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REVIEW

Biochemistry and turnover of lung interstitium

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ABSTRACT: The lung contains a host of extracellular matrix components that comprise the supporting and adhesive elements of conducting airways, alveoli and the vascular tree. While none of these components is unique to the lung, their peculiar distribution determines the architecture and function of this gas exchange organ. Cells and tissues of the lung interact with the matrix through a variety of surface receptors, especially the integrins and adhesive molecules, some of which may play important roles in lung injury and repair. Collagen type I is the predominant determinant of tensile strength, but as many as 11 other genetic types of collagen with specialized adhesive and connecting functions can be found in various lung structures, including cartilage and basement membranes. Excessive matrix accumulation in the lung is the result of a complex set of influences on gene regulation, part of which may be due to the presence of inflammatory cytokines that directly stimulate matrix synthesis. However, degradation and turnover of the matrix are also critical processes influenced by many of the same mediators. Collagenase and gelatinase (type IV collagenase) are tightly-regulated metalloenzymes that, together with a set of specific inhibitors of metalloproteinases, determine the net abundance and distribution of collagen. Elastases of several biochemical types are also under tight regulation by proteinase inhibitors. Elastin is essential to lung function at the level of alveolar wall resiliency and patency, and loss of elastin in emphysema appears to be due to uncontrolled degradation of the embryologically-established pattern of elastic fibres accompanied by nonfunctional replacement as a response to injury. Injury to the vascular endothelium of the lung, as well as other physiological insults that elevate pulmonary blood pressure, can lead to the excessive accumulation of collagen and elastin in the conductance and resistance arteries of the pulmonary circulation. Mechanical stress and endothelial injury may mediate the medial hypertrophy of these vessels. Extracellular matrix components are critically involved in every stage of lung biology: development, normal function and acute and chronic disease states. To date, only glucocorticoids, cross-linking inhibitors, and protease inhibitors have been used in a general attempt to suppress either excessive matrix accumulation or loss. More detailed understanding of the regulation and specific interactions of matrix components is central to the analysis of disease states and the development of appropriate therapeutic strategies.
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The biomechanical properties of the lung are largely dependent on the correct distribution and abundance of interstitial connective tissue components. [1, 2] In addition, connective tissues are critical to the maintenance of the polarity and differentiated state of numerous epithelial cell layers lining the airways, [3] blood vessels, lymphatic walls, and pleural surfaces. This brief, general review is intended to remind the reader of the complexity of the connective tissue biochemistry of the lung as well as the recent advances made in the descriptive and experimental information available on a variety of matrix macromolecules.

Collagens

The most abundant macromolecules in the lung are the interstitial collagens the fibrillar nature of which confers tensile strength properties to all of the distensible surfaces of the tissue: large airways, blood vessels and alveolar interstitium. However, there is a wide variety of collagen molecules, currently, as many as 13 genetically distinct types, some of which have multiple isoforms [4–6]. The majority of these molecules are immunologically distinct and can be specifically stained in tissues at the light and electron microscopic levels [7].

Interstitial collagens

This term is used to refer to the classic, fibril-forming collagen molecules [8] known as types I, II, III, V and XI collagen. Every collagen molecule consists of three α-chains, and they may consist of homotrimers or heterotrimers of α1, α2, and even α3 chains, coded by separate genes. Each of these molecules consists of long, continuous triple helical domains containing a regular repeat of Gly-X-Y triplets (about 330) where X and Y are frequently proline, and the prolines in the Y position are frequently hydroxylated [9] to hydroxyproline. Each molecule begins to form as three α-chains are wound into a left-handed helix, while still being translated on membrane-bound polysomes. Co-translational hydroxylation of proline by prolyl hydroxylase [10] in the endoplasmic reticulum is essential to thermal stability of the collagen helix and requires several co-factors: ascorbic acid, oxygen and α-ketoglutarate. Nonhelical molecules are largely degraded intracellularly as part of an error correction mechanism [11–13]. Hydroxyproline is also found in non-collagenous lung proteins such as elastin and the apoprotein of lung surfactant, but at much lower concentrations. An amino acid unique to collagen is hydroxylysine, which, together with lysine, plays a role in the formation of certain intermolecular cross-links between collagen chains [14]. In addition, collagen contains a further unusual modification of this residue due to o-galactosylation of hydroxylysine and glycosylation of this glycopeptide to produce glucosylgalactosylhydroxylysine [15]. Each of the fibrillar collagens is synthesized as a larger precursor, procollagen, which contain both amino and carboxy-terminal extensions or propeptides. These extensions serve a number of roles, including initiation of chain association, stabilization of nascent helical molecules and retardation of molecular aggregation. Cleavage of each terminus is catalysed by separate metalloproteinases [16–18]. Failure to cleave precursor regions of the molecule or imperfections in the helix formation due to mutations result in either partial or complete inhibition of fibrillogenesis [19–21]. Collagen synthesis is under complex regulation by many factors including cytokines, steroid hormones and matrix components [22–25].

"Minor" collagens

In the last decade, an increasing number of collagenous proteins have been discovered that are collectively termed minor collagens because of their low abundance in connective tissue [26]. Their actual biological roles may be quite critical, however. Many of this class fall into the category of non-fibrillar collagens, largely due to the fact that the helical regions of the constituent chains are interrupted at one or more positions, thus introducing regions of increased flexibility between rodlike triple-helical domains, those globular domains perhaps also serving other functional roles. A very extensive description of the diversity of collagens has recently been published by MAYNE and BURGESON [4] and should be referred to for much greater detail on the structure and biology of the minor collagens. With the exception of type X collagen, all of these molecules are likely to occur in lung tissue. Best understood of this class is type IV collagen, which appears to be exclusively a constituent of basement membranes below epithelial surfaces and the pericellular matrix of mesenchymal cells such as vascular smooth muscle. Type IV collagen, the molecules of which are composed of three different alpha chains in varying proportions, is the principal collagen of basement membranes. Type IV is not organized in parallel fibrillar arrays, but is arranged in a loose meshwork within the basal lamina along with several other glycoproteins. This mode of assembly is based in part on the molecular arrangement of type IV molecules: two triple-helical segments of unequal length connected by a hinge region and a relatively large, globular domain at the carboxyl (COOH)-terminus that promotes end-to-end chain associations rather than lateral aggregation. Protein and deoxyribonucleic acid (DNA) sequence analysis as well as rotary shadowing studies have led to the proposed "chicken wire" model of molecular arrangement.

Type VI collagen is another species with a short, central helix containing two imperfections and N- and C-terminal globular ends that appear to promote linear aggregation of tetrameric aggregates that predominantly assemble by end-to-end association, to produce linear microfibrillar aggregates. Type VI is distributed widely in interstitial tissues, and its biological role is largely unknown. The α1 and α2 chains of type VI collagen are hybrid molecules, containing type A repeat units representative of von Willebrand factor [27]. A third, less homologous and much larger (250–350 kDa) α3 chain has recently been described in the chick. This is an unusually large and heterogeneous molecule, containing peptide domains resembling the platelet glycoprotein Ib, the type II domain of fibronectin, and a motif characteristic of Kunitz-type proteinase inhibitors. Both a collagen-binding domain and an arg-gly-asp cell adhesion are present, suggesting that this chain may be a multifunctional adhesive protein [28]. Type VI has been suggested to form a microfibrillar connection between other extracellular matrix components.

Type VII collagen is localized to anchoring fibrils and thus plays a critical role in the attachment of epithelial sheets to underlying connective tissue [29]. Blistering diseases such as recessive dystrophic epidermolysis bullosa can arise as a result of the destruction or impaired synthesis of this collagenous protein. Although this unusually long (180 kD) collagen aggregates laterally into fibrils, ultrastructure suggests that these fibrils then associate in antiparallel pairs; thus, type VII occurs as short bundles of fibres which appear to interlace in the upper dermis with other matrix components, particularly type I collagen. Type VIII collagen is incompletely characterized but is synthesized by endothelial cells in an unusual trimeric form and is located in some basement membranes. Type IX collagen is unusual in that it is also a proteoglycan, containing one glycosaminoglycan

side chain. It is co-distributed with type II collagen in cartilage, possibly more closely associated with the surface of type II fibrils. An alternate (shorter form of type IX is expressed in cornea, due to use of a second transcription start site [30]. Also associated with the cartilage collagens is type XI collagen, which has a strong homology to type II (α3[XI] is identical to the α1[II] chain). Like type IX, type XI is apparently a minor component of most type II fibrils. In bone, type X collagen is associated with the hypertrophic zone, of ossifying cartilage, and is thus not likely to exist in the lung. The status of type XII is still somewhat uncertain, but it has been proposed to be a minor component of the type I fibre, somewhat analogous to the proposed role of type IX collagen in type II fibres of cartilage. Type XIII collagen appears to be a member of the basement membrane family. Type V collagen also is frequently located in pericellular sites, but it fulfils all the criteria of a fibrillar collagen: both N- and C-termini are processed and it consists of an approximately 1000-amino acid continuous triple helix. Type XIII collagen is listed as pericellular only because of its partial sequence homology with type IV collagen [31]. This protein is only identified from its complementary DNA (cDNA) sequence, and its pattern of synthesis or tissue distribution is presently under investigation.

Elastic fibres

Lung compliance is largely governed by the abundance and arrangement of interstitial collagen and elastin [32]. Collagen fibres determine the maximal limits of expansion of the various lung structures, including alveoli, bronchioles and blood vessel walls, while elastic fibres provide the resiliency needed to restore the lung structures to their original shape and volume after inspiration. Increased elastic fibres in peripheral arterioles are characteristic of pulmonary hypertension. Elastin also increases in pulmonary fibrosis. Loss of elasticity is a feature of pulmonary emphysema. Both the destruction of elastic fibres and lack of their functional replacement contribute to this pathology. Therefore, it is important to consider their composition and biology.

Elastin

Elastin is a biological rubber [33]. It is synthesized as a soluble precursor called tropoelastin (M_R=70,000), which is rapidly cross-linked after secretion into a highly polymerized and randomly oriented network of elastin molecules [34]. The cross-linking process is initiated by the same mechanism in collagen and elastin: oxidative deamination of epsilon amino groups of lysine to form reactive aldehydes (alpha-amino adipic-semialdehyde) through the action of lysyl oxidase [35] in the presence of copper and oxygen and the co-factor paraquinoline quinone. Unlike collagen, paired lysine residues are frequently found embedded in sequences such as ala-lys-ala-ala-lys-ala, and elastin cross-links are frequently formed between four lysyl residues on at least two tropoelastin molecules to form a cyclic condensation product termed desmosine or isodesmosine. These two cross-links are virtually unique to elastin and can be used as a measure of elastin concentration or elastin breakdown, since they are resistant to hydrolysis or proteolysis [36].

The rubbery properties of elastin [37] derive from its unusual primary structure which consists of alternating cross-link domains and hydrophobic domains containing repeating units of small, hydrophobic amino acids. Although structural models of elastin can be built from synthetic peptides resembling elastin [38] there is little evidence for structure in the protein itself, and the elasticity of the protein can be more easily understood as deriving from random coil behaviour of a heavily cross-linked material.

Elastin is the product of a single gene [39] located on human chromosome 7 but evidence from cDNA cloning [40] and biosynthetic studies [41] suggests that there is considerable protein polymorphism due to alternative splicing [42]. This could contribute to the randomness of tropoelastin chain association or signify distinct forms of the protein for either tissue specific or developmentally-related functions.

