Pulmonary Perspective

Hyaluronan in Respiratory Injury and Repair

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Over the past two decades, much of the research on the cellular and biochemical reactions to injurious agents of the lung has focused on specific cells and cytokines and their appearance or disappearance in the alveolar milieu. Little emphasis has been given to the connective tissue matrix itself, in which specific responses take place. Early on, the underlying premise was that the matrix substances such as glycosaminoglycans and the fibrous structure of the lung related to collagen and elastin are functionally relatively unresponsive and structurally fixed. This is far from true, and a primary purpose of this "perspective" is to describe the dynamic nature of the constituents of the interstitial space.

The pulmonary matrix, with its cellular constituents and fluctuating cytokines, should more realistically be considered as an integrated, interactive unit rather than separate unrelated entities. Also, it is becoming more apparent that the integrity and balance of matrix components themselves, which undergo degradation and reconstruction, are essential for normal lung function and the response to injury (1).

In recent years, new insights have been gained into the functional role of matrix components per se, modulating injury to the pulmonary structure and altering function. One of the components that has received increasing study in the last decades is hyaluronic acid (HA) (2). Since the discovery of HA in 1934 by Dr. Karl Meyer (3), voluminous literature has evolved from both basic and clinical studies. For the purposes of this review, HA will be considered from five aspects: (*1*) the chemical characteristics of HA, (*2*) HA in embryogenesis and response to injury, (*3*) binding proteins and receptors, (*4*) molecular size and the effect on biologic function, and (*5*) HA in respiratory disease. This perspective, it is hoped, will indicate the extraordinary versatility of biologic reactions of this molecule, including its receptors and the evolving applicability of these reactions to diseases of the respiratory system.

CHEMICAL CHARACTERISTICS OF HYALURONAN

Hyaluronan is used as a generic name for any hyaluronate in which a specific salt is not indicated. This nomenclature was more formally developed in the 1980s by Dr. A. Balazs (4). Strictly speaking, the HA used in medical products is this in salt form, and many people now use "hyaluronan" instead of hyaluronic acid as the terminology. HA is a naturally occurring, linear polysaccharide with repeating disaccharide units composed of glucuronic acid and N-acetyl glucosamine. Both monosaccharides have the β-D-anomeric configuration of C-1. The linkage from the glucuronic acid to N-acetyl glucosamine is (1 → 3), and the linkage from N-acetyl glucosamine to glucuronic acid is (1 → 4) (Figure 1).

HA belongs to a family of structurally similar polysaccharides called glycosaminoglycans, which includes hyaluronan, chondroitin sulfate, dermatan sulfate, keratan sulfate, heparan sulfate, and heparin. These polysaccharides all contain repeating disaccharide units that are characterized by 1-amino sugar and at least one negatively charged group of carboxylate or sulfate. Hyaluronan differs from the other glycosaminoglycans in that it does not contain sulfur.

A unique characteristic of HA, which relates to its variable functions, is its hydration. HA exists in solution in a flexible, coiled configuration (5–6), which is well hydrated and contains approximately 1000-fold more water than polymer (6). Also, the molecular weight of HA varies in specific tissues. Normal synovial fluid has HA of an average of 7,000 kD (7). In cartilage and lung, it has 2,000 (8) and 220 kD (9), respectively.

The concentration of HA in a tissue can determine its water content. This relationship has been demonstrated for normal animal lungs, where reductions in lung HA induced by hyaluronidase have been directly correlated with reductions in the extravascular lung water volume (10). This correlation has also been demonstrated in experimental alveolitis (9).

HYALURONAN IN EMBRYOGENESIS AND RESPONSE TO INJURY

Studies of HA content of rabbit lungs in the perinatal period indicate the highest content in the youngest fetuses (11). The HA content decreases just before birth and then increases again in the days after birth. Before term, it is likely that HA facilitates morphogenesis, but in the neonatal period, HA's major role is most likely regulation of fluid balance in the interstitium through its high water-binding capacity (11). This high fetal content of HA has also been noted by Schmid and colleagues (12) in human and bovine lungs. The decrease in HA content just before birth may be a necessary adjustment to reduce the water content of the lung and facilitate ventilation and gas exchange. Failure of this adjustment may complicate ventilatory function of the newborn. In the normal human adult lung, the total HA content is approximately 160 mg (12).

