Expression of hydrophilic surfactant proteins by mesentery cells in rat and man

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Human peritoneal dialysis effluent (PDE) contains a phosphatidylcholine-rich compound similar to the surfactant that lines lung alveoli. This material is secreted by mesothelial cells. Lung surfactant is also characterized by four proteins essential to its function. After having long been considered as lung-specific, some of them have been found in gastric and intestinal epithelial cells. To explore further the similarity between lung and peritoneal surfactants, we investigated whether mesothelial cells also produce surfactant proteins. We used rat transparent mesentery, human visceral peritoneum biopsies and PDE. Surfactant proteins were searched for after one- and two-dimensional SDS/PAGE and Western blotting. On a one-dimensional Western blot, bands at 38 and 66 kDa in rat mesentery, and at 38 and 66 kDa in human peritoneal mesothelial cells (*in vivo* and *in vitro*) and PDE, corresponded to monomeric and dimeric forms of lung surfactant protein A (SP-A). On two-dimensional Western blots, the 32 and 38 kDa spots in mesentery and PDE localized at the acidic pH appropriate to the SP-A monomer's isoelectric point. SP-D was also identified at the same 43 kDa molecular mass as in lung. SP-B was not detected in mesenteric samples. Expression of SP mRNA species was also assessed by reverse transcriptase–PCR, which was performed with specific primers of surfactant protein cDNA sequences. With primers of SP-A and SP-D, DNA fragments of the same size were amplified in lung and mesentery, indicating the presence of SP-A and SP-D mRNA species. These fragments were labelled by appropriate probes in a Southern blot. No amplification was obtained for SP-B. These results show that mesentery cells produce SP-A and SP-D, although they are of embryonic origin (mesodermal) and are different from those of the lung and digestive tract (endodermal) that secrete these surfactants.

INTRODUCTION

The normal peritoneal cavity contains a serous fluid that is essentially an ultrafiltrate of plasma. It has been shown that peritoneal dialysis effluent (PDE), which recovers this fluid, contains a relatively large quantity of phospholipids, more especially of phosphatidylcholine, which confers surface-active properties on the material [1]. This led Dobbie et al. [2] to point out the similarity of composition to that of pulmonary surfactant, in which the major constituent is also phosphatidylcholine. It was shown that, in peritoneum, mesothelial cells are the source of these phospholipids *in vivo* [3] and *in vitro* [4] and that, similarly to alveolar type II cells, they display lamellar bodies in their cytoplasm and at their surface. Lamellar bodies have been shown in the lung to represent the storage and exocytosis form of surfactant. These similarities between secreted materials of both organs led the authors to designate the phospholipidic compound of peritoneum as a mesenteric surfactant.

Pulmonary surfactant lines the alveolar epithelial surface, on which it confers remarkable low-tension and anti-adhesive properties. Although phospholipids are the predominant components of pulmonary surfactant, proteins account for approx. 10 % in mass. Among these, four characteristic proteins called surfactant proteins (SP) -A, -B, -C and -D have been identified [5,6]. These proteins can be classified into two categories. The first class includes SP-A and SP-D, which are structurally related hydrophilic collagenous glycoproteins. These proteins belong to the 'collectin' class of C-type lectins characterized by the presence of a collagen-like domain and of a lectin-like domain in their peptidic chain. SP-A participates in the formation of tubular myelin, a transient extracellular structure of surfactant that gives rise to the alveolar lining [7], and in the regulation of surfactant secretion and re-uptake [8]. In addition, both SP-A and SP-D seem to be involved in host defence, particularly through a phagocytosis-facilitating activity [6]. The second group consists of the hydrophobic proteins SP-B and SP-C, two small peptides that facilitate the formation of a surfactant monolayer by accelerating the spreading of phospholipids and stabilizing the pulmonary alveoli [8]. Lastly, the various components of surfactant also seem to modulate the immune response [9].

Whereas phospholipids have been studied in detail in the peritoneal secretion, nothing is known about the possible presence of surfactant-related proteins in peritoneal fluid as well as in mesothelial cells. To characterize mesenteric surfactant better, we have searched for the presence of SP-A, SP-B and SP-D in rat and human mesentery and in human PDE and for the presence of their mRNA species in the tissue. SP-C was not searched for because it has clearly been shown that a transgene placed under the control of SP-C promoter is exclusively expressed in the lung [10].

