Glycosaminoglycans support cellular and fibrous components of tissues in the developing lungs

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Abstract - Glycosaminoglycons (GAG'S) are anionic polysaccharides wldely dlstributed in animal tissues, hyoluronic Acid (HA) is a component of sinonial flnid and connective tissues, chondrotin sulfates (Ch-4s and Ch-6s) are aboundant in connective tissues, dermatan sulfate (DS) in skin and intertinol mucosa hepran sulfate (HS) is a matrix constituent of many tissues and seems to be an uliquitors component of cell surfaces. The amorphous ground substance fills the space between cells and fibers of the connective tissue. It is colourless, transparent and Homogereous. It iscomposed hioinly of two macro molecular components glycosominoglycans and structural glycoprotecirs. Three mole components of connective tissues connective tissue is the most abundant and widely distributed of the primery tissues. It has three mole component cells, fibers and ground substance together the ground substance and fibers makes up the extra cellular matrix. Connective tissue connects supports binds and separates organs and tissues forming a framework to support body tissues and organs for structural and metabolic purposes. In connective tissue cells and few and dispersed. They are motion close contact as in epitneliol tissues. Most connective tissues are voscularized (except cartilage). The extracellular spaces (space out side of cells) in connective tissue are referred to as the extracellular matrix. Connective tissue, therefore is made up of cells and extracellular matrix the extracellular matrix is composed of glycosaminoglycans and proteoglycoms. It is vaniahiwns in the compostion of the extracellular matrix that determine properties of the connective tissues. Proteoglycans (mucoproteins) are formed of glycosaminoglycons (GAG'S) covolently attached to the core proteins. They are found in all connective tissues, extracellular matrix (ECM) and on the surfaces of many cell types. The function of lung is fundamentally linked to the connective tissue composition of the alveolar interstitium. The composition and synthesis of one class of interstitial connective tissue components, the glycosaminoglycans (GAG), was determined in lung parenchyma of rabbits at different stages of development. Parenchymal GAG content ranged between 0.2 and 0.4% (wt/wt) of dry weight, with highest concentration in adult lung. There were significant changes in types of GAG present at different ages. Fetal lungs contained a relatively high proportion of chondroitin 4-sulfate while the GAG in lung parenchyma of older animals was predominantly dermatan sulfate, heparin sulfate, and heparin. Methods were developed for the study of rates of synthesis of GAG by incorporation of [1-'4C]glucosamine into lung explants. The rate of synthesis of total GAG per cell increased with development to a maximum in lung from weanling rabbits and fell to low rates of synthesis in mature rabbits. Fetal rabbit lung parenchyma synthesized mostly hyaluronic acid and heparan sulfate, while in weanling rabbit parenchyma hyaluronic acid and chondroitin 4/6-sulfate synthesis was greatest. In mature animals, the rates of synthesis of all types of GAG were relatively low but there was a relatively greater emphasis on synthesis of dermatan sulfate and heparin. These results may have significance in changes in lung function during development and in effects on other connective tissue components.

Keywords – Cellular, GAG, ECM.

I. INTRODUCTION

The interstitium of the lung alveolus is composed of mesenchymal cells and connective tissue. The latter is of fundamental importance in the maintenance of lung structure, determination of lung mechanical properties,

interchange of nutrients and metabolites between the capillary and epithelial cells, and diffusion of 02 and C02 between the alveolar gas and the blood. The two major

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constituents of interstitial connective tissue, collagen and elastin, comprise more than 90% of the noncellular interstitial dry weight. The remainder has been termed the "amorphous ground substance" The term "amorphous" is used because this material is poorly defined morphologically. It is known to include serum proteins, glycoproteins, cellular metabolites, small solutes (,e.g., glucose, urea, salts), and proteoglycans. The latter are macromolecules composed of a protein backbone with multiple, large polysaccharide side chains termed glycosaminoglycans (GAG). The GAG also include hyaluronic acid, which may not be part of a protein-polysaccharide complex. In tissues other than lung and in simplified model systems with purified components, it has been shown that specific types of GAG will: (a) associate with other connective tissue elements (b) influence the rate of synthesis of connective tissue components (c) affect the hydration of connective tissue and (d) influence the rate of collagen fibril formation and subsequent stability of these fibrils Thus, the quantity and type of GAG in the lung interstitium may have significant effects on the mechanical properties of the lung as well as on gas, solute, and fluid movements between the alveolar space and capillary. It is the purpose of this study to describe methods to quantitate the composition and synthesis of GAG in the lung interstitium and to define the normal pattern of GAG accumulation and synthesis in the growing lung.

