, plates were thoroughly washed, and the specific bound radioactivity was recovered in 10% SDS and measured with a gamma counter. In selected experiments, assay buffers without NaCl, or with 2 mM EDTA instead of $CaCl_2$, were used. Each condition was assayed in triplicate in at least three independent experiments, and data are presented as means ± the standard deviation of the resulting values.

*Immunofluorescent Staining.* These experiments were performed as previously described (*23*). Rat myometrial cells plated on coverslips were washed with HBSS, fixed for 15 min in a 4% formaldehyde/HBSS mixture, washed with 50 mM $NH_4Cl$, and permeabilized for 15 min in 0.2% Triton X-100 (v/v, HBSS) (washing buffer). Cells were first incubated with Texas Red-labeled SP-A (TR-SP-A) (20 μg/mL) in washing buffer containing 1% BSA (w/v) for 1 h at room temperature. After being washed with an excess of TR-SP-A, cells were incubated with mouse anti-desmin (1:150) or isotype control (1:2) for 1 h at room temperature in the same buffer. FITC-labeled anti-mouse antibody (1:100) was used as a secondary antibody. After samples had been washed with an excess of antibody at each step, coverslips were mounted in ProLong Antifade. Specificity of TR-SP-A staining was confirmed when prior incubation of the cells with a 10-fold excess of unlabeled SP-A abolished the binding of TR-SP-A. Conventional microscope observations were carried out with a Carl Zeiss (Le Pecq, France) Axiophot 2 microscope equipped with epi-illumination and specific filters as previously described (*23*). Photographs were taken with a Zeiss AxioCam camera and processed with AxioVision.

## RESULTS

*SP-A Binds to Desmin Present in Myometrial Cell Extracts.* We have previously described the presence of two major SP-A binding proteins of 200 and 50 kDa in detergent extracts from rat myometrial tissues (*23*). To determine the identity of the ~50 kDa SP-A binding protein, we prepared myometrial cell extracts as previously described (*23*). After detergent had been removed by dialysis, total myometrial proteins were precipitated with ammonium sulfate (AS) at 65% saturation and dialyzed again. This fraction was found to be enriched in the ~50 kDa SP-A binding protein. Two samples of this material were then subjected to two monodimensional SDS−PAGE runs, carried out under identical conditions, and one of the twin gels was electroblotted on a PVDF membrane. Incubation of blotted proteins with [$^{125}$I]SP-A revealed the presence of the 50 kDa SP-A binding protein (Figure 1A) as previously described (*23*), but only small amounts of the 200 kDa SP-A binding protein were detectable, because it was lost during the AS precipitation step. The ~50 kDa SP-A reactive band was also clearly localized by staining the PVDF membrane with Coomassie Blue. The corresponding band in the twin gel was then cut out (Figure 1A) and analyzed by LC−MS/MS as described in Experimental Procedures. Gel digestion of the cut band and further LC−MS/MS allowed us to identify 22 peptides corresponding to desmin (43.3% of the desmin sequence covered by the analysis) and other intracellular proteins (Table 1). The identity of the 50 kDa SP-A binding protein was confirmed when ligand blot experiments were performed with myometrial extracts from desmin knockout mice (Figure 1B). Whereas [$^{125}$I]SP-A recognized two bands at 200 and ~50 kDa in wild-type mouse myometrial extracts, as previously described in rats (*23*), in desmin −/− myometrial extracts, the ~50 kDa SP-A binding protein was absent. Furthermore, the binding of SP-A to human desmin monomers was studied by ligand blotting. Results in Figure 1C show that [$^{125}$I]SP-A bound to human recombinant desmin but not to BSA, used as a negative control. The presence of BSA on the PVDF membrane after blotting was confirmed by Coomassie Blue staining.

*SP-A Recognizes the Rod Domain of Desmin.* Intermediate filaments (IFs) consist of an N-terminal head domain, a C-terminal domain, and a long and highly conserved central domain (rod domain) which is mainly α-helical and forms a coiled coil with the homologous polypeptide of another IF molecule (*32*). The mature filament is built up by association of monomers in higher superstructures. To localize the SP-A binding site in the desmin polypeptide chain, we performed a limited proteolytic digestion of the desmin proto-filament as described elsewhere (*31*). Desmin is extremely sensitive to proteolytic attack, particularly at the non-α-helical regions of the molecule. In the presence of chymotrypsin, the desmin proto-filament first loses its non-α-helical head and tail domains, resulting in a 38 kDa, α-helical rod domain. Further chymotryptic digestion cleaves the rod domain at its middle non-α-helical spacer, generating an amino-terminal half (helix 1; 21 kDa) and a carboxy-terminal half (helix 2; 17 kDa) (see Figure 2A). Therefore, human desmin proto-filaments obtained from recombinant monomers (see the next paragraph) were digested under conditions described previously (chymotrypsin/desmin molar ratio of 1/400 and 23 °C). Products from digestion at different times were applied on two identical gels and analyzed by SDS−PAGE (16% acrylamide). One gel was stained with Coomassie Blue to follow the digestion of desmin. Its duplicate was blotted on a PVDF membrane, which was incubated with [$^{125}$I]SP-A.

