Review

Bench-to-bedside review: The role of glycosaminoglycans in respiratory disease

Alba B Souza-Fernandes[1], Paolo Pelosi[2] and Patricia RM Rocco[3]

[1]Laboratory of Pulmonary Investigation, Carolos Chagas Filho Biophysics Institute, Federal University of Rio de Janeiro, Ilha do Fundão, 21949-900, Rio de Janeiro, Brazil
[2]Department of Ambient, Health and Safety, University of Insubria, Viale Borri 57, 21100 Varese, Italy
[3]Laboratory of Pulmonary Investigation, Carlos Chagas Filho Biophysics Institute, Federal University of Rio de Janeiro, Ilha do Fundão, 21949-900, Rio de Janeiro, Brazil

Correspondence: Patricia RM Rocco, prmrocco@biof.ufrj.br

Published: 10 November 2006
This article is online at http://ccforum.com/content/10/6/237

Critical Care 2006, **10:**237 (doi:10.1186/cc5069)

Abstract

The extracellular matrix (ECM) plays a significant role in the mechanical behaviour of the lung parenchyma. The ECM is composed of a three-dimensional fibre mesh that is filled with various macromolecules, among which are the glycosaminoglycans (GAGs). GAGs are long, linear and highly charged heterogeneous polysaccharides that are composed of a variable number of repeating disaccharide units. There are two main types of GAGs: nonsulphated GAG (hyaluronic acid) and sulphated GAGs (heparan sulphate and heparin, chondroitin sulphate, dermatan sulphate, and keratan sulphate). With the exception of hyaluronic acid, GAGs are usually covalently attached to a protein core, forming an overall structure that is referred to as proteoglycan. In the lungs, GAGs are distributed in the interstitium, in the sub-epithelial tissue and bronchial walls, and in airway secretions. GAGs have important functions in lung ECM: they regulate hydration and water homeostasis; they maintain structure and function; they modulate the inflammatory response; and they influence tissue repair and remodelling. Given the great diversity of GAG structures and the evidence that GAGs may have a protective effect against injury in various respiratory diseases, an understanding of changes in GAG expression that occur in disease may lead to opportunities to develop innovative and selective therapies in the future.

Introduction

The alveolar wall is composed of an epithelial cell layer and its basement membrane, the capillary basement membrane and endothelial cells, and a thin layer of interstitial space lying between the capillary endothelium and the alveolar epithelium, which is the extracellular matrix (ECM) [1]. In some areas, the two basement membranes are physically fused to reduce the diffusion distance as much as possible. In the segments where the two basement membranes are not fused, the interstitium is composed of cells, a macromolecular fibrous component and the fluid phase of the ECM; here the ECM functions as a three-dimensional mechanical scaffold characterized by a fibrous mesh consisting mainly of collagen types I and III (providing tensile strength) and elastin (conveying elastic recoil) [2,3]. The three-dimensional fibre mesh is filled with other macromolecules, mainly glycosaminoglycans (GAGs), which are the major components of the nonfibrillar compartment of the interstitium [4].

The structure of the lung ECM plays several important roles, including mechanical (it provides tensile and compressive strength and elasticity, with a strong and expandable framework that supports the fragile alveolar-capillary intersection), gas exchange (it offers a low resistive pathway, allowing effective gas exchange), protective (it acts as a buffer against retention of water) and organizational (it controls cell behaviour by binding of growth factors and interaction with cell surface receptors) [2,4].

Although many studies have described the roles played by proteoglycans in a wide range of pulmonary diseases [5-8], the actions of GAGs in the lung parenchyma are much less well understood. Study of the ECM and GAGs is important because it may improve our pathophysiological knowledge on the development of oedema and specific interstitial lung diseases, it may permit early diagnosis of ECM alterations and lung remodelling processes, and it may promote development of ventilatory and pharmacological therapeutic strategies.

APC = activated protein C; ARDS = acute respiratory distress syndrome; ATIII = antithrombin III; DIC = disseminated intravascular coagulation; ECM = extracellular matrix; FGF = fibroblast growth factor; GAG = glycosaminoglycan; GAS = group A streptococci; IL = interleukin; LPS = lipopolysaccharide; PG = proteoglycan; Pip = pulmonary interstitium pressure; PLA_2 = phospholipase A_2; TFPI = type 1 tissue factor pathway inhibitor; TLR = Toll-like receptor; TNF = tumour necrosis factor.

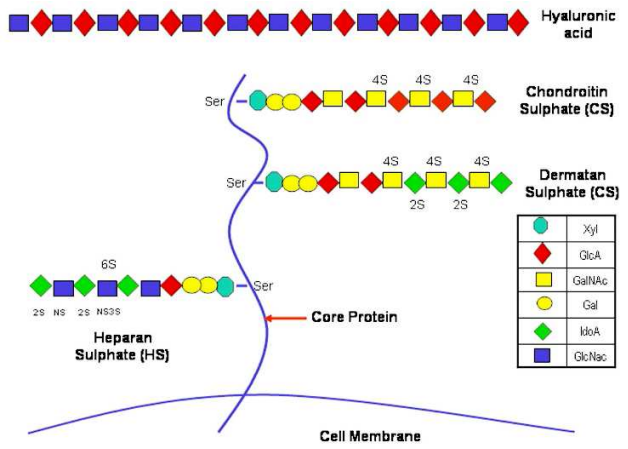
The present review discusses the biochemical characteristics of GAGs, their biological roles and mechanisms of action in several respiratory diseases, and their potential therapeutic effects.

Glycosaminoglycans

GAGs are long, linear and heterogeneous polysaccharides, which consist of repeating disaccharide units with sequences that vary in the basic composition of the saccharide, linkage, acetylation, and N-sulphation and O-sulphation; these disaccharide units are galactose, galactosamine, N-acetyl-galactosamine-4-sulphate and galacturonic acid. The chain length of GAGs can range from 1 to 25,000 disaccharide units, and their molecular weights vary over three orders of magnitude, implying that the polymer chains can contain as many as 10^4 units with great variability in size and structure [9].

There are two main types of GAGs: nonsulphated GAG (hyaluronic acid) and sulphated GAGs (heparan sulphate and heparin, chondroitin sulphate, dermatan sulphate and keratan sulphate). With the exception of hyaluronic acid, GAGs are usually covalently attached to a protein core, forming an overall structure referred to as proteoglycans [10] (Figure 1).

Figure 1



Schematic structure of glycosaminoglycan and proteoglycan. Note that the hyaluronic acid is not linked to a protein core. Heparan sulphate, dermatan sulphate and chondroitin sulphate are connected to proteoglycan via a serine residue.

Hyaluronic acid

Hyaluronic acid is the most abundant nonsulphated GAG in the lung ECM. Hyaluronic acid differs from the other GAGs because it is spun out from the cell membrane, rather than being secreted through the Golgi, and because it is enormous (10^7 Da, which is much larger than other GAGs). Hyaluronic acid is a naturally occurring, linear polysaccharide that is composed of up to 10,000 disaccharides constituted by an uronic acid residue covalently linked to an N-acetyl-glucosamine, with a flexible and coiled configuration. It is a ubiquitous molecule of the connective tissue that is primarily synthesized by mesenchymal cells. It is a necessary molecule for the assembly of a connective tissue matrix and is an important stabilizing constituent of the loose connective tissue [11]. A unique characteristic of hyaluronic acid, which relates to its variable functions, is its high anion charge, which attracts a large solvation volume; this makes hyaluronic acid an important determinant of tissue hydration [5]. Excessive accumulation of hyaluronic acid in the interstitial tissue may therefore immobilize water and behaves as a regulator of the amount of water in the interstitium [11]. Hyaluronic acid is present in the ECM, on the cell surface and inside the cell, and its functions are related to its localization [12]. Hyaluronic acid is also involved in several other functions, such as tissue repair [13,14] and protection against infections and proteolytic granulocyte enzymes [15].

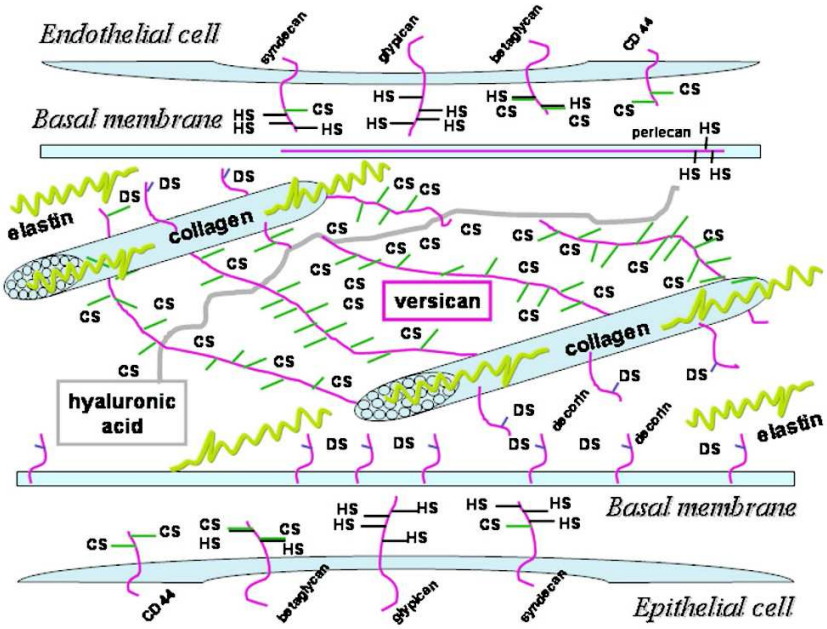
Sulphated glycosaminoglycans

GAGs of this type are synthesized intracellularly, sulphated, secreted and usually covalently bound into proteoglycans. They are sulphated polysaccharides composed of repeating disaccharides, which consist of uronic acid (or galactose) and hexosamines. The proteoglycan core proteins may also link carbohydrate units including O-linked and N-linked oligosaccharides, as found in other glycosylated proteins. The polyanionic nature of GAGs is the main determinant of the physical properties of proteoglycan molecules, allowing them to resist compressive forces and simultaneously to maintain tissue hydration. They are much smaller than hyaluronic acid, usually being only 20 to 200 sugar residues long [16,17].

Within the lung parenchyma the most abundant sulphated GAG is heparan sulphate, a polysaccharide that is expressed on virtually every cell in the body and comprises 50% to 90% of the total endothelial proteoglycans [18]. Heparan sulphate has the most variable structure, largely because of variations in the sulphation patterns of its chains. In addition to sequence diversity, its size ranges from 5 to 70 kDa. Although it is initially produced in a cell surface bound form, it can also be shed as a soluble GAG. The mechanism of action of heparan sulphate includes specific, noncovalent interactions with various proteins; this process affects the topographical destination, half-life and bioactivity of the protein. Furthermore, heparan sulphate acts on morphogenesis, development and organogenesis [19]. It is also involved in a variety of biological processes, including cell-matrix interactions and activation of chemokines, enzymes and growth factors [19,20].