MATERIALS AND METHODS

Preparation of tissue samples

Wistar rats (350 g; Charles River, St-Aubin-Lès-Elbeuf, France) were anaesthetized by intraperitoneal injection of undiluted sodium pentobarbital (80 mg/kg). The abdominal wall was incised, and the intestinal tube was removed and transparent

Abbreviations used: HPES, human peritoneal epithelial sheet; PDE, peritoneal dialysis effluent; SP-A, -B, -C, -D, surfactant protein A, B, C, D.

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mesenteric tissue (approximately 30 mg per rat) was carefully dissected free of adipose and large vascular tissues from the mesentery and immediately frozen in liquid nitrogen. Biopsies of surgical waste human visceral peritoneum and lung were obtained during elective surgery; the peritoneal epithelial sheet, i.e. mesothelial cells, was mechanically separated from the connective and adipose tissues; isolated cultured human peritoneal mesothelial cells [11] were also examined; PDE was sampled from patients starting a continuous ambulatory peritoneal dialysis (Service de Néphrologie, Hopital Tenon, Paris, France). Part of PDE was enriched in proteins by concentration on Amicon concentrators. Samples were kept at −80 °C until they were used either for protein electrophoresis or for RNA analyses.

One- and two-dimensional electrophoresis

Samples were sonicated in PBS, pH 7.4, containing 10 mM PMSF. The sample protein concentration was determined by Bradford's assay [12] with BSA as standard. Sonicated samples were solubilized in reducing buffer and heated at 100 °C for 5 min before they were subjected to one-dimensional SDS/PAGE [12 % (w/v) gel] by the method of Laemmli [13] with a Mini-Protean II apparatus (Bio-Rad, Ivry sur Seine, France). For SP-B, electrophoresis was also performed with a Tris/Tricine/SDS/PAGE [15 % (w/v) gel] system [14]. To enrich the samples with hydrophobic proteins, they were extracted by organic solvent [chloroform/methanol/water (1:2:0.8, v/v).]

Other samples were diluted in 10–20 μl of buffer containing 9.5 M urea, 2 % Nonidet P40, 2 % ampholines and 100 mM dithiothreitol as described by O'Farrell [15] for two-dimensional isoelectric focusing PAGE with pH 3–5 and pH 5–8 ampholines (Pharmacia Biotech, Orsay, France).

Antibodies

A rabbit polyclonal antibody directed against rat SP-A was kindly provided by Dr. M. Post (Hospital for Sick Children, Toronto, Ontario, Canada). A guinea-pig polyclonal antibody directed against human SP-A was prepared in our laboratory [16]. A rabbit polyclonal antibody directed against rat SP-B was kindly provided by Dr. J. A. Whitsett (Children's Hospital Center, Cincinnati, OH, U.S.A.). A rabbit polyclonal anti-silicotic rat SP-D was kindly provided by Dr. E. Crouch (Jewish Hospital, St. Louis, MO, U.S.A.). A mouse monoclonal antibody directed against rat SP-D [17] was purchased from BMA (Biomedicals AG, Augst, Switzerland). Peroxidase-labelled anti-rabbit and anti-mouse IgG antibodies were obtained from Pasteur Diagnostics (Marnes la Coquette, France). Peroxidase-labelled anti-guinea-pig IgG was obtained from Sigma (L'Isle d'Abeau-Chesnes, France), biotinylated anti-rabbit IgG and streptavidin peroxidase from Amersham (Les Ulis, France), and anti-guinea-pig IgG from Vector (Biosys, Compiègne, France).

Western blotting

Proteins were transferred to nitrocellulose membranes with a semi-dry blotting apparatus (Pharmacia Biotech). After blocking in 5 % (w/v) non-fat milk in Tris-buffered saline containing 0.05 % Tween-20 (TBS-T), immunoblotting was performed with antibodies raised against SP-A, SP-B or SP-D, or with a non-immune serum used as control. Incubation was performed overnight at 4 °C. After extensive washing with TBS-T, a secondary appropriate antibody conjugated with horseradish peroxidase was applied. After a further wash in TBS-T, immunoreactive products were detected by enhanced chemiluminescent-peroxidase detection reagents (ECL; Amersham) and recorded on hyperfilm-ECL (Amersham).