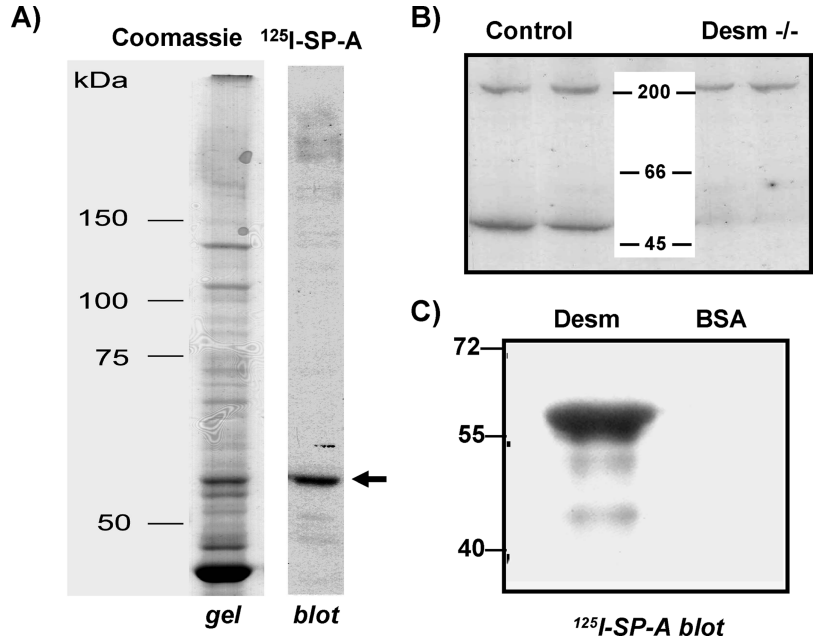


FIGURE 1: Binding of SP-A to blotted desmin monomers. (A) Rat myometrial proteins (20−30 $\mu$g) precipitated in ammonium sulfate (65% saturation) were analyzed by SDS−-PAGE. The [$^{125}$I]SP-A reactive band (see the arrow) in the ligand blot (right panel) was used to localize the SP-A binding protein of 50 kDa in gels (left panel), for further identification by LC−MS/MS (Table 1). (B) Ligand blot of [$^{125}$I]SP-A reactive bands in myometrial extracts (20 $\mu$g) from wild-type mice (Control; left) and from desmin-deficient mice (Desm −/−; right). (C) Ligand blot analysis with [$^{125}$I]SP-A of human recombinant desmin (Desm) and bovine serum albumin (BSA) (2 $\mu$g).

Table 1: LC−MS/MS Analysis of the ~50 kDa SP-A Reactive Band in Rat Myometrium[a]

| accession | description | *M* | coverage | peptides |
|---|---|---|---|---|
| P48675 | desmin, *Rattus norvegicus* (rat) | 53.4 | 43.3 | 22 (16) |
| P68369 | tubulin α-1 chain, *Mus musculus* (mouse) | 50.1 | 25.3 | 10 (8) |
| Q6P6V0 | glucose phosphate isomerase, *R. norvegicus* (rat) | 62.8 | 13.1 | 4 (4) |
| P04785 | protein disulfide-isomerase precursor, *R. norvegicus* (rat) | 56.9 | 13.9 | 4 (3) |
| Q6P736 | polypyrimidine tract binding protein 1, *R. norvegicus* (rat) | 59.3 | 11.2 | 3 (3) |

[a] Accession, accession number of the protein (Swiss-Prot or TrEMBL); description, description of the protein; *M*, molecular mass of the protein (kilodaltons); Coverage, percent of the protein sequence covered by the analysis; peptides, number of spectra (number of unique peptides) identified.

As shown in Figure 2B, recombinant human desmin migrates as two major and very close bands around 55 kDa (bands "a") and a minor band around 40 kDa (band "b") which can hardly be detected at the starting time (Figure 2B). Bands "a" correspond to intact desmin. The presence of two closely spaced bands in intact desmin has been previously described and attributed to isoelectric variant forms of desmin with slightly different electrophoretic mobilities (*33*). Band "b" corresponds to desmin's rod domain and represents less than 5% of the recombinant protein according to the manufacturer's information. After digestion for 5 min under the conditions used in our experiments, intact desmin was hardly detectable in Coomassie Blue-stained gels (Figure 2B). At that digestion time, most of the remaining polypetide corresponds to the rod domain, as already reported by other authors (*34*). Increasing digestion time to more than 5 min (15−60 min) resulted in the degradation of the rod domain in a close double band of 40 kDa, and in the time-dependent appearance of bands around 24 kDa (band "c") and 15 kDa (band "d") (helices 1 and 2, respectively), both resulting from excision of the rod domain. The appearance of a double band around 40 kDa in band "b" with long digestion times could be attributed to partial degradation of the rod domain by traces of trypsin, present as a contaminant in chymotrypsin, and leading to CT-desmin (chymotrypsin/trypsin desmin-digested fragment) which presents a slightly faster mobility in SDS−PAGE than the complete rod domain (*34*). When desmin fragments obtained at different digestion times were blotted on a PVDF membrane (0.2 $\mu$m pore size) and incubated with [$^{125}$I]SP-A, we observed a strong reactivity of SP-A with the rod domain (band "b") but neither with helix 1 (band "c") nor with helix 2 (band "d") (Figure 2C). Reactivity of SP-A with the rod domain was reduced when the rod domain was sequentially degraded over time. The presence of blotted bands "c" and "d" onto the membrane was confirmed after Coomassie Blue staining of the membrane. These data indicate that SP-A recognizes the rod domain of desmin. This is in accordance with the detection of a second SP-A reactive band of 40 kDa present in some preparations from rat myometrial tissues, identified as desmin by mass spectrometry (data not shown). This band might correspond to the rod domain of the protein. Therefore, these results show that SP-A binds also to rod domain forms produced during natural degradation of desmin.

