State of the Art

Lung Epithelium–specific Proteins
Characteristics and Potential Applications as Markers

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The lung interfaces with the environment across a continuous and heterogeneous epithelium. The proximal conducting airways are lined with a pseudostratified epithelium that is progressively replaced by a simple cuboidal cell layer in the more distal airways and by a very thin epithelial lining coating more than 95% of the lung's surface area in the alveoli. Whereas numerous cell types, including ciliated, basal, goblet and Clara cells, are present along the airways, only squamous type I and cuboidal type II cells cover the extensive alveolar surface (1–3).

The primary function of the lung, and of the alveolar epithelium in particular, is to provide an extensive and thin surface for gas exchange. The pulmonary epithelium serves a number of additional functions that basically act to preserve the capacity for such gas exchange. It provides a barrier that protects the host from the outside environment by segregating inhaled foreign agents, and it controls the movement of solutes and water, contributing to the maintenance of lung fluid balance. The lung epithelium also plays an active role in the metabolism of endogenous mediators and xenobiotic agents, and is capable of regeneration, allowing normal cell turnover and restoration of airway and alveolar functions after lung injury. Beyond this, the lung epithelium produces complex secretions, among which is the mucus blanket, a surface-active agent (surfactant), as well as several proteins important for host defense (4–7).

Sampling of the epithelial lining fluid (ELF) by bronchoalveolar lavage (BAL) is the common means of studying the proteins secreted by the lung epithelium and investigating their changes in lung diseases (8). Recent studies have shown that some proteins secreted by airway or alveolar epithelial cells are present not only at the surface of the respiratory tract, but also and normally in small amounts in the bloodstream. Among these proteins are the 16-kD Clara cell secretory protein (CC16, CC10), three surfactant-associated proteins (surfactant protein [SP]-A, SP-B, and SP-D) and mucin-associated antigens, as recognized by monoclonal antibodies (KL-6, 17-B1, 17-Q2). Because these proteins are mainly, if not exclusively, secreted within the respiratory tract, their occurrence in the vascular compartment can only be explained by assuming their leakage from the lung into the bloodstream. Interestingly, these proteins show variations in the serum of patients with different lung diseases and subjects exposed to lung toxicants, suggesting that their assay might represent a new approach in the assessment of lung disorders.

The purpose of this review is to summarize the current state of knowledge about these lung secretory proteins and their potential value as lung peripheral biologic markers. The review is divided in three sections, as follows: (1) A brief summary of the structural and functional characteristics of CC16, CC10, SP-A, SP-B, SP-D, and mucin-associated antigens; (2) a review of available data on the occurrence of these lung-specific proteins in pulmonary and extrapulmonary biologic fluids; (3) a discussion of the mechanisms likely to be involved in the passage of these proteins into the bloodstream and the potential clinical usefulness of their assay in blood.

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STRUCTURAL AND FUNCTIONAL CHARACTERISTICS OF LUNG EPITHELIUM–SPECIFIC PROTEINS

Surfactant-associated Proteins

Pulmonary surfactant is a complex material covering the alveolar surface of the lung. It is composed mainly of structurally heterogeneous phospholipids. A major function of pulmonary surfactant is to reduce the surface tension at the air–liquid interface of the alveolus, thereby preventing alveolar collapse on expiration. In 1973, King and associates demonstrated that the surfactant also contains specific proteins (9, 10). Four surfactant-specific proteins, with different structural and functional properties, have so far been identified. They were named SP-A (9, 11, 12), SP-B (13, 14), SP-C (15, 16), and SP-D (17–19) according to the chronologic order of their discovery (20). Extraction with organic solvents of the lipid-rich pellet recovered after ultracentrifugation of bronchoalveolar lavage fluid (BALF) allows the distinction of two families of surfactant-associated proteins: the low-molecular-weight hydrophobic SP-B and SP-C, and the high-molecular-weight hydrophilic SP-A and SP-D (20–22). The surfactant proteins have been the subject of several reviews (21, 23–38). The present review will consider only the structural aspects and localization of SP-A, SP-B, and SP-D, which have been shown to occur in the serum. Although it is an important surfactant component, SP-C, whose presence in serum has never been reported, will not be considered (39).

SP-A. SP-A is the major surfactant-associated protein, constituting approximately 3 to 4% of the total mass of isolated surfactant and 50% of the total surfactant protein (6, 40). As shown in Table 1, SP-A has also the highest molecular weight of the surfactant-associated proteins, consisting in its mature form of a complex hexameric structure with a total molecular mass of about 650 kD (41, 42). Each of the six subunits of SP-A is composed of three polypeptide chains, the amino-acid sequence of which has been deduced from the corresponding complementary DNA (cDNA) (11, 12). The primary translation product is a 247- to 248-residue–long polypeptide with a molecular mass of 28 kD (11). After removal of a 20-residue signal peptide, each polypeptide chain includes four discernible domains: a short amino-terminal region; a collagenlike domain consisting of 23 Gly–Xaa–Yaa triplet repeats, where Yaa is often proline; a segment of approximately 30 residues, termed the "neck region"; and a carboxy-terminal domain (11, 30, 43–49). A number of posttranslational modifications are made to the SP-A amino-acid chain, including acetylation (50), sulfation (51), hydroxylation (52), and addition of complex oligosaccharides (53–55), some of which share strong antigenic determinants with a major blood group A antigen (56). Because of this multitude of modifications, SP-A actually constitutes a heterogeneous family of molecules with respect to size (26 to 38 kD in the reduced state) and charge (isoelectric points varying between 4 and 5) (40, 47, 48, 54, 57–60).

In humans, two functional genes of SP-A (SP-A1 and SP-A2), as well as a pseudogene, have been identified on the long arm of chromosome 10 (11, 12, 61–64). SP-A2 is expressed only in airways cells, whereas both SP-A1 and SP-A2 are expressed in type II cells (65). The products of both genes, which present minor heterogeneities (11, 12, 66), appear to be required for the formation of mature SP-A (12, 63, 64, 67). To form the trimeric subunit, three polypeptide chains are associated through disulfide bonds between the cysteine residues of their amino-terminal portions, while their collagenous regions are arranged to form a left-handed triple helical structure (41–43). The noncollagenous C-terminal extremities form a globular head that can bind carbohydrate in a calcium-dependent manner, and is currently named the carbohydrate recognition domain (CRD) (43, 68–70). The secreted form of SP-A is assembled into a large asymmetric oligomer with a rodlike amino-terminal stem consisting of six closely associated triple helices, each connected to a globular head. This overall plan closely resembles the "tulip bunch" organization of the first component of the complement system, C1q (42, 71, 72). The association of a collagenous tail with a globular head containing a CRD is a structural and functional characteristic of an ancient family of proteins present in prokaryotes: the lectins or collectins (collagenous lectins) (68, 70, 73–76).

Immunocytochemical studies have shown SP-A to be present in alveolar type II and nonciliated bronchiolar Clara cells, as well as in alveolar macrophages (AM) (77–79). The pattern of distribution of SP-A secretion within the respiratory tract is, however, much larger. *In situ* hybridization has

TABLE 1

CHARACTERISTICS OF LUNG EPITHELIUM–SPECIFIC PROTEINS

Name	Pulmonary Producing Cells	Pro-protein (*kD*)	Posttranslational Modifications	Protein (*kD, reduced*)	Quaternary Structure	Hydrophobicity	Isoelectric Point	Functions
CC16-CC10	Clara Bronchial	> 8	Signal peptide cleavage Dimerization	8	Dimer (15.8 kD)	+	4.6	Immunosuppression Antiinflammation
SP-A	Type II Clara Bronchial	28	Signal peptide cleavage Acetylation Glycosylation Hydroxylation Sialylation, sulfation Oligomerization	28–36	18-mer (650 kD)	++	4.4–5	Tubular myelin Formation Surfactant turnover Host defense
SP-B	Type II Clara	40–42	Signal peptide cleavage Glycosylation Proteolytic processing Dimerization	5–8	Dimer (15–18 kD)	+++	5 (precursor) > 7 (mature SP-B)	Tubular myelin Formation Surfactant adsorption
SP-D	Type II Clara	40	Signal peptide cleavage Glycosylation Oligomerization	42	12-mer (600 kD)	+	6–8	Host defense
KL-6	Type II Bronchiolar	> 200	Glycosylation	> 200	—	+	< 6	Glycocalix formation Host defense
17-Q2 17-B1	Mucous	HMW	Glycosylation, sulfation Polymerization	HMW	Polymeric Gel	+	< 6	Mucus layer Host defense

Definition of abbreviation: HMW = high-molecular-weight mucins.

revealed expression of the human SP-A gene in alveolar type II cells and Clara cells, and focally in cells of the larger conducting airways (80, 81). Even if the lung appears as the major site of SP-A synthesis (75, 82), expression of SP-A has been detected in several extrapulmonary tissues. Reverse transcription–polymerase chain reaction (RT–PCR) has revealed SP-A transcripts in small intestine and colon of the rat, which secrete a surfactantlike material (83–85), and in the human thymus, prostate (75), and mesentery (86). A surfactant protein, probably SP-A, has also been detected in human middle-ear effusion (87), serosal mesothelium (peritoneum, pleura, and pericardium), and joints (88). Most SP-A is released in the lumen of the respiratory tract by constitutive secretion, independently of lamellar bodies, which contain only small amounts of SP-A (35, 89, 90). In the alveolar compartment, SP-A binds with high affinity to surfactant lipids (91–95), most likely in a calcium-dependent manner (95), as suggested by the dissociation of SP-A from surfactant lipids by calcium-chelating agents (95). A simple and rapid purification procedure for SP-A, using calcium chelation of isolated surfactant, has recently been described (96).

Functionally, SP-A is involved in numerous aspects of the surfactant system. SP-A promotes, in collaboration with SP-B, the structural transformation of lamellar bodies into tubular myelin, a transitional, lamellarlike structure consisting of surfactant-associated proteins, phospholipids, and calcium (97, 98). SP-A has long been considered to collaborate with SP-B and SP-C to spread and stabilize the phospholipid layer at the alveolar surface, and to be essential for the reduction of alveolar surface tension (91). However, no obvious effect on respiratory function has recently been observed in SP-A gene knockout mice (99). Another postulated function of SP-A would be to regulate the alveolar surfactant concentration by inhibiting the secretion and enhancing the uptake of surfactant phospholipids by alveolar type II cells (100–104). This regulation would be mediated by an alveolar type II cell receptor (104–107). Recent *in vivo* and *in vitro* studies with SP-A–deficient mice and showing only minimal effects on surfactant lipid metabolism are, however, inconsistent with such a regulatory role (108). SP-A has also been implicated in alveolar defense mechanisms through opsonization of microorganisms and promotion of phagocytosis (27, 38, 71, 109, 110). This role is supported by the greater susceptibility to group B streptococcal infection recently demonstrated in SP-A–deficient mice (111).

SP-B. SP-B appears as a minor constituent of the pulmonary surfactant, which because of its extreme hydrophobicity has long been very difficult to purify and characterize (21, 112). The main features of SP-B are summarized in Table 1. Mature SP-B is a disulfide-linked homodimer composed of two 79-residue polypeptide chains (14, 112, 113), with a molecular mass estimated by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) of approximately 15 to 18 kD under nonreducing conditions and of about 5 to 8 kD after reduction (13, 114–117). Specialized and complex processes are required to synthesize and release this highly hydrophobic protein into the extracellular alveolar compartment (118–120). The primary translation product of human SP-B has been identified as a hydrophilic 40-kD preproprotein of 381 amino acids (13, 14, 121, 122). Removal of the amino-terminal hydrophobic segment, which presumably acts as a signal peptide, and glycosylation, results in a proprotein of 42 kD (119). Further processing involves at least two distinct proteolytic events: (*1*) removal of 176 amino acids of the amino-terminal propeptide, to afford a 25-kD intermediate; and (*2*) cleavage of 102 amino acids of the carboxy-terminal peptide to yield the fully active form, consisting of 79 amino acids (113, 118–120, 123). By contrast with the SP-B precursor, which is an anionic protein (isoelectric point between 5.1 and 5.4), the mature protein features a net positive charge at physiologic pH (21, 112, 119). Mature SP-B contains three intrachain disulfide bridges, which connect distant parts of the polypeptide in a tightly folded structure (112). It also exhibits a high degree of α-helical structure, with positively charged residues predominating on one surface and hydrophobic residues on the opposite surface (124). The α-helices interact preferentially with the superficial parts of the lipid bilayers, whereas the positively charged basic residues are thought to be important for the interactions of SP-B with anionic phospholipids (112, 125).

The human SP-B gene encompasses approximately 9.5 kb and is located on chromosome 2 (121, 126). Restriction mapping of human genomic DNA is consistent with the existence of a single SP-B gene (121). Some internal positions of the SP-B amino-acid sequence exhibit polymorphisms, which do not appear to have major structural or functional implications (113, 127). Like SP-A and SP-D, SP-B is synthesized by alveolar type II and Clara cells (79, 81). SP-B precursor is present in the endoplasmic reticulum, Golgi complex, and lamellar bodies, whereas mature SP-B is found only in lamellar bodies (120). Study of the tissue distribution of SP-B synthesis through Northern blot analysis demonstrates strict specificity of this protein to pulmonary tissues in the mouse (128). By contrast, positive immunohistochemical labeling for SP-B has been reported in the rat intestinal brush border (84). Functionally, SP-B has no immunomodulatory or regulatory function, but seems to be a key protein in the organization of lamellar bodies and tubular myelin (129, 130) as well as in the formation of a functionally optimal and stable surfactant monolayer on the alveolar surface (14, 125, 131, 132). Indeed, SP-B markedly reduces the surface tension at the alveolar surface by squeezing out unsaturated phospholipids from the monolayer (133, 134). The importance of SP-B to the function of the pulmonary surfactant has been highlighted by the fatal respiratory distress suffered by infants homozygous for a mutation in codon 121 of the SP-B gene (135, 136), as well as by the decreased lung compliance in heterozygous (137, 138) and respiratory failure in homozygous SP-B-deficient mice (137).