Recent studies of HA in embryonic development of the heart (13) have demonstrated a crucial role for HA synthesized by specific HA synthases for the earliest development of the cushion of the heart, which then gives rise to the valvular structures.

A remarkable characteristic of HA is the rapidity of the changes in content and concentration after varying types of injury.

An early study in newts (14) established a pattern of change

(*Received in original form May 21, 2002; accepted in final form February 6, 2003*)

Supported by funds from the James P. Mara Center for Lung Disease, the Charles A. Mastronardi Foundation, the Ned Doyle Foundation, the Franklyn Bracken Fund, and the Alpha One Foundation.

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Am J Respir Crit Care Med Vol 167. pp 1169–1175, 2003
DOI: 10.1164/rccm.200205-449PP
Internet address: www.atsjournals.org

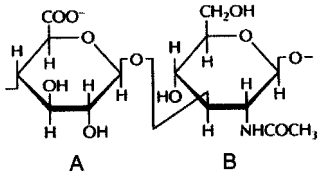

Figure 1. Hyaluronan is composed of repeating disaccharide units containing glucuronic acid (*A*) and *n*-acetylglucosamine (*B*).

in HA associated with amputation and regrowth of a forelimb. The increase and decrease in the concentration of HA in the limb stump before regrowth appears with great rapidity and sets the stage for reorientation and differentiation of cells to allow regrowth of the forelimb.

HA functions as a major matrix substance in which cells and fibrous constituents of the matrix such as elastin and collagen are embedded. Specifically, HA has been recognized to be structurally an integral part of the elastin fiber (15) and the microfibrils of collagen (16). It also is a coating substance for cells in the matrix. HA increases quickly and markedly in response to physical injuries such as wounding or tissue expurgation (17–18), as well as cytokine injury by endotoxin (19). The latter responses may be related to the ability of HA to stimulate and facilitate cellular movement within the matrix.

HYALURONAN ADHERING MOLECULES

Tissue Hyaldherins

To function in tissue modeling during development, as well as in normal tissue homeostasis and remodeling in disease, HA interacts with a number of HA-binding proteins. These binding proteins have been grouped together as suggested by Toole and are called hyaldherins (20). Their occurrence in tissues is widespread, which can be taken as an indication of their importance in tissue organization and cellular behavior. The hyaldherin molecules include structural matrix HA-binding proteins as well as cell-surface HA receptors that bind with high affinity to HA. In different tissues, HA exists as bound complexes with other macromolecules (21). The hyaldherins in cartilage have been well characterized (21). The aggregating chondroitin sulfate proteoglycan of cartilage has a high affinity for HA and has been termed aggrecan. Also, link protein, a glycoprotein that has strong affinity binding with HA, is noncovalently bound to both HA and aggrecan within cartilage to form a large molecular complex with over 100 aggrecan and link protein molecules bound to a single filament of HA (21).

In noncartilaginous tissue, HA-binding proteins exist that form structural complexes with HA. Examples are versican, a proteoglycan synthesized by fibroblasts (22), hyaluronectin, a proteoglycan widely distributed in nervous tissue and soft connective tissue (23), glial-HA binding protein in the central nervous system (24), and neurocan, a chondroitin sulfate in brain (25). In noncartilaginous tissues, link proteins have also been isolated that may stabilize the adherence of neurocan or versican with HA (25). In tissues such as cartilage, HA may function to retain and organize proteoglycans within the extracellular matrix to provide load-bearing functions. In other tissues, these binding proteins may modulate HA functions. Thus, it has been demonstrated that human fibrinogen specifically binds to HA, which would facilitate wound healing and tissue repair (26).

Also, HA binding has been demonstrated for fibronectin, laminens, and collagen (27–28), which suggests that through such binding, HA may be able to influence extracellular matrix structure as well as cell membranes.

Cell Surface Hyaldherins (Receptors)

Many cell surface receptors for HA have been detected on a variety of cells and a variety of tissues (20, 29). Among these, the CD-44 family has been better characterized. The first such receptor described was the 85-kD protein present on the surface of SV-40–transformed 3T3 cells, which are also called baby hamster kidney cells (30). Antibody studies later identified the 85-kD receptor as CD-44. Multiple isoforms of CD-44 have been identified, indicating that different forms of CD-44 mediate different cellular functions (20, 31–32). However, other cell surface HA receptors have been identified that do not belong to the CD-44 family (33–34).