*SP-A Impairs in Vitro Desmin Assembly.* Mutations in the rod domain of desmin in humans are associated with the aberrant polymerization and accumulation of desmin in vivo (*35*), in line with the participation of the rod domain in the maintenance of the stability of desmin filaments. Thus, our results showing that SP-A recognizes the rod domain in ligand blot experiments (Figure 2) suggested that SP-A could interfere with the association of subunits and therefore could disturb the assembly of desmin filaments. As a consequence, we induced polymerization of desmin filaments from monomers in vitro to study the effects of SP-A during the process of desmin assembly. Recombinant desmin was supplied with

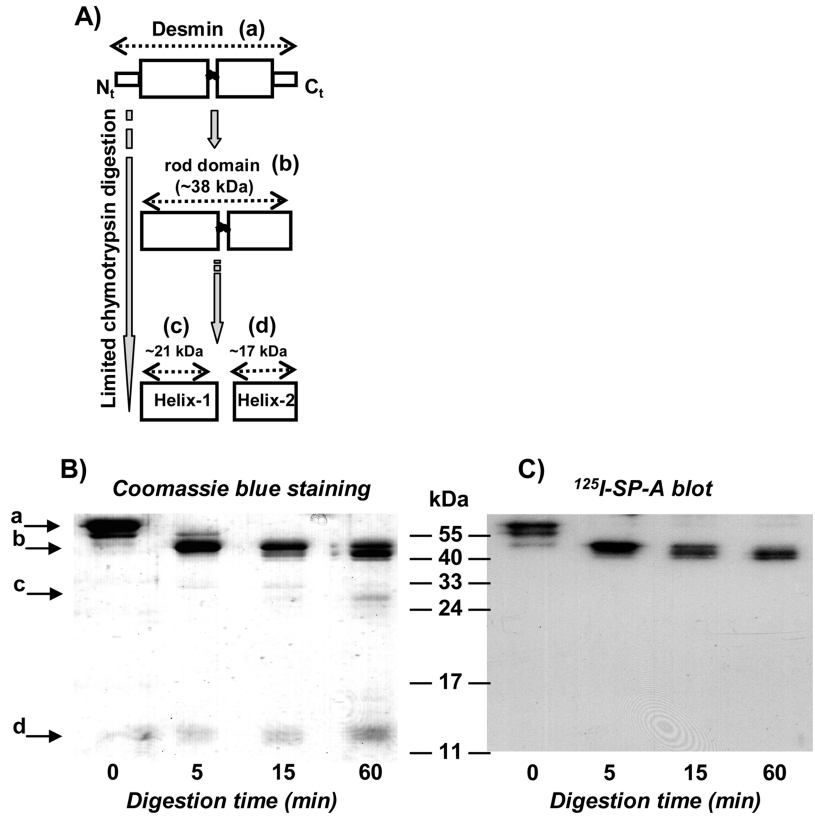


FIGURE 2: Identification of chymotryptic fragments of desmin recognized by [$^{125}$I]SP-A. (A) Diagram of chymotrypsin digestion of intact desmin. At short digestion times, desmin (a) gives the rod domain (b). Longer digestion times produce two fragments of >24 (c) and <17 kDa (d). (B) Coomassie Blue-stained SDS−PAGE (15% polyacrylamide) of fragments a−d from chymotrypsin-digested desmin at various digestion times. (C) A similar gel was blotted, and SP-A binding fragments were detected with [$^{125}$I]SP-A.

9 M urea after reconstitution. Under these conditions of high urea concentration, desmin tends to form monomers (*36*). Desmin monomers (53 kDa) were diluted 1/10 (final concentration of desmin, 1.88 $\mu$M) in 5 mM Tris-HCl (pH 8.5) and incubated at 37 °C for 1 h. Transmission electron microscopy (TEM) images (negative staining; 1% uranyl acetate) showed homogeneous rod-shaped structures 32 ± 0.6 nm wide and 120 ± 8.3 nm long ($n$ = 25) (Figure 3A). These structures should correspond to individual proto-filaments (formed by association of two dimers) and associated proto-filaments. It has been established that an increase in NaCl concentration in the medium induces a polymerization of IFs (*36*). When we diluted desmin monomers in a polymerization buffer [5 mM Tris, 150 mM NaCl, and 2 mM $CaCl_2$ (pH 7.4)] and incubated the mixture at 37 °C for 1 h, TEM images showed filament-like forms (30 ± 1 nm in diameter) with some lateral aggregation of desmin filaments (typically 10 nm in diameter) (Figure 3B). The length distribution of the filaments was not homogeneous, lengths of <300 nm corresponding to incompletely assembled filaments (Figure 3B). We then polymerized desmin monomers in polymerization buffer containing SP-A (25 nM). As shown in Figure 3C, we did not observe under these conditions the formation of filaments or parts of filaments as we did in the absence of SP-A. Instead, there were abundant grizzly forms associated with darker spots (arrow a in the inset of Figure 3C) and small filament-like forms (arrow b in the inset of Figure 3C). Thus, our data indicate that assembly of desmin in vitro was disturbed in the presence of SP-A. This effect was observed with SP-A concentrations between 5.6 and 25 nM. To exclude the possibility that the effect of SP-A was due to a dilution of desmin below the concentration required for polymerization, we used BSA (25 nM) instead of SP-A and found indeed that BSA did not affect the assembly of desmin (data not shown).