RNA isolation

Total cellular RNA was isolated from frozen mesenteric tissues with the RNAzol B kit (Bioprobe Systems, Montreuil, France) by the technique of Chomczynski and Sacchi [18]. RNA concentration was estimated from A_{260}, and its integrity was checked electrophoretically.

Oligonucleotide primers and probes

Oligonucleotide primers and probes (Genosys Inc., Biotechnologies Europe, Cambridge, U.K.), for rat and human SP-A, SP-B and SP-D were designed from previously published sequences. To avoid specious amplification of genomic DNA, each primer set was selected from different exons with at least one intervening intron. Surfactant-protein primer and probe sequences were as follows: rat SP-A [19] primer I (5′-GGAAGCCCTGGGATCCCTGGA-3′) (exon 2), primer II (5′-TGGGTACCAGTTGGTGTAGT-3′) (exon 5) and probe (5′-GTCTCCACGTTCTCCAGG-3′); human SP-A [20] primer I (5′-TGGAGAGTGTGGAGAGAAGG-3′) (exon 4), primer II (5′-TGGGTACCAGTTGGTGTAGT-3′) (exon 5) and probe (5′-AATGACAGTAGGAGAGAAGG-3′); human SP-B [21] primer I (5′-TGAGCACCTGGTTACCAC-3′) (exon 4) and primer II (5′-TTAGACGTAGGCACTGCG-3′) (exon 5); human SP-D [22] primer I (5′-ATACGCCAGCACAGATTCTTCC-3′) (exon 2), primer II (5′-CCACTGTATTGATGGCAGGC-3′) (exon 5) and probe (5′-TGAGTGTGTGAGTGCTGG-3′).

Reverse transcriptase–PCR

First-strand cDNA species were synthesized from 2 μg of RNA species by the random hexanucleotide priming method with Moloney murine leukaemia virus reverse transcriptase (Gibco–BRL, Cergy Pontoise, France). One-quarter of the cDNA products was used in the amplification reaction. The 50 μl total PCR volume consisted of 10 μl of cDNA, 1 unit of *Taq* polymerase (Eurogentec, Seraing, Belgium), 1 × amplification buffer, 3 mM $MgCl_2$ (Eurogentec), 25 mM deoxynucleotides triphosphate (Boehringer Mannheim, Meylan, France) and 250 ng of each primer (Genosys). The PCR reaction was then performed in a Crocodile II thermocycler (Appligen, Strasbourg, France) under the following conditions: 35 amplification cycles, denaturation for 1.5 min at 94 °C, annealing for 1 min at 55 °C (rat SP-A and human SP-B), 56 °C (human SP-A), or 57 °C (human SP-D), extension for 1 min at 72 °C.

Southern blotting

After completion of the amplification cycles, 10 μl of each PCR product was run on a 1.5 % (w/v) agarose gel containing ethidium bromide and examined under UV illumination. For hybridization, probes were labelled with [γ-^{32}P]dATP (ICN Pharmaceuticals, Orsay, France) by using T4 kinase (Gibco–BRL). PCR products were transferred overnight to a positively charged nylon membrane (Hybond N; Amersham) by alkaline capillary blotting with 0.4 M NaOH. Prehybridization was run in a solution containing 2 × SSC (0.3 M NaCl/0.03 M sodium citrate), 1 % SDS, 1 mg/ml denatured salmon sperm DNA at 42 °C for 3 h. After this time the labelled probe was added to the solution, and hybridization was proceeded for 16 h. Filters were subsequently washed twice in 2 × SSC at 42 °C for 15 min and

then twice in 2 × SSC plus 0.1 % SDS for 15 min at room temperature. Exposure of the film was for 16 h at −70 °C.

