



Matrix proteoglycans as effector molecules for epithelial cell function

C.W. Frevert* and P.L. Sannes#

ABSTRACT: Matrix proteoglycans are complex molecules composed of a core protein and glycosaminoglycan side chains. Once thought to be the molecular glue providing structural support and imparting biomechanical properties to lung tissue, it is now apparent that proteoglycans are important biological modifiers which regulate processes such as lung development, homeostasis, inflammation and wound healing. The diverse roles of proteoglycans in the extracellular matrix suggest that these molecules play a critical role in normal and diseased lungs. This short article will discuss the role extracellular matrix proteoglycans play in regulating epithelial cell function in the lungs.

KEYWORDS: Chondroitin sulphate, epithelial cell, heparan sulphate, perlecan, proteoglycans, versican

The primary function of the lungs is to allow for the diffusion of oxygen and carbon dioxide into and out of the blood. Therefore, the preservation of normal lung function is critical. The alveolar epithelium plays an important role in maintaining lung function through a number of different processes including production of surfactant and surfactant proteins [1], pulmonary host defences [2–5] and the formation of a tight barrier which keeps the airspaces dry through the active transport of sodium and chloride and the passive movement of water [6, 7]. Because of the importance of the pulmonary epithelium in maintaining normal lung function, it is critical to understand the mechanisms responsible for maintaining epithelial integrity. Recent evidence suggests that extracellular matrix plays an important role in regulating epithelial cell function. The extracellular matrix is found at the basal surface of epithelial cells in the lungs (fig. 1). It is composed of a number of components, including laminin, collagens, fibronectin, tenascin and proteoglycans. These extracellular matrix molecules are required for the development of a highly organised three-dimensional microarchitecture that provides structural support for epithelial cells. For many years it was considered that the primary role of extracellular matrix was its structural and biomechanical properties. It is now known to have many physiological and pathophysiological roles through the regulation of cellular processes such as migration, adhesion, proliferation, apoptosis and differentiation. An important element of the lung's extracellular matrix is a group of molecules called

proteoglycans. The goal of this article is to discuss the role of extracellular matrix proteoglycans in regulating epithelial cell function in normal and diseased lungs.

PROTEOGLYCANS IN NORMAL LUNGS

Proteoglycans

Proteoglycans are a family of charged molecules that contain a core protein and one or more covalently attached glycosaminoglycan side chain. The complexity and diversity of proteoglycans are derived from ~30 different core proteins of varying size and structure (10–500 kDa), and the considerable variability in the number (1–>100) and types of glycosaminoglycan side chains attached [8]. Proteoglycans are found in the extracellular matrix, the plasma membrane of cells and in intracellular structures.

Extracellular matrix proteoglycan families

There are three families of extracellular matrix proteoglycans, which include the large aggregating chondroitin sulphate proteoglycans (lecticans), the small leucine-rich chondroitin sulphate proteoglycans, and the heparan sulphate proteoglycans [8–11]. Lecticans include aggrecan, neurocan, brevican and versican. This family of proteoglycans has an N-terminal globular domain that contains a hyaluronan binding site and a C-terminal globular domain. The central domains of these proteoglycans are of variable length and contain multiple sites for the addition of chondroitin sulphate and dermatan sulphate. Versican, a chondroitin sulphate proteoglycan found in the extracellular matrix of the lungs, has four isoforms, V0, V1, V2 and V3,

AFFILIATIONS

*Pulmonary and Molecular Pathology Research Laboratories at the Seattle, Dept of Veterans Affairs Medical Center, and the Division of Pulmonary/Critical Care Medicine, Dept of Medicine, University of Washington School of Medicine, Seattle, WA, and

#Dept of Molecular Biomedical Sciences, College of Veterinary Medicine, North Carolina State University, Raleigh, NC, USA.