*SP-A Binds to Desmin Filaments in a Calcium-Dependent Manner at a Physiological Ionic Strength.* To study the interaction of SP-A with desmin filaments, desmin monomers were assembled in vitro as described above and used to coat Maxisorb microplates. Afterward, we analyzed the ability of [$^{125}$I]SP-A to bind to these desmin-coated plates. Figure 4A shows that [$^{125}$I]SP-A binds to desmin filaments in a concentration-dependent manner in a buffer (pH 7.4) consisting of 5 mM Tris, 150 mM NaCl, and 2 mM $CaCl_2$. The binding proved to be specific since it was inhibited by unlabeled SP-A (Figure 4D), and since [$^{125}$I]SP-A did not bind to plates coated with gC1qR (gC1qR/p33), a receptor for the globular head of C1q, the first component of complement (Figure 4B). Furthermore, C1q did not recognize desmin filaments under our experimental conditions (Figure 4C), although the radioactive reagent used ([$^{125}$I]C1q) was fully functional since it bound to plates coated with gC1qR (Figure 4C). It was then important to analyze the dependence of NaCl and calcium in the SP-A−desmin interaction. Indeed, calcium and ionic strength both play a role in modulation of SP-A conformation in solution (*4*) and in the stability of desmin filaments (*37*). Thus, we analyzed the requirements of ionic strength and calcium for the ability of SP-A to bind desmin. We observed that under physiological ionic strength conditions (150 mM NaCl), calcium proved to be essential for desmin−SP-A interaction. Indeed, the

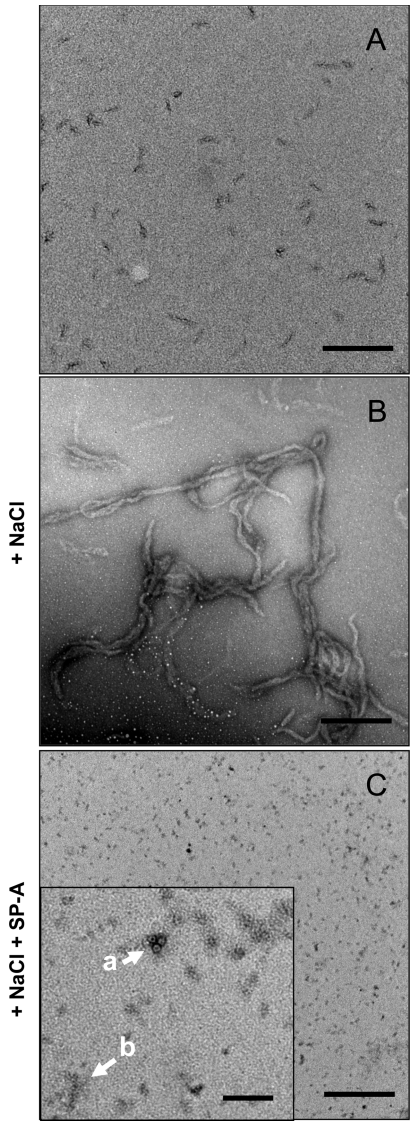


FIGURE 3: Effect of SP-A on desmin polymerization. Recombinant desmin monomers were diluted (final desmin concentration, 1.8 $\mu$M) in a 5 mM Tris-HCl buffer (pH 8.5) (A), with buffer A supplemented with 150 mM NaCl and 2 mM $CaCl_2$ (B), or with buffer B supplemented with 25 nM SP-A (C). An aliquot of the sample was laid on the grid of a Philips EM208 electron microscope and stained (1 min) with 1% uranyl acetate. Pictures were taken at 80 kV and are representative of the fields observed from three independent preparations. The scale bar is 500 nm; the inset scale bar in panel C is 100 nm.

presence of EDTA dramatically reduced the level of binding of SP-A to desmin (Figure 4D). In addition, the low ionic strength (absence of NaCl) impaired the binding of [$^{125}$I]SP-A to desmin, even in the presence of calcium (Figure 4D). The specificity of the binding of [$^{125}$I]SP-A to desmin-coated plates was assessed by the observation that unlabeled SP-A efficiently inhibited the binding (Figure 4D).

*SP-A Colocalizes with Desmin Filaments in Rat Myometrial Cells.* To determine whether SP-A associates with the desmin filamentous network present in the living cells, we performed double immunofluorescence studies in myometrial cells using anti-desmin antibodies and SP-A labeled with Texas Red (TR-SP-A) (Figure 5). We know from a previous study that TR-SP-A binds to living myometrial cells (*23*). Thus, we incubated myometrial cells with the TR-SP-A preparation, and after the excess of TR-SP-A had been washed out, the cells were incubated with the monoclonal mouse anti-desmin antibody and anti-mouse antibody coupled to FITC. Images from FITC emission showed a conspicuous desmin network in myometrial cells (Figure 5, desmin). As expected from the data on SP-A−desmin interactions mentioned above, images from TR emission also showed a network distribution of SP-A (Figure 5, SP-A). When images from FITC and TR fluorescence were overlaid, we observed that most of the TR staining coincided with that of FITC (Figure 5, overlay), thus indicating that SP-A colocalizes with desmin filaments present in myometrial cells. To exclude the possibility that colocalization of SP-A and desmin could be due to interactions between SP-A and the antibody, the same experiment was performed using an unrelated control antibody of the same isotype instead of the anti-desmin antibody. We did not observe colocalization of FITC and TR fluorescence in this control experiment (data not shown).