Heparin is the most highly modified form of heparan sulphate. This GAG, which can be considered an over-sulphated intracellular variant of heparan sulphate, is commonly used as an anticoagulant drug [19]. Heparin and heparan sulphate are closely related and may share many structural and functional activities. The lung is a rich native source of

Figure 2



Extracellular matrix components in lung parenchyma. CS, chondroitin sulphate; DS, dermatan sulphate; HS, heparan sulphate.

heparin. This abundance of heparin may be accounted for by the fact that the lung is rich in mast cells, which may be heparin's sole cell of origin [21]. Mast cell heparin resides in secretory granules, where most of the GAG chains are linked to a core protein (serglycin), forming macromolecular proteoglycans that are much larger than commercial heparin. Very little heparin is incorporated into cell surface proteoglycans of epithelial and endothelial cells; these are more likely to contain heparan sulphate, which is under-sulphated compared with heparin. Some heparan sulphate chains of vascular endothelium contain short heparin-like sequences [20]. However, most native lung heparin is locked up in mast cells as large proteoglycans. This does not necessarily mean that heparin's physiological action resides exclusively within cells, because stimulated mast cells secrete heparin outside of the cell along with granule-associated mediators, such as histamine, chymase and tryptase [22].

Proteoglycans

In the lung, three main proteoglycan (PG) families may be distinguished based on GAG composition, molecular weight and function: chondroitin suphate containing PG (versican), heparan sulphate containing PGs (perlecan and glypican), chondroitin and heparan sulphate containing PGs (syndecan) and dermatan sulphate containing PGs (decorin). They are localized in different areas of the ECM: versican resides in the pulmonary interstitium, perlecan in the vascular basement membrane, decorin in the interstitium and in the epithelial basement membrane linked with collagen fibrils, and syndecan and glypican in the cell surface (Figure 2).

Versican is a large molecule (>1000 kDa) that is found around lung fibroblasts and blood vessels in regions not occupied by the major fibrous proteins collagen and elastin. It is localized mainly in the interstitium, creating aggregates with hyaluronic acid [17]. The precise function of versican is unclear but it is thought to be involved in tissue hydration. It may form aggregates with hyaluronic acid, fibronectin and various collagens, playing an important role in cell-matrix interaction. It has been shown that versican is linked with smooth muscle cells in the walls of airways and pulmonary vessels, inhibits cell-matrix adhesion [23], regulates differentiation of mesenchymal cells and plays a specific role in matrix synthesis, favouring wound healing.

Perlecan is the largest PG in the lung, with its core possessing about 4400 amino acids. Perlecan is a typical component of vascular basement membrane [24], although it has been also identified within the ECM of some tissues, close to the basement membrane. Indeed, its complex core protein has the potential to interact with numerous proteins. In the basement membranes it provides a filtration barrier interacting with collagen IV, limiting the flow of macromolecules or cells between two tissue compartments. It also regulates the interaction of the basic fibroblast growth factor (FGF) with its receptor and modulates tissue metabolism.

Syndecan and glypican are densely arranged in the cell surface [25]. The function of syndecan is commonly associated with its heparan sulphate chains and its interaction with heparin binding growth factors or extracellular

proteins such as fibronectin and laminin, and it plays a role in wound healing [26].

Decorin is the smallest dermatan sulphate containing PG. The presence of decorin alters the kinetics of fibril formation and the diameter of the resulting fibril [17,25], modulating tissue remodelling. Indeed, its name was derived from its surface decoration of collagen fibrils when viewed under an electron microscope.

These findings indicate that the function of PGs and GAGs in the lung is not limited to maintenance of mechanical and fluid dynamic properties of the organ. These molecules also play roles in tissue development and recovery after injury, interacting with inflammatory cells, proteases and growth factors. Thus, the ECM transmits essential information to pulmonary cells that regulates their proliferation, differentiation and organization. The structural integrity of the pulmonary interstitium depends largely on the balance between the regulation of synthesis and degradation of ECM components.

Glycosaminoglycans and interstitial pressure

The efficiency of the alveolar-capillary membrane mostly depends on the hydration of the interstitial layer in the alveolar septa. In the tissue, fluid is partitioned into two components that are in equilibrium with each other: water molecules that are chemically bound to the polyanionic hyaluronic acid and proteoglycans; and water that freely moves across the porous mesh of extracellular fibrous macromolecules.

The very thin alveolar-capillary membrane reflects a condition of minimum hydration volume of the interstitial compartment. Lung water content depends on several factors, such as transcapillary balance of pressures (Starling balance), tissue forces transmitted through the interstitial matrix related to the degree of lung expansion, forces arising from surface tension phenomena at the alveolar-air interface, and lymph fluid drainage [27]. GAGs are responsible for two important aspects of microvascular and interstitial fluid dynamics, namely the sieving properties of the capillary membrane and of the matrix, and the compliance of the interstitial tissue. For example, the relatively high number of chondroitin sulphate chains imparts a high anion charge to the macromolecule, allowing it to exhibit marked hydrophilic properties and to control the hydration of the interstitial tissues. Heparan sulphate chains account for specific interaction properties in basement membrane organization, receptor functions, and cell-cell and cell-matrix interactions [27].

The hydraulic pressure of the liquid phase of the pulmonary interstitium (pulmonary interstitium pressure [Pip]) depends on the total tissue hydration as well as other mechanical factors such as the tissue stress related to lung volume and the alveolar surface tension phenomenon [28]. In addition, regional differences in Pip can be caused by the following: the interdependence phenomenon (the stress that acts on the outer surface of rigid structures such as bronchi and vessels is greater than that on the pleural surface), the gravity distribution of regional lung expansion and the interaction between lung and chest wall. Thus, Pip reflects the dynamic situation resulting from the complex interaction between these factors. Any change in one set of forces will influence the others. The result of this complex interaction is that a change in one set of forces might cause a perturbation in the extravascular water balance, leading to lung oedema [27].

Glycosaminoglycans and interstitial plasma protein distribution

The ionic solute concentration of free interstitial fluid essentially mirrors the plasma content; indeed, because these solutes have a molecular radius that is smaller than that of the endothelial intercellular clefts, they freely equilibrate between plasma and extravascular fluid. In fact, the three dimensional 'porous-like', water-filled mesh established by GAGs constitutes a selective sieve of variable porous size and charge density [29]. The functional result of this phenomenon, termed 'volume exclusion', is a restriction of the interstitial fluid volume available for proteins that, because of their large size, cannot diffuse through the fibrous, porous mesh [30]. In the normal lung, the mean albumin excluded fraction (the percentage of interstitial fluid volume not available to protein distribution) is about 70% [31]. Consequently, proteins are allowed to equilibrate in only 30% of the available interstitial fluid volume. Thus, the normal lung behaves differently from other tissues such as skeletal muscle or skin, whose normal albumin distribution volume is as low as about 30% [31]. Hence, compared with other tissues, the normal lung parenchyma exhibits a tight fibrous structure that is highly restrictive with respect to plasma proteins.

Glycosaminoglycans and lung oedema

The early phase of interstitial oedema implies an increase in interstitial fluid pressure with no significant change in interstitial fluid volume because of the low tissue compliance. A low compliance conferred by the structure of the matrix represents an important 'tissue safety factor' to counteract further progression of pulmonary oedema. As the severity of oedema progresses, Pip drops back to zero and subsequently remains unchanged, despite a marked increase in the wet weight:dry weight ratio of the lung. As oedema develops into a more severe condition, fluid filtration occurs down a transendothelial Starling pressure gradient that is less than that in the basal state, because of the progressive increase in interstitial fluid pressure. Hence, at least two factors interact to determine the development of pulmonary oedema, namely loss of the tissue safety factor and augmented microvascular permeability [27].

In hydraulic oedema, biochemical analysis of tissue structure reveals an initial fragmentation of chondroitin sulphate proteoglycan caused by mechanical stress and/or proteolysis. In

lesional oedema, the partial fragmentation of heparan sulphate proteoglycan is mainly due to enzymatic activity. The progression toward severe oedema is similar for both types of oedema because the activation of tissue metalloproteinases leads to extended fragmentation of chondroitin sulphate proteoglycan, causing a marked increase in tissue compliance and therefore a loss in tissue safety factor, and of heparan sulphate proteoglycan, leading to an increase in microvascular permeability [27,32,33].

Recent data also suggest that the integrity of the heparan sulphate proteoglycan is required to maintain the three-dimensional architecture of the matrix itself, which in turn guarantees its mechanical response to increased fluid filtration [34].

Glycosaminoglycans and the mechanical properties of lung parenchyma

Lung parenchymal tissues exhibit prominent viscoelastic behaviour. The anatomical elements potentially responsible for this behaviour include the collagen-elastin-proteoglycan matrix, the surface film and contractile elements in the lung periphery [2,35].

The viscoelastic characteristics of the parenchymal tissues may be attributed, at least in part, to GAGs [36]. For instance, GAGs are highly hydrophilic and have the ability to attract ions and fluid into the matrix and thus affect tissue viscoelasticity; furthermore, the arrangement of fibres within the connective tissue matrix associated with GAGs also enhances viscoelasticity. It seems that the energy dissipation occurs not at the molecular level within collagen or elastin but rather at the level of fibre-fibre contact and by shearing of GAGs, which provide the lubricating film between adjacent fibres [37].

In order to study the effects of different GAGs on the mechanical tissue properties of lung parenchyma, specific degradative enzymes to digest GAGs have been used. Tissue resistance and hysteresivity increased in lung tissues treated with chondroitinase or heparitinase, whereas the quasi-static elastance was augmented only by chondroitinase. Conversely, exposure to hyalurodinase yielded no effect on mechanical behaviour of the lung parenchyma. These data suggest that the resistive properties of lung parenchyma are influenced mainly by both chondroitin sulphate and heparan sulphate [38], and elastance by chondroitin sulphate only.

Glycosaminoglycans and mechanical ventilation

Changes in the components of ECM play an important role in ventilation-induced lung injury. Berg and coworkers [39] and Parker and colleagues [40] observed that abnormal ventilation regimens induced activation of matrix components. Furthermore, mechanical ventilation with increased tidal volumes led to increased levels of versican, heparan sulphate proteoglycans and byglican [38]. These studies suggest that abnormal ventilation induces changes in ECM components, including GAGs, even in normal lungs. In Figure 3 we summarize the effects of hydraulic oedema and lesional oedema on spontaneous breathing, and on physiological and injurious mechanical ventilation, both early and late in the course of lung injury. During hydraulic oedema and in the early phase of lung injury, the prevalent lesion is fragmentation of chondroitin sulphate, whereas in lesional oedema heparin sulphate is more damaged. Mechanical ventilation at 'physiological' tidal volume (7 ml/kg) led to fragmentation mainly of chondroitin sulphate proteoglycan. However, the ongoing mechanical ventilation resulted in fragmentation of both GAGs, leading to ECM disorganization. Interestingly, although the lymphatic flow drainage is reduced, the wet weight:dry weight ratio remained unaltered [41]. On the contrary, with 'injurious' mechanical ventilation, at the early phase of lung injury, fragmentation of both chondroitin sulphate and heparan sulphate proteoglycans occurs, which is partially compensated for by an increase in the synthesis of new GAGs. During the course of lung injury greater fragmentation of GAGs takes place, with an increase in the wet weight:dry weight ratio and progressive fibrogenesis [42] (Figure 3).