II. METHODS

Materials. An inbred strain of New Zealand white rabbits (B and H Rabbitry, Rockville, Md.) were used as a Abbreviations used in this paper: CPC, cetylpyridinium chloride; GAG, glycosaminoglycans. The animals were exsanguinated by decapitation and the lungs were dissected free. No lungs had evidence of infection. The hila were widely excised and the parenchyma was minced into 1-2-mm pieces. Enzymes utilized in preparation and quantitation of GAG included: papain (twice crystallized) and testicular hyaluronidase (Sigma Chemical Co., Inc., St. Louis, Mo.); partially purified streptococcal hyaluronidase isolated from Varidase chondroitinase ABC and chondroitinase AC GAG standards of hyaluronic acid, chondroitin 6-sulfate and dermatan sulfate, heparin, and heparan sulfate Chondroitin 4-sulfate was obtained from Isolation of GAG front lung. Lung minces were homogenized at 4VC and then placed on a boiling water bath for 3 min to denature proteins. The homogenate was then made to 0.1 M sodium acetate, 20 mM EDTA, and 20 mM cystine, and digested at 60'C by two separate additions (18 h apart) of papain (1 mg papain/20 mg dry wt lung). After papain digestion, trichloroacetic acid was added at 4°C to 6% concentration to precipitate nucleic acids and remaining protein. After 30 min, the precipitate was collected (10,000 g, 10 min) ; it contained less than 2% of the total uronic acid. The supernate was dialyzed against 0.03 M NaCl (4°C, 24 h) and the GAG were precipitated by addition of 10% cetylpyridinium chloride (CPC) in drops until no further precipitate formed. The CPC precipitate was pelleted (2,000 g, 10 min) and dissolved

in 2.0 M NaCl. Occasionally, a small amount of material would not dissolve in 2 M NaCl; this residue contained no hexuronic acid and was discarded. The GAG were reprecipitated from the 2 M NaCl solution with ethanol and dried. Total GAG content was determined by measurement of uronic acid or hexosamine content after hydrolysis of the dried GAG with 4 N HC1 for 16 h at 100°C. Identification and quantitation of GAG types in lung. Total lung GAG was isolated by CPC precipitation as described above and dissolved in 0.4 M NaCl, and 10% CPC was added until no further precipitate formed. The precipitate (containing all GAG types except hyaluronic acid) was pelleted (2,000 g, 10 min), dissolved in 2 M NaCl, and reprecipitated with 80% ethanol to remove CPC and other salts. The supernate from the 0.4 M NaCl solution (containing hyaluronic acid) was made 70% in ethanol and the precipitate was pelleted, washed with ethanol, and dried. The two dried precipitates were then analyzed for total uronic acid. Identification and quantitation of hyaluronic acid, chondroitin 4-sulfate, chondroitin 6-sulfate, dermatan sulfate, heparin, and heparan sulfate in these precipitates was done by enzymatic methods. Hyaluronic acid was determined by the method of Greiling utilizing partially purified streptococcal hyaluronidase. Chondroitin 4-sulfate, chondroitin 6-sulfate, and dermatan sulfate. The sum of heparin plus heparan sulfate was determined by subtracting total hyaluronic acid, chondroitin 4-sulfate, chondroitin 6-sulfate, and dermatan sulfate from total GAG hexuronic acid. The validity of this method for quantitating heparin plus heparan sulfate was proven by demonstrating that the total GAG hexuronic acid resistant to testicular hyaluronidase plus chondroitinase ABC was degraded by nitrous acid. The products after nitrous acid were quantitated by chromatography on columns of Bio-Gel P10 (1 X 100 cm) and equaled the heparin plus heparan sulfate as measured by difference. Rate of total GAG synthesis by lung explants. diluted 1: 1 with phosphate-buffered saline (17). D-[l-"C]Glucosamine (2 ,gCi, 37.5 nmol/incubation, added and the incubation (370C, 5% C0-95% 02) was continued from 2 to 10 h. At different time periods, the contents of each vial were diluted with 0.4 ml of cold 0.4 M EDTA and homogenized. 1 mg each of hyaluronic acid,heparan sulfate, heparin, chondroitin 4-sulfate, and dermatan sulfate were added as carriers and aliquots were taken for total GAG synthesis, DNA, specific activity of "Clabeled GAG precursors in the tissue, and specific GAG types synthesized.