SP-D. SP-D is a large, multimeric, water-soluble collagenous glycoprotein with a structural organization similar to that of SP-A (17, 18, 139–143). In its mature state, SP-D is made up of four subunits, each of them composed of three apparently identical polypeptides of about 43 kD (18, 19, 142, 144). The primary translation product is 355 residues long, with different domains consisting of a short *N*-terminal section followed by a collagenlike region of 59 Gly–Xaa–Yaa repeats and a C-terminal section of 153 amino acids (19, 140). Several posttranslational modifications have been described for SP-D, including cleavage of the signal peptide, hydroxylation of lysine and proline, and glycosylation within the collagenlike region (145). To form the 160-kD trimeric subunit, three polypeptidic chains are associated in a manner similar to that in SP-A, in a collagenlike triple helix at one extremity and a globular head at the other (146). The secreted form of SP-D is assembled into a large dodecamer of approximately 600 kD, with a rodlike amino-terminal stem consisting of four closely associated triple helices, each connected to a globular head (19, 145, 146). The occurrence of higher-order oligomers with a molecular mass exceeding 900 kD has also been reported (145, 146). In contrast to the bouquet organization of SP-A, SP-D forms a cruciform structure similar to that described for its cousin in the collectins family, conglutinin (145, 146). SP-D is encoded

by a single gene of approximately 11 kb, localized on human chromosome 10, in proximity to the genes for two other C-type lectins, SP-A and mannose-binding protein (MBP) (147–149).

Immunohistochemical studies have localized SP-D in alveolar type II and Clara cells (150–152). Regarding its subcellular localization, SP-D does not localize to lamellar bodies, which suggests that SP-D is synthesized independently of the surfactant pathway (150). Even if the lung appears as the major site of synthesis of SP-D, its expression is not restricted to the respiratory tract. Northern blotting and RT–PCR analysis have revealed SP-D transcripts in the rat gastric mucosa at expression levels threefold lower than those observed in the lung (153). In the mouse, SP-D synthesis has been detected in stomach and kidney tissues, as well as in the heart, with levels of expression 24-fold lower than those in the lung (154). SP-D transcripts have also been detected by RT–PCR in human extrapulmonary organs, including the small intestine and colon (75), as well as the stomach, but not in the heart (154). In contrast to SP-A, which is tightly bound to phospholipids, SP-D exists in lipid-bound and lipid-free forms, with the latter predominating in the extracellular alveolar compartment (144). The functional properties of SP-D in the surfactant system have not been fully elucidated, but SP-D apparently does not contribute to the surface-active properties of the surfactant complex (142). Structural analogies with other collagenlike proteins, including C1q, MBP, conglutinin, and SP-A, suggest that SP-D may play a role in local host defense, perhaps by functioning as an opsonin (27, 76, 146).

Clara Cell Protein (CC16, CC10)

The Clara cell protein is a homodimer consisting of 70 amino-acid subunits in antiparallel orientation and connected by two disulfide bonds (155). This protein has been studied in a wide variety of species including rats, mice, rabbits, dogs, and humans (155–159). Depending on the species and the source of isolation, it has been referred to in the literature by various names, including uteroglobin, human protein 1, urine protein 1, polychlorinated biphenyl-binding protein, Clara cell secretory protein (CCSP), Clara cell 10 kD protein (CC10), and Clara cell 16 kD protein (CC16) (160). The exact molecular mass of this protein, as determined by electrospray ionization/mass spectrometry, is 15,840, which justifies the abbreviation of CC16 used hereafter to designate the protein (161). However, because of an anomalous electrophoretic mobility, the protein, upon SDS–PAGE, shows an apparent molecular size of both 10 kD and 7.8 kD, under nonreducing and reducing conditions, respectively, and is frequently abbreviated as CC10 (162, 163).

The production of CC16 by nonciliated bronchiolar Clara cells in humans and rodents was first described in the mid-1980s by Singh and colleagues (164–171). Only minute amounts of CC16 were detected in BALF, and it was concluded that CC16 was a minor constituent of BALF, accounting for only 0.14% of the total protein content (168). In 1992, a new urinary protein called protein 1, initially isolated and purified from the urine of patients with renal tubular dysfunction, was identified as the lung secretory protein CC16 (172–175). The identity of this protein with CC10 was based on amino-acid sequencing and was confirmed by the finding of increased concentrations of protein 1 in sputum and BALF (172–174). CC16 shares several features with uteroglobin, a secretory protein of the lung and endometrium in rabbits (158, 162, 176–179). The two proteins show 53%, 55%, and 61% sequence homology in monkey, rat, and human, respectively, as well as similarities in their tertiary structure (155, 156, 158, 176, 180). Genetically, both proteins are encoded by single-copy genes of similar structures (i.e., two introns and three exons) (178, 179, 181), whose promoter regions contain transcriptional regulatory elements conserved between species. In humans, the CC16 gene has been localized to chromosome 11, at p12–q13, a region occupied by genes involved in the regulation of inflammation (*see the subsequent discussion*) (179, 182).

In both humans and rodents, immunohistochemical studies done with anti-CC16 antibodies have revealed that CC16 is essentially localized to Clara cells in terminal bronchioles (183–186). CC16, however, is not an entirely specific and exclusive product of Clara cells, or even of the lung. Through *in situ* hybridization, CC16 has been shown to be expressed by nonciliated cells along the tracheobronchial epithelium (80) and, like uteroglobin, in the urogenital tract, and particularly in the prostate (187), which explains its sex-dependent urinary excretion as well as its presence in semen (174, 188, 189). In the rat, Northern blot analysis indicates that the expression of CC16 is confined to the lung and the trachea (190).

The exact functions of CC16 are still unknown but there is increasing evidence that, like uteroglobin, CC16 plays an important immunosuppressive and antiinflammatory role in the lung (158, 160, 176, 177, 191–193). CC16 has been shown to inhibit the activity of cytosolic phospholipase A_2 (PLA_2), a key enzyme in inflammatory phenomena (177, 183). By inhibiting PLA_2, CC16 could also prevent the degradation of lung surfactant phospholipids (194). Additionally, CC16 can inhibit production of interferon-γ (IFN-γ) by peripheral blood mononuclear cells (193). The biologic actions of IFN-γ, namely its antiviral activity as well as its phagocytosis-stimulating activity, are also diminished by the addition of CC16 (193). The potential role of CC16 as a downregulator of inflammation is supported by the increased sensitivity to hyperoxic or ozone-induced lung injury and an exaggerated inflammatory response of CC16-deficient mice (195, 196). CC16 produces a dose-dependent inhibition of platelet-derived growth factor-induced chemotaxis of fetal lung fibroblasts, and decreased availability of CC16 may facilitate the recruitment of fibroblasts in fibrosing lung disorders (183). Beyond these other properties, CC16 contains a central hydrophobic cavity that binds phospholipids, progesterone, and xenobiotics, among them the widespread pollutants polychlorinated biphenyls (156, 197–199). CC16 could thus also play an important role in the sequestration or clearance of some harmful substances deposited in the respiratory tract.

Mucin-associated Antigens

Mucins are major components of the mucus layer covering the airway epithelium (200). They consist of high-molecular-weight glycoproteins belonging to a broad family of mucin peptides and characterized by different carbohydrate side chains (200–203). Nine mucin genes, named MUC1, 2, 3, 4, 5AC, 5B, 6, 7, and 8, have so far been identified, and three of them have been fully cloned (204–206). Four of these genes, MUC1 (207, 208), MUC2 (209, 210), MUC4 (211, 212) and MUC5 (213, 214), are expressed in the respiratory tract. All mucin genes are characterized by repetitive amino-acid structures, most commonly long stretches of tandemly repeated peptides, which can extend over more than 2,000 amino-acid residues (200). The tandem repeat units are rich in serine and threonine, and contain at least one proline residue per repeat. After translation, these proteins undergo extensive modification by glycosyltransferase, sialotransferase, and sulfotransferase, yielding diverse oligosaccharide structures joined primarily through O-glycosidic linkages to serine and threonine residues of the protein backbone (200). Various cells synthe-

size the same peptide backbone, but, through differential expression of glycosyltransferase, produce oligosaccharidic side chains showing cellular specificity (215).

Mucins are either associated with membranes or secreted at the surface of the respiratory tract (216, 217). Secretory mucins contribute to the viscid mucus of the tracheobronchial tree, and typically form extremely large polymers through linkage of their protein monomers by disulfide bonds. Secreted proteins form the mucous gel, which becomes an integral part of the mucociliary escalator that protects the epithelial surface from injury and facilitates the removal of material entering the lung. By contrast, membrane-associated mucins have a hydrophobic membrane-spanning domain and do not form oligomeric complexes. Membrane-associated mucins may form part of the cell surface glycocalyx and play a role in facilitating or inhibiting bacterial adherence (218).

KL-6 was initially described as a high-molecular-weight human glycoprotein detected by a murine monoclonal antibody that recognizes a mucinous sialylated sugar chain (219). The gene for KL-6 has been classified as belonging to the MUC1 gene family (220, 221), which encodes membrane-associated mucins (204, 222, 223). High concentrations of KL-6 have been detected in sera from patients with lung, pancreatic, and breast cancers, and more recently in patients with diffuse interstitial lung diseases, suggesting that KL-6 is released from neoplastic or pulmonary cells in the circulation (221). The diagnostic value of KL-6 as a tumor marker is, however, lower than that of other mucinous epitopes detected by monoclonal antibodies (219, 221). By contrast, KL-6 is apparently a sensitive indicator of damage to alveolar type II cells, which strongly express this mucin at their surface. The biochemical characteristics of KL-6 have recently been established. The glycoprotein has a molecular weight of approximately 200 kD on SDS–PAGE, and presents a high proportion of proline, alanine, threonine, serine, and glycine residues (224). The sugar content of KL-6 is characterized by high levels of *N*-acetylneuraminic acid, galactose, *N*-acetylgalactosamine, and *N*-acetylglucosamine (224). These biochemical features are quite similar to those previously reported for mucins deriving from the MUC1 gene (217, 225).

The MUC1 gene is expressed at high levels in the epithelium of the bronchi and bronchioles, and at slightly lower levels in alveoli (207, 226). Immunohistochemistry has mainly detected KL-6 in alveolar type II and epithelial cells of the respiratory bronchioles (219). No reaction has been obtained with other lung cell types (i.e., alveolar type I cells, goblet cells, and mucous cells of the bronchial glands). Interestingly, proliferating and regenerating alveolar type II cells in idiopathic pulmonary fibrosis (IPF) and radiation pneumonitis express the antigen more strongly than do normal alveolar type II cells (227, 228). In these conditions, a strong reaction for KL-6 was obtained with the apical region of regenerating alveolar type II cells and macrophages, but not with interstitial components. However, KL-6 is not entirely lung-specific, since it is also present on other somatic cells such as esophageal epithelial cells, ductal epithelial cells of the pancreas, and fundic gland cells of the stomach (219). This distribution is in agreement with the low level of expression of the MUC1 gene in many extrapulmonary epithelial tissues. Functionally, KL-6 has recently been shown to promote the migration of human lung and skin fibroblasts, suggesting that this membrane-associated mucin might be implicated in lung fibrogenic process (224).

In contrast to KL-6, which is mainly associated with cellular membranes, the oligosaccharidic epitopes recognized by the monoclonal antibodies 17-Q2 and 17-B1 belong to mucinous glycoproteins that are secreted in the tracheobronchial lumen (229–233). The amino-acid sequence of the protein backbone of the mucins recognized by the 17-Q2 and 17-B1 antibodies is rich in serine and threonine (229). The exact nature of the oligosaccharidic epitope has not yet been elucidated. Its sensitivity to β-endogalactosidase and periodic acid treatment suggests a structure at or near a nonsulfated glycoside bond such as Gal(β1–4)Glc in lacto-*N*-tetrose, which is present in respiratory tract mucins and is not structurally related to A, B, or O blood-group determinants (229, 231, 234). The specificity of these antibodies has been assessed with Western blot analysis and immunohistochemistry (229, 230): 17-Q2 and 17-B1 react with high-molecular-weight mucinous glycoproteins purified from airway secretions, and specifically label secretory granules of the surface epithelium and submucosal glands of monkey and human conducting airways.

CONCENTRATIONS IN BIOLOGIC FLUIDS

Pulmonary Fluids

SP-A. Numerous studies have been published since 1991 on the levels of SP-A in BALF of healthy subjects and patients with lung diseases. As shown in Table 2 (235–253), the mean concentrations of SP-A in BALF show a great dispersion, ranging from 0.2 to 120 mg/L. These large variations most likely result from the use of different antibodies and standards in the immunochemical measurement of SP-A, as well as from differences in BAL protocols and the preanalytical treatment of BALF samples. Because SP-A is tightly bound to phospholipids in a calcium-dependent manner, its concentrations in BALF differ markedly when measured in the surfactant phospholipid-rich pellet or in the BALF supernatant. Kuroki and associates have indeed shown that 99.1% of SP-A is associated with the crude surfactant pellet, whereas 0.9% is present in the supernatant (144). The calcium-dependent phospholipid binding of SP-A also explains why BALF supernatant obtained by low-speed centrifugation and stored at −70° C contains much less SP-A levels than does the same supernatant stored in the presence of ethylenediamine tetraacetic acid (EDTA) (254), which probably contributes to exposing SP-A antigenic determinants by disrupting the tubular myelin network (96, 255).

Surfactant-associated proteins have been particularly studied in lung injury, a situation known to be associated with alveolar type II cell damage and alterations of the surfactant system (Table 2). Decreased SP-A levels in BALF have been reported in patients with acute respiratory distress syndrome (ARDS) or at risk of developing ARDS (236, 237, 256, 257), in patients with traumatic parenchymal lung injury (238), and in a small group of patients who had undergone high-dose hemithorax irradiation (249). Of note is that in traumatic lung injury, SP-A decreases transiently and returns to normal levels with a time-course that depends on the severity of the lung injury (238). By contrast with ARDS, no alteration of SP-A in BALF has been detected in a subgroup of patients with hydrostatic pulmonary edema (237). Patients with pneumonia and cystic fibrosis (CF) show inconsistent changes of SP-A in BALF. Decreased levels have been found in human immunodeficiency virus (HIV)-negative patients with bacterial pneumonia (237, 239), whereas higher levels have been reported in HIV-positive patients with *Pneumocystis carinii* pneumonia (240, 241) or tuberculosis (258). In CF, SP-A levels in BALF are either reduced or unchanged in clinically stable patients (252, 253), but are increased in the presence of infection and/or inflammation (253).