*SP-A Binds to Vimentin, Another IF Related to Desmin.* SP-A modulates several macrophage functions acting through specific receptors present in the cell membrane (*2*). Therefore, using [$^{125}$I]SP-A, we studied by ligand blot assays the presence of SP-A binding proteins in detergent extracts from the pro-monocytic U937 cell line. In a previous analysis of this cell type, when we blotted total proteins from undifferentiated U937 cells, we observed that radioiodinated SP-A recognized a single SP-A binding protein at approximately 200 kDa (*23*) as also shown by others (*12*). This protein might correspond to SP-A receptor SPR210 isolated by Chroneos and co-workers from U937 cells, recently identified as the unconventional myosin 18A (*9*). Interestingly, when similar experiments were performed with phorbol myristate acetate (PMA)-differentiated U937 cells, we observed a second SP-A binding protein of 55 kDa (Figure 6A). We analyzed this 55 kDa SP-A binding protein by LC−MS/MS as described above for desmin, and we observed that one candidate could be vimentin (Table 2). Further experiments designed to corroborate that the 55 kDa SP-A binding protein corresponds to vimentin were based on specific differentiation of U937 cells depending on the inducer used. Indeed, undifferentiated U937 cells do not express vimentin, the expression of the protein being dramatically enhanced when cells are exposed to PMA (*38*). In parallel, they acquire a macrophage-like phenotype, including the capacity to adhere to substrates. On the other hand, 1,25-dihydroxyvitamin $D_3$ ($VD_3$) also confers a macrophage-like phenotype to U937 cells, including the expression of CD14 (*39*), but without inducing vimentin expression (*38*). Western blot analyses, using a monoclonal antibody against vimentin, confirmed the presence of vimentin in PMA-treated U937 cells but not in $VD_3$-treated U937 cells or undifferentiated U937 cells (data not shown). When the same cellular extracts were blotted and exposed to [$^{125}$I]SP-A, we observed the presence of the 55 kDa SP-A reactive protein only in PMA-treated U937 cells and not in $VD_3$-treated U937 cells (Figure 6A), in accordance with the vimentin expression pattern. Furthermore, [$^{125}$I]SP-A bound to pure human recombinant vimentin which migrates with an electrophoretic mobility similar to that of the 55 kDa SP-A binding protein present in PMA-U937 extracts (Figure 6A). Therefore, our data provide evidence that the SP-A binding protein of 55 kDa present in protein extracts of PMA-induced U937 cells corresponds to vimentin, another IF structurally related to desmin. To

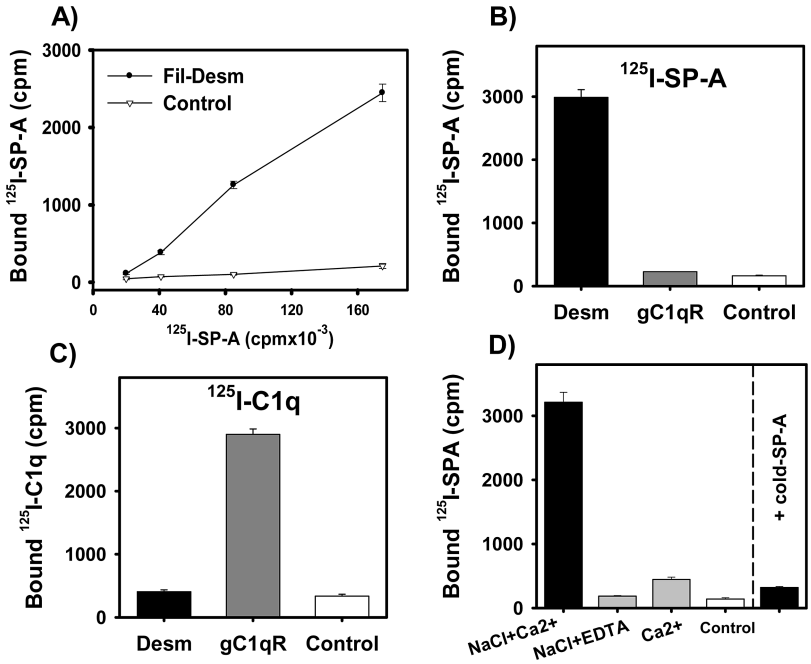


FIGURE 4: Binding of [$^{125}$I]SP-A and [$^{125}$I]C1q to plates coated with polymerized desmin. (A) Maxisorb plates uncoated (▽) or coated with desmin filaments (1 $\mu$g) (●) were blocked with 5% nonfat milk and incubated with [$^{125}$I]SP-A at different concentrations per well (*x*-axis) in an assay buffer [5 mM Tris, 150 mM NaCl, and 2 mM $CaCl_2$ (pH 7.4)] containing 0.05% nonfat milk. After the washings, bound radioactivity was recovered with 10% SDS and counted in a gamma counter (*y*-axis). Under the same experimental conditions, [$^{125}$I]SP-A did not bind to gC1qR/p33 (2 $\mu$g)-coated plates (B), and [$^{125}$I]C1q bound to plates coated with gC1qR/p33 but not to plates coated with desmin filaments (C). In panel D, binding of [$^{125}$I]SP-A (180000 cpm/well) to uncoated (control) or desmin-coated plates was assayed using a 5 mM Tris buffer (pH 7.4) containing 150 mM NaCl and 2 mM $CaCl_2$ (NaCl+Ca2+), 150 mM NaCl and 2 mM EDTA (NaCl+EDTA), or 2 mM $CaCl_2$ (Ca2+). For the bar delimited by the dashed line, binding of [$^{125}$I]SP-A was performed in assay buffer (NaCl+Ca2+) containing unlabeled SP-A (25 $\mu$g/mL). Results represent the means ± standard deviation from three independent experiments performed in triplicate.

confirm the interaction of SP-A with vimentin under nondenaturing conditions, vimentin filaments were prepared as described above for desmin and the ability of [$^{125}$I]SP-A to bind vimentin-coated plates was assayed. As shown in Figure 6B, [$^{125}$I]SP-A bound to vimentin in a concentration-dependent manner, and unlabeled SP-A inhibited the binding of radioactive SP-A (Figure 6B, right panel). The binding of SP-A to vimentin, like that to desmin, was calcium-dependent (Figure 6B, right panel).