CORRESPONDENCE

C.W. Frevert
Seattle VAMC Pulmonary Research Group
Seattle VA Medical Center
151L
1660 S. Columbian Way
Seattle
WA 98108
USA
Fax: 1 2067685289
E-mail: cfrevert@u.washington.edu

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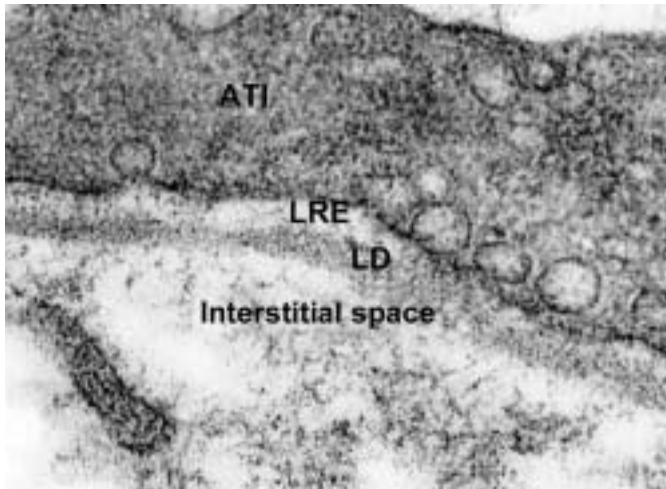


FIGURE 1. Ultrastructure of the extracellular matrix of a type I epithelial cell (ATI). Adjacent to the epithelial cell is the basement membrane with the lamina rara externa (LRE) and the lamina densa (LD), as seen in routinely prepared tissues by electron microscopy.

which carry varying amounts of chondroitin sulphate side chains [11]. The mRNA for versican isoforms V0, V1 and V3, but not V2, are found in normal lungs [12]. Small leucine-rich proteoglycans are extracellular molecules that bind to transforming growth factor (TGF)- β , surfactant protein D, collagens and other extracellular matrix molecules [11, 13, 14]. The small leucine-rich chondroitin sulphate proteoglycans have relatively small core proteins (30–50 kDa) and examples in the lungs include decorin, biglycan and lumican.

The core proteins of decorin and biglycan are highly homologous, but the core protein of decorin has an attachment site for one chondroitin sulphate/dermatan sulphate side chain, whereas biglycan has two attachment sites for chondroitin sulphate/dermatan sulphate side chains [8]. Decorin was named as such because it is usually found in association with or decorating collagen fibrils [15]. Lumican, a keratin sulphate proteoglycan, is present diffusely in peripheral lung tissue, mainly in vessel walls [16, 17]. The third family of extracellular matrix proteoglycans is the family of heparan sulphate proteoglycans, which includes perlecan, collagen XVIII and agrin. Perlecan is a large proteoglycan consisting of five domains, which contain low density lipoprotein receptor class A modules, immunoglobulin-like repeats, laminin-like repeats and epidermal growth factor-like repeats [18]. Electron micrographs of perlecan showed a protein with the appearance of beads on a string, which resulted in the name perlecan [8]. Whereas heparan sulphate is the predominant glycosaminoglycan on perlecan, chondroitin sulphate chains are found on domain V of recombinant perlecan [19]. Collagen XVIII is a heparan sulphate proteoglycan with features typical of collagen, including sensitivity of the core protein to collagenase [20]. This heparan sulphate proteoglycan is of particular interest because of the presence of endostatin, a 22 kDa anti-angiogenic peptide located in the C-terminal domain of collagen XVIII [21]. Agrin is a heparan sulphate proteoglycan found in the alveolar and capillary basement membrane, which contains nine follistatin-like domains which share

similarity to Kazal-type protease inhibitors, including pancreatic trypsin inhibitor, follistatin, thrombin inhibitor and elastase inhibitor [22, 23].

Glycosaminoglycans

Glycosaminoglycans are linear polymers of repeating disaccharides with a high negative charge imparted by sulphate and/or carboxyl groups in their structure. There are four classes of glycosaminoglycans: 1) hyaluronan, 2) chondroitin sulphate/dermatan sulphate, 3) heparan sulphate/heparin, and 4) keratan sulphate. All four classes of glycosaminoglycan are found in normal lungs and all except hyaluronan are bound to core proteins [9]. The predominant glycosaminoglycan in normal lungs is heparan sulphate (40–60%) followed by chondroitin sulphate/dermatan sulphate (31%), hyaluronan (14%) and heparin (5%) [24]. Sulphation of glycosaminoglycans, a structural modification that occurs in the Golgi complex during chain elongation, has important biological consequences, as specific sulphation patterns on glycosaminoglycans form the binding sites for a number of proteins, including morphogens, growth factors, adhesion molecules, cytokines and chemokines [25–27].