Turley and colleagues have characterized HA receptors that mediate HA-induced cell locomotion (33–36). The receptor protein is part of an HA-receptor complex that occurs on the cell surface of fibroblasts and has varying molecular weights ranging from 52 to 72 kD. The 58 kD protein of HA-receptor complex has an HA-binding component that has been termed RHAMM (receptor for HA-mediated motility). RHAMM is structurally different from CD-44 and is not recognized by anti–CD-44 antibodies.

Underhill and Lacey and Underhill (29–30, 37) have demonstrated a chemically significant relationship between a receptor for HA and the cytoskeleton of cells through actin filaments. Several lines of evidence in their work showed that the receptor for HA is directly or indirectly associated with cytosolic actin filaments. This association between actin and the HA receptors suggests that there is a transmembrane interaction between HA on the outside of the cell and actin filaments on the inside. Such a structural relationship could explain the effect of HA on such cellular activities as migration and phagocytosis.

Banerjee and Toole (38) have recognized another cell surface receptor by using a monoclonal antibody that they have termed IVd4. The relationship between the IVd4 group of hyaldherins receptors and CD-44 remains to be defined. However, when detected in cell culture, these receptors are usually in an occupied state, which may be evidence of their functional importance.

Another family of HA receptors, not related to CD-44, is involved in the clearance of HA from the circulation and is primarily located on the endothelium of liver sinusoids (39–40). These receptors have been designated as liver endothelial receptors. The temporal and spatial patterning of HA during embryogenesis tissue remodeling and tumor invasion indicates that the sequence of synthesis and the removal of HA are important regulatory factors in tissue reconstruction conducted by hyaldherins. In this regard, an HA receptor on the endothelium of lymph vessels has been identified (41) (lymphatic vessel endothelial-1 hyaluronan receptor), which is involved in HA degradation.

A model of pericellular matrix assembly has been proposed by Knudson and Knudson (42) that includes three components: a cell-surface hyaldherin receptor, HA, and a matrix hyaldherin. The specific matrix hyaldherin required may vary depending on the tissue being assembled. Examples of other hyaldherins are decorin (43), lumican (44), laminin (27), versican (22), as well as fibronectin (27–28) and fibrinogen (26).

Day and Prestwich provide a more complete discussion of recently identified cellular and extracellular hyaldherin molecules, including their chemically active domains (45).

Molecular Size and Biological Activity of HA

In several studies, Noble and coworkers (46–48) have demonstrated that low molecular weight fragments of HA can stimulate activated mouse alveolar macrophages to express RNAs of numerous chemokines and cytokines, including production of metalloelastase (49). High molecular weight fragments of HA had an opposite effect and suppressed such chemokine expression.

Small molecular size HA fragments 4–25 disaccharides units (approximately 1,600 to 10,000 Da) produced by the action of testicular hyaluronidase have been shown to induce angiogenesis in chick chorioallantoic membrane (50). Larger HA fragments outside that range were not angiogenic. It is hypothesized that stimulation of angiogenesis may result from the ability of such lower molecular weight fragments to loosen or disorganize the cell/cell or cell/matrix interactions to facilitate angiogenesis (50).

A recent report indicates a role for small molecular weight fragments of HA to enhance interferon-γ and monokine induced by interferon-γ via transcription of nuclear factor-κB. Thus, small molecular weight fragments of HA would serve to modulate macrophage functions through nuclear factor-κB signaling synergistically with interferon-γ (51).

Thus, several studies have demonstrated that the biologic effects of HA appear to vary depending on the average molecular mass. Ohkawara and colleagues (52) have demonstrated that HA of an average mass of 0.2×10^6 Da increases survival of peripheral blood eosinophils *in vitro*. They also demonstrated that higher molecular weight molecules (3.6×10^6 Da) were much less effective. The suggested mechanism is an increased expression of granulocyte macrophage colony stimulating factor, which can augment eosinophil survival *in vitro*.