Several studies have also been done on SP-A in interstitial

TABLE 2

CONCENTRATIONS OF SURFACTANT-ASSOCIATED PROTEIN A IN LUNG LAVAGE FLUID OF HEALTHY SUBJECTS AND PATIENTS WITH LUNG DISORDERS

	Controls (*mg/L*)		Patients (*mg/L*)				
	n	Mean ± SEM or Range	n	Mean ± SEM or Range	Comments	Statistical Significance	Reference No.
Healthy subjects							
Adults	17	3.6 [0.2–16.0]			No difference between adults and children	NS	235
Children	35	6.9 [0.1–33.7]					235
ARDS	29	123.6 ± 20.6	66	29.9 ± 8.5	Established ARDS	S	236
			20	49.3 ± 21.7	At risk of ARDS	S	236
	20	1.5 ± 0.2	16	0.8 ± 0.1	Without pneumonia	S	237
			36	0.7 ± 0.08	Associated pneumonia	S	237
Lung trauma	10	2.7 ± 0.3	10	1.0 ± 0.16	Severe trauma	S	238
			9	1.9 ± 0.2	Moderate trauma	S	238
Pneumonia	11	15.0 [5.8–36] (HIV−)	12	1.9 [0.5–6.7]	Gram-positive organisms	S	239
			10	5.5 [0.6–12.7]	Gram-negative organisms	S	239
	20	1.5 ± 0.2 (HIV−)	88	0.9 ± 0.07	Severe pneumonia	S	237
	21	1.5 ± 0.2 (HIV+)	22	5.2 ± 0.5	AIDS-related pneumonia	S	240
	29	0.2 [0.06–3.8] (HIV+)	16	10.3 [2.8–24.3]	HIV+, *Pneumocystis carinii*	S	241
	11	14.1 [3.7–23.2] (HIV−)	6	5.8 [1.5–13.1]	HIV−, *Pneumocystis carinii*	S	241
Pulmonary fibrosis	25	0.8 ± 0.1	28	0.4 ± 0.1	Idiopathic fibrosis	S	242
	8	1.4 [0.7–4.8]	28	0.4 [0.05–4.3]		S	243
	11	15.0 [5.7–26.5]	10	18.4 [6.5–45.6]		NS	239
	16	12.1 ± 6.7	11	7.6 ± 3.6	Idiopathic fibrosis	S	244
Sarcoidosis	10	2.8 [0.9–5.2]	20	3 [0.7–7]		NS	245
	21	4.0 [2.0–6.3]	35	8.0 [2.4–17.4]	X-ray stages I and II	S	246
			22	8.3 [3.3–15.6]	Stage I		246
			13	7.6 [2.4–17.4]	Stage II		246
	16	12.1 ± 6.7	23	16.5 ± 5.6		S	244
Asbestosis	39	1.6 ± 0.4	34	4.8 ± 1.4		S	247
Hypersensitivity pneumonitis	21	4.0 [2.0–6.3]	10	9.0 [3.0–19.1]		S	246
	14	0.7 ± 0.11	20	1.4 ± 0.3	Symptomatic subjects	S	248
			16	0.6 ± 0.09	Asymptomatic subjects	NS	248
	16	12.1 ± 6.7	12	15.9 ± 5.4		S	244
Radiation injury	4	0.5 ± 0.06	4	0.06 ± 0.03		S	249
Asthma	10	2.8 [0.9–5.2]	13	1.9 [0.4–2.9]		S	245
Alveolar proteinosis	13	3.5 [1.3–5.2]	6	39.3 [25.0–62.8]		S	250
Tobacco smoking	10	3.4 [2.0–5.2]	11	4.5 [2.2–6.3]		NS	246
	8	3.1 ± 0.4	12	1.8 ± 0.4		S	251
Cardiac lung edema	20	1.5 ± 0.17	13	1 ± 0.1		NS	237
Cystic fibrosis	17	5.4 [0.9–13.0]	20	1.9 [0.1–4.6]	No infection	S	252
	6	4.5 [2.6–8.4]	15	6.7 [4.8–8.0]	No infection	NS	253
			12	9.3 [8.4–14.6]	Infection	S	253

lung disorders. In a group of patients with various interstitial lung diseases, including a majority of patients with IPF, the SP-A concentration of BALF was significantly decreased (242–244), and in one study this reduction was correlated with the clinical outcome and survival time (243). These results were, however, not confirmed by Baughman and coworkers (239). Whereas levels of SP-A in BALF of IPF patients are reduced or unchanged, elevated levels have been reported in patients with various interstitial conditions including sarcoidosis (244, 246, 259), hypersensitivity pneumonitis (244, 246), acute farmer's lung disease (248), and asbestosis (247). In the last-named group, the levels were not significantly different in exposed workers without lung involvement and those with early and advanced asbestosis (247). As expected, SP-A levels were significantly increased in patients with pulmonary alveolar proteinosis (PAP) (250). Inconsistent results have been reported with regard to the effect of smoking on levels of SP-A in BALF, which have been found to be decreased (251, 260, 261), slightly increased (246), or unchanged (247). In asthma, Van de Graaf and associates (245) have reported a significant decrease in SP-A in BALF.

SP-A levels have also been determined in tracheal aspirates and sputum. In a group of 120 mechanically ventilated children with respiratory failure, SP-A levels were found to be decreased in tracheal aspirates of children with bacterial pneumonia and viral pneumonitis, but unchanged among children with cardiopulmonary bypass (262). In ventilated infants with respiratory distress syndrome, SP-A in tracheal aspirate was either unchanged (262) or decreased (263, 264). SP-A has also been found to be markedly increased in airways secretions of adults with cardiogenic pulmonary edema, and to a lesser extent in those with ARDS and stable congestive heart failure (265). The average SP-A level in sputum of patients with PAP was found to be 400-fold higher than in other pulmonary diseases (266).

As in humans, SP-A levels have been determined in lung lavage fluid isolated from other mammals (144, 267–273). The average concentration has been estimated at 0.7 mg/L in sheep (270), 2 mg/L in rats (274, 275), and 350 mg/L in guinea pigs (276). Interestingly, an increase in lung lavage SP-A content has been reported in animals with lung inflammation induced by hyperoxia (268, 272), bacterial lipopolysaccharide (LPS) (267, 277), and silica (270, 275), as well as in septic rats with and without lung injury (271). However, no change has been

observed in rats with bleomycin-induced lung fibrosis (274) or exposed either to ozone (276) or cigarette smoke (278).

SP-B. Immunologic quantification of SP-B has long been hampered by the fact that this surfactant-apoprotein occurs in BALF in forms of varying size and hydrophobicity, whose proportions are largely unknown. Proteolytic processing of the SP-B precursor into mature protein appears to take place not only intracellularly, as previously suggested (279), but also extracellularly (120), resulting in the occurrence in ELF not only of the mature protein, but also of the SP-B precursor and processing intermediates (280). Because of differences in their hydrophobicity, mature SP-B is intimately associated with the phospholipid-rich surfactant pellet (281), whereas the less hydrophobic SP-B precursor and processing intermediates are less tightly bound to phospholipids and are therefore predominantly recovered in the hydrophilic BALF supernatant (280).

The concentrations of SP-B in BALF of healthy subjects were first measured by Gregory and colleagues (236). Using a sandwich-type enzyme-linked immunosorbent assay (ELISA) based on monoclonal and polyclonal antibodies directed against a synthetic human SP-B peptide and calibrated with purified bovine SP-B, these investigators found a mean value for SP-B of 1.3 ± 0.3 mg/L lavage fluid (117, 236). As shown in Table 3 (236, 237, 251–253, 282, 283), similar estimates have been made by Krämer and colleagues and Griese and associates with an immunoassay based on a monoclonal antibody against porcine SP-B that cross-reacts with the human apoprotein (252, 282), as well as by Lesur and coworkers in sheep (270). These estimates most probably do not represent the real concentrations of SP-B in BALF, since the treatment of BALF samples with EDTA and detergent results in much higher values, presumably by exposing SP-B epitopes normally hidden by phospholipids (253, 280, 281). So far, few studies have investigated the changes in SP-B in patients with lung disorders or in animal models of lung injury. As shown in Table 3, inconsistent changes have been observed in patients with ARDS (236, 237). Concentrations of SP-B in BALF were unchanged in patients with CF (252, 253), pneumonia (237), and cardiogenic lung edema (237). Normal levels were also found in tracheal aspirates from mechanically ventilated children with respiratory failure resulting from bacterial pneumonia, viral pneumonitis, ARDS, and cardiopulmonary bypass (262). A decrease in the concentration of SP-B in lung lavage has been reported in rats exposed to cigarette smoke (278), whereas an increase was observed in sheep with silicosis (270).

SP-D. The concentrations of SP-D in BALF have been determined so far with a single ELISA, using monoclonal antibodies directed against SP-D purified from BALF, and human recombinant SP-D as a standard (283, 284). This recombinant protein, produced by the baculovirus expression system, migrates as a diffuse band of approximately 41 kD on SDS–PAGE under reducing conditions (283). The concentration of SP-D in BALF of healthy subjects averages 0.88 ± 0.13 mg/L, which is approximately one-fourth that of SP-A assayed by the same investigators (251, 283) (Table 3). By contrast with SP-A and mature SP-B, most SP-D appears to occur in a soluble form not associated to surfactant lipids (144), suggesting that pretreatment with EDTA and detergent is less critical in its measurement in BALF. Few studies have been done of SP-D levels in lung diseases. The protein was markedly increased in patients with PAP (BALF concentrations 22-fold higher than in healthy volunteers) (283), but its levels were unchanged in patients with pulmonary sarcoidosis or pulmonary fibrosis, either idiopathic or associated with collagen vascular disease (283). A significant decrease in the concentration of SP-D has been reported in BALF of smokers (251). In animals, a marked increase in SP-D in BALF has been reported following lung inflammation induced by silica (285), LPS (267), and *Pneumocystis carinii* infection (286).

Clara cell protein (CC16, CC10). CC16 levels in human BALF were initially determined in 1989, with a nonisotopic immunoassay developed against an unknown urinary protein named protein 1. In BALF from healthy subjects, mean CC16 concentrations range from 0.5 to 1.5 mg/L, depending on the procedure used for lung lavage (161, 174, 185, 287). This concentration represents an average of 6.3% of that of albumin and 2.3% of that of the total lavage protein content (287). The concentrations of CC16 in BALF of healthy subjects show a great dispersion that cannot be explained solely by the variable dilution of BALF samples, suggesting interindividual variation in the synthesis/secretion of CC16 in the respiratory tract (287).

Levels of CC16 in BALF have been determined in various lung disorders. As shown in Table 4 (161, 174, 183, 185, 247, 287–289), a significant reduction has been found in BALF from smokers and patients with lung cancer or chronic ob-

TABLE 3

CONCENTRATIONS OF SURFACTANT-ASSOCIATED PROTEINS B AND D IN LUNG LAVAGE FLUID OF HEALTHY SUBJECTS AND PATIENTS WITH LUNG DISORDERS

	Controls (*mg/L*)		Patients (*mg/L*)				
	n	Mean ± SEM or Range	n	Mean ± SEM or Range	Comments	Statistical Significance	Reference No.
SP-B							
ARDS	29	1.3 ± 0.3	66	0.6 ± 0.2	Established ARDS	S	236
			20	0.9 ± 0.5	At risk for ARDS	NS	236
	20	0.74 ± 0.1	15	0.86 ± 0.1	Established ARDS	NS	237, 282
			35	0.81 ± 0.08	With pneumonia	NS	237, 282
Cardiac lung edema	20	0.74 ± 0.1	13	0.6 ± 0.04		NS	237, 282
Pneumonia	20	0.74 ± 0.1	88	0.73 ± 0.04		NS	237, 282
Cystic fibrosis	20	2.9 ± 0.6	17	4.0 ± 0.7	No infection	NS	252
	6	15.3 [5.6–18.1]	15	10.7 [7.0–18.5]	Infection	NS	253
			12	10.6 [8.1–17.8]	Infection	NS	253
SP-D							
Alveolar proteinosis	28	0.9 ± 0.13	9	19.3 ± 9.3		S	283
Tobacco smoking	12	1.3 ± 0.2	8	0.5 ± 0.1		S	251
Sarcoidosis	28	0.9 ± 0.13	60	0.9 ± 0.08		NS	283
Pulmonary fibrosis	28	0.9 ± 0.13	7	0.6 ± 0.16	With collagen disease	NS	283
			33	0.6 ± 0.06	Idiopathic fibrosis	NS	283

structive pulmonary disease (COPD) (185, 287). By contrast, pulmonary sarcoidosis did not affect the values of CC16 in lung lavage (287). CC16 content was also significantly decreased in BALF from patients with IPF or bleomycin-induced lung injury (183). A slight diminution of CC16 has been reported in BALF of asthmatic individuals, but this decrease was not significant after adjustment for the BALF albumin content (289). Increased CC16 levels have been found in BALF from patients at risk for or with full-blown ARDS (288). Very interestingly, among ARDS patients, CC16 levels in BALF were on average higher in survivors than in nonsurvivors (288). A slight increase in levels of CC16 has also been found in BALF of asbestos-exposed workers (247). Interestingly, CC16 is present in high concentrations in sputum, which average one order of magnitude higher than in BALF (mean: 10 to 20 mg/L) (290).

A sensitive latex immunoassay, applicable to both rat and mouse CC16, has recently been developed (186). High levels of CC16 have been measured in BALF of these rodents (on average, 2 to 4 mg/L), in which, as in humans, CC16 emerges as one of the most abundant lung secretory proteins, with a concentration averaging 7% of that of albumin (186). Treatment of rats with systemic toxicants that damage Clara cells is associated with a decreased level of CC16 in BALF (186, 291).

Mucin-associated antigens. Despite the development of different monoclonal antibodies for mucin-associated antigens, very few studies have so far investigated the levels of these antigens in BALF. In a group of nine healthy subjects, the mean KL-6 value was found to be 200 ± 60 U/ml (292). A significant increase in KL-6 has been observed in BALF of patients with various interstitial lung diseases. Among patients with IPF, 70% (seven of 10) had levels above the control levels, as did 64% (nine of 14) patients with sarcoidosis and 100% (eight of eight) with hypersensitivity pneumonitis (293). By contrast, no significant difference in KL-6 in BALF could be evidenced in controls and patients with beryllium sensitization or chronic beryllium disease (292).