III. RESULTS

Concentration of lung parenchyma GAG. The average concentration of total GAG in rabbit lung parenchyma is relatively constant (3.5-3.9 ytmol hexosamine/ g dry wt)

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from late in gestation through the weanling period but rises approximately 60% as the animal reaches maturity. Based on an average GAG hexosamine content of 30% this corresponds to 0.2-0.4% (wt/wt) of the lung parenchyma being composed of GAG. Since rabbit lung collagen concentration rises rapidly in the perinatal period and then levels off the ratio of lung GAG concentration to collagen concentration decreases 150% between fetal and the weanling period There are marked differences in the percent distribution of lung parenchyma GAG concentration with growth The late fetal lung has approximately equal concentrations of hyaluronic acid, chondroitin 6-sulfate, dermatan sulfate, and heparan sulfate plus heparin, but almost twice as much chondroitin 4-sulfate as any other GAG type. These relationships change abruptly after birth, when dermatan sulfate concentration has increased almost threefold while the relative oncentrations of hyaluronic acid, chondroitin 6-sulfate and heparan sulfate plus heparin have fallen. In the weanling rabbit parenchyma, the relative chondroitin 4-sulfate concentration has decreased even more, with concomitant relative elevations of heparin sulfate heparin. The increase in this latter fraction is even

IV. DISCUSSION

Approximately 86% of the total cells of the lung and 62% of the total connective tissue of the lung are in the parenchyma, with the remaining 14% of cells and 38% of connective tissue in the conductive blood vessels and airways. When a wide excision is used to remove the hilar structures, the majority of the large blood vessels and airways are removed, so that any biochemical measurements in the remaining tissue reflect the cellular and connective tissue of the parenchyma. The cells of the parenchyma include primarily alveolar type I and II epithelial cells, endothelial cells, and interstitial mesenchymal cells, while the connective tissue of the parenchyma almost entirely reflects the connective tissue of the alveolar interstitium. The concentration of parenchymal GAG (0.2-0.4% dry wt GAG/dry wt parenchyma) is low compared to collagen (15-20%, wt/wt) and elastin (5-10%, wt/wt). Thus, total parenchymal GAG is less than 2% of the total interstitial connective tissue. Even so, the demonstrated interaction of GAG with other connective tissue elements influence on connective tissue synthesis and cellular differentiation, and effect on the state of the ground substance "gel" suggest that this relatively small amount of material could have a major impact on interstitial mechanical properties and on the rates of gas, solute, and fluid transfer through the interstitium. The control of the amounts of GAG in the interstitium seems at least in part to be related to the rates of synthesis of GAG by the cells comprising the parenchyma, since maximum rate of GAG synthesis, appears to precede the increase in GAG concentration found in the adult. However, the relative changes in the concentration of each type of GAG in the parenchyma cannot be due only to changes in rates of synthesis of GAG, since the rates of synthesis of specific types of GAG do not necessarily parallel the dramatic alterations in GAG types found with age. Presumably, interstitial GAG concentration is controlled by the balance of GAG synthesis and degradation by mechanisms as yet undefined in lung. Undoubtedly, these mechanisms include the changing populations of cell types in parenchyma with lung growth. Although it is known that epithelial, endothelial, and mesenchymal cells derived from organs other than lung synthesize and degrade GAG there are no data available of GAG synthesis or degradation by isolated lung parenchymal cells. It is possible that each of the four major cell types in parenchyma contributes to the control of interstitial GAG accumulation. The increase in heparin plus heparan sulfate concentration found in adult lung may also be related to other cell types, such as mast cells.

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