Biological roles of glycosaminoglycans

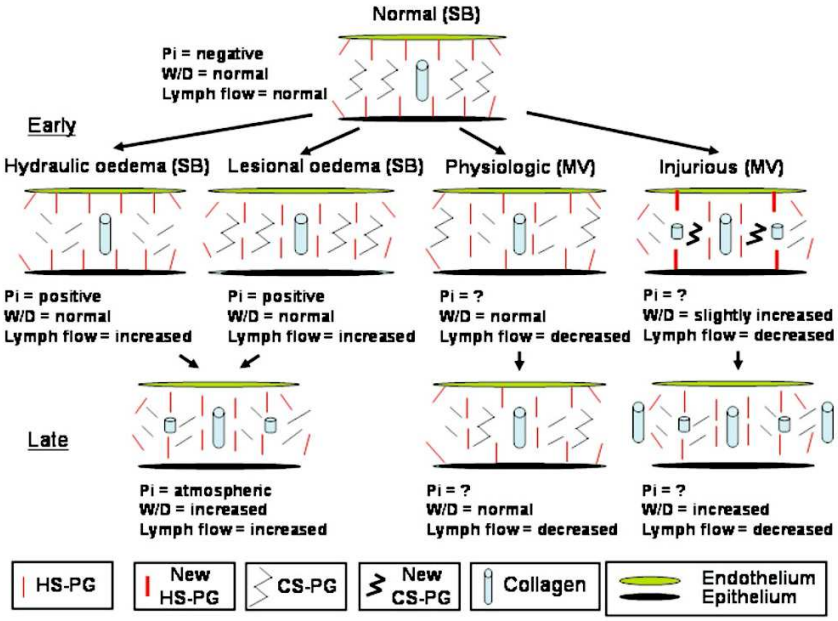
GAGs interact with an enormous number of proteins, ranging from proteases, extracellular signalling molecules, lipid-binding and membrane-binding proteins, and cell-surface receptors on viruses. Their functions include modulating signal transduction associated with processes such as development, cell proliferation and angiogenesis; and adhesion, localization and migration of cells. In addition, they act directly as receptors and assembly factors, and they are used by many pathogens for localization and entry into cells [18]. Furthermore, extracellular GAGs can potentially sequester proteins and enzymes, and present them to the appropriate site for activation [43] (Table 1).

Interactions of specific proteins with glycosaminoglycans

GAGs interact with proteins to modulate their activity. In this context, the interaction between FGFs and their tyrosine kinase receptors depends on the sequence of the heparan sulphate chain [18]. Heparan sulphate plays a critical role in FGF signalling by facilitating the formation of FGF-FGF receptor complexes (and/or stabilizing these complexes) and enhancing (and/or stabilizing) FGF oligomerization [43]. In addition, in the ECM heparan sulphate binds FGF, storing it in an inactive form until needed, thereby allowing rapid response to stimuli [44].

Another well studied example of protein-GAG interaction involves the binding of antithrombin to heparin/heparan sulphate, which results in the inactivation of the coagulation cascade. Heparan sulphate also regulates other aspects of the cardiovascular homeostasis by interacting with additional proteins, including apolipoproteins and lipoprotein lipase

Figure 3



Changes in extracellular matrix. Illutrated are changes in the extracellular matrix that occur during hydraulic and lesional oedemas in spontaneous breathing (SB) and physiological and injurious mechanical ventilation (MV) early and late in the course of lung injury. Bold lines represent the new synthesis of heparan sulphate (HS)-proteoglycan (PG) or chondroitin sulphate (CS)-PG. During hydraulic oedema and in the early phase, the prevalent lesion is fragmentation of CS, whereas in the lesional oedema HS is damaged. In physiological MV, mainly CS-PG was fragmented, but the ongoing MV yields the fragmentation of both glycosaminoglycans. During injurious MV, although HS-PG and CS-PG are injured, collagen fibre content increases early and late in the course of lung injury. Thus, we hypothesize that in the early phase of lung injury collagen fibre synthesis could be beneficial in avoiding the rupture of glycosaminoglycans, minimizing interstitial oedema formation. Pi, interstitial pressure; W/D, wet weight:dry weight ratio.

[45]. Growth factors such as hepatocyte growth factor, platelet-derived growth factor, epidermal growth factor, and vascular endothelial growth factor [46-48] also bind heparin and heparin sulphate, although the physiological consequences of this binding are unclear.

Heparan sulphate interacts with cytokines such as interleukin (IL)-5, IL-6, IL-8, IL-10, tumour necrosis factor (TNF)-α and platelet factor-4 [49-51]. The interaction of heparan sulphate with IL-8 promotes the activity of the cytokine, whereas, in the case of platelet factor-4, the interaction inhibits the activity. Heparan sulphate has been shown to interact with various ECM proteins, including fibronectin, laminin, thrombospondin, collagen types I, II, IV, V, VI, XIII and XVIII, and endostatin. The binding of endostatin by heparan sulphate is important for its antiangiogenic function [52]. The interaction between heparan sulphate and laminin could be important in determining the integrity of basement membranes [53]. The interaction between heparan sulphate and collagen V plays an important role in the modulation of cell adhesion to the substratum [54]. Furthermore, it has been demonstrated that chondroitin sulphate could also bind to collagen V, participating in the regulation of cell adhesion to the ECM [55].

Chemokines are a subset of cytokines that are known to interact with GAGs. Although chemokines bind to high-affinity G-protein-coupled receptors on migrating cells, it has been hypothesized that they bind to immobilized GAGs as a mechanism for cell-surface retention and possibly for presentation to circulating leucocytes. Without such a mechanism, chemokine gradients would be disrupted by diffusion, especially in the presence of shear forces in the blood vessels and draining lymph nodes. Chemokine immobilization is necessary because soluble chemokines could haphazardly bind and activate leucocytes prior to selectin-mediated adhesion, subsequent arrest and firm adhesion, and therefore transmigration of the leucocyte would not occur. Furthermore, interactions with GAGs may also provide another level of specificity and control over cell migration [56].

GAGs have also been shown to protect chemokines from proteolysis and may serve as an additional layer of regulation. Similarly, some chemokines are released as high-molecular-weight complexes associated with proteoglycans, and heparin and heparan sulphate can inhibit chemokine function; these findings suggest that some GAG interactions can prevent inappropriate chemokine activation. Such complexes

Table 1

The main characteristics of glycosaminoglycans

GAG	Structure	Function [references]
HA	D-glucuronate + GlcNAc	Stabilization of the connective tissue [11] Organization of the ECM [11] Hydration and water homeostasis [11] Receptor-mediated signalling [12] Morphogenesis and tissue homeostasis [13,14] Regulation of the inflammatory response [15] Tissue modelling and remodelling [72] Cellular migration and fagocytosis [5]
DS	L-iduronate + GalNAc-4-sulphate	Collagen organization [18] Regulation of TGF-β activity [5] Stabilization of the basement membrane [18] Regulation of cell-cell and cell-matrix interactions [5]
CS	D-glucuronate + GalNAc-4- or 6-sulphate	Prevention of inflammation [55] Immune modulation [43] Maintenance of the structure and function of cartilage [55] Cartilage shock-absorbing properties [55] Regulation of cell adhesion to the ECM [55]
HS	D-glucuronate-2-sulphate (or iduronate-2-sulphate) + *N*-sulfo-D-glucosamine-6-sulphate	Interaction with cytokines, chemokines and interleukins [18,44-52] Morphogenesis, development and organogenesis [19] Coreceptors for various receptor tyrosine kinases [27]
Heparin	D-glucuronate-2-sulphate (or iduronate-2-sulphate) + *N*-sulfo-D-glucosamine-6-sulphate	Anticoagulant effects [19] Stabilization of some mast cell tryptases [22] Modulation of the activity of various mast cell chymases [59] Regulation of the inflammatory response [57] Remodelling of the airway wall in asthma [58]
KS	Galactose + GlcNAc-6-sulphate	Tissue hydration [115] Cell biology [115] Most abundant GAG in airway secretion [116]

CS, chondroitin sulphate; DS, dermatan sulphate; ECM, extracellular matrix; EGF, epidermal growth factor; FGF, fibroblast growth factor; GAG, glycosaminoglycan; GalNAc, N-acetylgalactosamine; GlcNAc, N-acetylglucosamine; HA, hyaluronic acid; HGF, hepatocyte growth factor; HS, heparan sulphate; KS, keratan sulphate; PDGF, platelet-derived growth factor; TGF, transforming growth factor; VEGF, vascular endothelial growth factor.

may also serve as storage forms for rapid mobilization of chemokines without the need for protein synthesis [18].

Heparin and inflammation

There is increasing evidence that heparin has a wide range of biological properties that can be considered beneficial in the context of the regulation of the inflammatory response [57]. Heparin can inhibit the influx of neutrophils into certain tissues and inhibit T-cell trafficking, partly by an inhibitory effect on the heparinase enzyme secreted by T cells [58]. Furthermore, heparin has been shown to be released from human lung mast cells in response to allergen exposure, and increased levels of a heparin-like substance have been reported in the plasma of asthmatic individuals [59]. Heparin can also inhibit allergen-induced eosinophil infiltration into the airways of experimental animals [60].

After the inflammatory cells have passed through the lung tissue, it is recognized that there is a number of stages involved, including adhesion to the vascular endothelium, diapedesis across the endothelial cells and chemotaxis within tissues. It is clear that heparin can inhibit all stages of cell migration, including the carbohydrate-selectin interactions between endothelial cells and leucocytes, the presentation of specific chemoattractants to activated leucocytes, and leucocyte trafficking. Although the mechanisms that underlie the effect of heparin on neutrophil migration are well understood, the ability of heparin to interfere with eosinophil adherence is less well understood. Nonetheless, heparin is able to inhibit the actions of several important eosinophil chemoattractants, such as platelet factor-4 [58].

Although the precise mechanism of the anti-inflammatory effects of heparin is not established, it has been suggested that inhibition of the interaction between proinflammatory cytokines and membrane-associated GAGs may provide a mechanism for inducing clinically useful immunosuppression. Whereas immobilized heparin is essential for the biological activity of chemokines, soluble heparin has been shown to inhibit the biological effects of chemokines [56]. It is therefore

likely that the anti-inflammatory effects of heparin are mediated, at least in part, by interference with the chemokine system [18].