Microfibrillar components

The other morphological component of the elastic fibre is the so-called microfibrillar component, a 10–14 nM aggregation of filaments, the deposition of which at sites of elastic fibres precedes the accumulation of amorphous elastin [43]. The principal component of these fibres appears to be a 350 kDa glycoprotein, fibrillin [44]. This molecule is relatively insoluble, cysteine-rich (10–12%), and extensively cross-linked by disulphide bonds. Although all normal elastic fibres contain microfibrils, fibrillin is distributed more widely in structures such as the ciliary zonules of the eye. The microfibril may well consist of more than one structural protein [45].

Adhesion molecules

In the past decade, the roles of cell attachment factors, or adhesive proteins, have expanded. These molecules, all glycoprotein in nature, are involved in cell-matrix interaction by virtue of the fact that they act as ligands for cell surface receptors, as well as possessing binding sites for various components of the extracellular matrix. These molecules would appear to be crucial in processes such as cell attachment/detachment, cell migration, morphogenesis and matrix organization.

Fibronectin

The best studied adhesion protein is fibronectin, a 440,000 dimer of a large glycoprotein with binding sites for many components including collagen, fibrinogen, heparan sulphate (heparin), streptococcal walls and its

cell surface receptor [46, 47]. The protein molecule is divided into a number of functional and structural domains. A single fibronectin gene encodes multiple forms of the protein through an elaborate mechanism of alternative splicing [48]. The same mechanism is responsible for differences between cellular and plasma forms of the glycoprotein, which had been originally identified as the circulating molecule, cold-insoluble globulin [49]. Fibronectin is critical in mediating cellular attachment to fibrillar collagen; however, it also appears to be involved in opsonization of denatured collagen, as its affinity for gelatin is substantially higher. Fibronectin can also bind to the collagenous domain of complement, C1q [50].

Primary sequence analysis and peptide mapping studies lead to the definition of the cell binding region of the fibronectin molecule, which is surprisingly small, consisting of an arg-gly-asp-ser (RGDS) sequence [51]. Several other attachment factors, including vitronectin and even type I collagen, contain the RGD sequence, indicating that these attachment factors can use the same type of cell surface receptor. The receptors for fibronectin and vitronectin are members of a growing family of integrins [52] transmembrane complexes that possess sites which interact with the cytoskeleton on their cytoplasmic face. This structural arrangement probably forms the basis for the coupling of the cytoskeleton with the extracellular matrix [53].

Laminin

Laminin is a distinct attachment factor for epithelial and endothelial cells. It is a very large, trimeric glycoprotein consisting of three genetically distinct chains: A, B1 and B2 [54]. The A chain of laminin is about 400 kDa in size, while the two B chains are somewhat smaller, around 280 kDa. Laminin molecules consist of combinations of these three chains that assemble into a cruciate structure with binding sites for the cell surface, type IV collagen, and heparan sulphate proteoglycan. Laminin is a component of all basement membranes [55] and presumably acts as a bridge between epithelial cell plasma membranes and the pericellular matrix (basal lamina). Two cell surface receptors for laminin have been characterized. The larger species is a member of the integrin family [56, 57] and is thus likely to be involved in cell-substrate interactions *via* an arg-gly-asp (RGD) domain present in the laminin A chains. The smaller receptor molecule is a 67 kDa moiety [58, 59] which also appears to interact with elastin, possibly through a leu-gly-thr-ile-pro-gly sequence [60] and may be involved in chemotactic or phagocytic responses. A smaller laminin-binding molecule (32 kDa) has also been identified in tumour tissue [61]. The 67 kDa laminin-binding protein is also a galactose lectin, and sugar binding apparently displaces the glycoprotein from its binding site. Different regions of laminin have been shown to promote neurite outgrowth [62], heparin binding [63], and cell adhesion [64]: one of the cell adhesion sequences, tyr-ile-gly-ser-arg; (YIGSR), can also modulate the metastatic potential of certain melanoma cell lines [65].

Nidogen

This protein (M_R=150,000), also known as entactin, is present as a complex with laminin, binding in equimolar quantities to the so-called "cross" region of the molecule [66].

Vitronectin

Subsequent to the characterization of fibronectin, a second attachment factor was described in serum [47]. Because this molecule promotes the attachment of cells to glass and other non-proteinaceous surfaces, it was termed serum-spreading factor or vitronectin. This glycoprotein has an apparent molecular weight of 78 kDa and binds to cell surfaces through the same arg-gly-asp sequence *via* a distinct member of the integrin receptor system.

Thrombospondin

Platelets contain significant quantities of this adhesive glycoprotein, which shows specificity of binding for the platelet glycoprotein GP IIIb and sulphated glycolipids. Thrombospondin is also elaborated as a matrix component by various mesenchymal cells and acts as an adhesion molecule. In the lung, thrombospondin is located beneath the glandular epithelium [67].

Cell surface receptors

Collagen recognition

Every component of connective tissue is likely to encounter and interact with a variety of cells during its lifetime in the extracellular matrix. Recognition of each of the matrix components occurs through cellular receptors, some of which are rather specific for their ligands [68]. Several collagen receptors have been described [69, 70]. Another functional receptor is implied from observations on fibroblast and mononuclear cell chemotaxis to collagen or collagen fragments [71, 72]. Since the recognition system is present on phagocytic cells, this receptor could be involved in scavenging of collagen degradation products. A third recognition complex is present on the surface of platelets [73] where it provides a trigger for the discharge of the platelet when it comes into contact with exposed connective tissue as the result of traumatic injury [74]. In the lung fibroblast, interferon-γ is reported to increase the efficiency of collagen deposition by increasing the number of collagen receptors [75]. Recognition molecules for each of the thirteen collagen types have not been discovered, although anchorin [76] has specificity for binding of type II

collagen on the chondrocyte cell surface. However, it is conceivable that the nonhelical portions of some of the other, nonfibrillar collagens might have unique epitopes recognized by yet another receptor class. Epithelial cells possess a unique transmembrane proteoglycan that acts as a matrix receptor, discussed below.

Elastin receptors

An elastin receptor associated with fibroblast plasma membranes has recently been characterized [77, 78]. Although its recognition site appears to be an unusual hydrophobic sequence in elastin (val-gly-val-ala-pro-gly), the same molecule apparently binds to laminin as well. This receptor may be present on monocytes, which are specifically attracted to elastin peptides [79]. The so-called receptor molecule is not an integral membrane protein, but it appears to exist in a complex with two other transmembrane components. An unusual feature of this binding protein is its lectin properties. It appears to be related to another galactose-binding lectin (galaptin, 14 kDa lectin) described by others [80]. Possible functions include chemotactic response, scavenging of degradation products, organization of elastic fibres in the matrix, and cell attachment. The role of this molecule is still controversial [81]. Another protein, termed elastonectin [82], has been implicated in attachment of elastic fibre fragments to cell surfaces. Elastonectin activity is reported to be induced by the presence of elastin peptides [83]. Elastin itself is not known to be a good substrate for cell attachment, probably because of its highly hydrophobic character. Despite this fact, cells such as smooth muscle are intimately entwined in an elastic fibre network and elastic fibres can be visualized very near the plasma membrane [84]. Thus, other pericellular molecules may facilitate the association of cells with surrounding elastic fibres.

Integrins

Integrins are a family of transmembrane glycoproteins involved in recognition of matrix components and some other circulating elements [51, 52]. Integrins were also independently revealed as a group of Very Late Antigens (VLA) appearing on the surface of cultured T-cells [85], apparently involved in lymphocyte homing to target organs [86, 87]. They each consist of a heterodimer of alpha and beta subunits that are each representatives of gene families. Thus, the fibronectin and vitronectin receptors appear to share the same beta subunit and both recognize the same arg-gly-asp sequence in their respective ligands, but the alpha subunits are each unique and specific for the ligand. The vitronectin receptor is apparently identical to the platelet surface glycoprotein gpIIb/IIIa. Laminin and type IV collagen, in contrast, have recently been shown to bind to a distinct integrin with a 140–180 kDa alpha subunit and an 120 kDa beta subunit on the surface of epithelial cells. Integrins are membrane-embedded bridges between the actin cytoskeleton and fibrous elements of the extracellular matrix [88]. However, the exact linkage mechanism is not known; both vinculin and talin have been shown to rapidly redistribute at focal contacts induced by attachment of cells to substrates containing fibronectin or vitronectin. While integrins are not classical signal transducers, interactions of fibronectin with its integrin receptor can alter protease production [89].

Proteoglycans

In the lung, these molecules are most prominent in the cartilaginous elements of the bronchial tree, but their distribution is ubiquitous. Proteoglycans consist of families of core proteins [90] that can be extensively modified by the addition of glycosaminoglycan polysaccharide side-chains at serine residues *via* a xyloside linkage [91]. Numerous glycosaminoglycans are distinguished by their repeating disaccharide subunits, each consisting of an amino sugar and a uronic acid. Sulphation of these sugars also varies as a result of post-translational modification. Because of the high density of charged sulphate and carboxylate groups, the resultant hybrid structures are extremely hydrophilic. Proteoglycans are likely to have a very extended conformation in tissues to maintain their hydration. In cartilage, proteoglycans associate with long chains of hyaluronic acid in co-operation with a link protein to form enormous multimolecular complexes.

Cell surface receptors for hyaluronate have been identified [92], and one species of heparan sulphate proteoglycan can exist as a transmembrane glycoprotein with adhesive activity. The membrane-bound form of this molecule is referred to as syndecan [93], and possesses binding activity for interstitial collagens [94], fibronectin [95] and thrombospondin [96]. Syndecan expression on developing epithelia has been noted in association with mesenchymal expression of a large, adhesive, matrix glycoprotein variously termed tenascin, cytotactin or hexabrachion [97], that is involved in early morphogenetic interactions between epithelium and mesenchyme such as seen in lung development as well as sites of tissue repair [98]. Heparan sulphate proteoglycan is also a characteristic component of basement membranes together with laminin and type IV collagen. Proteoglycans have the capacity to bind other macromolecules, including collagen and growth factors such as fibroblast growth factor [99] and transforming growth factor-ß [100].

Matrix turnover

Matrix turnover is a critical element of lung biology. The net accumulation and distribution of any matrix component is governed by a dynamic balance between synthesis and degradation [101, 102]. Many of the pathological processes in pulmonary tissue result from the expression and discharge of matrix-degrading enzymes

into the extracellular space. This proteolysis can produce irreversible damage to lung architecture and, thereby, lung function.

Collagen degradation-neutral metalloproteinases

Initial degradation of fibrillar collagen is initiated by a very specific enzyme expressed by fibroblasts and mononuclear cells [103, 104]. Cleavage of collagen triple helices in a unique locus partially denatures the triple helix and allows access by other general proteinases. Thus, collagenase activity is the rate-limiting step in degradation of collagen types I, II and III. Activity of the enzyme is in turn regulated at at least three levels: expression of the proenzyme, activation by autocatalytic or endoproteolytic mechanisms and the relative abundance of inhibitor species. Other collagens with either less thermostable triple helices or intervening non-helical domains may be degraded by other, more generalized, proteinases. Collagenase is synthesized as a zymogen that can be activated *in vitro* by limited proteolysis with serine proteases such as trypsin or plasmin [105]. The enzyme is also capable of autoactivation without scission of any peptide bonds or by mercurial compounds such as aminophenyl mercuric acetate, presumably through a conformational change. It has been suggested that plasminogen activator might be the physiological activator of collagenase [106].