RESULTS

SP-A immunodetection

Characterization of SP-A in rat and human mesentery was performed by Western blotting after one- and two-dimensional gel electrophoresis under reducing conditions by comparison with rat lung homogenate. Indirect immunolabelling in two steps was used because of the non-specific binding of streptavidin in the amplified system. Figure 1 shows results obtained with an antibody directed against rat pulmonary SP-A. This antibody strongly labelled the characteristic SP-A monomeric bands (26, 32 and 38 kDa) in a lung cell extract enriched with lamellar bodies (Figure 1, lane 1), whereas in a lung homogenate a 66 kDa band was strongly labelled and monomeric forms (32 and 38 kDa) were weakly labelled (Figure 1, lane 2). Western blot analyses of rat mesentery showed a strong 60–66 kDa band, weaker bands around 50 kDa and one band at 38 kDa (Figure 1, lane 3). Characterization of SP-A was also done in isolated human peritoneal mesothelial cells, in homogenate of human peritoneal epithelial sheet (HPES) and in PDE with an antibody directed against human SP-A. A major band at 60 kDa and a weaker band at 50 kDa were detected (Figure 1, lanes 4–7). A band at 38 kDa was less strongly labelled and was more easily discerned when a larger amount of proteins was run (Figure 1, lane 7). Controls in the presence of a non-immune serum or in the absence of the first antibody were not labelled (results not shown).

As pulmonary SP-A is characterized by its acidic isoelectric point, two-dimensional electrophoreses of rat mesentery and HPES homogenates and of human PDE were performed. After labelling of blotted proteins with anti-SP-A, two spots at 60–66 and 38 kDa were revealed in rat mesentery (Figure 2B) and three spots at 60, 38 and 32 kDa in HPES (results not shown) and PDE (Figure 2C). The 38 and 32 kDa spots were observed at the pI 5 expected for pulmonary SP-A, whereas the 60–66 kDa spots were less acidic (pI approx. 6.5) (Figure 2A). Bands and spots of high range appear strongly labelled because it was necessary to increase the development time to reveal the low-range proteins.

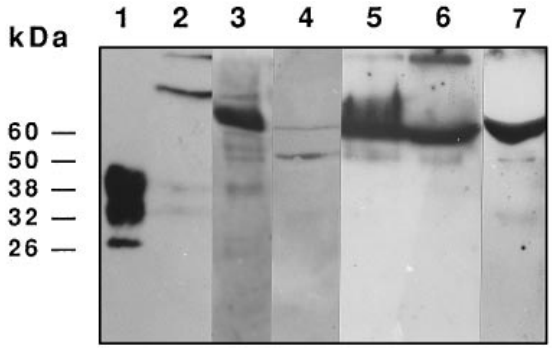

Figure 1 Western immunoblotting of SP-A

After SDS/PAGE [12% (w/v) gel], samples were probed with anti-(rat SP-A) or anti-(human SP-A) antibodies. Lane 1, rat lung cell extract enriched in lamellar bodies (5 μg of proteins); lane 2, rat lung homogenate (15 μg); lane 3, rat mesentery homogenate (22 μg); lane 4, isolated human peritoneal mesothelial cells (30 μg); lane 5, HPES homogenate (90 μg); lanes 6 and 7, human PDE (90 and 150 μg respectively).

SP-B immunodetection

The same samples were also examined for SP-B with either the glycine/SDS/PAGE or the Tricine/SDS/PAGE systems, the latter being especially adapted to the detection of low-molecular-mass proteins. After silver staining of the glycine/SDS/PAGE (12 % gel) under non-reducing conditions, an organic-solvent extract of rat lung lavage material enriched in SP-B/SP-C showed a minor band close to the dye front of the gel that was likely to correspond to SP-C but no labelling at the expected level for SP-B (Figure 3A, lane 1). In different human lung lavage materials, staining of SP-B with silver varied in intensity according to the sample but always appeared at 18 kDa (Figure 3A, lanes 2–4). A few bands in the rat mesentery sample were stained with silver; one of these appeared at the expected level for SP-B (Figure 3A, lane 5). No immunostaining for SP-B was observed for this protein (Figure 3A, lane10), however, whereas in rat lung lavage material as well as in each of the human lung lavage materials a band was labelled at 18 kDa (Figure 3A, lanes 6–9). No more labelling was observed in rat and human mesentery samples after concentration of the putative hydrophobic proteins by organic extraction (Figure 3A, lanes 11–13). With a Tricine/SDS/PAGE (15 % gel) system, SP-B immunoreactivity was not observed in rat mesentery (Figure 3B, lanes 3 and 4), in