Distribution of matrix proteoglycans

Basement membrane

The basement membrane or basal lamina of pulmonary epithelium contains proteoglycans that interact directly with epithelial cells and is divided into the lamina rara externa and the lamina densa, as seen in routinely prepared tissues by electron microscopy (fig. 1). Proteoglycans found in the basement membrane include the heparan sulphate proteoglycans, perlecan, agrin and collagen XVIII. Collagen IV is the only collagen which is more abundant in the basement membrane than collagen XVIII. Studies performed using high iron diamine (HID), a cationic probe that detects sulphated carbohydrates, indicate that there are microdomains of varying amounts of sulphation in the basement membranes of the lungs. For example, heparan sulphate in the basement membranes of alveolar type I and type II cells have differences in the amount of sulphation, with twice as much sulphation found in the lamina rara externa of type I cells as type II cells (fig. 2) [28]. A second example of these microdomains is shown by differences in sulphation of the basement membrane of pulmonary endothelial cells. The basement membrane underlying the endothelium of pulmonary capillaries has significantly less sulphation (fig. 2) when compared with the basement membrane underlying endothelial cells of post-capillary venules (fig. 3). The mechanisms that regulate the formation of these microdomains of high and low sulphation are not known.

Interstitial space

The large chondroitin sulphate proteoglycan, versican, is located in the interstitial spaces in the lungs where it interacts with hyaluronan, which fixes this proteoglycan in tissue as a very high molecular weight aggregate. Decorin and biglycan are also located in the interstitial spaces of the lungs where decorin binds collagen fibres with a regular periodicity. Staining of collagen fibrils with HID reaction products is most likely to be due to the binding of this cationic probe with the chondroitin sulphate side chains of decorin (fig. 3).