Concentrations in epithelial lining fluid. Classically, the concentrations of proteins in the thin layer of ELF are estimated on the basis of their levels in BALF, after adjustment for the dilution caused by the lavage procedure. Regarding CC16, its concentration in ELF has been estimated as approximately 140 mg/L in rat, using the urea dilution method (186), and 100 mg/L in humans, considering a 100-fold dilution by the lavage procedure (287). The average concentration of KL-6 in ELF as determined in eight subjects was 33,600 ± 18,500 U/ml, which is on average 100 times higher than in BALF (292, 293). The ELF concentrations of surfactant-associated proteins can also be derived from the pool size and protein composition of whole isolated extracellular surfactant. This approach is probably more reliable in view of the uncertainties in the recovery and concentrations of hydrophobic surfactant-associated proteins in BALF. As estimated in humans by extensive lung washing performed at autopsy, the extracellular surfactant pool size is 4 mg/kg (294), corresponding to a total mass of 280 mg for a 70-kg person. Interestingly, the surfactant pool size determined in humans is lower than the value of about 15 mg/kg found in sheep (295), which are mammals of similar size to humans. The protein content of isolated surfactant is difficult to estimate precisely, since it varies considerably according to the purification procedure used (6). Moreover, variable amounts of nonspecific proteins, predominantly originating from serum, contaminate surfactant preparations (6). According to most estimates, however, specific surfactant-associated proteins represent approximately 5 to 10% of whole isolated surfactant, the rest being contributed by lipids (6, 10, 25, 296). Because SP-A has been considered to constitute from 25 to 50% of the total surfactant protein pool, one may infer that normal lung contains approximately 3.5 to 14 mg of SP-A, which is in agreement with the value derived from the ELISA of SP-A in a total lung washing (7 mg for a 70-kg person) (294). From this estimate, it can be inferred that the concentration of SP-A in ELF ranges from 0.18 to 0.7 mg/ml, assuming an ELF volume of approximately 20 ml for an average adult (297). The corresponding concentrations in BALF are 1.75 to 7 mg/L, considering a 100-fold dilution due to the lavage procedure. Applying the same calculations to SP-B and SP-D, which apparently comprise 10 and 20% of the total surfactant protein pool, respectively, total amounts of these proteins in the normal lung can be estimated as ranging from 1.4 to 2.8 mg and 2.8 to 5.6 mg, respectively, and the corresponding levels in ELF as ranging from 0.07 to 0.14 mg/ml for SP-B and 0.14 to 0.28 mg/ml

TABLE 4

CONCENTRATIONS OF THE CLARA CELL PROTEIN (CC16, CC10) IN LUNG LAVAGE FLUID OF HEALTHY SUBJECTS AND PATIENTS WITH LUNG DISORDERS

	Controls (*mg/L*)		Patients (*mg/L*)				
	n	Mean ± SEM or Range	n	Mean ± SEM or Range	Comments	Statistical Significance	Reference No.
Healthy subjects	16	1.2 [0.2–2.7]					174
	25	1.3 [0.2–5.9]					161
Tobacco smoking	13	0.8 [0.8–1.9]	14	0.35 [0.1–0.8]		S	185
	25	3.6*	14	1.7*		S	287
Chronic bronchitis	25	3.6*	6	1.0*		S	287
Sarcoidosis	25	3.6*	17	3.5*		NS	287
Lung cancer	25	3.6*	15	1.4*	Affected side	S	287
			16	2.7*	Unaffected side	NS	287
Lung fibrosis	23	2.0	29	0.6		S	183
Bleomycin injury	23	2.0	11	0.5		S	183
Asbestosis	15	1.9 ± 0.2	16	3.1 ± 0.5	Nonsmokers	S	247
	24	0.6 ± 0.1	18	1.7 ± 0.3	Smokers	S	247
ARDS	13	0.16 [0.1–0.6]	14	0.5 [0.2–2.0]		S	288
			8	0.4 [0.2–0.9]	Nonsurvivors	S	288
			6	1.1 [0.2–2.5]	Survivors	S	288
			21	0.9 [0.1–5.5]	At risk for ARDS	S	288
Asthma	24	3.2 ± 0.3	24	2.1 ± 0.3		S	289

* Because of the use of different standards, these values should be divided by 3.2 to be comparable with other values (161).

for SP-D. The corresponding values in BALF (100-fold dilution) agree well with concentrations of surfactant-associated proteins reported in BALF (Tables 2 and 3). In rats, the concentrations of SP-A and SP-D in ELF have been estimated at 0.3 to 1.8 mg/L and 0.03 to 0.2 mg/L, respectively (38, 144).

Little is known about the distribution of these secretory proteins at the epithelial surface of the lung, but recent evidence suggests that it parallels that of the secreting cells. This is well illustrated with CC16, for which the concentrations have been found to be 10 times higher in bronchial than in alveolar lavage fluid, an observation that is in good agreement with the specific airways localization of Clara cells (287). It cannot be determined, however, whether the presence of CC16 in alveolar lavage fluid is due to residual contamination during the lavage procedure or to a physiologic transfer of the protein from the distal airways down to the alveoli. Surfactant-associated proteins secreted mainly by type II cells appear to be more concentrated at the alveolar than at the airway surfaces, since in humans, the SP-A/total protein ratio in bronchial lavage fluid is approximately 20 times lower than in BALF (298). In pig, isolated tracheal surfactant contains no measurable amounts of SP-B and considerably less SP-A than alveolar surfactant (299). However, no information is available about the relative contributions of alveolar and Clara cells to the amounts of surfactant-associated proteins in ELF, or about the possibility of these proteins' transfer along the ELF of the bronchioles and alveoli.

Factors influencing the concentrations of epithelial secretory proteins in pulmonary fluids. Factors influencing the levels of epithelial secretory proteins at the surface of the lung epithelium are largely unknown. It can be assumed that under steady-state conditions, the concentrations of these proteins in ELF reflect the balance between their rate of synthesis and/or secretion and their rate of clearance from the air spaces. Changes in both of these processes may result in an increase or a decrease in the levels of these proteins, which further complicates the understanding of the mechanisms leading to changes in BALF.

Regarding the synthesis and/or release of epithelial secretory proteins it is very likely that alteration of this process accounts for the reduction of surfactant-associated proteins in BALF in several inflammatory and pathologic states characterized by acute or chronic injury of the lung epithelium, such as ARDS (236, 237), bacterial pneumonia (237, 239), IPF (239, 242, 243), asthma (245), or tobacco smoking (251, 260, 261). A decrease in the production of CC16 has also been postulated to account for the reduced levels of this protein in BALF observed in several lung disorders, such as bleomycin-induced lung injury (183), asthma (289), IPF (183), and tobacco smoking-related illnesses (185, 287). In smokers, this explanation is supported by the well-documented decrease of Clara cells caused by tobacco smoking (185, 300, 301). By contrast, increased synthesis and/or release appears to be the most plausible mechanism for the increase of surfactant-associated proteins observed in BALF of patients with asbestosis (247), sarcoidosis (246), and hypersensitivity pneumonitis (244, 246). This is supported by several experimental studies showing an increased synthesis of SP-A and/or SP-D in different rat models of lung inflammation induced by the instillation of LPS (267, 302) or crystalline silica (285, 303, 304) and infection by *Pneumocystis carinii* (305). An increase of the local synthesis of CC16 has also been proposed to account for the increased BALF concentrations of this protein in asbestosis (247) and ARDS (288), although no alteration of CC16 synthesis has been found in an experimental model of lung injury induced by LPS (190). It has also been hypothesized that the release of some lung proteins is enhanced as a result of cytotoxicity. This mechanism has been postulated to explain the increase in KL-6 in BALF in various interstitial diseases characterized by alveolar type II cell damage (292, 293).

Different mechanisms have been proposed for the clearance of proteins present in the respiratory tract, such as transport by the mucociliary escalator, endocytosis by AM, degradation within the alveoli or airways, and absorption across the alveolar and bronchial epithelium into the circulation (306). These processes, depending on whether they are stimulated or inhibited, may also affect ELF levels of lung secretory proteins. Because AM take up and degrade surfactant-associated proteins (307), increased phagocytosis has, for instance, been suggested as responsible for the diminution of SP-A and SP-D in BALF of smokers (251). This is corroborated by a recent study of rats chronically exposed to cigarette smoke, which showed decreased SP-B levels in BALF despite unchanged messenger RNA (mRNA) levels for SP-B (278). Contrastingly, impaired removal/degradation of surfactant is the commonly accepted mechanism for the severe pulmonary accumulation of surfactant phospholipids and surfactant-associated proteins such as SP-A and SP-D in PAP (250, 308). Alteration of surfactant recycling by alveolar type II cells and/or AM is the explanation usually proposed for the increased levels of some surfactant-associated proteins in hypersensitivity pneumonia (246), sarcoidosis (246), and asbestosis (247), even though other mechanisms, such as decreased surfactant synthesis, cannot be ruled out. In addition to the foregoing mechanisms, Doyle and associates have suggested that increased leakage of some surfactant-associated proteins into the bloodstream might contribute to their reduction in BALF from patients with ARDS (280, 297).

Extrapulmonary Fluids

In 1986, Strayer and coworkers reported the presence of circulating immune complexes of surfactant proteins and antisurfactant antibodies in plasma from children with respiratory distress syndrome (RDS) treated with intratracheal homologous human surfactant (309). To interpret this finding, these investigators suggested that components of exogenous surfactant might leak into the circulation and elicit an immune response (309), although a reaction between surfactant blood-group antigens and isoimmune antibodies has been proposed as an alternative explanation (56). In 1989, Rubin and colleagues reported the occurrence of a surfactant antigen in blood from rabbits whose thoraces had been irradiated (310). These investigators also suggested that surfactant-associated proteins might penetrate into the circulation and represent an early manifestation of endothelial injury caused by radiation (310). In humans, the first evidence that endogenous lung-specific proteins occur in the blood was brought by Chida and associates, who identified SP-A and SP-B in the sera of infants with RDS (311). In 1992, Bernard and colleagues described the occurrence in serum of another lung secretory protein, Clara cell protein (287). For the first time, these investigators put forward the concept that a lung secretory protein might diffuse into the serum down a huge concentration gradient between the surface of the respiratory tract, in which CC16 is secreted in large amounts, and the bloodstream (287). Since these initial reports, a number of studies have confirmed the occurrence of lung secretory proteins in the blood of healthy subjects and patients with lung diseases. These results are summarized in the following section.

SP-A. The first attempt to measure SP-A in human serum with two different ELISAs was made by Van de Graaf and co-

workers (245). These investigators were able to detect SP-A in the serum of three patients with PAP, but failed to find the protein in serum from healthy subjects or patients with asthma and sarcoidosis (245). A very weak staining was, however, present on Western blotting analysis, suggesting the presence of SP-A in the bloodstream, but probably in amounts too small to be detected by the immunoassays (245). The presence of SP-A in serum was further confirmed by Kuroki and colleagues and Doyle and associates, who demonstrated immunoreactivity to SP-A in serum fractions of patients with PAP or IPF isolated by lectin-affinity chromatography (mannose-sepharose 6B column) (312), and in the serum of patients with acute pulmonary edema and ARDS (297). As shown in Table 5, the mean value of SP-A measured in serum is either in the range of 20 to 50 μg/L or above 200 μg/L (297). This difference is most likely due to the use of different types of antibodies (297, 312, 313). The high values are obtained with immunoassays based on polyclonal antibodies reacting with all forms of SP-A present in serum (*see the subsequent discussion*), whereas the low values are found with immunoassays using monoclonal antibodies that predominantly recognize the high-molecular-weight forms of SP-A (297, 312). Part of the variation is probably also due to other analytic factors, among which the pretreatment of samples with EDTA and detergent appears to play an important role, probably by dissociating the protein from other surfactant components (297).

There is increasing evidence that SP-A does not circulate freely in serum, but instead circulates as oligomers that can also bind to immunoglobulins (312). Western blot analysis of serum of patients with PAP shows that under nonreducing conditions, most of the SP-A migrates with a molecular weight of about 150 kD, whereas under reducing conditions, most of the SP-A immunoreactivity is localized to bands of about 25 and 60 kD, corresponding to the free monomer and dimer (297, 312). Study of sera of patients with PAP suggests that high-molecular-weight forms of SP-A correspond to complexes with immunoglobulin G (IgG), and to a lesser extent with IgM (312), with which SP-A can bind via its lectin prop-

TABLE 5

CONCENTRATIONS OF SURFACTANT-ASSOCIATED PROTEINS A, B, AND D IN SERUM OF HEALTHY SUBJECTS AND PATIENTS WITH LUNG DISORDERS

	Controls (μg/L)		Patients (μg/L)				
	n	Mean ± SEM or Range	n	Mean ± SEM or Range	Comments	Statistical Significance	Reference No.
SP-A*							
ARDS	15	175 [123–248]	15	378 [215–1,378]		S	297
	46	39.0	21	61.0		S	314
Pneumonia	57	45 ± 3	14	65 ± 11		NS	312
Tuberculosis	57	45 ± 3	14	49 ± 23		NS	312
Cardiac lung edema	15	175 [123–248]	10	250 [180–364]		S	297
Pulmonary fibrosis	57	45 ± 3	32	205 ± 23	Idiopathic fibrosis	S	312
	53	22.8 ± 1.4	20	49.9 ± 5.6	With collagen vascular disease	S	315
			65	79 ± 6		S	316
	89	28.1 ± 1.02	11	66.8 ± 9.2	Idiopathic fibrosis	S	317
	46	39.0	143	92.0		S	314
	89	28.1 ± 1.02	17	69.9 ± 13.3	With collagen vascular disease	S	317
Collagen disease	53	22.8 ± 1.4	46	24.3 ± 2.1	Without pulmonary fibrosis	NS	315
Sarcoidosis	57	45 ± 3	16	52 ± 27		NS	312
Asbestosis	89	28.1 ± 1	34	56.9 ± 6.2	Asbestosis	S	317
			169	30.2 ± 2	Asbestos-exposed	NS	317
Alveolar proteinosis		< 7	3	4–33		S	245
	57	45 ± 3	6	285 ± 23		S	312, 318
Tobacco smoking	29	18.35 ± 1.1	23	26.22 ± 2.1		S	319
SP-B							
ARDS	33	1,685 ± 58	22	8,007 ± 1,654		S	280
Cardiac lung edema			10	3,646 ± 635		S	280
Ventilated subjects			7	1,829 ± 184	No cardiopulmonary disease	NS	280
SP-D							
Healthy subjects							
Children	17	79 ± 13			0–2-mo-old children	NS	283, 320
	27	55 ± 9			2–11-mo-old children	NS	
	51	47 ± 4			4–6-yr-old children	NS	
	28	67 ± 8			10–12-yr-old children	NS	
	31	64 ± 7			13–18-yr-old children	NS	
Adults	179	66 ± 3			No difference with children	NS	314
Pulmonary fibrosis	179	66 ± 3	59	339 ± 38		S	283
	145	98	143	391		S	283
	179	66 ± 3	16	472 ± 144	With collagen vascular disease	S	283
Alveolar proteinosis	179	66 ± 3	8	461 ± 148		S	283
Tuberculosis	179	66 ± 3	24	119 ± 20		S	283
Sarcoidosis	179	66 ± 3	32	104 ± 15		S	283
Asthma	179	66 ± 3	28	59 ± 6		NS	283
Bacterial pneumonia	179	66 ± 3	11	82 ± 18		NS	283
Emphysema	179	66 ± 3	14	81 ± 18		NS	283
Bronchiectasis	179	66 ± 3	6	91 ± 14		NS	283
Panbronchiolitis	179	66 ± 3	6	89 ± 15		NS	283

* SP-A immunoassays are based either on polyclonal (297) or monoclonal (312, 314–319) antibodies.

erty or in the same manner as C1q (297). The hypothesis of autoantibodies directed against SP-A cannot be ruled out, since it has been reported that autoantibodies directed against blood group A antigenic determinants can cross-react with some SP-A oligosaccharides (56). However, such autoantibodies have not been detected in blood of patients with IPF, PAP, or ARDS (297, 312).