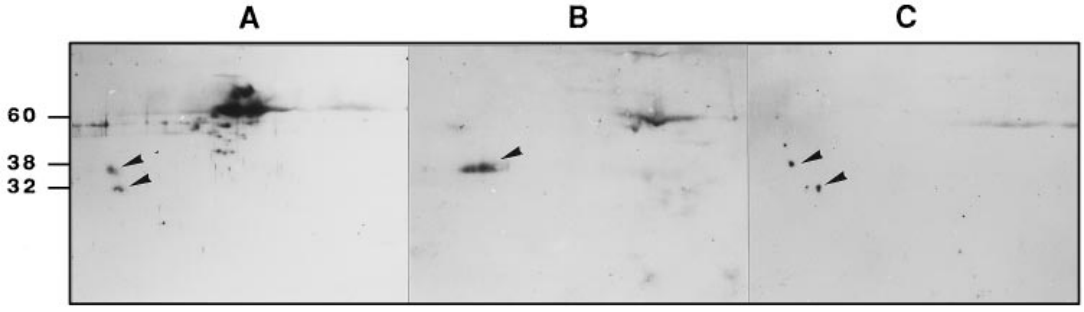

Figure 2 Western immunoblotting of SP-A after two-dimensional electrophoresis

The antibodies used were the same as those for Figure 1. (**A**) Delipidated rat lung homogenate (30 μg of proteins) (ampholines 3–10); (**B**) rat mesentery homogenate (20 μg); (**C**) human PDE (25 μg) (in **B** and **C**, mixed ampholines 3–10 and 5–8 were used). Spots (arrowheads) with apparent molecular masses consistent with SP-A monomeric forms were detected at the same characteristic acidic pI as lung SP-A.