Therapeutic aspects of heparin

The effectiveness of heparin in blocking fibrin deposition in the lung, with subsequent improvement in lung function, has been studied. Large doses of unfractionated heparin (500 units/kg) have been demonstrated to block fibrin deposition effectively in the lungs and to prevent an increase in extravascular lung water in dogs after microembolization [61]. Other authors, using higher doses of heparin (3000 units/kg) or fibrinogen depletion by viper venom, could not demonstrate a reduction in extravascular lung water in a microemboli model in sheep [62].

Heparin has also been evaluated in an ovine smoke inhalation model [63]. Smoke inhalation induces tracheobronchial obstruction and increases pulmonary microvascular permeability and oedema. This is thought to be mediated by the release of proteases, such as elastase, and free oxygen radicals. High-dose heparin (400 units/kg bolus), followed by continuous infusion to maintain an activated clotting time of 250 to 300 s, was associated with an improvement in partial arterial oxygen tension/fractional inspired oxygen ratio at 12 to 72 hours compared with controls. Tracheobronchial casts and pulmonary oedema were reduced, but leucocyte lung infiltration and oxygen free radical activity were unaffected by heparin [64].

In a lavage and volutrauma-induced lung injury model in piglets, heparin (30 units/kg) was compared with anti-thrombin alone and with antithrombin combined with heparin [65]. Surprisingly, gas exchange was improved and hyaline membrane formation was reduced by heparin alone compared with antithrombin alone or antithrombin combined with heparin. However, these data were not confirmed by two other studies that explored the effects of intravenous or inhaled heparin in endotoxin or smoke inhalation induced lung injury models [66,67]. Heparin prophylaxis (5000 units every 8 hours for 7 days) before and after lobectomy for non-small-cell lung carcinoma was associated with reduced plasma levels of neutrophil elastase, which may protect the lung from complications such as acute respiratory distress syndrome (ARDS) [68].

Hyaluronic acid

Hyaluronic acid in the pulmonary alveolus

The duplex nature of the lining of the pulmonary alveolus has long been appreciated. Surfactant is present at the interface with air, where it prevents the alveolar collapse by lowering surface tension. Surfactant rests on an aqueous subphase and forms a smooth, continuous surface over the projections of the epithelial cells. There is a constant requirement to replace losses to the surfactant layer brought about by the cyclic compression and expansion of breathing. Type II cells in the wall of the alveolus are specialized to produce surfactant and they also secrete hyaluronic acid into the subphase [18]. Hyaluronic acid is also known to attract the polar heads of surfactant phospholipids and has hydrophobic regions, which could bind to the hydrophobic surfactant proteins B and C. These direct interactions of hyaluronic acid and surfactant phospholipids contribute to the stability of the surfactant layer [69]. Thereafter, hyaluronic acid interacts with itself and with proteins in the subphase to form a hydrophilic gel. At the epithelial cell layer, the components are concentrated because of tethered hyaluronic acid molecules and the gel smoothes over cell projections. At the air interface, the components are so dilute that a layer of essentially water is present [7].

Hyaluronic acid in response to injury

During the maturation of tissue and organs there is a fall in water concentration, suggesting that mature organs are adapted to function in an environment with a considerably lower amount of water than in early foetal life. Based on this, and on the observation that synthesis of hyaluronic acid is a very early response to connective tissue cell activation *in vitro*, a rather attractive hypothesis is that inflammation and tissue repair (processes that involve migration and proliferation of cells and that require a vast array of paracrine mechanisms) require an environment with a water concentration considerably higher than that of many mature organs. From this point of view, both the permeability increase in the microvasculature and the increased synthesis of hyaluronic acid would synergize to achieve an overhydration of the interstitium, contributing to the inflammatory mechanism [11].

That the overall synthesis of hyaluronic acid in the organism is considerable and that the hyaluronic acid pool of the interstitium has a very short half-life [70,71] suggest that the concentration of hyaluronic acid in the interstitium is in a dynamic equilibrium, where synthesis and elimination are in balance. Because various cytokines have been observed to influence the synthesis of hyaluronic acid by connective tissue cells *in vitro* [72], an attractive hypothesis is that the synthesis of hyaluronic acid *in vivo* is altered in inflammatory and immunological conditions where there is increased cytokine release. Other data suggest that the run-off or elimination of hyaluronic acid from the tissue compartments is enhanced by a common feature in the inflammatory process, namely increased interstitial water flux. These observations suggest that an ongoing inflammatory state is associated with an increased turnover of hyaluronic acid in the affected tissue compartment. Furthermore, these data suggest that modulation of the tissue concentration of hyaluronic acid might be a mechanism by which the organism can modulate the behaviour of the interstitium, and thereby create differences in the environment where inflammation, tumour growth and tissue repair take place.

To function in tissue modelling during development, as well as in normal tissue homeostasis and remodelling in disease, hyaluronic acid interacts with a number of hyaluronic acid-

binding proteins called hyaldherins [73]. The hyaldherin molecules include structural matrix hyaluronic acid-binding proteins as well as cell surface receptors that bind with high affinity to hyaluronic acid [74]. Many cell surface receptors for hyaluronic acid have been detected in a variety of cells and tissues [73]. Among these, the CD44 family has been better characterized. Turley and coworkers characterized hyaluronic acid receptors that mediate cell locomotion [35]. Lacy and Underhill [74] demonstrated a chemically significant relationship between a receptor for hyaluronic acid and the cytoskeleton of cells through actin filaments. They showed that the receptor for hyaluronic acid is directly or indirectly associated with cytosolic actin filaments. This association suggests that there is a transmembrane interaction between hyaluronic acid outside of the cell and the actin filaments inside, which could explain the effect of hyaluronic acid on cellular activities such as migration and phagocytosis [5].

Several studies have demonstrated that the biological effects of hyaluronic acid appear to vary depending on the average molecular mass [5,8,12,75-79]. In physiological conditions, hyaluronic acid is a polymer with high average molecular mass, in excess of 10^6 Da. However, following tissue injury, hyaluronic acid fragments of lower molecular mass accumulate. Ohkawara and coworkers [77] demonstrated that small-molecular-weight fragments increase the survival of peripheral blood eosinophils *in vitro*. They also observed that molecules of higher molecular weight were much less effective. Tammi and coworkers [12] showed that fragmented hyaluronic acid with an average molecular mass of 250,000 Da can induce the expression of inflammatory genes [12]. Low-molecular-weight fragments can stimulate activated macrophages to express RNAs of numerous chemokines and cytokines, including the production of metalloelastase [76]. However, fragments of higher molecular weight have an opposite effect and suppressed such chemokine expression [5]. Horton and coworkers [78] reported that small-molecular-weight fragments of hyaluronic acid would serve to modulate macrophage functions through nuclear factor-κB signalling synergistically with interferon-γ [78].

Nevertheless, biological relevance is suggested by reports showing that fragmented hyaluronic acid, which induces inflammatory gene expression *in vitro* is in the same size range as hyaluronic acid that accumulates under inflammatory conditions *in vivo* [76]. A common theme appears to be that low-molecular-weight hyaluronic acid can initiate gene transcription, influencing cell proliferation and migration. Generation of hyaluronic acid fragments under conditions of inflammation or tumourigenesis, or tissue injury as a result of hyaluronidases or oxidation [75] may then signal to the host that normal homeostasis has been profoundly disturbed.

Hyaluronic acid and mechanical ventilation

Mascarenhas and coworkers [79] observed that high tidal volume ventilation of rat lungs caused changes in their production of hyaluronic acid, with increased levels of fragments of lower molecular mass. They also showed that these fragments induced IL-8 production in a dose-dependent manner in human type II-like alveolar epithelial cells, contributing to the pathogenesis of ventilator-induced lung injury [79]. Furthermore, Bai and coworkers [8] demonstrated that high tidal volume ventilation induced the appearance of low-molecular-weight hyaluronic acid and resulted in neutrophil infiltration in the lungs. These effects were not observed in hyaluronic acid synthase-3 knockout mice. They concluded that high tidal volume induced low-molecular-weight hyaluronic acid production is dependent on *de novo* synthesis through hyaluronic acid synthase-3, and plays a role in the inflammatory response of ventilator-induced lung injury [8]. Thus, we can observe that an inflammatory reaction, irrespective of its cause, is followed by an increased synthesis of hyaluronic acid in the interstitium [11].

Hyaluronic acid in respiratory diseases

Sepsis

Sepsis is the leading cause of mortality in intensive care units and is generally considered to result from excessive activation of the host's inflammatory defence mechanisms. Disseminated intravascular coagulation (DIC) frequently complicates sepsis. DIC is an acquired syndrome characterized by the activation of intravascular coagulation, culminating in intravascular fibrin formation and deposition in the microvasculature. Fibrin deposition leads to a diffuse obstruction of the microvascular bed, resulting in progressive organ dysfunction, such as the development of renal failure and ARDS, hypotension and circulatory failure. Because DIC is involved in the pathogenesis of sepsis and the development of multiple organ dysfunction syndrome, inhibition of coagulation seems a valuable therapeutic option. The hallmark of the coagulation disorder in sepsis is the imbalance between intravascular fibrin formation and its removal. Anticoagulant mechanisms deprive the activated coagulation system of thrombin. Thrombin is quickly inactivated by antithrombin by formation of thrombin-antithrombin complexes, which are rapidly cleared from the circulation. Moreover, thrombomodulin expressed on endothelial cells binds thrombin and abrogates its procoagulant activity [80]. The thrombin-thrombomodulin complex activates protein C, and activated protein C (APC) rapidly dissociates from the thrombomodulin-thrombin complex and inactivates factors Va and VIIIa, thereby decreasing thrombin generation [81]. Moreover, APC enhances fibrinolysis by neutralization of plasminogen activator inhibitor type 1 [80]. During sepsis, several of these anticoagulant mechanisms are severely compromised. Inactivation of antithrombin by elastase released from activated neutrophils and consumption of antithrombin caused by the rapid clearance of thrombin-antithrombin complexes decrease the availability of functional antithrombin. The function of the APC system is also severely compromised during sepsis. Reduced thrombomodulin expression on endothelial cells to inflammatory mediators,

such as TNF-α, has been claimed to explain the decreased APC activity [80].