Metalloproteinase inhibitors. Inhibition of collagenase is likely to be a critical means of controlling enzyme activity. Tissue inhibitor of metalloproteinases (TIMP, MR=25,000), [107, 108] irreversibly binds to and inactivates all classes of metalloproteinases with high to moderate affinity. Its expression is frequently coordinate with collagenase expression, leading to the concept that TIMP may act as a scavenger of stray enzyme molecules and thereby allow only focal, pericellular digestive activity. TIMP levels in amniotic fluid may be an indicator of lung maturity [109]. Alpha-2 macroglobulin is also capable of inhibiting collagenase, although it is less likely that this high molecular weight serum factor is present in the interstitium except after traumatic injury.

Gelatinases. Native interstitial collagen molecules are only degraded by vertebrate or bacterial collagenases, but another metalloproteinase secreted by a variety of cells can further degrade the denatured collagen molecule. Gelatinase has a fairly broad spectrum of substrates but has a preference for denatured collagen [110]. As implied by the name, this class of metalloproteinases is capable of cleaving denatured collagen molecules. Since the initial scission of native, fibrillar collagen by collagenase reduces the thermostability of the triple helix below body temperature, it is reasonable that this class of enzymes acts in the second phase of collagen degradation to smaller peptides. The 72 kDa gelatinase is synonymous with type IV collagenase, which cleaves native type IV collagen molecules in a specific fashion. Another 92 kDa gelatinase is also produced by a variety of cell types. Many of the collagen molecules with discontinuous helices (types IV, VI, VIII, IX) show fragmentation with a variety of nonspecific proteases, including elastase and trypsin. Gelatinase has recently been cloned, [111], and sequence data show strong homology with the other metalloproteinases, collagenase and stromelysin. A new metalloproteinase inhibitor with greater affinity for gelatinase has recently been described [112].

Stromelysin. The third, significant metalloenzyme family [113] member is stromelysin, initially cloned as a gene the expression of which was induced in CHO cells by epidermal growth factor. It was subsequently identified as a distinct protease activity in a variety of mesenchymal cells. This enzyme degrades a broad range of substrates, including proteoglycan core protein, elastin, and other globular proteins. It has the broadest spectrum (*i.e.* lowest substrate specificity) of action of any of the neutral metalloproteases.

Elastin degradation

The reappearance of elastin at sites of injury is often very protracted [114], although acute destruction of pulmonary elastin in experimental models can elicit a rapid rebound in elastin accumulation [115]. More importantly, replacement of functional elastic fibres appears to be virtually impossible once the architecture of the alveolar wall is destroyed. Thus, control of elastin degradation is of key importance in managing pulmonary disease. The enzymes that degrade elastin are termed elastases [116], but it must be remembered that they are all very broad in their substrate specificity, unlike collagenase. The prototype for elastin degradation has been the elastases expressed in the pancreas, but the elastases involved in lung pathology are genetically and biochemically distinct molecules.

Serine elastases. Neutrophil elastase is a relatively efficient protease for elastin [117]. It is stored in azurophilic granules and released from granulocytes on stimulation. It is a typical serine protease [118], and the enzyme is strongly inhibited by alpha-1-antiprotease (α_1PI) present in serum. In humans, monocytic cells are known to be able to take up the neutrophil enzyme [119], but they can also produce a distinct metalloenzyme under certain circumstances. Because of the severe risk of emphysema in individuals genetically deficient in α_1PI, neutrophil elastase has been implicated as the key destructive element in the pathogenesis of pulmonary emphysema. This inhibitor is present in lungs and found in association with elastic fibres [120].

Metalloelastases. Murine macrophages are known to express a biochemically distinct elastase which has considerable elastolytic activity. Although initial studies with human cells failed to identify a similar activity in human monocytic lines, this activity has been detected

under conditions wherein masking of activity by the co-expression of TIMP or other inhibitors [121] has been reduced in macrophage cultures. It is uncertain whether this metalloproteinase is a completely novel enzyme or related to stromelysin. In the mouse, these are two distinct activities, however [122]. Since elastolysis can be seen in the absence of large granulocyte infiltrates, macrophage (or fibroblast) elastase activity may turn out to have an important role in lung pathology.

Pathologies of lung connective tissue

It is not an exaggeration to state that lung connective tissue will be altered by virtually any pathological condition, because lung function and lung matrix are so intimately intertwined. However, this essay will be concluded with a discussion of only three broad categories of lung disease which are of particular interest because the primary effects appear to be at the level of connective tissue or connective tissue cells. Moreover, they are conditions which appear to produce irreversible structural changes in the lung architecture.

Emphysema

From the biochemist's perspective, pulmonary emphysema is a disease characterized by excessive or uncontrolled breakdown of interstitial elastin, leading to loss of lung compliance and adequate ventilation of airspaces. According to the proposals of Janoff and others, the defect is the result of an imbalance between elastase activity and inhibitory capacity of antiproteases [123], principally α_1PI. Oxidant injury [124] can compound the problem, firstly by inactivation of α_1PI [125] and, secondly, by stimulating proteolysis of connective tissue [126, 127]. Clearest evidence for the role of enzyme inhibition in the aetiology of pulmonary emphysema comes from studies on the genetic deficiencies in α_1PI. A large number of human mutations either have reduced, inactive, or no inhibitor produced by liver and other tissues, and these affected individuals are at great risk for development of the disease [128]. The inhibitor appears to work by acting as a surrogate substrate for many serine proteases but, upon cleavage, the enzyme becomes trapped in the complex as an inactive intermediary complex. The active site of the molecule contains a methionine which is shown to be readily oxidized by a variety of mechanisms, including cigarette smoke and the peroxide intermediates released by neutrophils during activation. Two therapeutic strategies are being tested for treatment of α_1PI deficiency: direct replacement of the protein with natural or recombinant products and genetic therapy by direct introduction of a normal α_1PI gene into the patient's cells. Other antiproteases are detected in lung tissue and lung secretions [129], and synthetic inhibitors may have therapeutic value [130]. Since α_1PI-deficient patients develop overt emphysema over the course of many years, either activation of neutrophil elastase in the lung is a rare event or other protective mechanisms afford partial protection.

Interstitial elastin is destroyed or damaged in emphysema, and the pathology is due to the failure of the interstitium to restructure in a fashion that results in functional air exchange units. Instead, elastin is nonfunctionally accumulated in lung tissue, and the sites of resynthesis are not clearly defined. Although models of emphysema that use intratracheal instillation of elastases can destroy elastic fibres, [131] neither experimental nor naturally emphysematous lungs show a net loss of total insoluble elastin [110]. Pulmonary architecture is the culmination of a precise sequence of morphogenetic events, leading from a coarse glandular arrangement to the delicate framework of alveoli and terminal airways. Although the signal and response mechanisms for resynthesis of various components of the lung are apparently functional, pulmonary tissue cannot regenerate alveolar morphology after extensive destruction of the basement membrane and alveolar interstitium.

Fibrosis

Excess connective tissue accumulates in the lung as a sequel to numerous forms of injury, usually involving acute or chronic inflammation [132–134]. Collagen accumulation rises significantly in human pulmonary fibrosis [135] through a combination of its increased synthesis and decreased degradation [136, 137]. As a result of deposition of collagen [138, 139] and other molecules, including fibronectin [140] and elastin [141, 142] in the normally compliant regions of the parenchyma, gas exchange diminishes. Fibrotic deposits are also likely to change the gas diffusion efficiency of the alveolar wall and the fluid dynamics of the lung microcirculation. Fibrotic changes are pathological in a number of organs, and it is reasonable to assume that common mechanisms act to stimulate the over-accumulation of collagen, elastin, or proteoglycans. The distribution and abundance of collagen types may also change [143, 144]. There is experimental support for the concept that connective tissue cells are phenotypically modulated to produce increased matrix in fibrotic lung diseases [145], since injury with agents such as bleomycin can produce persistently elevated collagen production [146]. Fibroblasts may also over-express matrix proteins due to increased sensitivity to fibrogenic stimuli such as transforming growth factor beta (TGF-ß) or reduced sensitivity to inhibitory signals such as hydrocortisone, as suggested in keloid fibroblasts [147]. It is probable that signals for matrix modification come from cells responding to injury: inflammatory cells, endothelial cells and epithelial cells. There are a host of potential mediators including cytokines that can affect collagen metabolism [148], many acting to modulate the degradative pathway by altering either protease production, activation, or inhibition. Alternatively, excessive fibroblast proliferation may be stimulated by mitogens, and excess matrix accumulation could directly

arise from increased numbers of connective tissue producing cells [149].

Increased collagen metabolism can be reflected in various markers. The N-propeptide of type III collagen in serum has frequently been used to detect fibrotic states [150], and collagen metabolites are present in increased amounts in pulmonary disease, including fibrotic lung disorders such as idiopathic pulmonary fibrosis, sarcoidosis [151–153] and cystic fibrosis [154, 155].

Pulmonary hypertension

An important subset of lung pathologies involving connective tissue are the changes which occur in vessel walls during periods of increased pressure in the pulmonary artery. This pathology has been experimentally induced by many forms of lung insult, including air embolization, hypoxia, bleomycin, and monocrotaline intoxication. Depending upon the nature of the stimulus, peripheral vascular resistance may rise, as in the injury models, whereas, in hypoxia, transformation of vessel walls may be directly driven by right heart output. Pathological changes in hypertension include the thickening of medial and adventitious layers in peripheral vessels and the accumulation of collagen and elastin. Experimental studies indicate that the structural changes in response to hypertension are quite rapid (2–4 days) [156], and one expects, therefore, that matrix gene expression is rapidly accelerated and then subsides. As in fibrosis [157–159], signals for matrix remodelling may be through soluble mediators expressed by endothelial or inflammatory cells; however, vascular smooth muscle cells can autogenously respond to increased mechanical stress by producing higher levels of matrix proteins, such as collagen and elastin.

There is abundant, undisputed evidence that chronic hypertension is accompanied by structural changes to blood vessel walls that include cellular hypertrophy, hyperplasia, and accumulation of interstitial matrix components, principally collagen and elastin [160–162]. The mechanisms leading to the development of this condition are uncertain but vascular injury is a precursor of many forms and models of hypertension [163]. Some of the vascular changes are irreversible [164]. Although there is evidence for a contribution by accessory, inflammatory cells [165], and overlying endothelium has a distinct effect on the pathogenesis of vascular injury [166], the pathology is manifested by the vascular smooth muscle cell [167–169]. This cell population may represent an altered smooth muscle cell (SMC) phenotype [170]. SMC involvement was demonstrated most recently and clearly by the work of MECHAM *et al.* [171] using a model of pulmonary hypertension, the hypoxic calf. This model, in addition to showing the typical morphological and physiological changes to pulmonary arterioles, illustrated three important concepts: matrix production by arterial tissue was increased in hypertensive animals, SMC derived from the pulmonary arteries of these animals were higher in elastin production, and these SMC appeared to modify serum in a way that increased its ability to stimulate elastin production by normal nuchal ligament fibroblasts. KEELEY and JOHNSON [172] have likewise evaluated elastin and collagen accumulation and synthesis in a rat model of renal hypertension that demonstrates the close coupling between the induction of a hypertensive state and the production of new connective tissue in the vessel wall, maintaining proper compliance and flow properties. Although relative concentrations of collagen and elastin are unchanged in the hypertrophic vessel wall, [171, 173] these matrix molecules are key markers of the physiological state of vascular tissue.