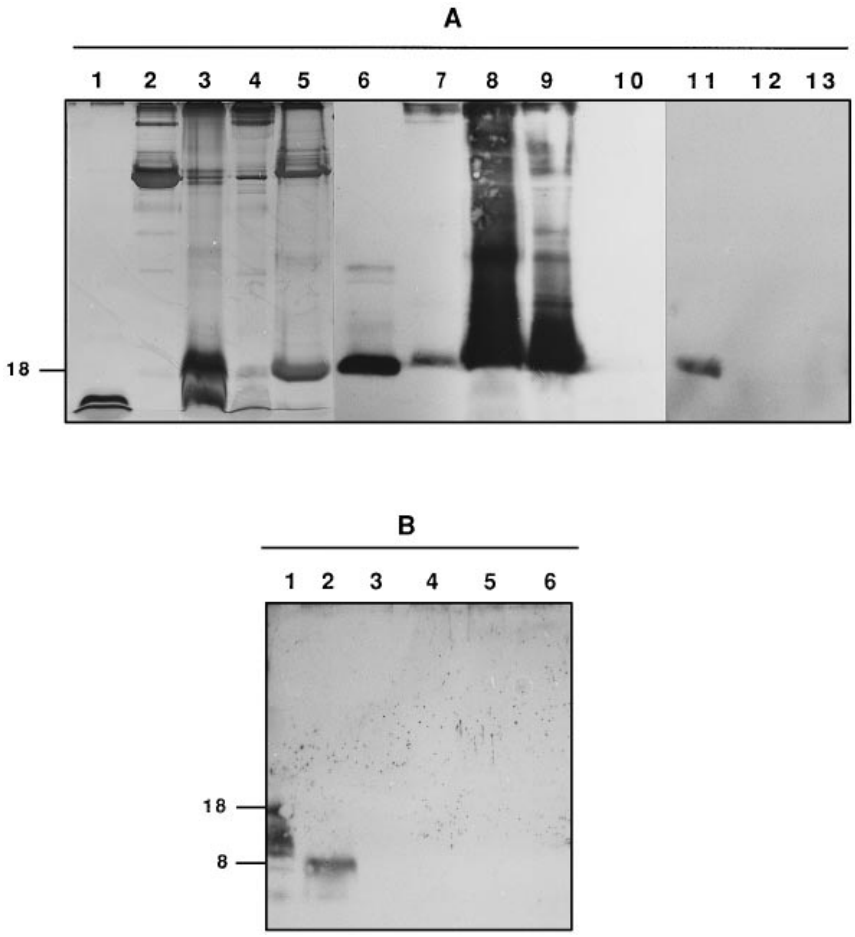

Figure 3 Western immunoblotting of SP-B

(**A**) Immunoblotting after SDS/PAGE [12% (w/v) gel] with glycine buffer (unreduced proteins), and (**B**) after SDS/PAGE [15% (w/v) gel] with Tricine buffer (unreduced and reduced proteins). Lanes 1–5 in (**A**), silver staining; (**B**) and lanes 6–13 in (**A**), immunoblotting. In (**A**): lanes 1, 6 and 11, organic solvent extract of rat lung lavage enriched in SP-B and SP-C (20 μg of proteins); lanes 2–4 and 7–9, human lung lavages (25 μg); lanes 5 and 10, rat mesentery homogenate (70 μg); lanes 12 and 13, organic solvent extracts of rat and human mesentery. In (**B**): lanes 1 and 2, organic solvent extract of rat lung lavage enriched in SP-B and SP-C (10 μg); lanes 3 and 4, rat mesentery homogenate (90 μg); lanes 5 and 6, HPES homogenate (90 μg). No immunoreactive SP-B was detected in mesentery samples.

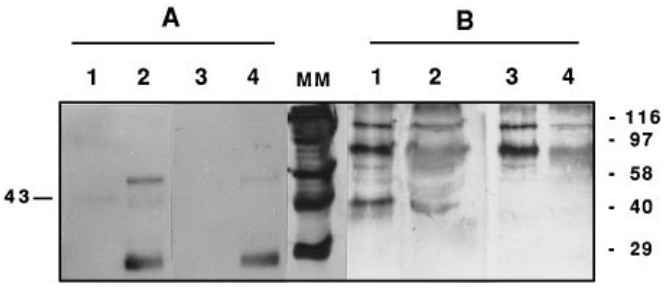

Figure 4 Western immunoblotting of SP-D

After SDS/PAGE [12% (w/v) gel], samples were probed with a mouse monoclonal antibody directed against rat SP-D (**A**, lanes 1 and 2) or with a rat polyclonal SP-D antibody (**B**, lanes 1 and 2). Respective controls in the absence of antibody and in the presence of non-immune serum are shown in lanes 3 and 4 in (**A**) and (**B**). Lanes 1 and 3 in (**A**) and (**B**), rat alveolar type II cell homogenate (56 μg of proteins); lanes 2 and 4 in (**A**), rat mesentery homogenate (56 μg); lanes 2 and 4 in (**B**), HPES homogenate (56 μg). Abbreviation: MM, molecular mass markers identified at the right (in kDa). A 43 kDa immunoreactive band was detected in mesentery samples and in alveolar type II cells.

HPES (Figure 3B, lanes 5 and 6) or in PDE (results not shown), under reducing as well as in non-reducing conditions, whatever the amount of proteins that was deposited. Only rat lung lavage organic extract used as a positive control displayed the characteristic bands of SP-B at 18 and 8 kDa under non-reducing and reducing conditions respectively (Figure 3B, lanes 1 and 2).

SP-D immunodetection

After one-dimensional electrophoresis under reducing conditions, a monoclonal anti-SP-D antibody detected only one specific band at 43 kDa in rat mesentery (Figure 4A, lane 2) as well as in rat alveolar type II cells (Figure 4A, lane 1). However, non-specific bands (45 and 25 kDa) in rat mesentery were not switched off by preincubation with normal sheep serum (host animal of the second antibody). These reacting bands seemed to be mesentery-specific because they were not labelled in lung (Figure 4A, lane 4). With the polyclonal anti-rat SP-D antibody, a 43 kDa band was detected in rat alveolar type II cells (Figure 4B, lane 1) and in HPES (Figure 4B, lane 2) but not in PDE or