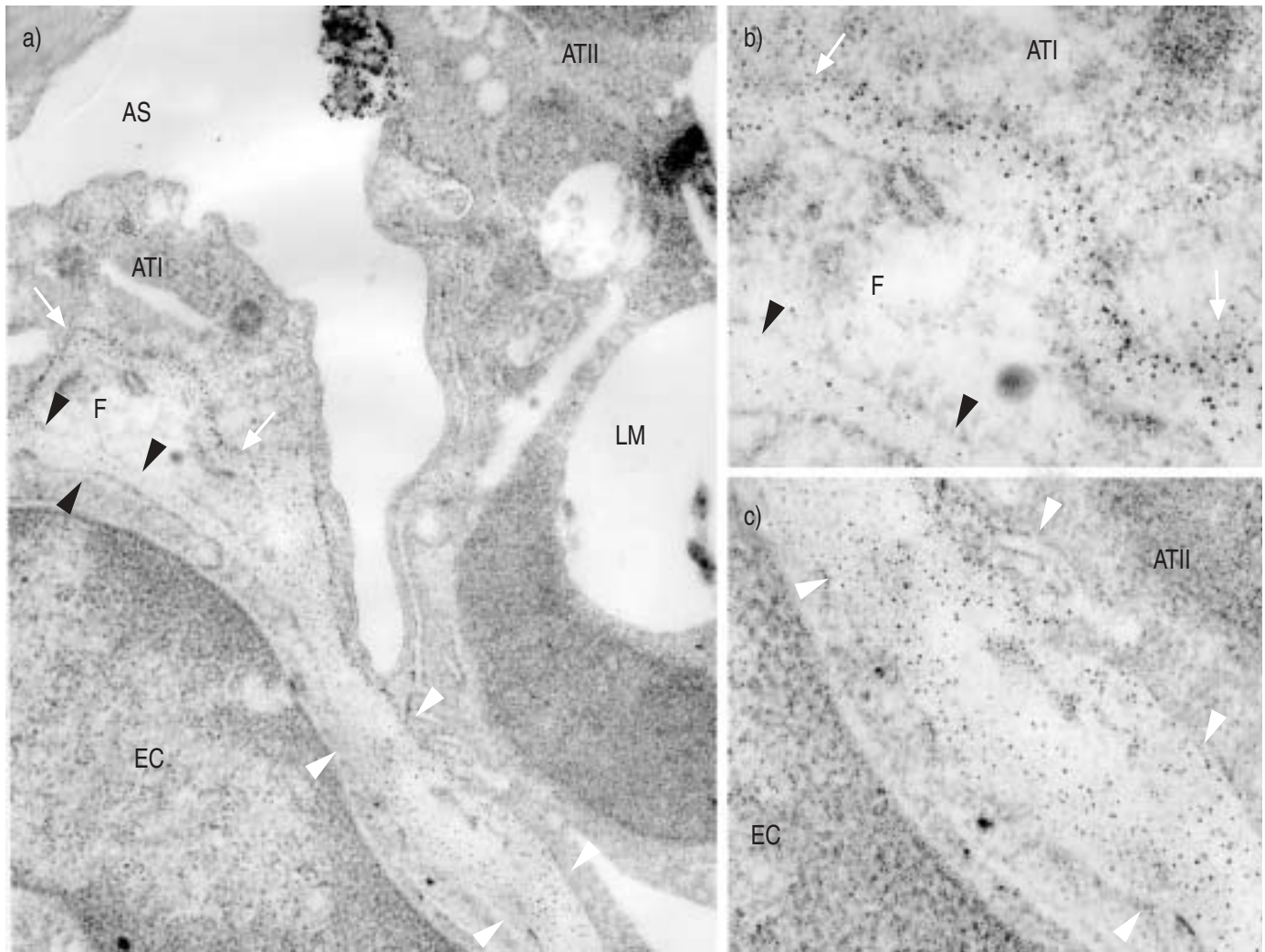


FIGURE 2. An electron micrograph of normal lung tissue showing microdomains in the extracellular matrix which contain varying amounts of sulphation. Cytochemical visualisation of sulphation in the extracellular matrix was performed using high iron diamine (HID), which binds to sulphates in lung tissue. The HID reaction product forms discrete 5–12 nm silver particles following appropriate intensification and is seen as discrete dark spots [29]. The basement membrane under a type I cell (ATI) and an adjacent fibroblast (F) is highly sulphated (white arrows in a and b). The microdomains surrounding the pulmonary fibroblast show this cell to have microdomains of high and low sulphation. The extracellular matrix adjacent to the type I epithelial cell is highly sulphated (white arrows in a and b) whereas the extracellular matrix adjacent to the endothelial cell (EC) is undersulphated (black arrowheads in a and b). In contrast to the ATI cell, this electron micrograph shows that the extracellular matrix underlying the type II cell (ATII) is undersulphated. White arrowheads in a) and c) define the extracellular matrix underlying the ATII cell and a pulmonary capillary EC. AS: alveolar space; LM: lamellar body.

Basilar surface of cells

Syndecans are a small family of transmembrane heparan sulphate proteoglycans that interact with a number of proteins in the extracellular matrix. Whereas syndecans are not considered extracellular matrix proteoglycans, and are therefore not discussed in detail here, their interaction with extracellular matrix transmits signals that are involved in focal adhesion and cell-death pathways [30]. Syndecan-1 and -4 have also been implicated in leukocyte migration [31–33].

Biomechanical properties of proteoglycans

Extracellular matrix proteoglycans confer important biomechanical properties to lung tissue. Glycosaminoglycans are strongly hydrophilic and their high negative charge attracts cations, such as Na^+ , which pulls water into the extracellular

matrix through osmotic activity. The large amount of water pulled into the matrix by glycosaminoglycans results in swelling of the extracellular matrix, which allows it to withstand compressive forces and probably promotes movement of solutes and cells. Proteoglycans interact with other extracellular matrix molecules, such as collagen, laminin and elastin, to form a structured matrix and this interaction contributes to lung elasticity and alveolar stability [34, 35]. In the kidney, anionic sites in the glomerular basement membrane are composed primarily of heparan sulphate proteoglycans, which provide the main charge-selective barrier to plasma proteins [36, 37]. The pulmonary microvascular barrier behaves as a net negative charge [38] and, although heparan sulphate is the most abundant of the negatively charged components in the alveolar basement membrane, it is