## DISCUSSION

Surfactant protein A is a lung collectin that regulates the activity of alveolar macrophages and alveolar type II cells. Although SP-A was originally reported to be synthesized and secreted by alveolar type II cells, recent publications show that this protein is also expressed in extrapulmonary tissues like fetal membranes (*19, 20*) or reproductive tissues, including uterus (*21*), suggesting a role of SP-A in the physiology of reproduction (*22*). In this context, we have previously detected two major SP-A binding sites of 200 and 50 kDa in myometrial extracts, in line with an ERK-1/2 activation induced by SP-A in rat myometrial cells (*23*). ERK-1/2 activation is associated with uterine contractility and preterm labor in rats (*40*). In the work presented here, using mass spectrometry, we identified the 50 kDa SP-A binding protein of myometrial cells as the IF desmin. In myometrial preparations derived from desmin-deficient mice, the binding of SP-A at the level of 50 kDa did not occur, confirming the identity of this SP-A interacting site as desmin. Our data provided evidence that SP-A bound to desmin monomers from rat, mouse, and human origin in ligand blot experiments. Binding of SP-A to desmin monomers was calcium-dependent (*23*), indicating that conformational changes in SP-A induced by calcium enhance its binding to blotted desmin. Using partial chymotryptic digestion, we further identified the rod domain as the binding site of SP-A in the desmin polypeptide. However, our data cannot exclude the possibility that SP-A interacts with the nonhelical linker region rather than with the helical region within the rod domain. In this case, the helical region might be required for the binding of SP-A to the linker regions. The rod domain and its linker region play a crucial role during assembly of IFs. Mutations in desmin's rod domain are associated with the formation of aberrant filaments in vitro and accumulation of desmin aggregates in vivo (*35*). In vitro, the first step in IF assembly is the formation of a parallel coiled-coil dimer, through stable interactions between central rod domain regions of two monomers. Afterward, two dimers associate in a half-staggered, antiparallel manner to form a tetramer. With an increase in ionic strength, tetramers associate laterally to form a so-called "unit-length filament". In the next step, these unit-length filaments anneal longitudinally to form loosely packed filaments that, in a further step, will be compacted by narrowing of the filament's diameter (*32*). Our results show that SP-A binding perturbs polymerization of desmin in vitro. Using a dilution method, we were able to polymerize desmin in protofibrils and filaments from recombinant monomers. In the presence of SP-A, we observed small electron dense forms instead of filament-like forms. The most likely possibility is that SP-A

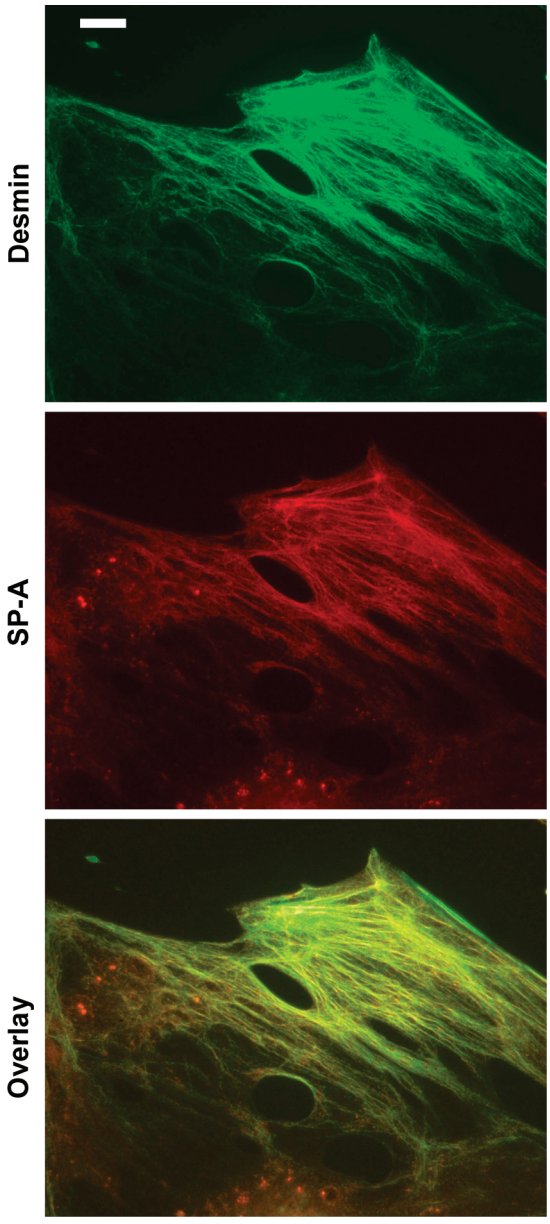


FIGURE 5: Colocalization of TR-SP-A with anti-desmin antibodies in myometrial cells. Fixed and permeabilized rat myometrial cells were doubly labeled at room temperature with TR-SP-A (20 $\mu$g/mL) and mouse monoclonal anti-desmin antibody (1/150) in HBSS containing 1% BSA and 0.2% Triton X-100. FITC-labeled anti-mouse antibody was used as a secondary antibody. Conventional epifluorescence microscopy was used to observe emission from TR (SP-A) or FITC (anti-desmin) using the corresponding emission filters. In the bottom panel, overlaid fluorescences are shown. The scale bar is 20 $\mu$M. Data are representative of the fields observed from three independent preparations.