No clear explanation exists for these differing biologic results of studies with high molecular weight HA as compared with HA of lower molecular mass. Central to an explanation may be the interaction of HA of differing size with its receptor, as suggested by Camenisch and McDonald (53). Also, as they pointed out, different HA preparations, the suppliers of which produce HA by different methods from different sources such as rooster comb, human umbilical cord, and Gram-positive streptococci, could have contaminants in the nanogram range, which could induce biologic effects apart from HA. In this regard, Filion and Phillips (54) tested low molecular weight fractions of HA obtained from seven different HA preparations for their ability to induce synthesis of interleukin-12 and tumor necrosis factor-α by human monocytic cells. They found that only two of the seven HA preparations tested stimulated the synthesis of interleukin-12 and tumor necrosis factor-α by human monocytic cells. They also found that the induction of these cytokines by HA preparations is due to the presence of contaminating DNA and not the low molecular weight fragments. Treatment of these two preparations with DNAse abrogated or reduced the production of interleukin-12 and tumor necrosis factor-α (54).

Also, *in vitro* experiments using isolated cell systems may not predict which HA effects may or may not be observed in more complex *in vivo* systems in the presence of other glycosaminoglycans, cell constituents, and their receptors. It is noteworthy that in hamster lungs, aerosols of HA in the average range of 150 kD have not induced inflammatory or abnormal histologic reactions (55). HA of higher molecular weights have also been administered to human subjects with no adverse effects observed (56–59). However, in recognition of proinflammatory transcriptional mechanisms and macrophage responses, the use of small molecular weight fragments of HA for therapies should be avoided.

THE ROLE OF HA IN RESPIRATORY DISEASE

HA in Experimental and Human Emphysema

In a series of studies, Cantor and associates (55, 60–63) have demonstrated that HA mitigates the action of elastases such as porcine pancreatic elastase, as well as human neutrophil elastase and human macrophage metalloelastase. This action has been demonstrated *in vivo* in models of pulmonary emphysema in hamsters, as well as in elastin matrices produced by pleural mesothelial cell culture (64). Interestingly, air space enlargement induced by intratracheal elastase is augmented by prior depletion of lung HA by intratracheally administered hyaluronidase (60–61). This effect of hyaluronidase has been duplicated by other investigators in a similar study in rats (65).

HA aerosol administered to hamsters with elastase-induced emphysema has been shown to significantly reduce the severity of the emphysema, as measured by alveolar mean linear intercept (62–63).

More recent insights into the mechanisms that produce emphysematous destruction of the lung in humans implicate a chronic inflammatory state in the lung parenchyma from an influx of neutrophils and macrophages with their associated elastases (66). In this regard, it is noteworthy that Akatsuka and colleagues (67) demonstrated a suppressive effect of HA on elastase release from rat peritoneal leucocytes. These investigators reported that HA indiscriminately inhibited leukocyte elastase release whether induced by opsonized zymosan, *N*-formyl-methionyl-leucyl-phenylalimine, cytochalasin B, or phorbol acetate.

Also, in an endotoxin induced peritonitis model in rats, the addition of high molecular weight HA to the peritoneal dialysate lowered levels of neutrophil elastase and increased levels of interferon-γ in the dialysate, along with a reduced loss of ultrafiltration (68).

An agent such as HA could protect against elastin injury, which might 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 HA content in the lungs of patients with emphysema have demonstrated significant reductions in HA (69). In this same study, other glycosaminoglycan components of the lung matrix such as chondroitin sulfate, heparan sulfate, dermatan sulfate, and heparin were not reduced in amount. One possible cause of the depletion of HA in human emphysema is the observation that exposure to the oxidants and hydroxyl radicals in tobacco smoke rapidly degrades HA to small molecular weight fragments and reduces its viscosity (70). Also, guinea pigs exposed to tobacco smoke injury have reduced levels of lung HA (71).

The depletion of HA in the emphysematous human lung could be a factor in the progression of pulmonary elastolysis and pulmonary emphysema with time. Exogenous replacement of depleted HA 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 fibers (55). It should be noted, however, that human emphysema is a complex disease in which elastic fiber degradation may be one of many factors causing alveolar destruction.

With respect to the protective effect of HA against elastase injury to elastin, it has been demonstrated that HA *in vitro* does not chemically inhibit neutrophil or pancreatic elastase activity (55). However, it should be noted that another glycosaminoglycan, Arteparan, a supersulphated chondroitin sulfate, reported by Rao and colleagues (72), has been demonstrated to inhibit elastase. This form of chondroitin sulfate, administered intratracheally, also was effective in reducing the severity of elastase-produced emphysema in a hamster model.