Because activation of coagulation during sepsis is mainly initiated through the extrinsic pathway, the tissue factor pathway inhibitor (TFPI) has attracted some interest. In the circulation, TFPI originates from at least three pools. The majority is bound via GAGs to endothelial cells in the microvasculature, and a small fraction circulates either associated with lipoproteins or in platelets. Although normal or even elevated levels of TFPI can be found in DIC and sepsis, elevated tissue factor levels can be measured in the plasma of these patients, suggesting a relative deficiency of TFPI to neutralize tissue factor, which finally results in unopposed thrombin generation [80]. Restoration of anticoagulant capacity as well as fibrinolysis might be a promising target for therapeutic strategies in sepsis. Thus, administration of coagulation inhibitors might be an attractive therapeutic approach to human sepsis. Based on these facts, a large, double-blind, placebo-controlled multicentre trial [82] was conoducted to investigate the effect of antithrombin, together with heparin, in patients with sepsis. Although considerable antithrombin levels 24 hours after administration had been achieved, no difference in mortality rate was found. Moreover, patients treated with antithrombin had significantly more bleeding complications compared with the placebo group. In the group of patients with antithrombin activity levels above 60%, a beneficial effect on 90-day mortality was found, which can be explained by the higher levels achieved by antithrombin administration in these patients, as compared with patients starting with levels below 60%. In subgroup analysis, patients without heparin exhibited a mortality risk reduction of approximately 15%. A probable explanation is that the concomitant use of heparin decreases the ability of antithrombin to bind to GAG on endothelial cells [80]. Antithrombin must bind to GAGs on endothelial surface or inflammatory cells to exert its anti-inflammatory effects. Heparin competitively inhibits the binding of antithrombin to other GAGs and eliminates the anti-inflammatory effects of antithrombin [83]. Moreover, patients treated with antithrombin receiving no heparin had fewer bleeding complications as compared with patients receiving heparin [80].

Patients with severe sepsis and with a predicted high risk of death have a treatment benefit from high-dose antithrombin III (ATIII). In this population, an absolute risk reduction in 56-day all-cause mortality was observed, and the treatment effect was maintained until 90 days after randomization [84]. The treatment effect in favour of ATIII was observed even with concomitant use of heparin, which has been shown to antagonize the anti-inflammatory activities of ATIII. ATIII may directly affect inflammatory cell functions by ligation of ATIII-binding GAGs, including members of the syndecan family of heparin sulphate proteoglycans. Syndecans are surface molecules in a variety of cell types, including leucocytes and endothelial cells, which mediate cell-cell adhesion and are involved in proliferation, migration, and differentiation. Heparins may prevent ATIII from binding to syndecans. The only serious adverse event significantly associated with ATIII administration was bleeding, but bleeding did not necessarily translate into increased mortality in the ATIII group.

Sepsis generates a procoagulant state by multiple other mechanisms. TNF-α is principally responsible for activation of the fibrinolytic pathways in systemic inflammatory states. Thrombin-antithrombin complex formation is accelerated by the heparan sulphate found on the endothelial surface. Through its interaction with this GAG, antithrombin may stimulate the production of prostacyclin by endothelial cells. Prostacyclin has anti-inflammatory properties, including diminishing TNF-α synthesis from monocytes, inflammatory mediator release from neutrophils and neutrophil adhesion to endothelial cells [85].

Sepsis induced by endotoxins such as lipopolysaccharide (LPS) is characterized by enhanced production of inflammatory mediators, including phospholipase A_2 (PLA_2), TNF-α, IL-1 and IL-6. It is known that LPS, either directly or mediated by TNF-α or IL-1, upregulates the expression of adhesion molecules on the endothelial surface and increases the production of chemokines *in situ*, thereby promoting an inflammatory response in organs such as kidney and lung [86].

Among the mediators involved in the pathophysiology of sepsis, PLA_2 appears to play a key role. PLA_2 hydrolyzes membrane phospholipids to produce fatty acids, including arachidonic acid and lyso-phospholipids, and thus it initiates the production of numerous inflammatory mediators including arachidonic acid derived eicosanoids (prostaglandins, thromboxane and leukotrienes), platelet-activating factor and the various lyso-phospholipids themselves. PLA_2 additionally synergizes with other proinflammatory mediators of tissue damage. It has been suggested that degradation of cell surface GAGs by reactive oxygen species and heparinase renders the cell membrane accessible to the action of exogenous PLA_2 and executes the actual cell lysis and tissue damage. Furthermore, PLA_2 also facilitates extravasation of inflammatory cells, which is a key process in the development of sepsis and inflammation in general [87].

Infant respiratory distress syndrome

Although surfactant replacement has revolutionized the therapy of respiratory distress syndrome of premature infants, the effects of surfactant therapy are less dramatic when it is used to treat lung diseases associated with ARDS. The less successful clinical response in these diseases may be due, in part, to surfactant inactivation caused by leakage of plasma and inflammatory products into the alveoli. In this context, because of the direct interactions of hyaluronic acid and surfactant phospholipids, the administration of hyaluronic acid together with surfactant is able to improve substantially the surface activity of surfactant, contributing to its stability [69].

Lu and coworkers [88] investigated the effects of using hyaluronic acid mixture *in vitro* and *in vivo* with meconium as the injury-producing substance. They observed that addition of hyaluronic acid to surfactant improves surface activity in the presence or absence of meconium. The degree of the effect depends on the concentration and molecular weight of hyaluronic acid. At the higher concentration, all molecular weights of hyaluronic acid were effective, whereas at the lower concentration only the hyaluronic acid with the greatest molecular weight had beneficial results. In animal experiments, hyaluronic acid added to surfactant improved gas exchange measurements and lung mechanics.

Various properties of hyaluronic acid may account for the alterations produced in surface activity of the surfactant. Hyaluronic acid is known to bind to water at many times its own weight. By binding to water molecules, hyaluronic acid may increase the concentration of other large molecules in the nonbound water, including the surfactant, and thus induce its inactivation. Furthermore, phospholipids can interact with hyaluronic acid to form complexes. The formation of phospholipid-hyaluronic acid aggregates may stabilize surfactant phospholipids at the air-water interface and/or increase surface adsorption of subphase lipids. Hyaluronic acid has hydrophilic properties and hydrophobic regions generated by its molecular shape that could potentially serve as interaction sites for the hydrophobic surfactant proteins B and C [88].

Pneumonia

Hyaluronic acid stimulates the activity of blood neutrophils such as phagocytosis and free oxygen radical formation and migration. Based of these findings, Venge and coworkers [89] tested the hypothesis that hyaluronic acid administration subcutaneously might reduce the number of bacterial infections in patients with an increased susceptibility to such infections. Patients with chronic bronchitis and recurrent acute exacerbation of their disease treated with hyaluronic acid had significantly fewer acute exacerbations than did placebo-treated patients. Those investigators concluded that hyaluronic acid reduces the consumption of antibiotics and the number of infectious exacerbations in patients with chronic bronchitis, possibly by enhancing cellular host defence mechanisms [5].

Cywes and coworkers [90] reported that CD44, a hyaluronic acid binding protein that mediates human cell-cell and cell-ECM binding interactions, functions as a receptor for group A streptococci (GAS) colonization of the pharynx *in vivo*. The recognition of CD44 as a receptor for a major microbial pathogen adds a new dimension to the multifaceted role of CD44 in cell-cell communication. The interaction between the GAS capsular polysaccharide and CD44 is a striking example of microbial adaptation to survival within the host through subversion of a host intercellular communication pathway. Interventions designed to disrupt that interaction represent a novel potential approach to the prevention of GAS infection [90].

Acute respiratory distress syndrome

During experimental induction of bleomycin-induced alveolar injury in rats, there was considerable augmentation of high-molecular-weight hyaluronic acid in the oedematous interstitial alveolar space during the alveolitis phase [91], which was attributed to alveolar interstitial fibroblast-like cells. Experimental studies demonstrated a link between the alveolar and interstitial accumulation of hyaluronic acid and the intra-alveolar oedema [92]. The hypothesis that interstitial water trapped by hyaluronic acid may result in impaired lung function is further supported by the clinical observation of a close relationship between large amounts of hyaluronic acid recovered by bronchoalveolar lavage fluid and reduced gas diffusion of the lung in patients with acute alveolitis or ARDS [93]. Another study of a bleomycin model of lung injury in mice [94] demonstrated a critical role for the hyaluronic acid receptor CD44 in the inflammatory processes. Clearance of hyaluronic acid fragments is crucial in resolving lung inflammation and is dependent on CD44, which is expressed on haematopoietic cells. In the absence of CD44, expression of genes involved in inflammation persists, suggesting that the signalling pathways, unlike hyaluronic acid-CD44, regulate macrophage responses to hyaluronic acid fragments. CD44 deficiency led to unremitting inflammation, increased mortality rate, accumulation of hyaluronic acid, prolonged inflammatory gene expression, decreased clearance of apoptotic neutrophils and impaired the ability to generate active transforming growth factor-β_1. CD44 can also mediate wound microvascular endothelial cell migration on fibrogen and invasion into fibrin matrix [95]. In addition, several observations suggest that CD44 may play a role in fibroblast migration in wound healing [96]. Whether hyaluronic acid of higher molecular weight influences the CD44 receptor in the clearing phase of inflammation is not clear [5]. Data have shown that CD44 is not sufficient to mediate hyaluronic acid signalling [97]. Thus, another receptor system must be required for hyaluronic acid signalling.

Jiang and coworkers [98] provided evidence of a requirement for both hyaluronic acid and Toll-like receptors (TLRs), which was stimulated by subnanomolar concentrations of LPS, in regulating tissue injury and repair. They described two major functions for hyaluronic acid-TLR interactions in these processes. Soluble hyaluronic acid degradation products generated during noninfectious lung injury can stimulate macrophages to produce chemokines and cytokines, through TLR2 and TLR4, which recruit neutrophils to the site of injury; this suggests that circulating hyaluronic acid fragments could contribute to unremitting inflammation. In addition, these data also support a previously unrecognized role for native cell surface high-molecular-mass hyaluronic acid and TLRs in limiting the extent of lung epithelial cell injury by providing a basal nuclear factor-κB activation and inhibiting apoptosis

and promoting the repair of parenchymal cell injury through TLR-dependent mechanisms. Altering the balance in favour of forms of high molecular mass could favour recovery from ARDS [98,99].

Pulmonary fibrosis

Lung fibrosis results from injury to the lung parenchyma, increased proliferation of mesenchymal cells, and excessive accumulation of connective tissue matrix in the interstitium and intra-alveolar space of the lung. A combination of several normal but exaggerated biological processes, including local overproduction of growth factor and cytokines, coagulation system activation, decreased fibrinolysis and excessive oxidative stress, are believed to be involved in the pathogenesis of pulmonary fibrosis. After injury, resident alveolar cells, including endothelial/epithelial cells and alveolar macrophages, secrete cytokines, chemokines, adhesion molecules and tissue factor. Under normal conditions, this reaction leads to temporary inflammation, tissue formation, remodelling and, finally, normal tissue repair. However, persistent injury and chronic inflammation may accelerate the fibroproliferative response by promoting the secretion of growth factors from resident lung cells, including alveolar macrophages. These growth factors stimulate the proliferation of fibroblasts and smooth muscle cells and the secretion of extracellular matrix components such as collagen, fibronectin and GAGs in the lung. The relative insufficiency of metalloproteinases, increased secretion of tissue inhibitors of metalloproteinases, and abnormal function of the fibrinolysis system also play critical roles in fibroproliferative processes in the lung [100].