As shown in Table 5 (245, 280, 283, 297, 312, 314–320), levels of SP-A have been measured in the serum of patients with various lung diseases. Increased levels have been found in patients with PAP (245, 312, 318) and IPF (312, 314–318). Higher SP-A levels have been found in patients with asbestosis (317). SP-A was also found to be significantly increased in sera of patients with ARDS (256, 297, 314) and those with acute pulmonary edema (297). A slight increase of SP-A in serum has also been found among smokers (319). By contrast, patients with pneumonia (312), tuberculosis (312), and sarcoidosis (312, 314) exhibited levels similar to those of controls.

SP-B. Apart from the preliminary report by Chida and colleagues of the occurrence of SP-B in the serum of a 3-d-old neonate with severe RDS (311), the first extensive study of SP-B in serum was recently done by Doyle and coworkers (280). These investigators showed that immunoreactivity to SP-B in serum is localized to two bands: one of about 42 to 45 kD, corresponding to the proprotein, and a second of about 24 to 26 kD, corresponding to the mature protein and processing intermediates (280). In serum, the SP-B proprotein appears as the major circulating form of SP-B, as opposed to BALF, in which the processing intermediates and the mature form predominate (280). These different proportions of immunoreactivity patterns of SP-B in BALF and serum are probably due to the much higher hydrophobicity of mature SP-B than of the immature form. First, because of its hydrophobicity, mature SP-B binds strongly to surfactant phospholipids, which might restrict its passage into the bloodstream. Second, when present in the circulation, mature SP-B might be rapidly removed after its adsorption to membranes, or might more simply be undetectable on serum protein blots (280). There is no evidence that plasma immunoreactive SP-B is associated with other proteins in the bloodstream (280). As shown in Table 5 (245, 280, 283, 297, 312, 314–320), the average concentration of SP-B in serum of healthy subjects is 1,700 $\pm$ 60 μg/L (280). Circulating levels of SP-B are significantly higher than those of SP-A, with a mean SP-B/SP-A ratio of approximately 10 (280). Few studies have so far investigated the changes in SP-B in patients with lung disorders. As compared with its levels in healthy controls and ventilated subjects with no cardiopulmonary disease, plasma immunoreactive SP-B levels have recently been found to be significantly increased in serum of patients with ARDS and acute cardiogenic pulmonary edema (280).

SP-D. The occurrence of SP-D in serum was first described by Honda and colleagues, on the basis of an ELISA using monoclonal antibodies prepared against human SP-D purified from BALF of patients with PAP, and recombinant human SP-D protein as standard (283, 284). In the serum of healthy adults and children of both sexes, the average level was about 60 $\pm$ 3 μg/L (283, 314, 320), which is about three times higher than the concentration of SP-A measured by the same investigators (283). As shown in Table 5 (245, 280, 283, 297, 312, 314–320), increased concentrations of SP-D have been reported in patients with various lung diseases such as IPF, collagen vascular disease-related interstitial pneumonitis (283, 314, 321), PAP (283), pulmonary tuberculosis (283), and sarcoidosis (283). No significant increase in SP-D has been observed in several lung diseases, including bronchial asthma, bacterial pneumonia, diffuse panbronchiolitis, chronic pulmonary emphysema, and bronchiectasis (283). Notably, the concentrations of serum SP-D from patients with IPF and patients with PAP were, respectively, 4.3-fold and 6-fold greater than those of SP-A (283). Moreover, serum SP-D levels showed higher assay positivities (91.5% in IPF and 54.5% in PAP) than did serum SP-A (71.4% in IPF and 54.5% in PAP) when the cutoff value of serum SP-A (mean $\pm$ 2 SD) was set at 43 μg/L based on the levels in 53 healthy volunteers (283).

Clara cell protein (CC16, CC10). The concentrations of CC16 in serum as measured with different immunoassays vary from 5 to 50 μg/L (185, 287, 322, 323). Much higher values, of up to 1,000 μg/L, have been reported by Nomori and colleagues (324–327), but the reliability of their assay has recently been questioned (323). CC16 concentrations in serum are about 50-fold lower than those in BALF (287). It should be noted that in contrast to major low-molecular-weight plasma proteins (e.g., β_2-microglobulin, cystatin-C, and retinol-binding protein), whose concentrations fluctuate within narrow ranges, CC16 shows considerable variations in the serum of healthy subjects, with values differing by a factor of 10 or more (185, 287, 323). There is currently no explanation for this great variability, which could represent interindividual differences in the lung–blood passage of CC16 or in the synthesis/secretion of CC16 in the respiratory tract, as suggested by the great dispersion of CC16 levels in BALF (287). Different extrapulmonary factors have been shown to influence the concentration of CC16 in serum. Like other low-molecular-weight proteins, plasma CC16 is rapidly eliminated by glomerular filtration and reabsorbed by the renal tubules (174, 189, 328–330). As a corollary, serum CC16 rises as the glomerular filtration rate (GFR) declines (322, 323, 331). The slight increase in serum levels of CC16 with aging (332) is probably due to the age-related decrease in GFR. CC16 in serum is, however, independent of the level of lipids, body mass index, and sex (323), and does not show nocturnal variations (333).

As shown in Table 6 (161, 174, 183, 185, 287, 290, 322, 323, 331–336), changes in CC16 concentrations in serum have been observed in patients with various lung diseases and in subjects exposed to lung toxicants. A reduction of CC16 in the serum of smokers of both sexes has been observed in several studies (185, 323, 332, 334). Interestingly, this decrease was negatively correlated both with current and lifetime cigarette consumption and with the 24-h urinary excretion of thiocyanate (332). After adjustment for age, a linear dose-response relationship was apparent between smoking history and serum CC16, the latter decreasing on average by about 15% for each 10 pack-yr of smoking history (332). Shijubo and associates have recently confirmed these observations by showing that CC16 concentrations are lower in sera and BALF from healthy smokers than in those of healthy nonsmokers (185). These investigators have also shown, by immunohistochemistry, that CC16-positive bronchiolar epithelial cell densities among total bronchiolar epithelial cells are significantly decreased in lung tissue specimens from smokers with normal results in pulmonary function tests (185). In patients with lung diseases, a significant reduction of CC16 has been found in serum from patients with COPD or lung cancer (287). By contrast, CC16 has been found to be increased in the serum of patients with IPF (183) and sarcoidosis (287, 335), but unchanged in the serum of patients with bleomycin-induced lung injury (183). Nomori and colleagues (325–327) have not confirmed the effects of tobacco smoking and lung cancer on circulating levels of CC16, but all evidence indicates that this discrepancy arises from interference by serum lipids. In particular, Nomori and colleagues found a remarkable correlation ($r = 0.93$, $p < 0.0001$)

TABLE 6

CONCENTRATIONS OF THE CLARA CELL PROTEIN (CC16, CC10) IN SERUM OF HEALTHY SUBJECTS AND PATIENTS WITH PULMONARY AND EXTRAPULMONARY DISORDERS

	Controls (μg/L) n	Mean ± SEM or Range	Patients (μg/L) n	Mean ± SEM or Range	Comments	Statistical Significance	Reference No.
Healthy subjects							
Male	9	11.8 [3.0–19.1]			No difference between males and females	NS	174
Female	9	13.4 [8.3–19.1]					
Male	55	21.7 [7.1–50.8]				NS	161, 322
Female	59	27.9 [4.0–77.4]					
Tobacco smoking							
	107	11.7 ± 3.9	104	7.9 ± 2.8		S	185
	61	16.1 [7.7–34.5]	35	11.1 [5.2–24.1]		S	323
Female	75	21.0 [8–53.9]	65	15.9 [5–63.5]	15% decrease/10 pack-year	S	332, 334
Male	60	23.9 [5.3–74]	69	15.7 [3.4–51]		S	332, 334
Chronic bronchitis	25	82.0*	6	29.0*		S	287
Sarcoidosis	25	82.0*	17	134.0*		S	287, 335
Lung cancer	25	82.0*	18	50.0*		NS	287
Pulmonary fibrosis	23	18	29	33		S	183
Bleomycin injury	23	18	11	19		NS	183
Silicosis	86	16.3 [2.2–53]	86	12.3 [4–40]		S	290
Smoke inhalation	6	19.5 ± 4.79	6	54.4 ± 14.3	20 min postexposure	S	336, 333
	6	17.7 ± 5.1	6	15.9 ± 1.13	10 d postexposure	NS	336, 333
End-stage renal failure				2,800 ± 200	Patients on peritoneal dialysis		331
Obesity	44	14.1 [5.6–29.6]	34	14.4 [5.2–34.4]	Body mass index > 30	NS	323

* Because of the use of different standards, these values should be divided by 3.23 to be comparable with other values (161).

between serum triglycerides and serum CC16 (324), which was not confirmed by two independent immunoassays (323).

Cross-sectional studies have been done on workers exposed to lung toxicants, to determine whether serum CC16 might be of value in detecting pulmonary effects of air pollutants (337, 338). A first study involved a group of 86 miners exposed to high concentrations of silica-rich particles for an average of 15.2 mo (290). These workers were compared with a group of 86 control subjects matched for age and smoking status. No difference between exposed and control workers was apparent in respiratory symptoms, radiography, or lung function tests. By contrast, mean serum concentrations of CC16 were significantly reduced in workers exposed to silica, among both smokers and never-smokers. A significant effect of tobacco smoking was also found to be additive to that caused by silica. Notably, serum concentrations of β_2-microglobulin, a low-molecular-weight protein similar in size to CC16, were unaffected either by silica exposure or by tobacco smoking, which testifies to the specificity of changes affecting serum CC16. Because the miners in the study were exposed for less than 2 yr and had no sign of silica-induced lung impairment, these data demonstrate that the assay of CC16 can detect very early toxic effects on the airways of asymptomatic subjects with normal radiographic and spirometric test results (290). More recently, CC16 was also assayed in the serum of six voluntary firefighters who had inhaled smoke from the combustion of polypropylene in a chemical plant for about 10 min. Five of the firefighters had complaints of airways irritation. Clinical examination was, however, normal for all six of the subjects, and no significant decrement in lung function test results was found. The concentration of CC16 in firefighters' serum at 1 h after the fire was significantly greater than that of six age-matched controls examined simultaneously. Ten days later, the values for the firefighters had returned to levels similar to those of the controls. Using the latter as baseline, the increase in serum CC16 averaged 328% (333, 336).

As in humans, mouse and rat Clara cell protein can easily be quantified not only in BALF but also in extrapulmonary fluids such as serum and urine (186). The CC16 concentrations in the serum of mice and rats (on average between 10 and 20 μg/L for the rat) are very similar to those reported in humans (323). In serum, levels of CC16 increase markedly after lung injury and inflammatory changes in mice exposed to ozone (291) and in rats challenged with systematically administered pneumotoxicants (186, 291).

Mucin-associated antigens. The specific membrane-associated mucin-antigen KL-6, which is present in high concentrations in BALF, also circulates in the bloodstream. In a group of 160 healthy subjects, Kohno and coworkers estimated the average KL-6 serum concentration as 260 ± 130 U/ml (221, 227, 292, 339). As shown in Table 7 (227, 228, 234, 292, 339–343), levels of KL-6 are increased in patients with interstitial lung disorders such as pulmonary fibrosis, either idiopathic (228, 341, 344–347) or related to collagen-vascular disorders (348, 349), hypersensitivity pneumonia (350–352), and *Pneumocystis carinii* pneumonia (353). Of interest, the levels are significantly higher in patients with active disease than in those with inactive disease (228, 341, 345–347, 349, 354). A significant increase in levels of KL-6 has also been found in patients undergoing radiation therapy for lung cancer who developed radiation pneumonitis, as compared to those who did not develop this disorder (340, 352, 355). A similar pattern of change has been observed in patients with interstitial granulomatous lung diseases (341). For instance, in sarcoidosis, serum KL-6 levels are increased and significantly influenced by the severity of the lung involvement (341). In patients with chronic beryllium-induced lung disease, KL-6 shows an increase that also correlates with the functional and radiologic abnormalities in the disease (292). However, such changes are not present in beryllium-sensitized subjects in the absence of lung disease (292). By contrast, patients with noninterstitial lung diseases, including alveolar pneumonia, bronchial asthma, chronic bronchitis, and aspergillosis do not show a remarkable elevation of the KL-6 antigen level in serum (227, 228). Similarly, only a

TABLE 7

CONCENTRATIONS OF MUCIN-ASSOCIATED ANTIGENS (KL-6, 17-B1, 17-Q2) IN SERUM OF HEALTHY SUBJECTS AND PATIENTS WITH LUNG DISORDERS

	Controls (*U/ml*) n	Mean ± SEM or Range	Patients (*U/ml*) n	Mean ± SEM or Range	Comments	Statistical Significance	Reference No.
KL-6							
Radiation pneumonitis	9	277 ± 125	6	392 ± 194		S	340
Sarcoidosis			10	333 ± 173	Stage 0	NS	341
			15	430 ± 225	Stage I	NS	341
			13	945 ± 725	Stage II	S	341
			9	1,179 ± 1,320	Stage III	S	341
Chronic beryllium disease	32	402 [335–699]	26	1,002 [436.5–1,741]		S	292
Interstitial pneumonitis	160	258 ± 12	51	1,187 [224–2,656]		S	339, 227, 228
Hypersensitivity pneumonitis			10/3	1,508 ± 499/393 ± 58	Active/inactive disease	S	228
Idiopathic pulmonary fibrosis			8/9	1,619 ± 250/445 ± 113	Active/inactive disease	S	228
Collagen vascular disease			18/3	1,437 ± 96/478 ± 82	Active/inactive disease	S	228
Noninterstitial lung diseases			67	309 [123–855]	Pneumonia, aspergillosis	NS	228
					Asthma, emphysema	NS	228
					Bronchiectasis	NS	228
Tuberculosis	38	227 ± 15	57	518 ± 115	Extensive disease	S	342
	n	(*ng/ml*) Mean ± SEM or Range	n	(*ng/ml*) Mean ± SEM or Range			
17-B1							
Cystic fibrosis	8	25 ± 1	25	13,853 ± 1,281		S	234
Chronic bronchitis			17	25.5 ± 1.9		NS	234
17-Q2							
Tobacco smoking	59	9.9 ± 0.8	29	12.7 ± 1.6		NS	343
Chronic bronchitis			28	21.8 ± 1.9		NS	343
Cardiac lung edema			5	9.0 ± 3.1		NS	343
ARDS			13	53.8 ± 6.6		S	343

minority of the patients with emphysema, bronchiectasis, and pulmonary tuberculosis studied so far have had elevated KL-6 levels in serum (228, 342).