binds to the rod domain of monomers and impairs formation of dimers and subsequently their assembly in higher superstructures. However, when we assayed the binding of SP-A to plates coated with desmin monomers, we observed that SP-A hardly bound to monomers (results not shown) in comparison with preformed filaments (Figure 4). This suggests that SP-A recognizes the rod domain only under appropriate presentation of the latter (monomers blotted on membranes but not adsorbed on plates). We cannot exclude the possibility that SP-A binding sites in the rod domain become accessible to SP-A only during the rearrangements that occur in some particular steps of the polymerization process. This hypothesis implies that once SP-A interacts with the rearranged intermediate, further assembly is impaired and/or assembled structures are disassembled. When we examined desmin samples in the presence of SP-A by TEM at high magnification, we observed the presence of electron dense forms that resemble the bouquet shape of SP-A and should correspond to aggregated forms of the collectin. This is consistent with the fact that the polymerizing buffer contains NaCl and calcium, two compounds that induce SP-A aggregation (*4*). These electron dense forms (SP-A aggregates) were found to be associated with darker points (probably unpolymerized desmin) and short unraveled filaments, indicating that SP-A interacts with desmin in solution and prevents or disrupts the assembly of the molecule in vitro. Moreover, we observed that SP-A was able to depolymerize in vitro preformed desmin filaments (data not shown). Considering the effects of SP-A on the stability of desmin IF, our data suggest a potential role of SP-A as an IF mucolytic agent which may be involved in protection from lung injury and in repair processes. Furthermore, SP-A could facilitate the clearence of IF from alveolar subphase following lung injury, as previously described for cellular myosin (*8*).

Because IFs are present as filamentous structures in the cytoplasm of the cells, we further studied whether SP-A binds to in vitro or in vivo assembled desmin filaments attached to a solid support. The binding of SP-A to in vitro formed filaments was calcium-dependent and occurs at a physiological ionic strength. Ionic strength might be necessary to compensate for the electrostatic repulsion between the negative surface charges of the two proteins at pH 7. Calcium, itself, induces conformational changes in SP-A (*4*) that affect further interactions of the protein with other ligands and receptors. In addition, NaCl and calcium improve the stability of desmin filaments (*37*). Therefore, the presence of both calcium and NaCl favors SP-A−desmin interaction.

Among members of the IF family, the rod domain is the most conserved region. Both vimentin and desmin exhibit a high degree of amino acid sequence identity in this domain. Vimentin is expressed in mesenchimal cells and is the most abundant IF in leucocytes. We have identified vimentin as a major SP-A reactive band present in PMA-U937 cell extracts. Other authors have previously studied the presence of SP-A receptors in this monocytic cell line. Studies of Chroneos and co-workers identified the SP-A receptor named SPR-210kDa (myosin 18A) in U937 cells. Radio-iodinated SP-A detected a single band of 210 kDa in U937 cell extracts using ligand blot assays (*12*). However, a 55 kDa SP-A binding protein was detected only after differentiation of U937 cells by PMA. Sano and co-workers have identified CD14 as another SP-A binding protein present in the membrane of PMA-differentiated U937 cells (*14*). CD14 has a molecular mass similar to that of vimentin. When we differentiated U937 cells with $VD_3$, an agent that increases the level of CD14 expression, we did not induce the expression of the ~50 kDa SP-A reactive band. Therefore, our data indicate that the ~50 kDa SP-A binding protein present in PMA-differentiated U937 cells is not CD14. Analysis by mass spectrometry identified this protein as vimentin. We further confirmed that vimentin is indeed an SP-A binding protein when ligand blots were performed using a recombinant form of human vimentin. SP-A bound to monomeric and poly-