The present hypothesis for the protective effect of HA against elastic fiber degradation is a coating and barrier function of HA against elastases. Visualization of elastin and fluoreceinated HA *in vivo* in hamster lungs by light microscopy, as well as *in vitro*, in a mesothelial cell elastic fiber matrix demonstrates their close

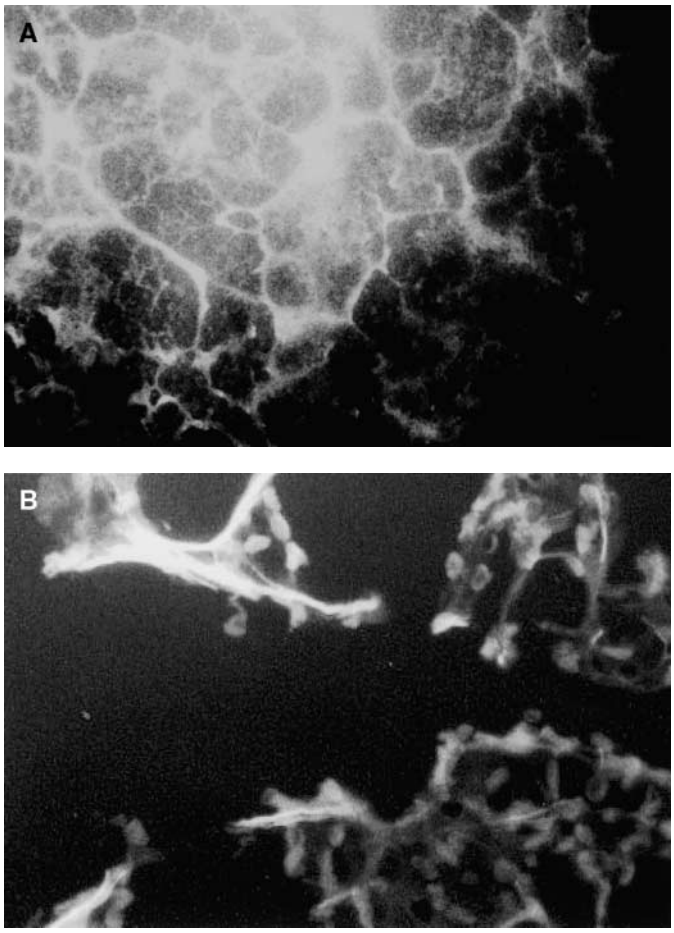

Figure 2. (*A*) Fluorescein-labeled HA is shown bound to elastin fibers in an elastin-rich cell-free matrix produced by rat pleural mesothelial cells (1,000×). (*B*) Aerosolized fluorescein-labeled HA in hamster lung shown binding to the pulmonary matrix in the linear configuration of elastin-containing alveolar walls (800×).

association (Figure 2). The precise mechanism of adherence of HA to elastin, which may involve specific receptors, remains to be determined.

Lysozyme and Pulmonary Emphysema

In consideration of other matrix components that may be part of the pathogenic process, producing pulmonary parenchymal destruction in emphysema is the role of lysozyme. It has been demonstrated that lysozyme is increased in human pulmonary emphysema and preferentially binds to elastic fibers in the pulmonary parenchyma (73). It is noteworthy that increased concentrations of lysozyme in the pulmonary parenchyma of patients with emphysema appear to be a finding specific for emphysema and have not been demonstrated in other pulmonary diseases such as interstitial pulmonary fibrosis (73).

Experimentally, using an *in vitro* model of the pulmonary matrix, it has been demonstrated that lysozyme impairs the ability of HA to prevent elastase injury to elastic fibers. *In vitro* elastin matrices sequentially treated with lysozyme and HA and then incubated with pancreatic elastase showed significantly increased elastolysis compared with those treated with HA alone (74). Because HA is closely associated with elastic fibers *in vivo*, attachment of lysozyme to these fibers in human pulmonary emphysema may make them more susceptible to injury. This hypothesis was further tested in an animal model of emphysema induced by intratracheal administration of porcine pancreatic elastase. Animals exposed to aerosolized lysozyme before elastase administration showed significantly increased lung injury (74).