Asthma

In addition to a role in protecting against alveolar destruction in experimental models of lung emphysema, hyaluronic acid has also been found to block bronchial obstruction induced by aerosol administration of pancreatic elastase in sheep [101]. Because *in vitro* experiments have demonstrated that hyaluronic acid can inactivate tissue kallikrein, it has been proposed that the protective effects of hyaluronic acid against elastase-induced bronchoconstriction are mediated through the inactivation of tissue kallikrein [102]. These studies suggest a possible therapeutic role for hyaluronic acid in mitigating the bronchial responses induced by elastases.

In this way, the first studies in humans with a single dose of inhaled hyaluronic acid were suggestive of a protective effect against exercise-induced bronchoconstriction [103,104]. The exercise-induced bronchoconstriction is mediated by hyperventilation, which leads to heat and/or water loss from the airway. This causes changes in osmolarity and temperature of the bronchial mucosa, which can stimulate airway epithelial cells, infiltrative cells and airway nerves. These pathways indirectly induce smooth muscle contraction and thereby bronchoconstriction. Through its barrier properties, hyaluronic acid may prevent heat and water loss from the airways during exercise and could thereby protect against exercise-induced bronchoconstriction.

Kunz and coworkers [104], however, showed that a single dose of hyaluronic acid administered before exercise did not protect against exercise-induced bronchoconstriction in asthmatic patients. Another GAG, namely heparin, has been shown to protect against exercise-induced bronchoconstriction. This inhibitory effect may be due to prevention of mediator release rather than direct effects on smooth muscle [104]. Furthermore, heparin may be capable of modulating the extent of remodelling of the airway wall seen in asthma by modulating the actions of a range of proteins including ECM proteins, growth factors and certain enzymes, and by inhibiting the proliferation of lung fibroblasts and airway smooth muscle cells [58]. Additionally, heparin is released in the airways physiologically as a homeostatic mechanism to limit the extent of the cellular adhesion and diapedesis, and the release of heparin provides a plausible homeostatic mechanism to limit tissue damage and remodelling following an inflammatory insult to the mucosal surface [57].

Mast cells are essential elements in allergic inflammation. Mediators produced by mast cells are packaged within secretory granules and, on activation, are released into the extracellular environment within minutes. Principal granule constituents include histamine, serine proteases, carboxypeptidase A and GAGs (heparin and chondroitin sulphate). Histamine in granules is found in ionic association with acidic residues of the side chains of heparin and chondroitin sulphate, and dissociates in extracellular fluids by exchanging with sodium ions. Moreover, mast cells might contribute to the downregulation of the allergic response in that they produce and release IL-1 receptor antagonist, heparin and other molecules with anti-inflammatory properties [105].

Hyaluronic acid is also important in regulating the remodelling process in asthma. It has been found to be elevated in asthma, and promotes a number of proinflammatory responses, including B cell activation, enhancement of antigen presentation, inflammatory mediator production in macrophages and eosinophil survival. In lung fibroblasts, a number of inflammatory mediators that are elevated in asthma, specifically TNF-β, interferon-β and IL-1β, can modulate hyaluronic acid production, both alone and in combination [106,107].

Moreover, post-transcriptional regulation may play a role in airway remodelling via upregulation of the receptors that mediate responses to hyaluronic acid. Deposition of hyaluronic acid and the expression of its receptor is a key ECM axis in which post-transcriptional regulation may play an important role in airway remodelling [105]. The expression CD44 is increased in areas of epithelial repair in asthmatic patients. In fact, CD44 may have an important function in

localizing chemokines and growth factors to disrupted epithelium [108]. Airway epithelium-derived cells adhere to ECM, particularly hyaluronic acid, through CD44 [109,110].

Pulmonary emphysema

In a series of studies, Cantor and coworkers [15,111,112] demonstrated that hyaluronic acid mitigates the action of elastases such as porcine pancreatic elastase, as well as human neutrophil elastase and human macrophage metalloelastase. Air space enlargement induced by intratracheal elastase is augmented by prior depletion of lung hyaluronic acid. In the same way, hyaluronic acid aerosol administered to hamsters with elastase-induced emphysema has been shown to reduce significantly the severity of the disease.

These properties of hyaluronic acid could protect against elastin injury, which may have significant therapeutic potential in diseases such as pulmonary emphysema related to α_1 antitrypsin deficiency or due to smoking, as well as cystic fibrosis. In a related observation, it is noteworthy that the few biochemical studies of hyaluronic acid content in the lung of patients with emphysema have demonstrated significant reductions of this GAG [113]. One possible cause of the depletion of hyaluronic acid in human emphysema is the observation that exposure to oxidants and hydroxyl radicals present in tobacco smoke rapidly degrades hyaluronic acid to small-molecular-weight fragments and reduces its viscosity [114].

The depletion of hyaluronic acid in the emphysematous human lung could be a factor in the progression of pulmonary elastolysis and pulmonary emphysema with time. Exogenous replacement of depleted hyaluronic acid could be an indication for its use in clinical emphysema, along with a protective function against elastolysis from neutrophil and macrophage elastases through a barrier mechanism for elastic fibres [112]. It should be noted, however, that human emphysema is a complex disease in which elastic fibre degradation may be one of many factors that cause alveolar destruction [5].

Conclusion

GAGs play a significant role in many pathophysiological processes that occur in the ECM of the lung: they regulate hydration and water homeostasis; they maintain structure and function; they modulate the inflammatory response; and they influence tissue repair and remodelling.

Given the great diversity of GAG structures and the evidence that GAGs may have a protective effect against injury in various respiratory diseases, an understanding of changes in GAG expression that occur in disease may lead to opportunities to develop innovative and selective therapies in the future. From this perspective, the structure of the GAG molecule deserves further investigation into its possible therapeutic role in a variety of pulmonary diseases.

Competing interests

The authors declare that they have no competing interests.

Acknowledgements

The authors should like to express their gratitude to Mr André Benedito da Silva and Mr Sérgio Cezar da Silva Junior for their skilful technical assistance. This work was supported by The Centers of Excellence Program (PRONEX-MCT and PRONEX-FAPERJ), The Brazilian Council for Scientific and Technological Development (CNPq), and Carlos Chagas Filho Rio de Janeiro State Research Supporting Foundation (FAPERJ).

References

1. West JB, Mathieu-Costello O: **Structure, strength, failure, and remodeling of the pulmonary blood-gas barrier.** *Annu Rev Physiol* 1999, **61:**543-572.
2. Suki B, Ito S, Stamenovic D, Lutchen KR, Ingenito EP: **Biomechanics of the lung parenchyma: critical roles of collagen and mechanical forces.** *J Appl Physiol* 2005, **98:**1892-1899.
3. Rocco PR, Negri EM, Kurtz PM, Vasconcellos FP, Silva GH, Capelozzi VL, Romero PV, Zin WA: **Lung tissue mechanics and extracellular matrix remodeling in acute lung injury.** *Am J Respir Crit Care Med* 2001 **164:**1067-1071.
4. Negrini D, Passi A, De Luca G, Miserocchi G: **Matrix proteoglycans in development of pulmonary edema.** In *Proteoglycans in Lung Disease*. Edited by Garg HG, Roughley PJ, Hales CA. New York: Marcel Dekker; 2000:143-168.
5. Turino GM, Cantor JO: **Hyaluronan in respiratory injury and repair.** *Am J Respir Crit Care Med* 2003, **167:**1169-1175.
6. Cantor J, Turino GM: **Modulation of air-space enlargement in elastase-induced emphysema by intratracheal instilment of hyaluronidase and hyaluronic acid.** *Exp Lung Res* 1995, **21:** 423-436.
7. Bray BA: **The role of hyaluronan in the pulmonary alveolus.** *J Theor Biol* 2001, **210:**121-130.
8. Bai KJ, Spicer AP, Mascarenhas MM, Yu L, Ochoa CD, Garg HG, Quinn DA: **The role of hyaluronan synthase 3 in ventilator-induced lung injury.** *Am J Respir Crit Care Med* 2005, **172:**92-98.
9. Scott JE: **Supramolecular organization of extracellular matrix** glycosaminoglycans, ***in vitro*** **and in the tissues.** *FASEB J* 1992, **6:**2639-2645.
10. Johnson Z, Proudfoot AE, Handel TM: **Interaction of chemokines and glycosaminoglycans: a new twist in the regulation of chemokine function with opportunities for therapeutic intervention.** *Cytokine Growth Factor Rev* 2005, **16:**625-636.
11. Gerdin B, Hallgren R: **Dynamic role of hyaluronan (HYA) in connective tissue activation and inflammation.** *J Intern Med* 1997, **242:**49-55.
12. Tammi MI, Day AJ, Turley EA: **Hyaluronan and homeostasis: a balancing act.** *J Biol Chem* 2002, **277:**4581-4584.
13. Skold CM, Blaschke E, Eklund A: **Transient increases in albumin and hyaluronan in broncoalveolar lavage fluid after quitting smoking: possible signs of reparative mechanisms.** *Respir Med* 1996, **90:**523-529.
14. Li Y, Rahmanian M, Widstrom C, Lepperdinger G, Frost GI, Heldin P: **Irradiation induced expression of hyaluronan (HA) synthase 2 and hyaluronidase 2 genes in rat lung tissue accompanies active turnover of HA and induction of types I and III collagen gene expression.** *Am J Resp Cell Mol Biol* 2000, **23:**411-418.
15. Cantor JO, Shteyngart B, Cerreta JM, Liu M, Armand G, Turino GM: **The effect of hyaluronan on elastic fiber injury in vitro and elastase-induced airspace enlargement *in vivo.*** *Proc Soc Exp Biol Med* 2000, **225:**65-71.
16. Hardingham T, Fosang AJ: **Proteoglycans: many forms and many functions.** *FASEB J* 1992, **6:**861-870.
17. Roberts CR, Wight TN, Hascall VC: **Proteoglycans**. In *The Lung*, 2nd ed. Edited by Crystal RG, West JB, Weibel ER, Barnes PJ. Philadelphia, PA: Lippincott-Raven; 1997:757-767.
18. Handel TM, Johnson Z, Crown SE, Lau EK, Proudfoot AE: **Regulation of protein function by glycosaminoglycans - as exemplified by chemokines.** *Annu Rev Biochem* 2005, **74:**385-410.
19. Whitelock JM, Iozzo RV: **Heparan sulfate: a complex polymer charged with biological activity.** *Chem Rev* 2005, **105:**2745-2764.