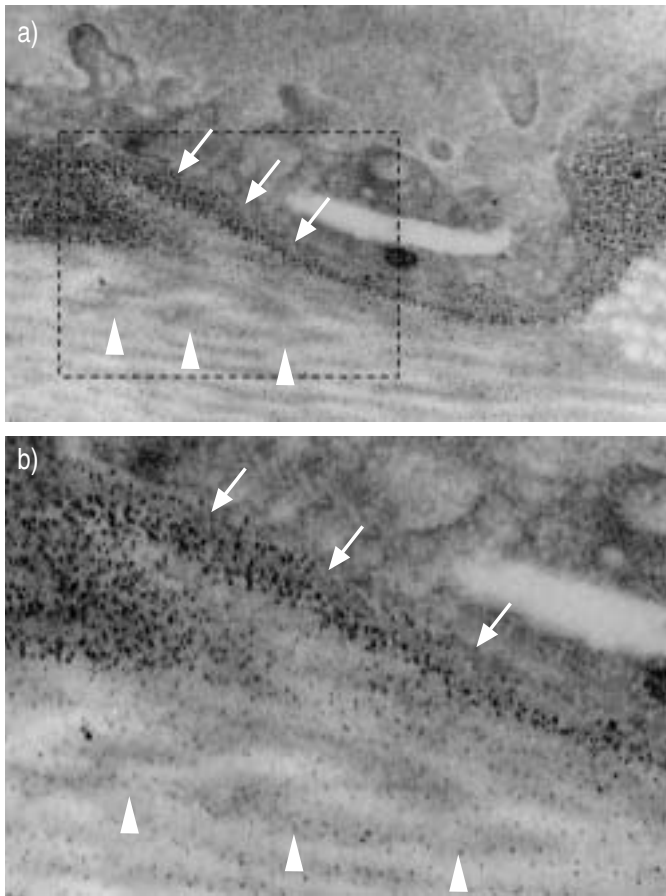


FIGURE 3. An electron micrograph of normal lung tissue showing a highly sulphated microdomain in the extracellular matrix underlying a postcapillary venule (a). An enlargement of the inset area enclosed by hatched lines is shown in (b). The extracellular matrix directly underlying the endothelial cell is highly sulphated (white arrows). The sulphation in the interstitial space has a regular periodicity (white arrow heads), which is consistent with decorin binding to collagen fibrils.

not known if it provides a charge-selective barrier to plasma proteins.

Proteoglycans as determinants of epithelial cell phenotype

Proteoglycans are important determinants of cellular phenotype, playing a role in development, cellular proliferation, cell death, inflammation and wound healing. A critical function of proteoglycans in the extracellular matrix is mediated by glycosaminoglycan side chains, which bind to growth factors, cytokines and chemokines [25–27, 39–42]. The binding of these proteins to glycosaminoglycans was initially believed to be a nonspecific ionic interaction between positively charged amino acids on proteins and negatively charged sulphates on glycosaminoglycans. It is now known that considerable specificity exists in the interaction between proteins and glycosaminoglycans, with unique binding sites being identified on glycosaminoglycans for proteins such as thrombin and growth factors [27, 43]. Despite a wealth of knowledge from studies showing a role for extracellular proteoglycans in regulating cellular function *in vitro*, very little is known about their role in regulating epithelial cell function in the lungs.

Five potential functions of protein–glycosaminoglycan interactions in regulating epithelial cell phenotype in the lungs are listed below.

Determination of protein binding sites

Because of the specificity observed in protein–glycosaminoglycan interactions, extracellular matrix proteoglycans localise proteins such as chemokines and fibroblast growth factors (FGFs) to distinct anatomical sites, *e.g.* basement membrane or interstitial spaces [25, 26]. FGF-2 and -4 provide excellent examples of this specificity because these two growth factors bind to different anatomical locations in the lungs, and this difference in binding is mediated by heparan sulphate. Whereas FGF-2 binds to heparan sulphate in lung tissue and on endothelial cells, FGF-4 binding in lungs is restricted, binding to heparan sulphate in lung tissue but not on endothelial cells. This suggests that FGF-4 and -2 bind to distinct sulphation patterns, and that the sulphation pattern required for FGF-4 binding is not expressed in most vascular tissues [25].