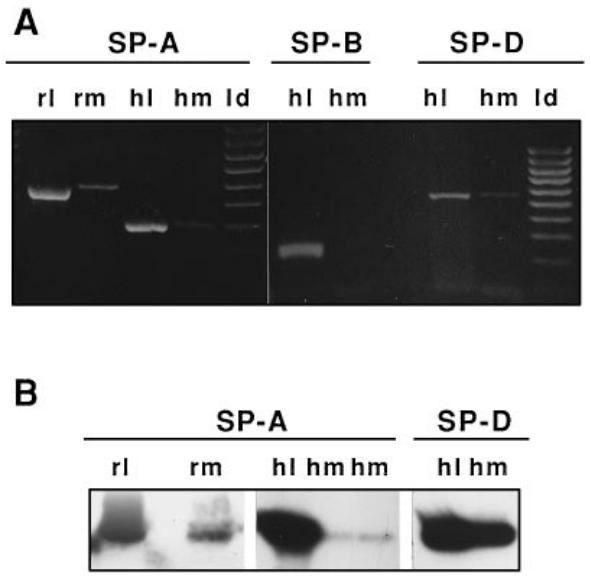

Figure 5 Reverse transcriptase–PCR analysis of SP-A, SP-B and SP-D transcripts in rat and human mesentery

Lung was used as positive control. (**A**) Ethidium bromide-stained agarose gel of reverse transcriptase–PCR products (35 cycles) electrophoresed in parallel with a 100 bp DNA ladder. Total RNA (2 μg) was reverse-transcribed and amplified. For SP-A, products of the expected sizes, 557 and 393 bp respectively, were amplified from rat and human samples. The SP-B-specific signal (225 bp) was observed in human lung, but not in human mesentery. The SP-D-specific signal (538 bp) was observed in both human samples. (**B**) Southern blot autoradiograms of SP-A and SP-D amplified products with appropriate probes. Abbreviations: rl, rat lung; rm, rat mesentery; hl, human lung; hm, human mesentery; ld, DNA ladder.

in cultured mesothelial cells (results not shown). Other bands with higher molecular masses were due to non-specific labelling as demonstrated in Figure 4B (lanes 4–6).

Detection of surfactant protein transcripts

The presence of SP-A, SP-B and SP-D transcripts was assessed by reverse transcriptase–PCR in rat and human mesentery. cDNA species from rat and human lung were used as positive controls. After 35 amplification cycles, specific SP cDNA species reverse-transcribed from total RNAs were amplified selectively and revealed signals of the expected sizes. Positive signals were detected for SP-A and SP-D, whereas no positive signal was detected for SP-B in rat (results not shown) and human mesentery (Figure 5A). In Southern blot analyses, amplified fragments reacted positively with labelled probes specific for SP-A and SP-D (Figure 5B).

DISCUSSION

In the present study, evidence is given for the first time that lung-characteristic hydrophilic surfactant proteins SP-A and SP-D are elaborated by mesentery. These results bring new elements to the characterization of the surfactant material. It had been previously reported that mesothelial cells synthesize phosphatidylcholine, the major component of lung surfactant, in amounts similar to those produced by cultured alveolar type II cells [2], and that phosphatidylcholine is also the major phospholipid in the PDE obtained from human subjects [23]. Moreover, lamellar bodies, which have been identified as the storage and secretion form of surfactant in type II cells, have been shown to be present also in the cytoplasm of human, rat and rabbit mesothelial cells, and to be secreted on the cell surface [3]. Various organs are known to elaborate materials with certain similarities to lung surfactant in their phospholipid composition, including the pleura, joints and digestive tract [24,25]. Among these, only a few have been studied for the presence of surfactant proteins. SP-A has been detected in small intestine and colon [26] and SP-D in stomach [27]. In the intestinal lumen, SP-A and SP-B have been shown to be associated with secreted surfactant-like particles [28,29]. Our study shows that SP-A and SP-D are also present in mesentery in addition to phospholipids similar to those in lung surfactant. The monomeric isoforms of SP-A present in mesentery might correspond to the glycosylated monomeric 32 and 38 kDa forms of pulmonary SP-A. However, as previously discussed [26], SP-A in cells is detected mainly as irreducible or hardly reducible multimeric forms. The 60–66 kDa form is consistent with a non-disulphide-linked dimer of the 32–38 kDa proteins, although the nature of the cross-links remains unclear. SP-D in mesentery appeared with the same molecular mass features as that in lung and can be considered to be similarly glycosylated.