Role of mechanical stress. There are a host of causes that can initiate the morphological and biochemical changes seen in hypertension, including endothelial injury [174, 175], vasoconstriction [176] and obstruction of blood flow [177]; however, little is known with certainty, at the mechanistic level, to link chronic elevation of blood pressure to medial wall changes. Obvious possibilities include mechanical stress, stress-induced tissue injury, [178], altered blood flow [179], altered interactions between leucocytes and the vessel wall [180], changes in matrix composition [181] and changes in levels of circulating hormones or vasoactive peptides [161]. Other studies have suggested that proteolytic damage to the vessel wall could initiate structural changes characteristic of hypertension [182]. A number of experiments suggest that mechanical stress alone is sufficient to initiate matrix accumulation [183–185]. The classic study of LEUNG *et al.* [186] showed that SMC, grown on an elastin matrix that was cyclically stretched, produced more protein and collagen than unstretched controls, more recently suggested to be the result of changing adenosine 3'5-cyclic phosphate (cAMP) levels [187]. More recent findings in other cell systems [188–190] have confirmed this observation as well as showing increased proliferation, and preliminary reports of the effects of monotonic stretching on aortic rings of chick aorta, with or without intact endothelium, indicate that stretched tissues can respond in a similar manner [191]. Below, data from this laboratory also support the hypothesis that matrix accumulation in vessel walls is due, at least in part, to mechanical forces.

Hormones and cytokines. In chronic hypertension, structural changes in the vessel wall can become permanent, suggesting that the phenotype of the SMC is shifted to one of enhanced wall thickening [170]. Part of this could arise from the observed polyploidy of hypertensive smooth muscle cells in experimental animals [192–194], but evidence from MECHAM *et al.* [171] and our own work suggest that SMC of hypertensive vascular tissue produce greater quantities of elastin per unit DNA than cells from normotensive vessels, even in the same culture environment [195]. Therefore, either the genes or the systems that regulate matrix genes are reprogrammed in some way to produce more (or degrade less) matrix. In addition, external signals derived either from the local or humoral environment of the vessel wall could play an important role in maintaining or amplifying

the hypertensive phenotype [160]. Among the potential effector molecules, TGFß is a prime candidate, since it is the most potent agonist of matrix production and an antagonist of matrix degradation [196]. Insulin-like growth factor-I has a positive effect on elastin production [197, 198]. Glucocorticoids can also be up-regulators of matrix production, particularly in developing connective tissue and derived cells, but also in certain adult-derived cells, including SMC [199]. Platelet-derived growth factor could also play an early role in SMC hyperplasia [200, 201] but it fails to stimulate matrix production [202], and is reported not to be significantly expressed in the hypertensive vessel wall [203].

TGFß is a multipotential inhibitor of cell division, present in tissues including the lung [204], that has been widely implicated as a mediator of fibrotic changes taking place during development [205], in tissue repair [206], and most importantly in the aorta of hypertensive animals [186]. TGFβ has properties consistent with a role in the pathogenesis of hypertension [207, 208]. Recent studies by our collaborator, E.A. Perkett, strongly suggest that TGFβ is present in the lung during the progression of hypertension [209] and our own findings are consistent with TGFβ being an elastogenic signal in pulmonary hypertension. TGFβ appears to act through receptors with protein kinase activity [196, 210], but further events leading to increased matrix expression are unknown. A site in the mouse α1(I) collagen promoter has been linked to TGFβ response [211] but others have suggested that matrix formation could be regulated by altered transcript stability [212, 213], as was previously suggested for glucocorticoid-mediated down-regulation of collagen transcript abundance [214].

Few studies have considered how mechanical distortion of cells could lead to increased matrix production. Possible direct effects of mechanical stress are changes in ion fluxes [215] (Ca^{++}, K^{+}) leading to phospholipid mobilization, [216] cyclic nucleotide metabolism, [187] and protein phosphorylation [217]. Stress may also induce the production of a cytokine signal that produces positive feedback. In wounds, we have shown that TGFβ1 induces it own production, for example [218]. Matrix regulation has not been tightly linked to the activation of a particular signal transduction pathway, although cyclic nucleotides can participate to some extent in the regulation of collagen and elastin [219, 220], and theophylline is reported to reduce stretch-induced stimulation of protein production [187]. Whether hypertension or mechanical stress mediate their effects through a TGFβ receptor and direct mechano-reception is still an open question.

Biochemical markers of lung disease

There are few clinical biochemical markers for vessel wall thickening or other fibroproliferative disorders, although the N-propeptides of collagen I and III have been used in some applications [221]. Elastin peptides detected in plasma and desmosines (elastin-specific cross-links) present in plasma and urine have been suggested as markers of elastin destruction [222–224] but they have not previously been measured under conditions of net elastin accumulation. These peptides may have significant biological activity in the lung [79]. Our preliminary data [225] lend support to the concept that elastin turnover is a hallmark of medial wall thickening [226], and that elastin peptides could be of diagnostic value in lung and vascular pathology [227].

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References

1. Clark JG, Kuhn CE, McDonald JA, Mecham RP. – Lung connective tissue. *Int Rev Connect Tissue Res*, 1983, 10, 249–331.
2. Turner WM. – Some connective tissue disorders of the lung. *Postgrad Med J*, 1988, 64, 497–504.
3. Rannels DE, Rannels SR. – Influence of the extracellular matrix on type 2 cell differentiation. *Chest*, 1989, 96, 165–173.
4. Mayne R, Burgeson RE eds. – *In*: Structure and function of collagen types. Academic Press, London, 1987, p. 317.
5. Miller EJ, Gay S. – Collagens, an overview. *Meth Enzymol*, 1987, 144, 3–41.
6. Nimni ME, ed. – *In*: Collagen: Biochemistry, Biomechanics, Biotechnology, Vol 1. CRC Press, Boca Raton, 1988, p. 312.
7. Mayne R. – Preparation and applications of monoclonal antibodies to different collagen types. *Clin Biochem*, 1988, 21, 111–115.
8. Bornstein P, Traub W. – *In*: The Proteins, H. Neurath ed., Academic Press, New York, 1979, Vol 4, pp. 411–632.
9. Bornstein P, Sage H. – Structurally distinct collagen types. *Ann Rev Biochem*, 1980, 49, 957–1003.
10. Kivirikko KI, Myllyla R, Pihlajaniemi T. – Protein hydroxylation, prolyl 4-hydroxylase, an enzyme with four cosubstrates and a multifunctional subunit. *Faseb J*, 1989, 3, 1609–1617.
11. Berg RA. – Intracellular turnover of collagen. *In*: Regulation of Matrix Accumulation. R.P. Mecham ed., Academic Press, New York, 1986, pp. 29–52.
12. Berg RA, Steinmann B, Rennard SI, Crystal RG. – Ascorbate deficiency results in decreased collagen production. Under-hydroxylation of proline leads to increased intracellular degradation. *Arch Biochem Biophys*, 1983, 226, 681–686.
13. Bienkowski RS, Wu CH, Wu GY. – Limitation on sensitivity of measuring collagen degradation: studies with cultured cells. *Connect Tiss Res*, 1986, 14, 213–219.
14. Eyre DR, Paz MA, Gallop PM. – Cross-linking in collagen and elastin. *Annu Rev Biochem*, 1984, 53, 717–748.
15. Kivirikko KI, Myllyla R. – Collagen glycosyltransferases. *Int Rev Connect Tissue Res*, 1979, 8, 23–72.
16. Kessler E, Adar R, Goldberg B, Niece R. – Partial purification and characterization of a procollagen C-proteinase from the culture medium of mouse fibroblasts. *Coll Relat Res*, 1986, 6, 249–266.
17. Hojima Y, McKenzie JA, Van DRM, Prockop DJ. – Type I procollagen N-proteinase from chick embryo tendons. Purification of a new 500 kDa form of the enzyme and identification of the catalytically active polypeptides. *J Biol Chem*, 1989, 264 (19), 11336–11345.