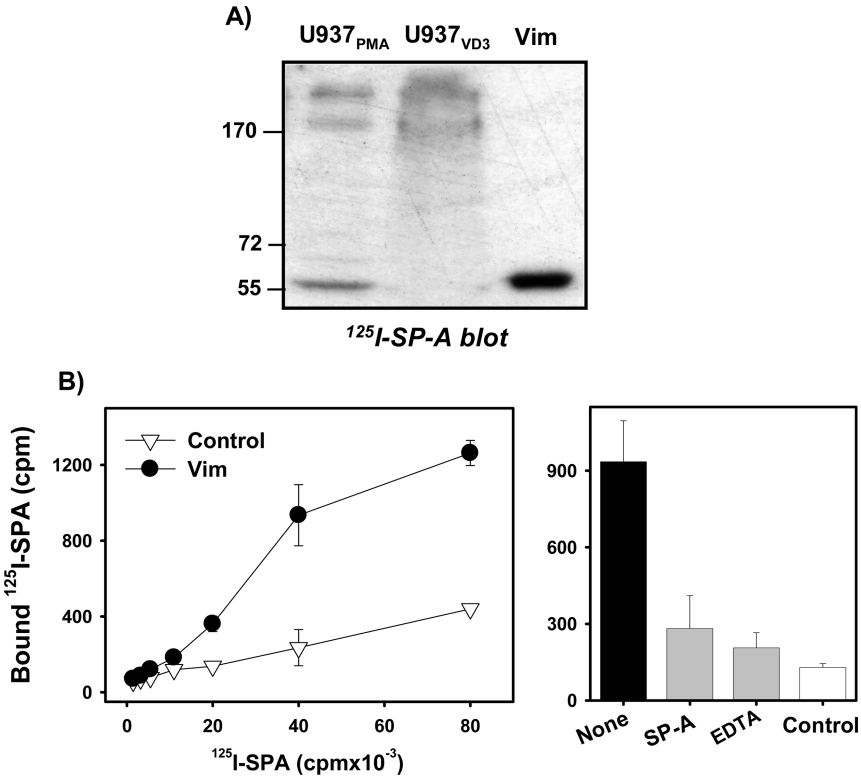


FIGURE 6: Binding of SP-A to vimentin. (A) [$^{125}$I]SP-A reactive bands in SDS−PAGE blots of extracts (20 $\mu$g) of U937 cells differentiated by incubation with PMA (1 nM, 24 h) or VD3 (10 nM, 72 h). Human recombinant vimentin (1 $\mu$g) was analyzed in parallel in the right lane. (B) To produce the data depicted in the left panel, Maxisorb plates uncoated (▽) or coated with 1 $\mu$g of vimentin filaments (●) were blocked (5% nonfat milk) and incubated with [$^{125}$I]SP-A at different concentrations per well (*x*-axis) in an assay buffer [5 mM Tris, 150 mM NaCl, and 2 mM $CaCl_2$ (pH 7.4)] containing 0.05% nonfat milk. After the washings, bound radioactivity was recovered with 10% SDS and counted with a gamma counter (*y*-axis). To produce the data depicted in the right panel, [$^{125}$I]SP-A (50000 cpm/well) was incubated with uncoated (white bar) or coated vimentin plates in the presence of 25 $\mu$g/mL unlabeled SP-A (SP-A) or in assay buffer in which 2 mM $CaCl_2$ was replaced with 2 mM EDTA (EDTA). Values are the means ± the standard deviation of three separate experiments performed in triplicate.

Table 2: LC−MS/MS Analysis of the ~50 kDa SP-A Reactive Band in U937 Cell Extracts[a]

| accession | description | *M* | coverage | peptides |
|---|---|---|---|---|
| P08670 | vimentin, *Homo sapiens* (human) | 53.6 | 45.5 | 24 (18) |
| P07237 | protein disulfide-isomerase precursor, *H. sapiens*(human) | 57.1 | 26.8 | 10 (10) |
| P06744 | glucose-6-phosphate isomerase, *H. sapiens* (human) | 63.1 | 19.7 | 8 (7) |
| Q9BUQ0 | polypyrimidine tract binding protein 1, *H. sapiens* (human) | 59.6 | 11 | 4 (3) |

[a] Accession, accession number of the protein (Swiss-Prot or TrEMBL); description, description of the protein; *M*, molecular mass of the protein (kilodaltons); coverage, percent of the protein sequence covered by the analysis; peptides, number of spectra (number of unique peptides) identified.

merized vimentin, and as for desmin, the binding was calcium- and NaCl-dependent, indicating that a similar mechanism governs interactions of both vimentin and desmin with SP-A. Recent experiments with SP-A-deficient mice demonstrate a role of SP-A in surfactant metabolism (*5, 6*). During surfactant production mediated by alveolar type II epithelial cells, precursors of surfactant (lipoproteins and lipids) accumulate in the lamellar bodies that will be fused with the plasme membrane to release the surfactant at the aqueous subphase of the alveoli. Afterward, AE-II and macrophage participate in surfactant clearance. Surfactant metabolism is dependent on the integrity of the cytoskeleton, including the IF framework. In fact, several reports suggest a role of IFs (e.g., keratins and vimentin) in the secretion process in epithelial cells (*41*). Moreover, vimentin has been implicated in intracellular lipoprotein transport (*42*). Our data suggest that intracellular SP-A−IF interactions could participate in an unknown role of SP-A in surfactant lipid processing.

Although IFs are cytoskeletal proteins present in the cytoplasm, several investigators observed an expression of vimentin on the surface of leukocytes (*43, 44*). Moreover, the level of expression of vimentin on neutrophil membranes is increased under apoptosis (*43*) and might help in the recognition of the apoptotic cell by inflammatory mediators or by the immune cells. Apoptosis is an important phenomenon in uterus physiology, especially after delivery. After parturition, the uterus undergoes a marked involution that involves an ordered series of events, including apoptosis and phagocytosis of cellular debris (*45, 46*). Under these physiological conditions, extracellular IFs could be overexpressed and therefore may contribute to the recognition and clearance of apoptotic cells and cell debris by immune cells. In this context, SP-A locally produced (*21*), or coming from amniotic fluid, could participate in the clearance of apoptotic cells through interactions with IFs. A parallel mechanism, initiated by the interaction of SP-A with its phagocyte receptor (the CD91−calreticulin complex) (*47*), occurs in

lung alveoli, where it triggers SP-A-induced enhancement of phagocytosis in apoptotic neutrophils (*48*).

Desmin is well-known as a marker of the contractile phenotype for smooth muscle cells. Additionally, desmin and other IFs have been shown to participate in the cytoskeletal architectures that are crucial for maintaining the structural and mechanical integrity of cells and tissues. Recently, a new notion emerged which puts forward the functional role of IFs as organizers or members of signaling platforms (*49*). In this context, our data provided the first evidence that desmin binds SP-A and may participate in the activation of the myometrial cell signaling pathway. Further investigations are needed to elucidate the mechanism by which SP-A−desmin complexes, with or without other interacting proteins, regulate smooth muscle cell functions.

## ACKNOWLEDGMENT

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