The presence of SP-A in the epithelial sheet of peritoneum HPES (likely to contain essentially mesothelial cells) separated from other cell types suggests that mesothelial cells are the source of this protein in mesentery. A weak SP-A signal was found consistently in isolated cultured mesothelial cells. The weakness of the signal compared with that of mesentery *in situ* can be accounted for by some loss of expression during culture, similar to that observed for alveolar type II cells cultured on plastic [30]. No SP-D was detected in the cultured cells but its presence in the epithelial sheet argues for its production by mesothelial cells also.

It has been reported that the plasma membrane of mesothelial cells has the same properties of lectin-binding as that of the type II cells because it also binds *Maclura pomifera* lectin [31]. The finding of SP-A in mesentery might provide a possible interpretation of this similarity. Taking into account on the one hand the lectin-like character of SP-A, and on the other hand the fact that putative type II cell membrane receptors to SP-A have been detected [32], it is possible that these similar lectin-binding properties might reflect the presence of the same receptors, including SP-A receptors, on both cell types.

In contrast with collectins, SP-B, a functionally crucial component of alveolar surfactant [33], seems to be absent from mesentery. In the lung, SP-B and SP-A are implicated together in the formation of tubular myelin, the extracellular transition structure between lamellar body content and the surface lining film. The absence of SP-B from mesentery is in keeping with the fact that no tubular myelin figures have been observed in thin sections at the surface of mesothelial cells [3]. It also suggests that the properties of mesenteric surfactant might be somewhat different from those of pulmonary surfactant and do not require the presence of SP-B.

Because the pulmonary and intestinal epithelia are of endodermal origin, whereas mesenteric cells are of mesodermal origin, it should be emphasized that the present findings represent the first report of hydrophilic surfactant protein production by a tissue of non-endodermal origin. The finding of SP-A and SP-D collectins in mesentery therefore indicates that these proteins can be present in tissues that elaborate a surfactant-like material, whatever the embryonic origin.

With regard to the possible function of SP-A and SP-D in mesentery, one can assume that they could have, in the peritoneal cavity, a role similar to that suggested for their presence in the lung as elements of the pre-immune response mechanism against pathogens. Both SP-A and SP-D opsonize micro-organisms and facilitate their phagocytosis by macrophages [6]. It has been

demonstrated that SP-D also enhances the production of oxygen radicals by rat alveolar macrophages [34]. Although the latter effect of SP-D was not demonstrated for peritoneal macrophages [34], it is likely that the other collectin functions might be operative in mesentery because macrophages are present in the peritoneal cavity in amounts making them available for recruitment to sites of inflammation. The presence of SPs in mesentery might improve our understanding of the mechanisms of defence in organs containing few immunocytes. Taking into account the properties of the collagenous C-type lectins as proteins interacting (1) with a variety of micro-organisms and (2) with phagocytic cells, SP-A and SP-D might therefore have functions in the protection of peritoneal cavity against pathogens.

Repeated peritoneal dialysis induces an inflammatory reaction. The presence of SP-A and SP-D in human and rat mesenteric tissue in the absence of dialysis treatment, however, indicates that they are normal components of mesenteric surfactant under basal conditions and that the presence of SP-A in dialysis effluent does not result from inflammation. The immunosuppressive properties of lung surfactant have long been recognized [9]. The protein moiety of lung surfactant, as well as its phospholipid components, have been demonstrated to inhibit lymphocyte proliferation *in vitro* [35]; recently, purified bovine SP-A has been shown not only to have this proliferation-inhibiting activity but also to inhibit the production of interleukin 2, an inflammation-promoting cytokine, by mononuclear cells in a dose-dependent manner [36]. Anti-inflammatory and immunosuppressive functions of mesenteric surfactant, which contains both phospholipids and SP-A, are therefore likely. In this respect, in the goal of protecting mesenteric surface against inflammation, it may be useful to enrich dialysis fluid with an exogenous surfactant containing SP-A and/or SP-D.

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