20. Nader H B, Dietrich C P, Buonassisi V, and Colburn P: **Heparin sequences in the heparan sulfate chains of an endothelial cell proteoglycan.** *Proc Nat Acad Sci USA* 1987, **84:**3565-3569.
21. Poole AR: **Proteoglycans in health and disease: structures and functions.** *Biochem J* 1986, **236:**1-14.
22. Ruoss SJ, Gold WM, Caughey GH: **Mast cell exocytosis: evidence that granule proteoglycan processing is not coupled to degranulation.** *Biochem Biophys Res Commun* 1991, **179:**140-146.
23. Iozzo RV, Murdoch AD: **Proteoglycans of the extracellular environment. Clues from the gene and protein side offer novel perspective in molecular diversity and function.** *FASEB J* 1996, **10:**598-614.
24. Yurchenko PD, Schittny JC: **Molecular architecture of basement membrane.** *FASEB J* 1990, **4:**1577-1590.
25. Zhao J, Sime PJ, Bringas P Jr, Gauldie J, Warburton D: **Adenovirus-mediated decorin gene transfer prevents TGF-beta-induced inhibition of lung morphogenesis.** *Am J Physiol* 1999, **277:**L412-L422.
26. Tumova S, Woods A, Couchman JR: **Heparan sulphate proteoglycans on the cell surface: versatile coordinators of cellular functions.** *Int J Biochem Cell Biol* 2000, **32:**269-288.
27. Miserocchi G, Negrini D, Passi A, De Luca G: **Development of lung edema: interstitial fluid dynamics and molecular structure.** *News Physiol Sci* 2001, **16:**66-71.
28. Miserocchi G, Negrini D, Gonano C: **Parenchymal stress affects interstitial and pleural pressure in situ lung.** *J Appl Physiol* 1991, **71:**1967-1972.
29. Wiig H, Tenstad O: **Interstitial exclusion of positively and negatively charged IgG in rat skin and muscle.** *Am J Physiol* 2001, **280:**H1505-H1512.
30. Aukland K, Reed RK: **Interstitial-lymphatic mechanisms in the control of extracellular fluid volume.** *Physiol Rev* 1993, **73:**1-78.
31. Negrini D, Tenstad O, Wiig H: **Interstitial exclusion of albumin in rabbit during development of pulmonary edema.** *J Physiol* 2003, **3:**907-917.
32. Passi A, Negrini D, Albertini R, De Luca G, Miserocchi G: **Involvement of lung interstitial proteoglycans in development of hydraulic- and elastase-induced edema.** *Am J Physiol* 1998, **275:**L631-L635.
33. Negrini D, Passi A, De Luca G, Miserochi G: **Proteoglycan involvement during development of lesional pulmonary edema.** *Am J Physiol* 1998, **274:**L203-L211.
34. Negrini D, Tenstad O, Passi A, Wiig H: **Differential degradation of matrix proteoglycans and edema development in rabbit lung.** *Am J Physiol Lung Cell Mol Physiol* 2006, **290:**L470-L477.
35. Farias LL, Faffe DS, Xisto DG, Santana MC, Lassance R, Prota LF, Amato MB, Morales MM, Zin WA, Rocco PR: **Positive end-expiratory pressure prevents lung mechanical stress caused by recruitment/derecruitment.** *J Appl Physiol* 2005, **98:**53-61.
36. Iozzo RV: **Matrix proteoglycans: from molecular design to cellular function.** *Annu Rev Bochem* 1998, **67:**609-652.
37. Mijailovich SM, Stamenovic D, Brown R, Leith DE, Fredberg JJ: **Dynamic moduli of rabbit lung tissue and pigeon ligamentum propatagiale undergoing uniaxial cyclic loading.** *J Appl Physiol* 1994, **76:**773-782.
38. Al Jamal R, Roughley PJ, Ludwig MS: **Effect of glycosaminoglycan degradation on lung tissue viscoelasticity.** *Am J Physiol Lung Cell Mol Physiol* 2001, **280:**306-315.
39. Berg JT, Fu Z, Breen EC, Tran HC, Mathieu-Costello O, West JB: **High lung inflation increases mRNA levels of ECM components and growth factors in lung parenchyma.** *J Appl Physiol* 1997, **83:**120-128.
40. Parker JC, Breen EC, West JB: **High vascular and airway pressures increases interstitial protein mRNA expression in isolated rat lungs.** *J Appl Physiol* 1997, **83:**1697-1705.
41. Moriondo A, Mukenge S, Negrini D: **Transmural pressure in rat initial subpleural lymphatic during spontaneous or mechanical ventilation.** *Am J Physiol Heart Circ Physiol* 2005, **289:**H263-H269.
42. Garcia CSN, Rocco PR, Facchinetti LD, Lassance RM, Caruso P, Deheinzelind D, Morales MM, Romero PV, Faffe DS, Zin WA: **What increases type III procollagen mRNA levels in lung tissue: stress induced by changes in force or amplitude?** *Respir Physiol Neurobiol* 2004, **144:**59-70.
43. Raman R, Sasisekharan V, Sasisekharan R: **Structural insights into biological roles of protein-glycosaminoglycan interactions.** *Chem Biol* 2005, **12:**267-277.
44. Aviezer D, Safran M, Yayon A: **Heparin differentially regulates the interaction of fibroblast growth factor-4 with FGF receptors 1 and 2.** *Biochem Biophys Res Commun* 1999, **263:**621-626.
45. Shriver Z, Liu D, Sasisekharan R: **Emerging views of heparan sulfate glycosaminoglycan structure/activity relationships modulating dynamic biological functions.** *Trends Cardiovasc Med* 2002, **12:**71-77.
46. Rubin JS, Day RM, Breckenridge D, Atabey N, Taylor WG, Stahl SJ, Wingfield PT, Kaufman JD, Schwall R, Bottaro DP: **Dissociation of heparan sulfate and receptor binding domains of hepatocyte growth factor reveals that heparan sulfate-c-met interaction facilitates signaling.** *J Biol Chem* 2001, **276:**32977-32983.
47. Raines EW, Ross R: **Compartmentalization of PDGF on extracellular binding sites dependent on exon-6-encoded sequences.** *J Cell Biol* 1992, **116:**533-543.
48. Ruhrberg C, Gerhardt H, Golding M, Watson R, Ioannidou S, Fujisawa H, Betsholtz C, Shima DT: **Spatially restricted patterning cues provided by heparin-binding VEGF-A control blood vessel branching morphogenesis.** *Genes Dev* 2002, **16:**2684-2698.
49. Lipscombe RJ, Nakhoul AM, Sanderson CJ, Coombe DR: **Interleukin-5 binds to heparin/heparan sulfate. A model for an interaction with extracellular matrix.** *J Leukoc Biol* 1998, **63:**342-350.
50. Mummery RS, Rider CC: **Characterization of the heparin-binding properties of IL-6.** *J Immunol* 2000, **165:**5671-5679.
51. Menart V, Fonda I, Kenig M, Porekar VG: **Increased in vitro cytotoxicity of TNF-alpha analog LK-805 is based on the interaction with cell surface heparan sulfate proteoglycan.** *Ann N Y Acad Sci* 2002, **973:**194-206.
52. Kreuger J, Matsumoto T, Vanwildemeersch M, Sasaki T, Timpl R, Claesson-Welsh L, Spillmann D, Lindahl U: **Role of heparan sulfate domain organization in endostatin inhibition of endothelial cell function.** *EMBO J* 2002, **21:**6303-6311.
53. Parthasarathy N, Gotow LF, Bottoms JD, Kute TE, Wagner WD, Mulloy B: **Oligosaccharide sequence of human breast cancer cell heparan sulfate with high affinity for laminin.** *J Biol Chem* 1998, **273:**21111-21114.
54. LeBaron RG, Hook A, Esko JD, Gay S, Hook M: **Binding of heparan sulfate to type V collagen. A mechanism of cell-substrate adhesion.** *J Biol Chem* 1989, **264:**7950-7956.
55. Takagaki K, Munakata H, Kakizaki I, Iwafune M, Itabashi T, Endo M: **Domain structure of chondroitin sulfate E octasaccharides binding to type V collagen.** *J Biol Chem* 2002, **277:**8882-8889.
56. Kuschert GS, Coulin F, Power CA, Proudfoot AE, Hubbard RE, Hoogewerf AJ, Wells TN: **Glycosaminoglycans interact selectively with chemokines and modulate receptor binding and cellular responses.** *Biochemistry* 1999, **38:**12959-12968.
57. Page CP: **Proteoglycans: the 'Teflon' of the airways?** *Thorax* 1997, **52:**924-925.
58. Tyrell DJ, Kilfeather S, Page CP: **Therapeutic uses of heparin beyond its traditional role as an anticoagulant.** *Trends Pharmacol Sci* 1995, **16:**198-204.
59. Green WF, Konnaris K, Woolcock AJ: **Effect of salbutamol, fenoterol, and sodium cromoglycate on the release of heparin from sensitized human lung fragments challenged with *Dermatophagoides pteronyssinus* allergen.** *Am J Respir Cell Mol Biol* 1993, **8:**518-521.
60. Ahmed T, Garrigo J, Danta I: **Preventing bronchoconstriction in exercise-induced asthma with inhaled heparin.** *N Engl J Med* 1993, **329:**90-95.
61. Malik AB, van der Zee H: **Time course of pulmonary vascular response to microembolization.** *J Appl Physiol* 1977, **43:**51-58.
62. Binder AS, Nakahara K, Ohkuda K, Kageler W, Staub NC: **Effect of heparin or fibrinogen depletion on lung fluid balance in sheep after emboli.** *J Appl Physiol* 1979, **47:**213-219.
63. Cox CS Jr, Zwischenberger JB, Traber DL, Traber LD, Haque AK, Herndon DN: **Heparin improves oxygenation and minimizes barotrauma after severe smoke inhalation in an ovine model.** *Surg Gynecol Obstet* 1993, **176:**339-349.
64. Laterre PF, Wittebole X, Dhainaut JF: **Anticoagulant therapy in acute lung injury.** *Crit Care Med* 2003, **Suppl:**S329-S336.