18. Kadler KE, Hojima Y, Prockop DJ. – Assembly of collagen fibrils de novo by cleavage of the type I pC-collagen with procollagen C-proteinase. Assay of critical concentration demonstrates that collagen self-assembly is a classical example of an entropy-driven process. *J Biol Chem*, 1987, 262 (32), 15696–15701.
19. Byers PH, Wenstrup RJ, Bonadio JF, Starman B, Cohn D. – Molecular basis of inherited disorders of collagen biosynthesis: implications for prenatal diagnosis. *Curr Probl Dermatol*, 1987, 16, 158–174.
20. Prockop DJ. – Osteogenesis imperfecta, phenotyplc heterogeneity, protein suicide, short and long collagen. *Am J Hum Genet*, 1984, 36, 499–505.
21. Cohn DH, Byers PH. – Osteogenesis imperfecta and other inherited disorders of the structure and synthesis of type I collagen, models for the analysis of mutations that result in inherited chondrodysplasias. *Pathol Immunopathol Res*, 1988, 7, 132–138.
22. Cutroneo KR, Sterling KM Jr, Shull S. – Steroid hormone regulation of extracellular matrix proteins. *In*: Regulation of Matrix Accumulation. R.P. Mecham ed., Academic Press, New York, 1986, pp. 119–176.
23. Kucharz EJ. – Hormonal control of collagen metabolism. Part II. *Endocrinologie*, 1988, 26, 229–237.
24. Bornstein P, Sage H. – Regulation of collagen gene expression. *Prog Nucleic Acid Res Mol Biol*, 1989, 37, 67–106.
25. Raghow R, Thompson JP. – Molecular mechanisms of collagen gene expression. *Mol Cell Biochem*, 1989, 86, 5–18.
26. Burgeson RE. – New collagens, new concepts. *Annu Rev Cell Biol*, 1988, 4, 551–557.
27. Chu M-L, Pan T-C, Conway D, Saitta B, Stokes D, Kuo H-J, Glanville RW, Timpl R, Mann K, Deutzmann R. – The structure of type VI collagen. *Ann NY Acad Sci*, 1990, 580, 55–63.
28. Bonaldo P, Colombatti A. – The carboxyl terminus of the chicken α2 chain of collagen VI is a unique mosaic structure with glycoprotein Ib-like, fibronectin, and Kunitz modules. *J Biol Chem*, 1989, 264, 20235–20239.
29. Burgeson RE. – The collagens of skin. *Curr Probl Dermatol*, 1987, 17, 61–75.
30. Nishimura I, Muragaki Y, Olsen B. – Tissue-specific forms of type IX collagen-proteoglycan arise from the use of two widely separated promoters. *J Biol Chem*, 1989, 264, 20033–20041.
31. Tikka L, Pihlajaniemi T, Henttu P, Prockop DJ, Tryggvason K. – Gene structure for the α1 chain of a human short-chain collagen (type XIII) with alternatively spliced transcripts and translation termination codon at the 5' end of the last exon. *Proc Natl Acad Sci USA*, 1988, 85, 7491–7495.
32. Schellenberg JC, Liggins GC, Kitterman JA, Lee CC. – Elastin and collagen in the fetal sheep lung. II. Relationship to mechanical properties of the lung. *Pediatr Res*, 1987, 22, 339–343.
33. Dorrington KL, McCrum NH. – Elastin as a rubber. *Biopolymers*, 1977, 16, 1201–1222.
34. Davidson JM, Giro MG. – Control of elastin synthesis, molecular and cellular aspects. *In*: Regulation of Matrix Accumulation. R.P. Mecham ed., Academic Press, New York, 1986, pp. 178–217.
35. Kagan HM. – Characterization and regulation of lysyl oxidase. *In*: Regulation of Matrix Accumulation. R.P. Mecham ed., Academic Press, New York, 1986, pp. 322–399.
36. Rucker RB, Dubick MA. – Elastin metabolism and chemistry, potential roles in lung development and structure. *Environ Health Perspect*, 1984, 55, 179–191.
37. Starcher BC. – Elastin and the lung. *Thorax*, 1986, 41, 577–585.
38. Urry DW. – Physicochemical properties of elastin and constituent peptides. *In*: Elastin and elastases, Vol 1, L. Robert, W. Hornebeck eds, CRC Press, Boca Raton, 1989, pp. 141–186.
39. Olliver L, Davidson JM, Luvalle PA, Rosenbloom J, Matthew C, Bester AJ, Boyd C. – The gene coding for tropoelastin is represented as a single copy sequence in the haploid sheep genome. *Collagen Rel Res*, 1987, 7, 77–89.
40. Fazio MJ, Olsen DR, Kuivaniemi H, Chu M-L, Davidson JM, Rosenbloom J, Uitto J. – Isolation and characterization of human elastin cDNAs, and age-associated variation in elastin gene expression in cultured skin fibroblasts. *Lab Invest*, 1988, 58, 270–277.
41. Wrenn DS, Parks WC, Whitehouse LA, Crouch EC, Kucich U, Rosenbloom J, Mecham RP. – Identification of multiple tropoelastins secreted by bovine cells. *J Biol Chem*, 1987, 262, 2244–2249.
42. Indik Z, Yeh H, Ornstein-Goldstein N, Sheppard P, Anderson N, Rosenbloom JC, Peltonen L, Rosenbloom J. – Alternative splicing of human elastin mRNA demonstrated by sequence analysis of cloned genomic and complementary DNA. *Proc Natl Acad Sci USA*, 1987, 84, 5680–5684.
43. Cleary EG, Gibson MA, Fanning JC. – The microfibrillar component of elastic tissue. *In*: Elastin and elastases, Vol 1, L. Robert, W. Hornebeck eds, CRC Press, Boca Raton, 1989, pp. 31–48.
44. Sakai LY, Keene DR, Engvall E. – Fibrillin, a new 350 kDa glycoprotein, is a component of extracellular microfibrils. *J Cell Biol*, 1986, 103, 2499–2509.
45. Colombatti A, Bressan GM, Volpin D, Castellani I. – Monoclonal antibodies against chick gp 115, a matrix glycoprotein with broad distribution. *Coll Relat Res*, 1985, 5, 181–191.
46. Hynes RO, Yamada KM. – Fibronectins, multifunctional modular glycoproteins. *J Cell Biol*, 1982, 95, 369–377.
47. Editorial. – Fibronectins and vitronectin. *Lancet*, 1989, 8636, 474–476.
48. Hynes RO. – Fibronectins, a family of complex and versatile adhesive glycoprotein derived from a single gene. *Harvey Lect*, 1985–1986, 81, 133–152.
49. Saba TM. – Plasma fibronectin and lung vascular injury. *Prog Clin Biol Res*, 1989, 299, 133–156.
50. Rennard SI, Chen YF, Robbins RA, Gadek JE, Crystal RG. – Fibronectin mediates cell attachment to C1q, a mechanism for the localization of fibrosis in inflammatory disease. *Clin Exp Immunol*, 1983, 54, 239–247.
51. Ruoslahti E, Pierschbacher MD. – New perspectives in cell adhesion, RGD and integrins. *Science*, 1987, 238, 491–497.
52. Hynes RO. – Integrins, a family of cell surface receptors. *Cell*, 1987, 48, 549–554.
53. Burridge K, Fath K. – Focal contacts, transmembrane links between the extracellular matrix and the cytoskeleton. *Bioessays*, 1989, 10, 104–108.
54. Martin GR, Timpl R. – Laminin and other basement membrane components. *Annu Rev Cell Biol*, 1987, 3, 57–85.
55. Grant DS, Leblond CP. – Immunogold quantitation of laminin, type IV collagen and heparan sulfate in a variety of basement membranes. *J Histochem Cytochem*, 1988, 36, 271–283.
56. Gehlsen KR, Dillner L, Engvall E, Ruoslahti E. – The human laminin receptor is a member of the integrin family of cell adhesion receptors. *Science*, 1988, 241, 1228–1229.
57. Tomaselli KJ, Damsky CH, Reichardt LF. – Purification and characterization of mamalian integrins expressed by a rat neuronal cell line (PC12): evidence that thet function as alpha/

beta heterodimeric receptors for laminin and type IV collagen. *J Cell Biol*, 1988, 107, 1241–1252.
58. Liotta LA, Rao CN, Wewer UM. – Biochemical interactions of tumour cells with the basement membrane. *Annu Rev Biochem*, 1986, 55, 1037–1057.
59. Van der Mark K, Kuhl U. – Laminin and its receptor. *Biochim Biophys Acta*, 1985, 823, 147–160.
60. Mecham RP, Hinek A, Griffin GL, Senior RM, Liotta LA. – The elastin receptor shows structural and functional similarities to the 67 kDa tumour cell laminin receptor. *J Biol Chem*, 1989, 264, 16652–16657.
61. Yow H, Wong JM, Chen HS, Lee C, Steele GD JR, Chen LB. – Increased mRNA expression of a laminin-binding protein in human colon carcinoma, complete sequence of a full-length cDNA encoding the protein. *Proc Natl Acad Sci USA*, 1988, 85, 6394–6398.
62. Sephel GC, Burrous BA, Kleinman HK. – Laminin neural activity and receptors. *Dev Neurosci*, 1989, 11, 313–331.
63. Charonis AS, Skubitz AP, Koliakos GG, Reger LA, Dege J, Vogel AM, Wohlheuter R, Furcht L. – A novel synthetic peptide from the B1 chain of laminin with heparin-binding cell adhesion-promoting activities. *J Cell Biol*, 1987, 107, 1253–1260.
64. Graf J, Iwamoto Y, Sasaki M, Martin GR, Kleinman HK, Robey FA, Yamada Y. – Identification of an amino acid sequence in laminin mediating cell attachment, chemotaxis, and receptor binding. *Cell*, 1988, 48, 989–996.
65. Iwamoto Y, Robey FA, Graf J, Sasaki M, Kleinman HK, Yamada Y, Martin GR. – YIGSR, a synthetic laminin pentapeptide, inhibits experimental metastasis formation. *Science*, 1987, 238, 1132–1134.
66. Paulsson M, Deutzmann R, Dziadek M, Nowack H, Timpl R, Weber S, Engel J. – Purification and properties of intact and degraded nidogen obtained from a tumor basement membrane. *Eur J Biochem*, 1986, 156, 467–478.
67. Wight TN, Raugi GJ, Mumby SM, Bornstein P. – Light microscopic localization of thrombospondin in human tissues. *J Histochem Cytochem*, 1985, 33, 295–302.
68. Buck CA, Horowitz AF. – Cell surface receptors for extracellular matrix macromolecules. *Annu Rev Cell Biol*, 1987, 3, 179–205.
69. Arenciba I, Sundquist KG. – Collagen receptor on T lymphocytes and the control of lymphocyte mobility. *Eur J Immunol*, 1989, 19, 929–934.
70. Gullberg D, Terracio L, Borg TK, Rubin K. – Identification of integrin-like matrix receptors with affinity for interstitial collagens. *J Biol Chem*, 1989, 264, 12686–12694.
71. Postlethwaite AE, Seyer JM, Kang AH. – Chemotactic attraction of human fibroblasts to type I, II, and III collagens and collagen-derived peptides. *Proc Natl Acad Sci USA*, 1978, 75(2), 871–875.
72. Postlethwaite AE, Kang AH. – Collagen- and collagen peptide- induced chemotaxis of human blood monocytes. *J Exp Med*, 1976, 143, 1299–1307.
73. Takada Y, Hemler ME. – The primary structure of the VLA-2/collagen receptor alpha 2 subunit (platelet GPIa), homology to other integrins and the presence of a possible collagen-binding domain. *J Cell Biol*, 1989, 109, 397–407.
74. Kunicki TJ. – Platelet membrane glycoproteins and their function, an overview. *Blut*, 1989, 59, 30–34.
75. Clark JG, Dedon TF, Wayner EA, Carter WG. – Effects of interferon-gamma on expression of cell surface receptors for collagen and deposition of newly synthesized collagen by cultured human lung fibroblasts. *J Clin Invest*, 1989, 1505–1511.
76. Von der Mark K, Mollenhauer J, Muller PK, Pfaffle M. – Anchorin CII, a type II collagen-binding glycoprotein from chondrocyte membranes. *Ann NY Acad Sci*, 1985, 460, 214–223.
77. Hinek A, Wrenn DS, Mecham RP, Barondes SH. – The elastin receptor, a galactoside-binding protein. *Science*, 1988, 239, 1539–1541.
78. Mecham RP, Hinek A, Entwistle R, Wrenn DS, Griffin GL, Senior RM. – Elastin binds to a multifunctional 67 kilodalton peripheral membrane protein. *Biochemistry*, 1989, 28, 3716–3722.
79. Hunninghake GW, Davidson JM, Rennard SI, Szapiel S, Gadek J, Crystal RG. – Mechanisms of pulmonary emphysema, attraction of macrophage precursors to sites of disease activity by elastin fragments. *Science*, 1981, 212, 925–927.
80. Cerra RF, Haywood Reed PL, Barondes SH. – Endogenous mammalian lectin localized extracellularly in lung elastic fibres. *J Cell Biol*, 1984, 98, 1580–1589.
81. Powell JT. – Evidence against lung galaptin being important to the synthesis or organization of the elastic fibril. *Biochem J*, 1988, 252, 447–452.
82. Hornebeck W, Tixier JM, Robert L. – Inducible adhesion of mesenchymal cells to elastic fibres. *Proc Natl Acad Sci USA*, 1986, 83, 5517–5520.
83. Robert L, Jacob MP, Fulop T, Timar J, Hornebeck W. – Elastonectin and the elastin receptor. *Pathol Biol (Paris)*, 1989, 37, 736–741.
84. Clark JM, Glagov S. – Transmural organization of the arterial media. The lamellar unit revisited. *Arteriosclerosis*, 1985, 5, 19–34.
85. Hemler ME. – Adhesive protein receptors on hematopoetic cells. *Immunol Today*, 1988, 9, 109–113.
86. Holtzmann B, Weissman IL. – Integrin molecules involved in lymphocyte homing to Peyer's patches. *Immunol Rev*, 1989, 108, 45–61.
87. Kishimito TK, Larson RS, Corbin AL, Dustin ML, Staunton DE, Springer TA. – The leukocyte integrins. *Adv Immunol*, 1989, 46, 149–182.
88. Akiyama SK, Yamada SS, Chen WT, Yamada KM. – Analysis of fibronectin receptor function, with monoclonal antibodies, roles in cell adhesion, migration, matrix assembly, and cytoskeletal organization. *J Cell Biol*, 1989, 109, 863–875.
89. Werb Z, Tremble PM, Behrendtsen O, Crowley E, Damsky CH. – Signal transduction through the fibronectin receptor induces collagenase and stromelysin gene expression. *J Cell Biol*, 1989, 109, 877–889.
90. Hassell JR, Kimura JH, Hascall VC. – Proteoglycan core protein families. *Annu Rev Biochem*, 1986, 55, 539–567.
91. Ruoslahti E. – Structure and biology of proteoglycans. *Annu Rev Cell Biol*, 1988, 4, 229–255.
92. Toole BP, Knudson CB, Munaim SI, Knudson W, Welles CHI-Rosso G. – Hyaluronate-cell interactions and regulation of hyaluronate synthesis during embryonic limb development. *In:* Cutaneous development, aging and repair. G. Abatangelo, J.M. Davidson eds, Fidia Res Ser, Vol 18, Liviana Press, Padova, 1989, pp. 41–56.
93. Saunders S, Jalkanen M, O'Farrell S, Bernfield M. – Molecular cloning of syndecan, an integral membrane proteoglycan. *J Cell Biol*, 1989, 108, 1547–1556.
94. Koda JE, Rapraeger A, Bernfield M. – Heparan sulfate proteoglycans from mouse mammary epithelial cells, cell surface proteoglycan as a receptor for interstitial collagens. *J Biol Chem*, 1985, 260, 8157–8162.
95. Saunders S, Bernfield M. – Cell surface proteoglycan binds mouse mammary epithelial cells to fibronectin and behaves as a receptor for interstitial matrix. *J Cell Biol*, 1988, 106, 423–430.