Storage/sequestration

Glycosaminoglycans provide a site to which proteins can bind; this sequesters a protein and prolongs its retention in lung tissue. The binding of chemokines to glycosaminoglycans provides a site for dimerisation of chemokines, which is a mechanism shown to increase the amount of chemokine binding in the lungs [26, 44]. Undersulphation of the lamina rara externa of basement membrane microdomains adjacent to alveolar type II cells compared with that of neighbouring type I cells is a mechanism that may account for some of the known morphological and functional differences between these alveolar epithelial cells (fig. 2) [28, 39, 40]. Increased sulphation of glycosaminoglycans under type I cells sequesters growth factors (*i.e.* FGF-2), limiting their effect on this cell. Injury or damage to these highly sulphated sites in the extracellular matrix results in the proteolytic liberation of FGF-2. In the undersulphated microenvironment in the extracellular matrix beneath type II cells, FGF-2 has access to specific cell surface receptors, thus promoting activation signalling pathways in this cell.

Formation and stabilisation of morphogen and chemokine gradients

The binding of morphogens and chemokines to glycosaminoglycans has been proposed as a mechanism whereby gradients develop in tissue. The binding affinity of morphogens and chemokines to glycosaminoglycans, which is in the low micromolar range, provides a mechanism whereby these molecules are able to diffuse and form gradients in tissue. This specific interaction is determined by the glycosaminoglycan-binding site on the protein and specific sulphation patterns found on glycosaminoglycans in tissue [28, 41, 45–49].

Cell signalling

The binding of growth factors and cytokines to glycosaminoglycans has been shown to play an important role in the activation of their receptors on the cell surface [50]. In contrast, the sequestration of FGFs by heparan sulphate in the basement membrane prevents the interaction with their receptors on epithelial cells [40]. Therefore, the ability of growth factors to

activate epithelial cells will depend on the location where the growth factor binds to heparan sulphate. Growth factors sequestered by heparan sulphate in the extracellular matrix will have limited effects on epithelial cell function, whereas ligands that bind to heparan sulphate on the cell surface will activate growth factor receptors and alter epithelial cell function.

Protection from proteolysis

The binding of a protein to a glycosaminoglycan may conceal proteolytic cleavage sites, which protects the protein from proteolysis. Glycosaminoglycans have been shown to protect chemokines and growth factors from proteolysis [51, 52].

MATRIX PROTEOGLYCANS AND LUNG DISEASE

Alterations in the composition of glycosaminoglycans

Changes in the composition of glycosaminoglycans and proteoglycans in the lungs have been reported in animal models and human lung disease. A consistent finding in animal models, such as exposure to lipopolysaccharide and bleomycin, is an increase in the synthesis of chondroitin sulphate and dermatan sulphate [53–55]. Gram-negative bacterial pneumonia results in the increased expression of chondroitin sulphate in the lungs of rabbits within 24 h (fig. 4). Hyaluronan is increased in a number of lung diseases, including acute respiratory distress syndrome, fibrosis, ventilator-induced lung injury and chronic obstructive pulmonary disease [56–60].

Changes in core protein expression

An increased deposition of the chondroitin sulphate proteoglycans versican, decorin and biglycan is associated with a number of lung diseases. Versican is increased in patients with asthma, acute respiratory distress syndrome, idiopathic pulmonary fibrosis, bronchiolitis obliterans organising pneumonia and lymphangioleiomyomatosis [61–64]. Often associated with the increased expression of versican in the lungs is an increased deposition of hyaluronan, which would result in the formation of large molecular weight complexes of hyaluronan and versican.

Matrix proteoglycans and epithelial cell phenotype in diseased lungs

Changes in the composition of extracellular matrix proteoglycans and glycosaminoglycans are likely to affect the clinical course of lung diseases. However, very little is known about the effect of altered extracellular matrix on epithelial cell function. Patients with severe asthma have increased expression of versican and biglycan in their airways, which is correlated with increased airway responsiveness [65]. Decorin decreases the fibrotic response of bleomycin in the lungs, possibly by inhibition of TGF- β bioactivity, suggesting that this chondroitin sulphate proteoglycan has anti-inflammatory properties [66, 67]. The biological effects of versican can be somewhat varied if not contradictory, because versican can both increase and decrease specific cellular functions. This is better understood in the context of the different isoforms of versican, the modular structure of the versican core protein and the binding partners for versican [68]. For example, the V1 isoform of versican increased the proliferation of 3T3 fibroblasts and protected these cells from apoptosis. In contrast, the