These findings suggest that lysozyme may not be an innocuous component of the inflammatory response associated with pulmonary emphysema. It may actually play a role in the pathogenesis of the disease. However, HA administered to the lysozyme-treated elastin matrix before the administration of pancreatic elastase was protective against elastin degradation by elastase (74).

HA and Airway Function

In addition to a role in protecting against alveolar destruction in experimental models of pulmonary emphysema, HA has also been found to block bronchial obstruction induced by aerosol administration of pancreatic elastase in sheep (75). Because *in vitro* experiments have demonstrated that HA can inactivate tissue kallikrein, it has been proposed that the protective effects of HA against elastase-induced bronchoconstriction is mediated through inactivation of tissue kallikrein (76). These studies suggest a possible therapeutic role for HA in mitigating the bronchial responses induced by elastases. In this regard, preliminary studies by Allegra and coworkers (77–78) have demonstrated that HA aerosol, administered before provocations of exercise or aerosolized distilled water, in known subjects with asthma, suppresses the bronchial obstructive response to each.

Forteza and colleagues (79) showed that tissue kallikrein and lactoperoxidase are bound to the airway epithelial surface by HA and are not cleared by ciliary beating. They also showed that the amino acid sequence of tissue kallikrein contains a specific HA-binding segment $B(x_1)B$ in the active site of tissue kallikrein. They also demonstrated the presence of the receptor for HA, RHAMM, which in turn is responsible for signaling ciliary beating by HA. This is thought to be similar to the effect of HA on sperm flagellar bending. Forteza and colleagues (79) proposed that HA serves a pivotal role in mucosal host defense by stimulating ciliary beating through the RHAMM receptor, increasing the clearance of foreign material. However, HA also retains and regulates enzymes at the apical, mucosal surface, to maintain homeostasis. Thus, HA and its receptors would play the role of maintaining an enzyme pool at the apical bronchial surface of cells in both health and disease.

Systemic Administration of HA in Chronic Bronchitis

On the basis that subcutaneous injections of HA stimulated the activity of blood neutrophils with respect to certain functions such as phagocytosis and free oxygen radical formation and migration, Venge and colleagues (80) tested the hypothesis that HA 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 exacerbations of their disease were studied. The patients were divided into two groups, one of which received HA for 6 months followed by 6 months of placebo. The other group started with placebo treatment followed by HA. The treatment periods occurred during two consecutive winter periods. HA-treated patients had significantly fewer acute exacerbations than did placebo-treated patients. They also noted that the consumption of antibiotics and signs of bacterial infections were reduced. These authors concluded that HA reduces the number of infectious exacerbations in patients with chronic bronchitis, possibly by enhancing cellular host defense mechanisms.

HA Receptors and Pharyngeal Streptococcus Infection

Recent studies (81–82) have demonstrated a significant role for the CD-44 receptor of HA in pharyngeal cell adherence of group

TABLE 1. EVIDENCE FOR POTENTIAL THERAPEUTIC EFFECTS OF HA IN RESPIRATORY DISEASE

Categories of Lung Disease	Therapeutic Mechanisms
COPD and pulmonary emphysema	Prevention of elastolysis by barrier function (63) Decreased chemotaxis through decreased elastin fragmentation (66) Blocking elastase secretion by neutrophils and macrophages (67) Blocking airway obstruction by Kallikrein (76) Hyaluronan content reduced in human emphysematous lung (69) Cigarette smoke degrades HA *in vitro* (70) and *in vivo* (71)
Cystic fibrosis	Prevention of elastolysis in airways (63) Decreased chemotaxis through decreased elastin fragmentation (6) Blocking elastase secretion by neutrophils and macrophages (67)
Asthma	HA reduces airway hyperreactivity to provocations of exercise and distilled water (77, 78) HA blocks airway obstruction by Kallikrein (76)
Pharyngeal streptococcal infection	HA blocks epithelial cell CD-44 receptor of HA to prevent streptococcal colonization (82)
Bleomycin lung injury	CD-44 clears HA small fragments which may be inflammatory (83)

Definition of abbreviations: COPD = chronic obstructive pulmonary disease; HA = hyaluronic acid.

A streptococcus via the formation of an HA capsule around the group A streptococcus organism. This then provides a mechanism for adherence of the streptococcus to pharyngeal surface cells through their CD-44 receptors and subsequent colonization *in situ* of this organism. Accordingly, exogenous HA, by occupying surface CD-44 receptors, could play a therapeutic role in preventing or limiting streptococcal colonization in animals and humans. In this regard, pretreatment of the oral pharynx of mice with a blocking antibody for the CD-44 receptor with exogenous HA blocked colonization of group-A streptococcus (82).