Respiratory mucins have also been detected in blood with the 17-B1 monoclonal antibody, which is directed against another epitope of secretory respiratory tract mucins than KL-6 (234). In healthy subjects, the mean concentration of this mucin-associated antigen, as determined with a double-sandwich ELISA method, has been estimated as 25 ± 1 μg/L (234). In patients with CF, the level is severely increased and correlated with the severity of the pulmonary involvement (234). Structurally, the mucins detected in serum of patients with CF have a lower molecular weight than those found in normal subjects, and probably correspond to proteolytic fragments (234, 356). Interestingly, levels of 17-B1 antigen decrease markedly after lung transplantation, suggesting that increased serum levels of this antigen in CF patients are mainly of respiratory rather than extrapulmonary origin (234). No change in 17-B1 level has been detected in patients with COPD (234).

In a group of 59 normal subjects, the level of the circulating mucin antigen recognized by the 17-Q2 monoclonal antibody has been estimated as approximately 10 ± 1 μg/L (343). This level is not significantly different than that found in chronic smokers and patients with acute cardiogenic pulmonary edema (343). A 3-fold increase of 17-Q2 antigen has, however, been found in patients with ARDS (343). As for the circulating mucin detected by 17-B1, the distribution in size of circulating mucins determined by gel-filtration chromatography showed that the fraction of high-molecular-weight mucins was significantly lower, and the amount of low-molecular-weight mucins higher, in patients with ARDS (343). The most likely explanation for this is a proteolytic fragmentation secondary to ARDS, occurring either in BALF or in serum (356).

ORIGINS OF LUNG SECRETORY PROTEINS IN BLOOD

The numerous studies described previously demonstrate that proteins synthesized by airway or alveolar epithelial cells are not only present at the surface of the respiratory tract, but also occur normally in small amounts in the bloodstream. Because these proteins are mainly, if not exclusively, secreted by polarized cells in the lumen of respiratory tract, their occurrence in the vascular compartment can only be explained by assuming their passage from the lung into the bloodstream following their transfer across the air–blood barrier. Although the possibility of a basolateral secretion of some proteins cannot be formally ruled out, there is no evidence that lung proteins enter the blood by this mechanism. One specific lung secretory protein that leaks into serum appears to be CC16. In humans, Northern blot analysis performed on 50 different tissues showed that CC16 is expressed almost exclusively in the respiratory tract, with the exception of a very weak synthesis in the prostate and the kidney (Figure 1). That CC16 concentrations in serum correlate with those in BALF in both the human (185, 287) and rat (186) further points to the lung as the major source of circulating CC16. This correlation, as well as the lack of a sex difference in serum CC16 levels (161, 174, 322), suggests that the postrenal secretion of CC16 contributes either not at all or only insignificantly to blood levels of CC16. The lung also appears to be the main source of surfactant-associated proteins detected in the circulation, since, as with CC16, surfactant-associated proteins are mainly (SP-A and probably SP-D) if not exclusively (SP-B) expressed by the respiratory tissues (Figure 1). Although their secretion by several extrapulmonary tissues has been reported, circulating mucin antigens recognized by the monoclonal anti-KL-6 antibody also appear to derive mainly from the respiratory tract. This is sup-

ported by the fact that KL-6 is a member of the MUC1 gene family, which is expressed in the lung, and by the behavior of this antigen in serum, which correlates with ELF levels among healthy subjects and increases markedly in several lung disorders (227, 228, 292, 339). Even if different data suggest that the lung is the major source of 17-Q2 and 17-B1 in the circulation, the lung specificity of these mucin-associated antigens has not been fully demonstrated.

EVIDENCE OF A LUNG–BLOOD BIDIRECTIONAL EXCHANGE

Although the lung–blood barrier has long been considered to be impermeable to most macromolecules (357–363), this view has been challenged by an increasing number of studies indicating that small quantities of proteins may normally be translocated in both directions across the air–blood barrier. Since

Figure 1. Master Blot analysis of poly(A)$^+$ RNA from various human tissues (Human RNA Master Blot; Clontech, San Diego, CA). For hybridization, ^{32}P-radiolabeled cDNA probes specific for CC16 (CC10), SP-A, SP-B, and β-actin (control probe) were used on the same Northern blot. The expression of CC16, SP-A, and SP-B was confined mainly to respiratory tract tissues. Extrapulmonary synthesis has been found in the prostate for CC16 and in the kidney for SP-A and CC16. In these tissues, the levels of expression average 20 times lower than in the lung (B. Knoops and A. Clippe, manuscript in preparation).

the first description more than 20 yr ago of the protein composition of lung lavage fluid, by Reynolds and associates (8), several studies have shown that intact plasma proteins with molecular weights ranging from 50 kD (such as albumin) to 900 kD (such as IgM and α_2-macroglobulin) are normally present in the ELF of the lower respiratory tract of animals and humans as a result of their leakage from plasma rather than from their local synthesis in the lung (364, 365). These observations, as well as the detection in BALF of labeled tracers introduced in the systemic circulation, demonstrate that proteins, even of considerable size, can reach the surface of the airways and the alveoli by successively crossing the capillary endothelium, the interstitium, and the pulmonary epithelium. These proteins, particularly those of high molecular weight, normally occur in minute amounts in ELF as compared with their levels in serum, which suggests that the blood–lung barrier is effective in restricting their leakage into the respiratory tract (365).

Movement of proteins in the opposite direction, from the lung into the blood, was suggested 50 yr ago when it was shown that intratracheally administered albumin appeared in the lymph and blood of intact rabbits at a rate proportional to the rate of ventilation (366). An alveolar-to-vascular permeation has since been demonstrated in different species with various tracer macromolecules (367–373). Altogether, these studies have provided strong evidence that a bidirectional protein flux across the air–blood barrier exists physiologically. These findings even led to the hypothesis that the lung epithelium might serve as a route of administration for some drugs that cannot be taken orally. For example, Hubbard and coworkers showed that an aerosolized synthetic analogue of the antiprotease α_1-antitrypsin is absorbed into blood from the pulmonary air space of sheep (374).

DETERMINANTS OF THE LUNG–BLOOD PASSAGE OF MACROMOLECULES

Our understanding of the mechanisms governing the selective permeability of the lung–blood barrier derives mainly from numerous studies of the transfer between the air spaces and the bloodstream of various heterologous proteins or tracer molecules, either instilled or aerosolized into the respiratory tract (359, 368, 375–378) or introduced into the circulation (359, 371, 379, 380). The efficiency of the passage in both directions of various tracers is inversely related to their molecular weight and influenced by their shape (380–387).

These studies have led to the conclusion that the lung–blood barrier behaves as a molecular sieve, allowing the passage of small solutes and macromolecules but restricting the passage of homologous or heterologous macromolecules the size of albumin or larger (360, 368, 376, 379, 380, 388–391). A pore theory has been proposed to explain the molecular sieving properties of the lung–blood barrier. According to this theory, the lung-capillary wall is perforated with water-permeable pores whose dimensions restrict the passage of proteins and macromolecules. There is, however, no general agreement on the exact size of these pores, or on whether their distribution is best represented by homoporous or heteroporous models. On the basis of the reflection coefficients of urea, glucose, and sucrose (hydrodynamic molecular radius estimated at 0.5 nm) in dog lungs, Taylor and coworkers initially suggested that the entire pulmonary epithelial barrier was characterized by a homogenous population of small pores with an equivalent pore radius of 0.5 to 1 nm (379). Similar estimates were made by Normand and colleagues on the basis of the transfer constants across the lung–blood barrier of a variety of radiolabeled markers introduced either into the blood or into the alveolar fluid (359). Subsequently, Theodore and associates found that larger molecules, such as high-molecular-weight dextrans (molecular weight between 60,000 and 90,000) could permeate the lung–blood barrier (380). These investigators suggested that the passage of macromolecules across the alveolar epithelial barrier was best accommodated by a heteroporous model with a large population of small pores and a smaller number of larger pores ($>$ 8 nm pore radius), representing a nonrestrictive pathway for large polar solutes (380). Similar observations were made in the rat by Berg and colleagues, who found that the pulmonary epithelial barrier could be represented by a majority of small pores (0.5 nm pore radius) and a minority of larger pores (3.4 nm pore radius) occupying 98.7% and 1.3% of the total pore area, respectively (392). By studying the interstitial concentrations of instilled, uniformly sized particle tracers in isolated dog lung, Conhaim and coworkers found that the lung epithelial barrier was best fitted by a three-pore-size model, including a very small number of large pores (400 nm pore radius), an intermediate number of medium-size pores (40 nm pore radius), and a very large number of small pores (1.3 nm pore radius) (393).

With the exception of the study by Robertson and colleagues showing a transfer into blood of human recombinant SP-A instilled in the lungs of immature newborn rabbits (394), no study has so far investigated the lung-vascular permeation of endogenous lung secretory proteins. However, one may logically postulate that the passage of endogenous proteins is governed by similar determinants as tracer molecules, and particularly by size. In view of the sieving properties of the air–blood barrier, with a predominant population of small pores (0.5 to 1.0 nm pore radius), even the low-molecular-weight proteins such as CC16 (with a molecular radius of approximately 1.9 nm) (331) and mature SP-B are probably hindered in their passage. This hindrance is probably much more pronounced for SP-A (41, 42) and SP-D (141, 395), which represent a much more heterogenous family including complex polymeric forms (Stokes radius between 10 and 40 nm for oligomeric SP-A). The proportion of these different forms of proteins is likely to influence their ability to cross the lung–blood barrier. The size restriction is probably even more severe for the complex high-molecular-weight mucinous glycoproteins, which are too large to permeate into the serum in their mature form, but are probably diffusible after proteolytic fragmentation (234, 343). The size-restrictive properties of the lung–blood barrier apparently provide an attractive explanation for the predominance in serum as compared with ELF of monomeric and oligomeric forms of SP-A and mucin fragments (234, 245, 297, 343). However, the occurrence in the circulation of high-molecular-weight SP-A oligomers and large mucin fragments can only be explained by assuming their transfer across the largest pores (245, 280, 297, 312).

In addition to steric hindrance, the passage of macromolecules through the lung-blood barrier is probably also influenced by electrostatic forces. Many studies indicate that the endothelial and epithelial cell surfaces and basement membranes, as well as the interstitial extracellular matrix, are invested by negatively charged proteoglycans that influence the movement of charged versus uncharged macromolecules (396–398). Because of these effects, the lung–blood barrier probably behaves as an electrostatic filter that limits the blood-to-lung exudation of plasma proteins that have a net negative charge at physiologic pH. This is reminiscent of the well-known influence of electrical charge on the filtration of plasma proteins by the renal glomeruli (399). Because most lung proteins have isoelectric points between 4 and 6, and are also negatively charged at physiologic pH (163, 174, 400–402), it is very

plausible that a similar electrostatic repulsion influences their transfer from the lung into the serum. This is especially the case for mucins, which are strongly anionic proteins (200, 234). As compared with its anionic precursor, the electrostatic repulsion for mature SP-B, which has a net positive charge, is probably less important (21, 112, 119).

Besides steric and electrostatic factors, the movement of macromolecules across the lung–blood barrier is probably also influenced by their hydrophobicity. The permeability of the lung–blood barrier for different polar tracers has indeed been shown to increase with their free diffusion coefficients in water (380, 384), which is consistent with a transfer through water-filled porous channels. However, lung secretory proteins, such as some surfactant-associated proteins (SP-A and mature SP-B), are tightly associated with surfactant lipids, a property that is likely to reduce their concentrations in the water-soluble exchangeable fraction of ELF, and their ability to breach the lung–blood barrier.

Additionally, the movement of proteins across the lung–blood barrier is probably directly or indirectly governed by dynamic factors, including the hydrostatic and oncotic pressures present in the alveoli, the interstitium, and the pulmonary capillaries. According to the Starling equation, the differences in oncotic and hydrostatic pressures across the epithelial and endothelial barriers in healthy subjects favor the outward flux of alveolar liquid, and contribute to keeping the alveolar surface dry (403). This liquid movement might favor the efflux of lung secretory proteins from the lumen of the respiratory tract into the interstitium. In addition, increased differences in transepithelial and transcapillary hydrostatic pressures may influence the permeability properties of the lung–blood barrier to macromolecules. Increased intravascular pressure is in fact associated with an increased permeability of the pulmonary endothelium to large molecules, most likely as a result of a distention of the endothelial barrier by a so-called stretched pore phenomenon (404). Similarly, increased subepithelial hydrostatic pressure, which dissociates epithelial cells by inducing a pressure load on their basolateral aspects, probably enlarges intercellular pathways of transepithelial passage (405, 406). On the opposite side of the air–blood barrier, lung overdistension probably also enhances the permeability of the epithelial barrier, as suggested by the linear increase with ventilatory pressure in the passage of protein from the lung into the blood (361, 363, 407).