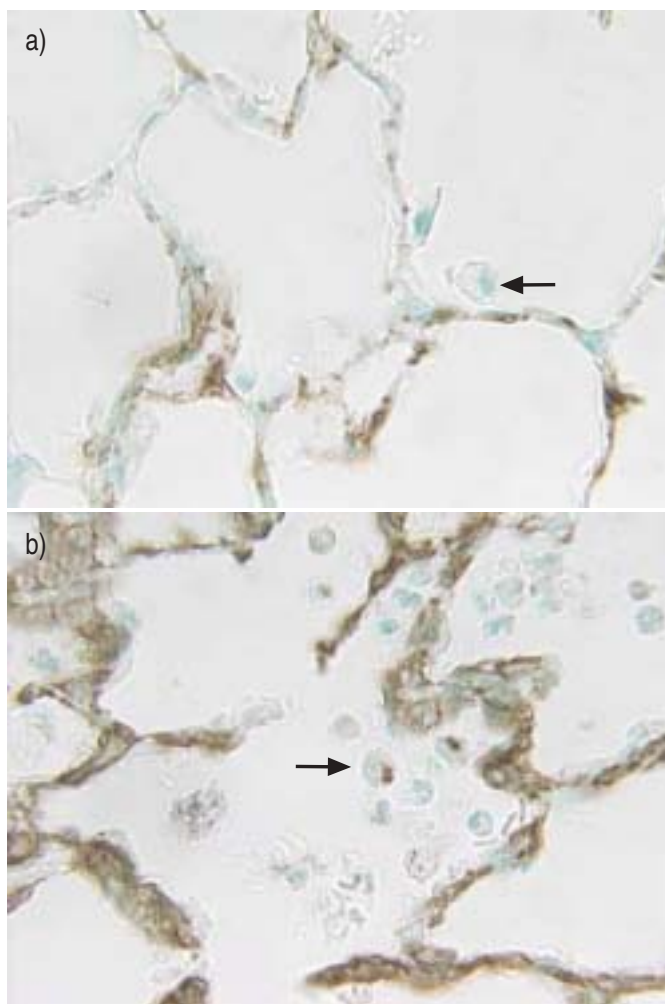


FIGURE 4. Increased expression of chondroitin sulphate proteoglycan in the lungs of a rabbit with *Escherichia coli* pneumonia. Immunohistochemistry performed using the mouse monoclonal antibody CS-56 (Sigma; St. Louis, MO, USA), which recognises chondroitin sulphate glycosaminoglycans, shows chondroitin sulphate (brown) in a) normal and b) inflamed rabbit lungs. In normal lungs, there is a multifocal distribution of chondroitin sulphate in the alveolar septa and no chondroitin sulphate in alveolar macrophages (a; arrow). In contrast, in inflamed lungs, chondroitin sulphate is increased in the alveolar septa and is found in alveolar macrophages (b; arrow).

V2 isoform of versican exhibited the opposite effect by inhibiting 3T3 cell proliferation and by not protecting against apoptosis [69]. Future studies will need to determine whether changes in the extracellular matrix proteoglycans during lung disease affect epithelial cell function in the lungs.

Activation of Toll-like pathways by degradation of the extracellular matrix

Mounting evidence suggests that Toll-like receptors not only recognise pathogen-associated molecular patterns but also endogenous factors, such as degradation products of fibronectin, heparan sulphate, hyaluronan and biglycan [70–74]. This has led to the hypothesis that matrix degradation acts as an endogenous biological “danger” signal. Pulmonary epithelial cells express Toll-like receptors and therefore may be activated upon recognition of matrix degradation products [75].

CONCLUSIONS

Extracellular matrix proteoglycans and glycosaminoglycans play an important role in the regulation of lung function in both normal and diseased lungs. New information about these diverse molecules has resulted in a shift in the paradigm whereby extracellular matrix proteoglycans modify lung function in health and disease. Initially, extracellular matrix proteoglycans were considered to be the molecular glue that provides structural support and maintains the extracellular microarchitecture. Whereas these are important functions of extracellular matrix proteoglycans, it has turned out that these diverse molecules regulate a variety of key biological processes, such as development, immune system function and tissue homeostasis, and pathological processes, such as inflammation, cardiovascular disease and cancer. This suggests that extracellular matrix proteoglycans play a critical role in regulating epithelial cell function in the lungs.

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