96. Sun X, Mosher DF, Rapraeger A. – Heparan sulfate-mediated binding of epithelial cell surface proteoglycan to thrombospondin. *J Biol Chem*, 1989, 264, 2885–2889.
97. Chiquet-Ehrisman R, Mackie EJ, Pearson CA, Sakakura T. – Tenascin: an extracellular matrix protein involved in tissue interactions during foetal development and oncogenesis. *Cell*, 1986, 47, 131–139.
98. Erickson HP, Bourdon MA. – Tenascin: an extracellular matrix protein prominent in specialized embryonic tissue and tumors. *Annu Rev Cell Biol*, 1989, 5, 71–92.
99. Bashkin P, Doctrow S, Klagsbrun M, Svahn CM, Folkman J, Vlodavsky I. – Basic fibroblast growth factor binds to subendothelial extracellular matrix and is released by heparitinase and heparin-like molecules. *Biochemistry*, 1989, 28 (4), 1737–1743.
100. Andres JL, Stanley K, Cheifetz S, Massagué I. – Membrane-anchored and soluble forms of betaglycan, a polymorphic proteoglycan that binds transforming growth factor-β. *J Cell Biol*, 1989, 109, 3137–3145.
101. Laurent GJ. – Dynamic state of collagen, pathways of collagen degradation *in vivo* and their possible role in the regulation of collagen mass. *Am J Physiol*, 1987, 252, 26–34.
102. Laurent GJ, Harrison NK, McAnulty RJ. – The regulation of collagen production in normal lung and during interstitial lung disease. *Postgrad Med J*, 1988, 64, 26–34.
103. Welgus HG, Campbell EJ, Bar-Shavit Z, Senior RM, Teitelbaum SL. – Human alveolar macrophages produce a fibroblast-like collagenase and collagenase inhibitor. *J Clin Invest*, 1985, 76, 219–224.
104. Wahl LM, Mergenhagen SE. – Regulation of monocyte/macrophage collagenase. *J Oral Pathol*, 1988, 17, 452–455.
105. Jeffrey JJ. – The biological regulation of collagenase activity. *In*: Regulation of Matrix Accumulation. R.P. Mecham ed., Academic Press, New York, 1986, pp. 53–99.
106. Moscatelli D, Rifkin DB. – Membrane and matrix localization of proteinases: a common theme in tumor cell invasion and angiogenesis. *Biochim Biophys Acta*, 1988, 948, 67–85.
107. Bunning RAD, Murphy G, Kumar S, Phillips P, Reynolds JJ. – Metalloproteinase inhibitors from bovine cartilage and body fluids. *Eur J Biochem*, 1984, 139, 75.
108. Stricklin GP. – Human fibroblast tissue inhibitor of metalloproteinases: glycosylation and function. *Coll Relat Res*, 1986, 6, 219–228.
109. Stricklin GP, Gast MJ, Welgus HG. – Amniotic fluid collagenase inhibitor, correlation with gestational age and fetal lung maturity. *Am J Obstet Gynecol*, 1986, 154, 134–139.
110. Murphy G, Docherty AJP. – Molecular studies of connective tissue proteinases. *In*: Cutaneous development, aging and repair, G. Abatangelo, J.M. Davidson eds, Fidia Res Ser, Vol 18, Liviana Press, Padova, 1989, pp. 333–338.
111. Collier IE, Wilhelm SM, Eisen AZ, Marmer BL, Grant GA, Seltzer JL, Kronberger A, He CS, Bauer EA, Godberg GI. – H-ras oncogene-transformed human bronchial epithelial cells (TBE-1) secrete a single metalloprotease capable of degrading basement membrane collagen. *J Biol Chem*, 1988, 263, 6579–6587.
112. Stetler-Stevenson W, Krutzch HC, Liotta LA. – Tissue inhibitor of metalloproteinase (TIMP-2). A new member of the metalloproteinase inhibitor family. *J Biol Chem*, 264, 1989, 17374–17378.
113. Birkedal-Hansen H. – From tadpole collagenase to a family of matrix metalloproteinases. *J Oral Path*, 1988, 17, 445–451.
114. Davidson JM, Giro MG. – Elastin repair. *In*: Wound Repair. I.K. Cohen, R. Diegelmann, W. Lindblad eds, Williams and Wilkins, Baltimore, 1990, in press.
115. Kuhn C III, Yu SY, Chraplyvy M, Linder HE, Senior RM. – The induction of emphysema with elastase. II. Changes in connective tissue. *Lab Invest*, 1976, 43, 372–380.
116. Bieth JG. – Elastases, catalytic and biological properties. *In*: Regulation of Matrix Accumulation. R.P. Mecham ed., Academic Press, New York, 1986, pp. 218–321.
117. Janoff A. – Elastase in tissue injury. *Annu Rev Med*, 1985, 36, 207–216.
118. Travis J. – Structure, function, and control of neutrophil proteinases. *Am J Med*, 1988, 84, 37–42.
119. Campbell EJ, Wald MS. – Fate of human neutrophil elastase following receptor-mediated endocytosis by human alveolar macrophages. Implications for connective tissue injury. *J Lab Clin Med*, 1983, 101, 527–536.
120. Kramps JA, Te Boekhorst AH, Fransen JA, Ginsel LA, Dijkman JH. – Antileukoprotease is associated with elastin fibers in the extracellular matrix of human lung. *Am Rev Respir Dis*, 1989, 140, 471–476.
121. Albin RJ, Senior RM, Welgus HG, Connoly NL, Campbell EJ. – Human alveolar macrophages secrete an inhibitor of metalloproteinase elastase. *Am Rev Respir Dis*, 1987, 135, 1281–1285.
122. Banda MJ, Werb Z, McKerrow JH. – Elastin degradation. *Meth Enzymol*, 1987, 144, 288–305.
123. Virca GD, Schnebli HP. – The elastase/alpha 1-antiprotease inhibitor balance in the lung. A review. *Scweiz Med Wochenschr*, 1984, 114, 895–898.
124. Cantin A, Crystal RG. – Oxidants, antioxidants and the pathogenesis of emphysema. *Eur J Respir Dis*, 1985, 139 (Suppl.) 7–17.
125. Stockley RA. – Alpha-1-antitrypsin and the pathogenesis of emphysema. *Lung*, 1987, 165, 61–77.
126. Kerr JS, Chae CU, Nagase H, Berg RA, Riley DJ. – Degradation of collagen in lung slices exposed to hyperoxia. *Am Rev Respir Dis*, 1987, 135, 1334–1339.
127. Riley DJ, Kerr JS. – Oxidant injury of the extracellular matrix, potential role in the pathogenesis of pulmonary emphysema. *Lung*, 1985, 163, 1–13.
128. Brantly M, Nukiwa T, Crystal RG. – Molecular basis of alpha-1-antitrypsin deficiency. *Am J Med*, 1988, 84, 13–31.
129. Hutchison DC. – The role of proteases and antiproteases in bronchial secretions. *Eur J Respir Dis*, 1987, 153 (Suppl.), 78–85.
130. Doherty JB, Ashe BM, Argenbright LW, Barker PL, Bonney PL, Chandler GO, Dahlgren ME *et al.* – Cepahlosporin antibiotics can be modified to inhibit human leukocyte elastase. *Nature*, 1986, 322, 192–194.
131. Morris SM, Kagan HM, Stone PJ, Snider GL, Albright JT. – Ultrastructural changes in hamster lung 15 min to 3 hr after exposure to pancreatic elastase. *Anat Rec*, 1986, 215, 134–143.
132. Saldiva PH, Delmonte VC, De Carvahlo CR, Kairalla RA, Auler-Junior JO. – Histochemical evaluation of lung collagen content in acute and chronic interstitial diseases. *Chest*, 1989, 95, 953–957.
133. Eisenberg H. – The interstitial lung diseases associated with the collagen-vascular disorders. *Clin Chest Med*, 1982, 3, 565–578.
134. Harmon KR, Leatherman JW. – Respiratory manifestations of connective tissue disease. *Semin Respir Infect*, 1988, 3, 258–273.
135. Kirk JM, Da CPE, Turner WM, Littleton RJ, Laurent GJ. – Biochemical evidence for an increased and progressive deposition of collagen in lungs of patients with pulmonary fibrosis. *Clin Sci*, 1986, 70, 39–45.
136. Selman M, Montano M, Ramos C, Chapela R. – Concentration, biosynthesis and degradation of collagen in idiopathic pulmonary fibrosis. *Thorax*, 1986, 41, 355–359.