ROUTES OF LUNG–BLOOD PASSAGE

Identification of the exact routes of passage of macromolecules from the air spaces into the blood is difficult because of the anatomic complexity and cellular heterogeneity of the lung–blood barrier. To reach the bloodstream, lung secretory proteins must cross the epithelial layer of the airways and/or the alveoli (Figure 2). These epithelial barriers present several differences that might affect the passage of macromolecules from the air space into the bloodstream. First, the alveolocapillary barrier has a surface area estimated at 50 to 100 m^2, which is considerably larger than the surface area of the bronchocapillary barrier, which is estimated at 2.5 m^2 (2, 408). Sec-

Figure 2. Schematic diagram of the pathways of transfer of lung-specific epithelium proteins from the lumen of the respiratory tract into the circulation. (Pore size estimate refers to equivalent pore radius.)

ond, the mean thickness of the air–blood barrier approximates 0.45 μm within the alveoli, but varies from several micrometers to millimeters in the airways, according to the airway level (409, 410). The distance from the air–liquid interface to the capillaries has therefore been estimated to be on the order of 10-fold thicker in the conducting airways than in the alveoli (411, 412). Third, the microvascular surface area is very much greater in the alveoli than in the conducting airways. These differences in surface area and thickness, as well as differences in blood flow, account for the 2-fold or more rapid absorption and clearance of small solutes such as ^{99m}Tc-diethylenetriamine pentaacetate (^{99m}Tc-DTPA) by the alveolar than by the bronchial epithelium (381, 413). By contrast, for large proteins that are subject to steric hindrance, the route of passage is most likely also determined by the relative size of the pores at the bronchial and alveolar levels. According to heteroporous models, the maximum equivalent pore radius estimated for the alveolar epithelium is on the order of 1 to 5 nm (360, 379, 413), whereas the large airways are occupied by much larger pores, with radii from 7 to 12 nm, coexisting with small pores of similar size to those of the alveolar epithelium (413, 414). In addition, the pores of the alveoli are probably not only smaller but also much less numerous, because of fewer intercellular contacts (where the pores are located) owing to the large surface covered by each alveolar type I cell. These anatomic and physiologic characteristics (i.e., surface, thickness, blood flow, estimated pore size) are probably responsible for differences in the amounts of lung secretory proteins leaking into the circulation, predominantly from the lumen of the airways (probably CC16, 17-Q2, and 17-B1) or the alveoli (probably SP-A, SP-B, SP-D, and KL-6) across the bronchoalveolar–blood barrier.

Proteins that have permeated the lung epithelium may enter the bloodstream not only by direct diffusion into alveolar capillaries, but also indirectly by lymphatic drainage into the venous system, as schematically represented in Figure 2. Because their concentrations in lung lymph have never been determined, it is difficult to evaluate the importance of this route of drainage for lung secretory proteins. According to several studies, the contribution of lung lymphatics to the removal of plasma proteins present in the lung interstitium appears to be minor as compared with leakage into the bloodstream (368, 377, 415). Studies with unanesthetized sheep have indeed shown that the lung lymph accounts for only 25% of total protein clearance over a 48-h period, and other short-term studies have also assigned a minor role to the lymphatics in removal of proteins from the alveoli (306, 415). By analogy, one may reasonably speculate that lung secretory proteins are mainly cleared by direct transfer into the bloodstream, rather than indirectly, by lymphatics. In addition, several studies have shown that 20 to 25% of acute pulmonary edema fluid drains into the pleural space, and that leakage could represent an additional route of drainage for proteins present in the interstitium (416–419). The detection of high concentrations of CC16 in human pleural transudates associated with congestive heart failure supports the hypothesis that not only fluid, but also lung secretory proteins, might be cleared by this route (420).

TRANSEPITHELIAL PATHWAYS

Analysis of the movement of various tracers and of the ultrastructure of the air barrier indicates that the epithelial layer provides the major resistance to the passage of macromolecules between blood and the air spaces, whether the tracer macromolecules are injected into the bloodstream or instilled (359, 371, 379, 380, 386, 415, 421–424). Most studies suggest that by contrast with respiratory gas exchange, which is accomplished largely through the lipid segments of the alveolar epithelial cells, the transfer of water-soluble solutes from the air spaces into the bloodstream occurs predominantly paracellularly, through water-filled porous channels. Ultrastructural studies indicate that these pores are located at the tight junctions linking the apicolateral borders of epithelial cells coating the alveoli and the conducting airways (Figure 2) (425–427). Comparable junctional complexes exist between pulmonary endothelial cells, but they are considerably less tight than those of the epithelium (421, 424). Although some water transfer may occur through these intercellular junctions, much of the water movement across the epithelium probably occurs through specific transcellular water channels. These so-called aquaporins have been shown to be very selective for water, since they do not permit the movement of any other molecule (428–431).

Transcellular passage has been proposed as an alternative pathway of macromolecule transfer across the epithelium. Several studies have shown that alveolar epithelial cells can take up different proteins from the alveolar space, such as albumin and IgG (432–435). A similar endocytic pathway, via pinocytic vesicles, has been demonstrated for the passage of albumin across the canine bronchial epithelium (436). For some proteins, such as human growth hormone (hGH), a receptor-mediated endocytosis has been shown to be a major route of passage from the lung into the circulation (437). This mechanism of transfer apparently explains why some proteins such as hGH are cleared from the lung much more rapidly than would be expected on the basis of their size (437). However, this pathway of transfer seems of minor importance, since removal of albumin or IgG from the alveolar space is unaffected by the inhibition of endocytosis (432). A receptor for SP-A has been described on alveolar cells, and receptor-mediated endocytosis has been shown to play a role in the alveolar turnover of SP-A (104–106). Whether this receptor is also partly implicated in the lung-to-blood passage of surfactant-associated proteins remains to be established. Regarding the other lung secretory proteins reviewed here, a receptor has recently been identified for SP-D, and has been localized on AM but not on alveolar epithelial cells (438).

Whatever the pathway of their passage, proteins have to cross the epithelial and the endothelial basal membranes, as well as the interstitium, to gain access to the bloodstream. Although this has not been extensively investigated, these two components might represent an additional barrier to the passage of macromolecules. Indeed, these basement membranes contain a fine fibrillar material composed of collagens and proteoglycans (439). In other capillary beds, most proteins have been shown to be retained by these membranes according to their size. By analogy with basal membranes in the glomerular filter, it is very likely that the lung basal membranes present size- and charge-selective properties that hinder the passage of proteins (439). For similar reasons, it is also quite conceivable that the pulmonary interstitium restricts the movement of high-molecular-weight macromolecules. Because of its complex three-dimensional collagenous architecture, with an interspersed ground matrix of proteoglycans and glycosaminoglycans (440), the interstitium might behave as a gel column across which macromolecules percolate.

MECHANISMS OF LUNG–BLOOD PASSAGE OF MACROMOLECULES

Health

Most molecules introduced into the respiratory tract have been found intact in the circulation, suggesting that their transfer across the lung–blood barrier occurs without significant degradation, and that their breakdown is not a prerequi-

site for their transfer (366, 368, 375, 377, 378, 382, 415, 433, 441, 442). Similarly, different molecules (e.g., thyrotropin releasing hormone, insulin) cross alveolar epithelial cell monolayers intact *in vitro* (443). Thus, in contrast with the tracheal, gastrointestinal, and proximal renal tubular epithelia, the respiratory epithelium does not appear to be a major site of significant protein breakdown (306). Furthermore, it is interesting to note that the lower respiratory tract surface is not a hostile environment to proteins. The lung does not normally contain large quantities of proteolytic enzymes, and has an adequate antiprotease defensive screen (444).

The concentrations of different tracers introduced in the respiratory tract have been shown to increase linearly in the blood with the dose instilled (377, 387, 445). This finding, as well as the absence of saturation kinetics for the lung–blood transfer of most tracers, is in agreement with the hypothesis of a passive protein transport, most likely occurring by diffusion through water-filled porous channels. By analogy, the driving force for the intravascular transfer of lung secretory proteins is provided by the huge concentration gradient between ELF and blood. For CC16 and surfactant-associated proteins, for instance, values in ELF exceed those in serum by more than three orders of magnitude (280, 287). The existence of a correlation between the levels in serum and those in BALF for CC16 (185, 186, 287) and KL-6 (292) further supports the hypothesis of a diffusional exchange. Even if passive diffusion predominates, the existence of a liquid flux outward from the alveoli suggests that an alveolar–vascular transport of macromolecules might occur by convection. Because the transfer of water across the air–blood barrier is mainly transcellular, through selective water channels (429–431), this mechanism of passage is probably less important in the absence of increased permeability of the air–blood barrier. Some investigators have, however, suggested that protein and fluid might, in some conditions, enter the interstitium by bulk flow and convection through very large and nonselective leaks or breaks, coexisting with porous channels in the epithelial barrier (390). According to tracer studies, transfer across the lung-blood barrier is considered to be slow, since tracers the size of albumin introduced in the respiratory tract are removed at rates ranging from 1% to 4% per hour (368, 376–378, 415, 446). Notably, the rate of clearance has been shown to be faster for smaller than for larger macromolecules (376, 415), which fits well with the concept of a size-selective transfer of these proteins.

Although it is not specifically documented for lung secretory proteins, there is some evidence that alveolar–vascular permeation is influenced by the degree of lung maturation and/or development. The bidirectional flux of albumin to and from the alveoli has, for example, been shown to be inversely related to gestational age in prematurely delivered lambs (447). Similarly, the permeability of the lung-blood barrier to small molecules decreases progressively after birth in different species (442, 448, 449). These observations are reminiscent of the age-dependent changes in intestinal permeability, which is high in the immediate postnatal period and then abruptly decreases (306). Whether the lung–blood barrier permeability to solutes is influenced by age later in life is currently unknown.

Lung Diseases

Many lung diseases are characterized by an exudation of plasma proteins into ELF, whereas in different experimental models of lung injury and/or inflammation, the flux of tracer proteins in the opposite direction, from the alveoli into the capillaries, is generally increased (382, 383, 450). These observations indicate that the physiologic and bidirectional flux of macromolecules across the air–blood barrier, and thus the intravascular leakage of endogenous lung secretory proteins, increases when the bronchoalveolar–blood barrier is disrupted. According to several studies, the markedly increased permeability of the air–blood barrier in ARDS is associated with a loss of the size-selectivity governing the lung–blood transfer of macromolecules. Large amounts of high-molecular-weight plasma proteins, such as IgM and α_2-macroglobulin, are indeed present in BALF of ARDS patients (451). Similarly, in experimental lung injury, high-molecular-weight tracers leak into the intravascular compartment more efficiently than do tracers of smaller size (383). Whether this loss of size-selectivity results from enlargement of the paracellular pores or from the appearance of nonselective transepithelial leaks remains to be determined. Whatever its exact mechanism, such a pathophysiologic process might explain the marked increase in high-molecular-weight lung secretory proteins such as SP-A and mucin fragments in the serum of patients with acute lung injury (234, 297) or various other lung disorders associated with increased permeability of the air–blood barrier to macromolecules. Alterations in determinants of the transepithelial passage of proteins other than porosity might also contribute to this lung–blood protein leakage, but this has not been investigated so far.

FATE IN THE CIRCULATION

Circulating levels of lung proteins are not only influenced by their rate of entry into the circulation, but also by their distribution and their rate of clearance from the vascular and extravascular compartments. Like any protein or exogenous substance introduced in the systemic circulation, lung proteins should partition themselves between the vascular and the extravascular compartments according to their size. Proteins the size of albumin or larger, such as polymeric forms of SP-A or large mucin fragments, are efficiently retained by the capillary endothelium and probably remain largely confined to the intravascular compartment. By contrast, smaller proteins, such as CC16, SP-B, and small mucin fragments, can leak out of the capillaries and have a much larger distribution volume than high-molecular-weight proteins. This is supported by the recent observation of a diffusional equilibrium of CC16 between the intravascular and different extravascular fluids (ascites and pleural fluids) (420).

Depending on their molecular size and hydrosolubility, plasma proteins are cleared from the circulation either by glomerular filtration or by metabolism in the liver, with ensuing secretion into the biliary tract. We have previously shown that, like other low-molecular-weight proteins, plasma CC16 is eliminated by glomerular filtration and is reabsorbed by the renal tubules. The renal disposal of CC16 takes place mainly in the cells of the proximal tubule, and a defect in tubule reabsorptive capacity results in a greatly increased excretion of this protein in both humans (322) and rodents (186). The half-life of CC16 in plasma has not yet been determined, but one may logically assume that it is of the same order of magnitude (i.e., a few hours) as that of other nonpulmonary, low-molecular-weight plasma proteins such has β_2-microglobulin or cystatin-C, which are rapidly eliminated by glomerular filtration. The exact route by which surfactant-associated proteins are cleared from the circulation remains to be determined. Despite the high molecular weight of SP-A–immunoglobulin complexes, there is at present no evidence that the liver is a major route of clearance for SP-A. No marked increase in SP-A has been detected in patients with hepatic failure (280). In patients with ARDS, there is no indication that the increase in SP-A is related to liver function. Although most circulating SP-A would be normally too large to be filtered through the glomeruli, Doyle

and coworkers have reported the occurrence of immunoreactive SP-A and SP-B in the urine of patients with ARDS (280). The presence of SP-A in urine of these patients might result from the glomerular proteinuria and increased glomerular permeability that complicate acute lung injury. By contrast with CC16, levels of SP-A and SP-B in serum have recently been found to be independent of the levels of different markers of the GFR, suggesting that the kidney is not a major pathway of clearance of serum SP-A and SP-B (452). In patients with ARDS, it has been estimated that SP-A, SP-B, and CC16 are rapidly cleared from the circulation with half-lives of 3 to 18 min (452). However, the extent to which the metabolism and the catabolism of these proteins are altered in patients with acute lung injury, and whether these estimates are valid for healthy subjects and patients suffering from other kinds of lung disorders, remains to be determined. The exact route by which SP-D and mucin antigens are eliminated from the circulation has never been investigated, and their half-lives in serum are completely unknown.

POTENTIAL CLINICAL APPLICATIONS

The evidence that lung-specific epithelial proteins secreted in large amounts in ELF are transferred into the circulation across a permselective barrier leads to different potential clinical applications.

Markers of Epithelial Cell Number and/or Integrity

Several studies suggest that the measurement of some lung-specific secretory proteins in serum might be used to detect changes in the number and/or integrity of epithelial secretory cells (185, 227, 228, 292, 323, 334). This application relies on the existence of a diffusional gradient between the concentrations of these proteins in ELF and in the circulation, so that the changes occurring in their concentrations in the respiratory tract are reflected in the serum. This hypothesis is supported by the decrease in serum levels of CC16 in conditions associated with a diminution in the number of Clara cells and/or in the secretion of CC16 into the lumen of the respiratory tract, such as with tobacco smoking (185, 323, 332, 334). By contrast with CC16, the level of KL-6 in BALF increases in various diseases characterized mainly by alterations in alveolar type II cells (227, 228, 292). This increase of KL-6 in ELF is also reflected by a concomitant increase of KL-6 in the serum (227, 228, 292). It is currently unknown whether the concentrations of surfactant-associated proteins in serum are related to the number and/or the integrity of secreting alveolar or bronchiolar cells. It is important to point out, however, that the use of lung-specific proteins as peripheral reporters of pulmonary epithelial cell number and/or integrity is valid only when the bronchoalveolar–blood barrier is intact or slightly compromised, so that protein exchange is governed mainly by the diffusional gradient existing between ELF and the intravascular compartment. As shown hereafter, a disruption of the barrier does in fact lead to an increase in the serum levels of lung secretory proteins that is unrelated to their levels in ELF.