65. Abubakar K, Schmidt B, Monkman S, Webber C, deSA D, Roberts R: **Heparin improves gas exchange during experimental acute lung injury in newborn piglets.** *Am J Respir Crit Care Med* 1998, **158:**1620-1625.
66. Uchiba M, Okajima K, Murakami K, Okabe H, Takatsuki K: **Attenuation of endotoxin-induced pulmonary vascular injury by antithrombin III.** *Am J Physiol* 1996, **270:**L921-L930.
67. Tasaki O, Mozingo DW, Dubick MA, Goodwin CW, Yantis LD, Pruitt BA Jr: **Effects of heparin and lisofylline on pulmonary function after smoke inhalation injury in an ovine model.** *Crit Care Med* 2002, **30:**637-643.
68. Tian Y, Gebitekin C, Martin P, Satur CM, Mearns A, Walker DR: **Influence of heparin thromboprophylaxis on plasma leucocyte elastase levels following lobectomy for lung carcinoma.** *Blood Coagul Fibrinolysis* 1995, **6:**527-530.
69. Lu KW, Goerke J, Clements JA, Taeusch HW: **Hyaluronan decreases surfactant inactivation in vitro.** *Pediatr Res* 2005, **57:**237-241.
70. Laurent TC, Fraser JR: **Hyaluronan.** *FASEB J* 1992, **6:**2397-2404.
71. Suzuki M, Asplund T, Yamashita H, Heldin CH, Heldin P: **Stimulation of hyaluronan biosynthesis by platelet-derived growth factor-BB and transforming growth factor-beta 1 involves activation of protein kinase C.** *Biochem J* 1995, **307:**817-821.
72. Toole BP: **Hyaluronan and its binding proteins, the hyaladherins.** *Curr Opin Cell Biol* 1990, **2:**839-844.
73. LeBaron RG, Zimmermann DR, Ruoslahti E: **Hyaluronate binding properties of versican.** *J Biol Chem* 1992, **267:**10003-10010.
74. Lacy BE, Underhill CB: **The hyaluronate receptor is associated with actin filaments.** *J Cell Biol* 1987, **105:**1395-1404.
75. Uchiyama H, Dobashi Y, Ohkouchi K, Nagasawa K: **Chemical change involved in the oxidative reductive depolymerization of hyaluronic acid.** *J Biol Chem* 1990, **265:**7753-7759.
76. McKee CM, Penno MB, Cowman M, Burdick MD, Strieter RM, Bao C, Noble PW: **Hyaluronan (HA) fragments induce chemokine gene expression in alveolar macrophages. The role of HA size and CD44.** *J Clin Invest* 1996, **98:**2403-2413.
77. Ohkawara Y, Tamura G, Iwasaki T, Tanaka A, Kikuchi T, Shirato K: **Activation and transforming growth factor-beta production in eosinophils by hyaluronan.** *Am J Respir Cell Mol Biol* 2000, **23:**444-451.
78. Horton MR, Boodoo S, Powell JD: **NF-kappa B activation mediates the cross-talk between extracellular matrix and interferon-gamma (IFN-gamma) leading to enhanced monokine induced by IFN-gamma (MIG) expression in macrophages.** *J Biol Chem* 2002, **277:**43757-43762.
79. Mascarenhas MM, Day RM, Ochoa CD, Choi WI, Yu L, Ouyang B, Garg HG, Hales CA, Quinn DA: **Low molecular weight hyaluronan from stretched lung enhances interleukin-8 expression.** *Am J Respir Cell Mol Biol* 2004, **30:**51-60.
80. Zeerleder S, Hack CE, Wuillemin WA: **Disseminated intravascular coagulation in sepsis.** *Chest* 2005, **128:**2864-2875.
81. Bernard GR, Vincent JL, Laterre PF, LaRosa SP, Dhainaut JF, Lopez-Rodrigues A, Steigrub JS, Garber GE, Helterbrand D, Ely EW, for the Recombinant human protein C Worldwide Evaluation in Severe Sepsis (PROWESS) study group: **Efficacy and safety of recombinant human activated protein C for severe sepsis.** *N Engl J Med* 2001, **344:**699-709.
82. Warren BL, Eid A, Singer P, Pillay SS, Carl P, Novak I, Chalupa P, Atherstone A, Penzes I, Kubler A, *et al.*; KyberSept Trial Study Group: **Caring for the critically ill patient. High-dose antithrombin III in severe sepsis: a randomized controlled trial.** *JAMA* 2001, **286:**1869-1878.
83. O'Brien JM Jr, Abraham E: **New approaches to the treatment of sepsis.** *Clin Chest Med* 2003, **24:**521-548.
84. Wiedermann CJ, Hoffmann JN, Juers M, Ostermann H, Kienast J, Briegel J, Strauss R, Keinecke HO, Warren BL, Opal SM: **High-dose antithrombin III in the treatment of severe sepsis in patients with a high risk of death: efficacy and safety.** *Crit Care Med* 2006, **34:**285-292.
85. LaRosa SP, Opal SM: **Tissue factor pathway inhibitor and antithrombin trial results.** *Crit Care Clin* 2005, **21:**433-448.
86. Beck GCh, Hermes WC, Yard BA, Kaszkin M, von Zabern D, Schulte J, Haak M, Prem K, Krimsky W, van Ackern K, *et al.*: **Amelioration of endotoxin-induced sepsis in rats by membrane anchored lipid conjugates.** *Crit Care Med* 2003, **31:**2015-2021.
87. Kunz LI, Rensen EL, Sterk PJ: **Inhaled hyaluronic acid against exercise-induced bronchoconstriction in asthma.** *Pulm Pharmacol Ther* 2006, **19:**286-291.
88. Lu KW, Goerke J, Clements JA, Taeusch HW: **Hyaluronan reduces surfactant inhibition and improves rat lung function after meconium injury.** *Pediatr Res* 2005, **58:**206-210.
89. Venge P, Pedersen B, Hakansson L, Hallgren R, Lindblad G, Dahl R: **Subcutaneous administration of hyaluronan reduces the number of infectious exacerbations in patients with chronic bronchitis.** *Am J Respir Crit Care Med* 1996, **153:**312-316.
90. Cywes C, Stamenkovic I, Wessels MR: **CD44 as a receptor for colonization of the pharynx by group A streptococcus.** *J Clin Invest* 2000, **106:**995-1002.
91. Nettelbladt O, Bergh J, Schenholm M, Tengblad A, Hallgren R: **Accumulation of hyaluronic acid in the alveolar interstitial tissue in bleomycin-induced alveolitis.** *Am Rev Respir Dis* 1989, **139:**759-762.
92. Nettelbladt O, Tengblad A, Hallgren R: **Lung accumulation of hyaluronan parallels pulmonary edema in experimental alveolitis.** *Am J Physiol* 1989, **257:**L379-L384.
93. Hallgren R, Samuelsson T, Laurent TC, Modig J: **Accumulation of hyaluronan (hyaluronic acid) in the lung in adult respiratory distress syndrome.** *Am Rev Respir Dis* 1989, **139:**682-687.
94. Teder P, Vandivier RW, Jiang D, Liang J, Cohn L, Pure E, Henson PM, Noble PW: **Resolution of lung inflammation by CD44.** *Science* 2002, **296:**155-158.
95. Henke CA, Roongta U, Mickelson DJ, Knutson JR, McCarthy JB: **CD44-related chondroitin sulfate proteoglycan, a cell surface receptor implicated with tumor cell invasion, mediates endothelial cell migration on fibrinogen and invasion into a fibrin matrix.** *J Clin Invest* 1996, **97:**2541-2552.
96. Clark RAF, Lin F, Greiling D, An J, Couchman JR: **Fibroblast invasive migration into fibronectin/fibrin gels requires a previously uncharacterized dermatan.** *J Invest Dermatol* 2004, **122:**266-277.
97. Jiang D, Liang J, Li Y, Noble PW: **The role of Toll-like receptors in non-infectious lung injury.** *Cell Res* 2006, **16:**693-701.
98. Jiang D, Liang J, Fan J, Yu S, Chen S, Luo Y, Prestwich GD, Mascarenhas MM, Garg HG, Quinn DA, *et al.*: **Regulation of lung injury and repair by Toll-like receptors and hyaluronan.** *Nat Med* 2005, **11:**1173-1179.
99. Termeer C, Benedix F, Sleeman J, Fieber C, Voith U, Ahrens T, Miyake K, Freudenberg M, Galanos C: **Oligosaccharides of hyaluronan activate dendritic cells via Toll-like receptor 4.** *J Exp Med* 2002, **195:**99-111.
100. Suzuki K, Gabazza EC, Hayashi T, Kamada H, Adachi Y, Taguchi O: **Protective role of activated protein C in lung and airway remodeling.** *Crit Care Med* 2004, **32:**S262-S265.
101. Scuri M, Abraham WM, Botvinnikova Y, Forteza R: **Hyaluronic acid blocks porcine pancreatic elastase (PPE)-induced bronchoconstriction in sheep.** *Am J Respir Crit Care Med* 2001, **164:**1855-1859.
102. Forteza R, Lauredo I, Abraham WM, Conner GE: **Bronchial tissue kallikrein activity is regulated by hyaluronic acid binding.** *Am J Respir Cell Mol Biol* 1999, **21:**666-674.
103. Petrigni G, Allegra L: **Aerosolised hyaluronic acid prevents exercise-induced bronchoconstriction, suggesting novel hypotheses on the correction of matrix defects in asthma.** *Pulm Pharmacol Ther* 2006, **19:**166-171.
104. Kunz LI, Rensen EL, Sterk PJ: **Inhaled hyaluronic acid against exercise-induced bronchoconstriction in asthma.** *Pulm Pharmacol Ther* 2006, **19:**286-291.
105. Prussin C, Metcalfe DD: **IgE, mast cells, basophils, and eosinophils.** *J Allergy Clin Immunol* 2006, **Suppl Mini-Primer:**S450-S456.
106. Ammit AJ: **The role of mRNA stability in airway remodelling.** *Pulm Pharmacol Ther* 2005, **18:**405-415.
107. Slade DJ, Kraft M: **Airway remodeling from bench to bedside: current perspectives.** *Clin Chest Med* 2006, **27:**71-85.
108. Holgate ST: **Epithelial damage and response.** *Clin Exp Allergy* 2000, **30:**37-41.
109. Leir SH, Baker JE, Holgate ST, Lackie PM: **Increased CD44 expression in human bronchial epithelial repair after damage or plating at low cell densities.** *Am J Physiol Lung Cell Mol Physiol* 2000, **278:**L1129-L1137.
110. Leir SH, Holgate ST, Lackie PM: **Inflammatory cytokines can enhance CD44-mediated airway epithelial cell adhesion inde-**

pendently of CD44 expression. *Am J Physiol Lung Cell Mol Physiol* 2003, **285:**L1305-L1311.
111. Cantor JO, Cerreta JM, Armand G, Keller S, Turino GM: **Pulmonary air-space enlargement induced by intratracheal instillment of hyaluronidase and concomitant exposure to 60% oxygen.** *Exp Lung Res* 1993, **19:**177-192.
112. Cantor JO, Cerreta JM, Armand G, Turino GM: **Aerosolized hyaluronic acid decreases alveolar injury induced by human neutrophil elastase.** *Proc Soc Exp Biol Med* 1998, **217:**471-475.
113. Konno K, Arai H, Motomiya M, Nagai H, Ito M, Sato H, Satoh K: **A biochemical study on glycosaminoglycans (mucopolysaccharides) in emphysematous and in aged lungs.** *Am Rev Respir Dis* 1982, **126:**797-801.
114. McDevitt CA, Beck GJ, Ciunga MJ, O'Brien J: **Cigarette smoke degrades hyaluronic acid.** *Lung* 1989, **167:**237-245.
115. Monzon ME, Casalino-Matsuda SM, Forteza RM: **Identification of glycosaminoglycans in human airway secretions.** *Am J Respir Cell Mol Biol* 2006, **34:**135-141.
116. de Medeiros Matsushita M, da Silva LF, dos Santos MA, Fernezlian S, Schrumpf JA, Roughley P, Hiemstra PS, Saldiva PH, Mauad T, Dolhnikoff M: **Airway proteoglycans are differentially altered in fatal asthma.** *J Pathol* 2005, **207:**102-110.