137. Laurent GJ, McAnulty RJ. – Protein metabolism during bleomycin-induced pulmonary fibrosis in rabbits. *In vivo* evidence for collagen accumulation because of increased synthesis and decreased degradation of the newly synthesized collagen. *Am Rev Respir Dis*, 1983, 128, 82–88.
138. Laurent GJ. – Biochemical pathways leading to collagen deposition in pulmonary fibrosis. *Ciba Found Symp*, 1985, 114, 222–233.
139. Last JA. – Changes in the collagen pathway in fibrosis. *Fundam Appl Toxicol*, 1985, 5, 210–218.
140. Bray BA, Osman M, Ashtyani H, Mandl I, Turino GM. – The fibronectin content of canine lungs is increased in bleomycin-induced fibrosis. *Exp Mol Pathol*, 1986, 44, 353–363.
141. Cantor JO, Osman M, Keller S, Cerreta JM, Mandl I, Turino GM. – Measurement of cross-linked elastin synthesis in bleomycin-induced pulmonary fibrosis using a highly sensitive assay for desmosine and isodesmosine. *J Lab Clin Med*, 1984, 103, 384–392.
142. Eskenasy A. – Morphogenesis and pathogenesis of chronic lung diseases. XIX. Hyperelastosis *versus* elastolysis in chronic pulmonary pathological processes. *Morphol Embryol (Bucur)*, 1982, 28, 225–235.
143. Kirk JM, Heard BE, Kerr I, Turner WM, Laurent GJ. – Quantitation of types I and III collagen in biopsy lung samples from patients with cryptogenic fibrosing alveolitis. *Coll Relat Res*, 1984, 4, 169–182.
144. Nerlich AG, Nerlich ML, Muller PK. – Pattern of collagen types and molecular structure of collagen in acute post-traumatic pulmonary fibrosis. *Thorax*, 1987, 42, 863–869.
145. McDonald JA, Broekelmann TJ, Matheke ML, Crouch E, Koo M, Kuhn C III. – A monoclonal antibody to the carboxyterminal domain of procollagen I visualizes collagen-synthesizing fibroblasts. Detection of an altered fibroblast phenotype in lungs of patients with pulmonary fibrosis. *J Clin Invest*, 1986, 78, 1237–1244.
146. Phan SH, Varani J, Smith D. – Rat lung fibroblast collagen metabolism in bleomycin-induced pulmonary fibrosis. *J Clin Invest*, 1985, 76, 241–247.
147. Russell SB, Trupin JS, Myers JC, Broquist AH, Smith JC, Myles ME, Russell JD. – Differential glucocorticoid regulation of collagen mRNAs in human dermal fibroblasts. Keloid-derived and fetal fibroblasts are refractory to down-regulation. *J Biol Chem*, 1989, 264, 13730–13735.
148. Agelli M, Wahl SM. – Cytokines and fibrosis. *Clin Exp Rheumatol*, 1986, 4, 379–388.
149. Martinet Y, Rom WN, Grotendorst GR, Martin GR, Crystal RG. – Exaggerated spontaneous release of platelet-derived growth factor by alveolar macrophages from patients with idiopathic pulmonary fibrosis. *N Engl J Med*, 1987, 317, 202–209.
150. Risteli J, Niemi S, Trivedi P, Maentausta O, Mowat AP, Risteli L. – Rapid equilibrium radioimmunoassay for the amino-terminal propeptide of human type III procollagen. *Clin Chem*, 1988, 34, 715–718.
151. Kirk JM, Bateman ED, Haslam PL, Laurent GJ, Turner WM. – Serum type III procollagen peptide concentration in cryptogenic fibrosing alveolitis and its clinical relevance. *Thorax*. 1984, 39, 726–732.
152. Low RB, Cutroneo KR, Davis GS, Giancola MS. – Lavage type III procollagen N-terminal peptides in human pulmonary fibrosis and sarcoidosis. *Lab Invest*, 1983, 48, 755–759.
153. Bjermer L, Lundgren R, Hallgren R. – Hyaluronan and type III procollagen peptide concentrations in bronchoalveolar lavage fluid in idiopathic pulmonary fibrosis. *Thorax*, 1989, 44, 126–131.
154. Ammitzbll T, Pedersen SS, Espersen F, Schiler H. – Excretion of urinary collagen metabolites correlates to severity of pulmonary disease in cystic fibrosis. *Acta Paediatr Scand*, 1988, 77, 842–846.
155. Bentsen KD, Hrslev PK, Junker P. – Connective tissue metabolites in tissue fluids. New possibilities for monitoring the fibrotic process. *Ugeskr Laeger*, 1986, 148, 2007–2011.
156. Stenmark KR, Orton EC, Reeves JT, Voelkel NF, Crouch EC, Parks WC, Mecham RP. – Vascular remodeling in neonatal pulmonary hypertension. Role of the smooth muscle cell. *Chest*, 1988, 93, 127s–133s.
157. Rennard SI, Bitterman PB, Ozaki T, Rom WN, Crystal RG. – Colchicine suppresses the release of fibroblast growth factors from alveolar macrophages *in vitro*. The basis of a possible therapeutic approach to the fibrotic disorders. *Am Rev Respir Dis*, 1988, 137, 181–185.
158. Schwartz DA, Rosenstock L, Clark JG. – Monocyte-derived growth factors in asbestos-induced interstitial fibrosis. *Environ Res*, 1989, 49 (2), 283–294.
159. Weissler JC. – Idiopathic pulmonary fibrosis: cellular and molecular pathogenesis. *Am J Med Sci*, 1989, 297, 91–104.
160. Dzau V, Gibbons GH. – Cell biology of vascular hypertrophy in systemic hypertension. *Am J Cardiol*, 1988, 62, 30G–35G.
161. Chobanian AV, Prescott MF, Haudenschild CC. – Recent advances in molecular pathology. The effects of hypertension on the arterial wall. *Exp Molec Pathol*, 1984, 41, 153–169.
162. Wolinsky H. – Response of the rat aortic media to hypertension, morphological and chemical studies. *Circ Res*, 1970, 26, 507–522.
163. Meyrick B, Reid L. – Pulmonary hypertension. Anatomic and physiologic correlates. *Clinics in Chest Med*, 1983, 4, 199–217.
164. Brecher P, Chan CT, Franzblau C, Faris B, Chobanian AV – Effects of hypertension and its reversal on aortic metabolism in the rat. *Circ Res*, 1978, 43, 561–569.
165. Gerrity RB. – The role of the monocyte in atherogenesis. Migration of foam cells from atherosclerotic lesions. *Am J Pathol*, 1981, 103, 191–200.
166. Campbell JH, Campbell GR. – Endothelial cell influences on vascular smooth muscle phenotype. *Annu Rev Physiol*, 1986, 48, 295–306.
167. Glagov S. – Hemodynamic risk factors, mechanical stress, mural architecture, medial nutrition, and the vulnerability of arteries to atherosclerosis. *In*: The Pathogenesis of Atherosclerosis. R. Wissler, J. Geer eds, Williams and Wilkins, Baltimore, 1972, pp. 164–199.
168. Olivetti G, Anversa P, Melissari M, Loud A. – Morphometry of medial hypertrophy in the rat thoracic aorta. *Lab Invest*, 1980, 42, 559–565.
169. Olivetti G, Melissari M, Marchetti G, Anversa P. – Quantitative structural changes of the rat thoracic aorta in early spontaneous hypertension. Tissue composition and hypertrophy and hyperplasia of smooth muscle cells. *Circ Res*, 1982, 51, 19–26.
170. Haudenschild CC, Grunwald J, Chobanian A. – Effects of hypertension on migration and proliferation of smooth muscle in culture. *Hypertension*, 1985, 7, 1101–1104.
171. Mecham RP, Whitehouse LA, Wrenn DS, Parks WC, Griffin GL, Senior RM, Crouch EC, Stenmark KR, Voelkel NF. – Smooth muscle-mediated connective tissue remodeling in pulmonary hypertension. *Science*, 1987, 237, 423–426.
172. Keeley FW, Johnson DJ. – The effect of developing hypertension on the synthesis and accumulation of elastin in the aorta. *J Biochem Cell Biol*, 1986, 64, 38–43

173. Cleary EG, Moont M. – Hypertension in weanling rabbits. *Adv Exp Med Biol*, 1976, 79, 477–490.
174. Todorovitch-Hunter L, Johnson DJ, Ranger P, Keeley FW, Rabinovitch M. – Altered elastin and collagen synthesis associated with progressive pulmonary hypertension induced by monocrotaline. *Lab Invest*, 1988, 58, 184–195.
175. Walker LN, Bowen-Pope DF, Ross R, Reidy MA. – Production of platelet-derived growth factor-like molecules by cultured smooth muscle cells accompany proliferation after arterial injury. *Proc Natl Acad Sci USA*, 1986, 83, 7311–7315.
176. Folkow B. – Physiological aspects of primary hypertension. *Physiol Rev*, 1982, 62, 348–504.
177. Owens GK, Reidy MA. – Hyperplastic growth response of vascular smooth muscle cells following the induction of acute hypertension in rats by aortic coarctation. *Circ Res*, 1985, 57, 695–701.
178. Boyd CD, Kniep AC, Pierce RA, Deak SB, Karboski C, Miller DC, Parker MI, Mackenzie JW, Rosenbloom J, Scott GE. – Increased elastin mRNA levels associated with surgically induced intimal injury. *Conn Tiss Res*, 1988, 18, 65–78.
179. Langielle BL, O'Donnell F. – Reductions in arterial diameter produced by chronic decreases in blood flow are endothelium-dependent. *Science*, 1986, 231, 405–407.
180. Schwartz SM, Ross R – Cellular proliferation in atherosclerosis and hypertension. *Prog Cardiovasc Dis*, 1984, 26, 355–372.
181. Majack RA, Cook SC, Bornstein P. – Control of smooth muscle growth by components of the extracellular matrix, autocrine role for thrombospondin. *Proc Nat Acad Sci USA*, 1986, 83, 9050–9054.
182. Keeley FW, Todorovitch L, Rabinovitch M. – Elastin and elastases in the pathology of the arterial wall. *In:* Elastin and Elastases, L. Robert, L. Hornebeck eds, Vol. 2, CRC Press, Boca Raton, 1989, pp. 169–184.
183. Helin P, Lorenzen I, Garbarsch C, Mathiessen ME. – Repair in arterial tissue morphological and biochemical changes after a single dilatation injury. *Circ Res*, 1971, 29, 542–545.
184. Sottiurai VS, Kollros P, Glagov S, Zarins CK, Mathews MB. – Morphologic alteration of cultured arterial smooth muscle cells by cyclic stretching. *J Surg Res*, 1983, 35, 490–497.
185. Vandenburgh H, Kaufman S. – *In vitro* model for stretch-induced hypertrophy of skeletal muscle. *Science*, 1979, 203, 265–268.
186. Leung DYM, Glagov S, Mathews MB. – Cyclic stretching stimulates synthesis of matrix components by arterial cells *in vitro*. *Science*, 1976, 191, 475–477.
187. Kollros PR, Bates SR, Mathews MB, Horwitz AL, Glagov S. – Cyclic AMP inhibits increased collagen production by cyclically stretched smooth muscle cells. *Lab Invest*, 1987, 56, 410–417.
188. Sumpio BE, Banes AJ, Link WG, Johnson G Jr. – Enhanced collagen production by smooth muscle cells during repetitive mechanical stretching. *Arch Surg*, 1988, 123, 1233–1236.
189. Sutcliffe MS, Davidson JM. – Effect of static stretching on elastin production by porcine aortic smooth muscle cells. *Matrix*, 1990, 10, 148, 153.
190. Tozzi CA, Poiani GJ, Harangozo AM, Boyd CJ, Riley DJ. – Pulmonary vascular endothelial cells modulate stretch-induced DNA and connective tissue synthesis in rat pulmonary artery segments. *Chest*, 1988, 93, 169S–170S.
191. Keeley FW, Alatawi A, Cho A. – The effect of wall stress on the production and accumulation of vascular elastin. *In*: Elastin, Chemical and Biological Aspects. A.M. Tamburro, J.M. Davidson eds, Congedo Editore, Galatine, 1990, pp. 341–356.
192. Barrett TB, Sampson P, Owens GK, Schwartz SM, Benditt EP. – Polyploid nuclei in human artery wall smooth muscle cells. *Proc Natl Acad Sci USA*, 1983, 80, 882–885.
193. Chobanian AV, Lichtenstein AH, Schwartz JH, Hanspal J, Brecher P. – Effects of deoxycorticosterone/salt hypertension on cell ploidy in rat aortic smooth muscle cells. *Circulation*. 1987, 75, 1102–1106.
194. Rosen EM, Goldberg ID, Shapiro HM, Zoller LC, Myrick KV, Levenson SE, Halpin PA. – Growth kinetics as a function of ploidy in diploid, tetraploid, and octaploid smooth muscle cells derived from normal rat aorta. *J Cell Physiol*, 1985, 125, 512–520.
195. Perkett EA, Sutcliffe MS., Davidson JM. – Unpublished observations.
196. Sporn MB, Roberts AB, Wakefield LM, Decrombrugghe BD. – Some recent advances in the chemistry and biology of transforming growth factor-beta. *J Cell Biol*, 1987, 105, 1039–1045.
197. Foster J, Rich CB, Florini JR. – Insulin-like growth factor, somatomedin C, induces the synthesis of tropoelastin in aortic tissue. *Coll Rel Res*, 1987, 7, 161–169.
198. Badesch DB, Lee PD, Parks WC, Stenmark KR. – Insulin-like growth factor I stimulates elastin synthesis by bovine pulmonary arterial smooth muscle cells. *Biochem Biophys Res Commun*, 1989, 160, 382–387.
199. Oikarinen AI, Uitto J, Oikarinen J. – Glucocorticoid action on connective tissue, from molecular mechanisms to clinical practice. *Med Biol*, 1986, 64, 221–230.
200. Ross R, Raines EW, Bowen-Pope DF. – The biology of platelet derived growth factor. *Cell*, 1986, 46, 155–169.
201. Fabisiak JP, Evans JN, Kelley J. – Increased expression of PDGF-B(c-sis) mRNA in rat lung precedes DNA synthesis and tissue repair during chronic hyperoxia. *Am J Respir Cell Mol Biol*, 1989, 1, 181–189.
202. Pierce GF, Mustoe TA, Lingelbach J, Masakowski VR, Griffin GL, Senior RM, Deuel TF. – Platelet-derived growth factor and transforming growth factor-beta enhance tissue repair activities by unique mechanisms. *J Cell Biol*, 1989, 109, 429–440.
203. Sarzani R, Brecher P, Chobanian AV. – Growth factor expression in aorta of normotensive and hypertensive rats. *J Clin Invest*, 1989, 83, 1404–1408.
204. Yamauchi K, Martinet Y, Basset P, Fells GS, Crystal RG. – High levels of transforming growth factor-β are present in the epithelial lining fluid of the normal human lower respiratory tract. *Am Rev Respir Dis*, 1988, 137, 1360–1363.
205. Roberts AB, Flanders KC, Kondaiah P, Thompson NI, Van Obberghen-schilling E, Wakefield L, Rossi P, Decrombrugghe B, Heine U, Sporn MB. – Transforming growth factor beta, biochemistry and roles in embryogenesis, tissue repair and remodeling, and carcinogenesis. *Recent Prog Horm Res*, 1988, 44, 157–197.
206. Broadley KN, Aquino AM, Hicks B, Ditesheim JA, McGee GS, Demetriou AA, Woodward SC, Davidson JM. – The diabetic rat as an impaired wound healing, stimulatory effects of transforming growth factor-beta and basic fibroblast growth factor. *Biotech Therap*, 1989, 1, 55–68
207. Owens GK, Geisterfer AT, Yang YW-H, Komoriya A. – Transforming growth factor-beta-induced growth inhibition and