Markers of Bronchoalveolar–Blood Barrier Integrity

Acute lung injury. Several lines of evidence suggest that the measurement of lung-specific secretory proteins in serum might be used to evaluate the integrity of the lung–blood barrier, which is compromised in several lung disorders. In acute lung injury characterized by increased permeability of the lung–blood barrier to albumin and other plasma proteins, circulating levels of SP-A, SP-B, the mucin-antigen 17-Q2, and CC16 have been reported to be markedly increased (256, 280, 297, 314, 343, 452). By contrast with their increased levels in serum, decreased levels of SP-A and SP-B in BALF are found in patients with acute lung injury, due most likely to changes in alveolar type II cells (236, 237, 256). This increase in serum levels, like that of albumin in BALF, most likely results from increased protein leakage resulting from disruption of the air–blood barrier (453). That SP-A and SP-B increase in serum despite a decrease in BALF indicates that the increased permeability of the alveolocapillary barrier in lung injury is more important in determining the circulating levels of these surfactant-associated proteins than are their absolute amounts in the alveoli (280). In ARDS, SP-A appears to be related to clinical outcome, since its blood levels are considerably higher in nonsurvivors than in survivors (280). The increase in SP-B in the serum of ARDS patients is greater than that of SP-A, indicating that surfactant-associated proteins respond with different sensitivity to lung injury and changes in lung permeability (280). The significance of the increase in levels of the mucin antigen 17-Q2 in the serum of patients with ARDS has not yet been clearly determined (343). Because 17-Q2 has not been measured in BALF in this condition, it cannot be established whether its increased leakage results mainly from an alteration of the air–blood barrier, as for SP-A and SP-B, from increased synthesis and/or release from damaged epithelial cells, or from protein degradation into more diffusible fragments (343). Whereas increased serum levels of SP-A and SP-B might reflect increased alveolocapillary permeability in patients with ARDS, independently of the presence of renal impairment, CC16 in serum is closely related to the GFR, so that part of the increase in its serum levels may be explained by the renal insufficiency frequently accompanying ARDS (452). Collectively, these findings suggest that the measurement of lung secretory proteins in serum might provide a useful tool for evaluating the permeability to proteins of the air–blood barrier in patients with acute lung injury.

Interstitial lung diseases. As in acute lung injury, increased levels of lung secretory proteins have been observed in the serum of patients with various kinds of interstitial lung diseases, and most likely again result from an altered permeability of the air–blood barrier. In IPF, increased circulating levels of SP-A (312, 314, 315, 317), SP-D (283, 314, 321), and CC16 (183) have been reported. As compared with that of SP-A, the serum level of SP-D is more frequently increased in IPF patients (283, 321). The most likely explanation for this is that SP-D, which is less tightly associated with surfactant lipids, reaches the bloodstream more easily than does SP-A (283). Interestingly, as for ARDS, the pattern of changes of these proteins in the serum of IPF patients is opposite to that found in BALF, in which levels of surfactant-associated proteins (242–244) and CC16 (183) have been found to be reduced. This suggests that as in ARDS, the permeability of the air–blood barrier is the most limiting factor in the transepithelial leakage of proteins in IPF. Moreover, several reports suggest that the increased levels of surfactant-associated proteins such as SP-A and SP-D in serum might also be used as prognostic indicators of IPF (283, 312, 314). Similarly, increased permeability of the lung–blood barrier seems to account for the significant elevation of serum SP-A levels in patients with asbestosis (317), as well as for the elevation of SP-D (283), KL-6 (341), and CC16 (287, 335) levels in the serum of patients with sarcoidosis. The latter two situations are known be associated with an increased permeability of the air–blood barrier to macromolecules, as evidenced by an increased albumin level in BALF (287, 454, 455).

Regarding the mucin-associated KL-6 antigen, increased

serum levels have been reported in several types of interstitial lung disease (227, 342, 348). These increases are usually found in patients with clinically active rather than inactive disease (227, 341). Among patients with chronic beryllium disease, the increase in serum levels of KL-6 has recently been found to correlate with that of albumin in BALF, suggesting that serum KL-6 may be a useful marker of the degree of permeability of the air–blood barrier (292). Moreover, in the group of patients in which this was found, levels of KL-6 in serum were superior to those in BALF in distinguishing patients with chronic beryllium disease from beryllium-sensitized subjects. However, it remains to be determined whether the increased serum levels of KL-6 in interstitial lung diseases mainly reflects epithelial cell injury with an ensuing release of KL-6 into ELF, damage to the air–blood barrier causing enhanced leakage into serum, or both processes. No data are available on possible changes in levels of 17-Q2 and 17-B1 in interstitial lung diseases. Altogether, the studies described here indicate that increased permeability of the air–blood barrier in lung injury and interstitial lung diseases is probably the main factor responsible for the elevation in levels of most lung secretory proteins in serum. Because of their common mechanism, these changes are not typical of a specific lung disorder, but may provide sensitive indices for the diagnosis and follow-up of changes in the air–blood barrier.

Noninterstitial Lung Diseases

PAP is the only noninterstitial lung disease known to be associated with an important increase in surfactant-associated proteins in serum. Serum levels of both SP-A (245, 312, 316, 318) and SP-D (283) are significantly increased in this condition. Very interestingly, a dramatic decrease in serum SP-D was found in four patients with PAP following therapeutic whole-lung lavage, suggesting that the serum SP-D level reflects the disease severity of PAP (283). The serum level of SP-D was significantly increased in all patients with PAP, whereas increased levels of SP-A where detected in 54% of PAP patients, which suggests that SP-D could have a greater sensitivity than SP-A in the assessment of PAP just as it does in IPF (283).

In smokers, the most consistent change is a significant decrease in serum CC16 (185, 323, 332, 334), which is ascribed to the well-documented diminution of the Clara cell number induced by tobacco smoke (185, 300, 301). By contrast, a slight increase in serum levels of SP-A has been reported, which probably arises from an increased epithelial permeability to macromolecules in smokers (319). As shown in Tables 5–7, lung secretory proteins do not appear to undergo major changes in the serum of patients with other noninterstitial lung diseases. The mechanisms that might lead to changes in

Figure 3. Schematic representation of the passage of CC16 (CC10) from the lung into the blood, followed by its renal elimination and postulated mechanisms leading to changes in serum levels of CC16 (sCC16) in the following situations: normal (*A*), following damage to Clara cell (*B*), injury to the bronchoalveolar–blood barrier (BA/BB) (*C*), damage to both Clara cells and the BA/BB (*D*), and renal impairment (*E*). Depending on the integrity of the lung and the kidney, CC16 in serum shows correlation with CC16 in BALF (*A*, *B*), with albumin in BALF (*C*, *D*) and with serum creatinine (*E*). These diagrams are based on observations in humans and experimental models of lung and renal injury (185, 186, 287, 291, 332, 334, 337, 338).

serum levels of CC16 are illustrated in Figure 3 (185, 186, 287, 291, 332, 334, 337, 338).

COMPARISON WITH CURRENT PULMONARY TESTS

In several lung disorders, there is evidence for relationships between changes in levels of lung secretory proteins in blood and abnormalities in various diagnostic tests. In ARDS, for instance, the increase in levels of SP-A, SP-B, and 17-Q2 correlates with the severity of the lung injury score (LIS) (280, 297, 343). In sarcoidosis, serum levels of both KL-6 and CC16 are markedly elevated in patients with more advanced chest X-ray abnormalities (335, 341), whereas higher KL-6 levels are observed in patients with IPF showing ^{67}Ga-citrate pulmonary uptake (227). In ARDS, the increase in levels of SP-A and SP-B as well as 17-Q2 correlates negatively with blood oxygenation (Pa_{O_2}/FI_{O_2} ratio) (Figure 4), but positively with the static respiratory system compliance ($\Delta V/\Delta P$) (280, 297, 343). Such correlation is not surprising, since the loss of integrity of the air–blood barrier typical of ARDS is responsible for the outward intravascular leakage of lung secretory proteins and the inward edematous flooding of the interstitium and air spaces, which impairs gas exchange and decreases lung compliance.

Lung lavage is a classic tool for studying the secretion of different secretory proteins and the exudation of plasma protein into the lumen of the respiratory tract (8, 364, 365). The measurement of lung-specific secretory proteins in serum avoids the intrinsic danger and artifacts associated with the instillation of fluid into the airways. As suggested earlier, the significance of the levels of these proteins in serum depends markedly on whether the air–blood barrier is intact or damaged. In the latter condition, the levels of lung secretory proteins in serum most likely reflect changes in the permeability of the air–blood barrier to macromolecules, as corroborated by the parallelism between the increased level of albumin in BALF and that of KL-6 in serum among patients with beryllium lung disease (292), and of CC16 in serum following experimental lung injury (186, 291). When the air–blood barrier is intact, the levels in serum of some proteins, such as CC16 mainly, reflect their levels in ELF (Figure 4) (186, 287).

Radioaerosol studies are a popular method for detecting and quantitating alterations of the permeability of the pulmonary epithelium by measuring the rate of disappearance from the lung of radiolabeled inhaled aerosols (456). Interestingly, many conditions with accelerated radioaerosol clearance, such as ARDS (457–459), asbestosis (460), IPF (461, 462), sarcoidosis (463, 464), and smoke inhalation (465, 466), are associated with increased leakage of different lung secretory proteins into serum. Such a similarity is not surprising, since most radiolabeled tracers used in clearance studies are hydrophilic indicators of small size (such as ^{99m}Tc-labeled DTPA) which, as endogenous lung secretory proteins, most likely permeate the epithelial barrier by passing through intercellular junctions (381). Moreover, many determinants of the lung–blood transfer of these tracers (e.g., molecular weight, surface area, site of deposition) are similar to those likely to be implicated in the leakage of lung secretory proteins (381, 467, 468). According to many studies, the acceleration in clearance of radioaerosols measured in many lung diseases mainly reflects changes in the extracellular permeability of the epithelial lung barrier (467). Compared with that of small-size radiolabeled tracers, the lung–blood passage of lung secretory proteins with many different physicochemical properties is probably more complex. However, both approaches appear to provide information about the presence and degree of lung damage in various conditions, by revealing the paracellular permeability to solutes.

Measurement of the diffusing capacity for carbon monoxide ($D_{L_{CO}}$) is another useful test for evaluating various lung disorders. Interestingly, conditions that tend to be associated with slow gaseous diffusion, such as IPF, sarcoidosis, and ARDS, are those that have been shown to be associated with increased levels of lung secretory proteins in serum and acceleration of the clearance of aerosolized radiolabeled tracers (468). Because carbon monoxide diffuses through the entire surface area of the alveoli by the transcellular route, a reduction in this surface is the main factor responsible for the decrease in $D_{L_{CO}}$. By contrast, in the case of endogenous lung secretory proteins and radiolabeled tracers, the extracellular permeability of the epithelial barrier to these solutes has no influence on the $D_{L_{CO}}$. These fundamental differences suggest that the tests of lung permeability described here represent complementary rather than competitive approaches (381, 468). For example, the decrease in $D_{L_{CO}}$ and the concomitant increase in radioaerosol clearance and serum levels of different lung secretory proteins documented in IPF presumably indicate that the air–blood barrier has a reduced effective surface area for gaseous exchange but is simultaneously much more permeable to aerosolized radioaerosols or endogenous lung secretory proteins, despite its increased thickness (468). The reduction in $D_{L_{CO}}$ is therefore predictive of the impairment of gas exchange, whereas acceleration of radioaerosol clearance and increased leakage of lung secretory proteins most likely indicate a change in permeability that might predispose to the passage of plasma into the lumen of the respiratory tract and the formation of edema (468). These changes in IPF are reminiscent of the concomitant decrease in the GFR and the high-molecular-weight proteinuria in renal diseases affecting the glomeruli. In these conditions, the decrease in GFR mainly reflects reduction of the glomerular surface area available for filtration, whereas the high-molecular-weight proteinuria results from an increased leakiness of the glomerular filter to proteins that are normally retained. As is true for the GFR, the $D_{L_{CO}}$ depends on some anthropometric variables such as age and sex. By contrast, radioaerosol clearance as well as the leakage of lung secretory proteins into serum are unaffected by these factors, resembling the situation in pathological proteinuria.

CONCLUSIONS

Evidence has accumulated that several proteins synthesized predominantly or exclusively by the respiratory tract epithelium occur normally in the serum of healthy subjects. In several disorders, these proteins show changes that appear to be related either to a loss of integrity of the bronchoalveolar–blood barrier and/or to changes in their secretion into the respiratory tract. We recently proposed the terms “pneumoproteins” and “pneumoproteinemia” to refer to such lung-specific proteins and their occurrence in serum (469). Although the exact mechanisms and pathways of lung–blood transfer remain to be clarified, there is now ample evidence that the bronchoalveolar–blood barrier allows a bidirectional flux of endogenous or exogenous macromolecules. As extensively documented, the leakage of proteins from the lung into the circulation appears to be governed by a number of factors, among which the permeability of the tight bronchoalveolar epithelium, and the molecular features of the proteins themselves (e.g., size and charge) and their exchangeable pool, appear to be critical. These observations open new perspectives on the biologic assessment of pulmonary diseases by suggesting a new approach based on the assay of lung-specific pro-

Figure 4. Relationship of plasma immunoreactive SP-B ($p < 0.0001$, $r_s = -0.74$, $n = 72$) and the plasma SP-B/SP-A ratio ($p < 0.0010$, $r_s = -0.30$, $n = 72$) to the $Pa_{O_2}/F_{I_{O_2}}$ ratio. The samples were obtained from normal subjects (*closed squares*) and from the following groups of ventilated patients: no evidence of cardiorespiratory disease (*closed circles*), acute cardiogenic pulmonary edema (*open squares*), and ARDS (*open circles*) (280).

teins in serum. Further studies are, however, needed to better understand the pulmonary and extrapulmonary determinants of pneumoproteinemia and to evaluate its significance and usefulness in the evaluation of patients with lung disorders and populations exposed to pulmonary toxicants.

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