The Role of HA in Bleomycin Injury

A recent study of a bleomycin model of lung injury and repair in mice (83) has demonstrated a critical role for the CD44 receptor of HA for clearing the inflammatory process. CD-44 deficiency in this model leads to unremitting inflammation, increased mortality, accumulation of low molecular weight fragments (HA), prolonged inflammatory gene expression, decreased clearance of apoptotic neutrophils, and an impaired ability to generate active transforming growth factor-B1. Whether HA of higher molecular weight plays a role in relationship to the CD-44 receptor in the clearing phase of inflammation is not clear from this model.

HA and HA Receptors in Experimental Atherosclerosis

Although not directly related to respiratory disease, but of possible significance for the pulmonary vasculature, Cuff and colleagues (84) have recently proposed a role for HA and HA receptors in atherosclerotic plaques in the arterial vasculature. They have demonstrated that HA, as the principle ligand for CD-44, is upregulated in atherosclerotic lesions of apolipoprotein E–deficient mice and that the low molecular weight proinflammatory forms of HA stimulate vascular cell adhesion molecule-1 expression and proliferation of cultured primary aortic smooth muscle cells, whereas high molecular weight forms of HA inhibit smooth muscle cell proliferation. To assess the potential contribution of CD-44 to atherosclerosis, these investigators bred CD-44-null mice to atherosclerotic-prone apolipoprotein E–deficient mice. A 50–70% reduction in the aortic lesions was found in CD-44-null mice compared with CD-44-heterozygote and wild-type littermates. They also demonstrated that CD-44 promotes the recruitment of macrophages through atherosclerotic lesions. These investigators conclude that CD-44 plays a critical role in the progression of atherosclerosis through multiple mechanisms. The authors further conclude that inhibitors of CD-44 function and CD-44 HA interactions may provide an effective means for inhibiting chronic inflammatory diseases, including atherosclerosis.

HA and Alveolar Surface Structure

In a recent publication, Bray (85) hypothesized that the duplex nature of the lining of the pulmonary alveolus is composed of surfactant and an aqueous subphase of HA. Type 2 cells in the wall of the alveolus, which produce surfactant, also secrete HA into the subphase. Also, HA is known to interact with phospholipids and has hydrophobic regions that could bind to the hydrophobic surfactant proteins B and C. The hypothesis is that HA interacts with itself and with proteins in the subphase to form a hydrophilic gel, which smoothes over epithelial cell projections to create a gas fluid interface where surfactant phospholipids spread on the water. From this hypothesis, depleting the lung of HA could alter the stability of the alveolar air interface, which deserves further study.

Conclusions

From the perspective of published work thus far, there is evidence that HA may exert a protective effect against injury in a number of respiratory diseases (Table 1). HA prevents elastin injury by elastases *in vivo* in hamster models of pulmonary emphysema and *in vitro* in elastin matrices. In addition, HA modulates neutrophil elastase secretion, stimulates or represses immunologic reactions, suppresses bronchial responsiveness, and may play a role in alveolar surface structure. From this perspective, the HA molecule is worthy of further investigation for its possible therapeutic role in a variety of respiratory diseases. However, many questions remain to be addressed, such as these: (*1*) What are the variations in molecular size and structure of HA, as well as HA receptors in various tissue components of the lung? (*2*) What are metabolic turnover rates of HA and receptors normally and in lung injury? (*3*) What is the nature of binding of HA to various lung tissues, including elastin and collagen? (*4*) Does exogenously administered HA by aerosol have therapeutic efficacy in patients with chronic obstructive pulmonary disease or emphysema in α_1 antitrypsin deficiency or in cystic fibrosis? (*5*) Does HA aerosol suppress bronchial hyperreactivity in patients with asthma or chronic obstructive pulmonary disease? (*6*) What are the chemical and cellular bases of the functional variability of HA of different molecular sizes?

Conflict of Interest Statement: G. M. T. and J. O. C. are consultants and founders of Exhale Therapeutics, a company attempting to develop hyaluronan as an aerosol medication for possible treatment of certain diseases of the lung.

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