cellular hypertrophy in cultured vascular smooth muscle cells. *J Cell Biol*, 1988, 107, 771–780.
208. Majack RA. – Beta-type transforming growth factor specifies organization behavior in vascular smooth muscle cell cultures. *J Cell Biol*, 1987, 105, 465–471.
209. Perkett EA, Lyons RM, Moses HL, Brigham KL, Meyrick B. – Transforming growth factor-β activity in sheep lung lymph during development of pulmonary hypertension. 1989, Submitted for publication.
210. Massague J, Cheifetz S, Ignotz RA, Boyd FT. – Multiple type-beta transforming growth factors and their receptors. *J Cell Physiol*, 1987, 5 (Suppl;), 43–47.
211. Rossi P, Karsenty G, Roberts AB, Roche NS, Sporn MB De Crombrugghe B. – A nuclear factor 1 binding site mediates the transcriptional activation of a type I collagen promoter by transforming growth factor-beta. *Cell*, 1988, 52, 405–414.
212. Raghow R, Postlethwaite AE, Keski-Oja J, Moses HL, Kang AH. – Transforming growth factor-beta increases steady state levels of type I procollagen and fibronectin messenger RNAs posttranscriptionally in cultured human dermal fibroblasts. *J Clin Invest*, 1987, 79, 1285–1288.
213. Penttinen RP, Kobayashi S, Bornstein P. – Transforming growth factor β increases mRNA for matrix proteins both in the presence and in the absence of changes in mRNA stability, *Proc Natl Acad Sci USA*, 1988, 85, 1105–1108.
214. Oikarinen J, Kivirikko KI. – Synthesis and degradation of type I procollagen mRNAs in cultured human fibroblasts and the effects of cortisol. *J Biol Chem*, 1985, 260, 720–725.
215. Kirber MJ, Walsh JV Jr, Singer JJ. – Stretch-activated ion channels in smooth muscle: a mechanism for the initiation of stretch-induced contraction. *Pflugers Arch*, 1988, 412, 339–345.
216. Binderman I, Zor U, Kaye AM, Shimshoni Z, Harrell A, Somjen D. – The transduction of mechanical force into biochemical events in bone cells may involve activation of phospholipase A2. *Calcif Tiss Int*, 1988, 42, 261–266.
217. Civitelli R, Hruska KA, Jeffrey JJ, Kahn AJ, Avioli LV, Partridge NC. – Second messenger signaling in the regulation of collagenase production by osteogenic sarcoma cells. *Endocrinology*, 1989, 124, 2928–2934.
218. Quaglino D Jr, Nanney LB, Kennedy R, Davidson JM. – Localized effects of transforming growth factor-beta on extracellular matrix gene expression during wound healing. I. Excisional wound model. *Lab Invest*, 1990, 67, 307–319.
219. Davidson JM, Berg RA. – Posttransitional events in collagen biosynthesis. *In:* Methods in Cell Biology. A. Hand, C. Oliver eds, Academic Press, New York, 1981, pp. 119–136.
220. Mecham RP, Levy, BD, Morris SL, Madaras JG, Wrenn DS. – Increased cyclic GMP levels lead to a stimulation of elastin production in ligament fibroblasts that is reversed by cyclic AMP. *J Biol Chem*, 1985, 260, 3255–3258.
221. Risteli L, Risteli J. – Analysis of extracellular matrix peptides in biological fluids. *Meth Enzymol*, 1987, 145, 391–411.
222. King GS, Starcher BC, Kuhn CT. – The measurement of elastin turnover by the radioimmunoassay of urinary desmosine excretion. *Bull Eur Physiopathol Respir*, 1980, 16 (Suppl.), 61–64
223. Janoff A, Chanana AD, Joel DD, Susskind H, Laurent P, Yu SY, Dearing I. – Evaluation of the urinary desmosine radioimmunoassay as a monitor of lung injury after endobronchial elastase instillation in sheep. *Am Rev Respir Dis*, 1983, 128, 545–551.
224. Pelham F, Wewers M, Crystal R, Buist AS, Janoff A. – Urinary excretion of desmosine (elastin cross-links) in subjects with PiZZ alpha-1-antitrypsin deficiency, a phenotype associated with hereditary predisposition to pulmonary emphysema. *Am Rev Respir Dis*, 1985, 132, 821–823
225. Perkett EA, Davidson JM, Curtis-Atchley P, Sutclifffe ML, Brigham KL, Meyrick B. – Lung lymph elastin concentration is increased in sheep with pulmonary hypertension secondary to air embolization. *Am Rev Respir Dis*, 1989, 139, A174.
226. Lkiw R, Todorivitch-Hunter L, Maruyama K, Shin J, Rabinovitch M. – SC-39026, a serine elastase inhibitor, prevents muscularization of peripheral arteries, suggesting a mechanism of monocrotaline-induced pulmonary hypertension in rats. *Circ Res*, 1989, 64, 814–825.
227. Schriver EE, Bernard GR, Swindell BB, Sutcliffe MS, Davidson JM. – Elastin fragment levels in human plasma, urine, and bronchoalveolar lavage fluid (BALF). *Chest*, 1989, 96, 153S.

Revue générale. Biochimie et rotation de l'interstitium pulmonaire. J.M. Davidson.
RÉSUMÉ: Le poumon contient une quantité de composantes de la matrice extra-cellulaire qui comportent les éléments de support et d'adhésion des voies aériennes de conduction, des alvéoles et de l'arbre vasculaire. Alors qu'aucun de ces composants n'est spécifique au poumon, leur distribution particulière détermine l'architecture et la fonction de cet organe d'échanges gazeux. Les cellules et les tissus du poumon interagissent avec la matrice par une variété de récepteurs de surface, en particulier les intégrines et les molécules adhésives, dont cetaines peuvent jouer des rôles importants dans l'agression et la réparation pulmoniare. Le collagène de type I est le déterminant prédominant de la force de tension; mais pas moins de 11 autres types génétiques de collagène, avec des fonctions d'adhésion et de connection spécialisées peuvent être observés dans différentes structures pulmonaires, y compris le cartilage et les membranes basales. Une accumulation excessive de matrice dans le poumon est le résultat d'une série complexe d'influences sur la régulation des gènes, dont une partie peut être due à la présence de cytokines inflammatoires qui stimulent directment la synthèse de la matrice. Toutefois, la dégradation et la rotation de la matrice sont également des processus critiques influencés par beaucoup des mêmes médiateurs. La collagènase et la gèlatinase (collagènase de type IV) sont des métallo-enzymes étroitement réglés qui, en accord avec un ensemble d'inhibiteurs spécifiques des métallo-protéinases, déterminent l'abondance nette et la distribution du collagène. Les élastases de différents type biochimiques sont de même étroitement réglés par les inhibiteurs des protéinases. L'élastine est essentielle à la fonction pulmonaire, au niveau de la capacité de rétraction et d'ouverture de la paroi alvéolaire; la perte de l'élastine dans l'emphysème semble due à une dégradation non contrôlée du type de fibres élastiques embryologiquement déterminées, accompagnée par un remplacement non fonctionnel comme réponse à l'agression. L'agression de l'endothélium vasculaire du poumon, ainsi que les autres agressions physiologiques qui élévant la pression sanguine pulmoniare, peuvent entraîner une accumulation excessive de collagène et

d'élastine dans les artères de conductance et de résistance de la circulation pulmonaire. Le stress méchanique et l'agression endothéliale peuvent médier l'hypertrophie de la média de ces vaisseaux. Les composantes de la matrice extra-cellulaire sont impliquées de façon critique à presque chaque stade de la biologie pulmonaire: développement, fonction normale, et états pathologiques aigus et chroniques. A ce jour, seuls les glucocorticoïdes, inhibiteurs "crosslinking" et les inhibiteurs des protéases ont été utilisés avec l'intention générale de supprimer, soit une accumulation, soit une perte excessive, de matrice. Une compréhension plus détaillée de la régulation et des interactions spécifiques des composantes de la matrice sont essentielles pour l'analyse des états pathologiques et pour le développement de stratégies thérapeutiques appropriées.

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