HHS Public Access

Author manuscript

*Matrix Biol*. Author manuscript; available in PMC 2019 November 01.

Published in final edited form as:

*Matrix Biol*. 2018 November ; 73: 122–134. doi:10.1016/j.matbio.2018.04.003.

# Pulmonary immunity and extracellular matrix interactions

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## Abstract

The lung harbors a complex immune system composed of both innate and adaptive immune cells. Recognition of infection and injury by receptors on lung innate immune cells is crucial for generation of antigen-specific responses by adaptive immune cells. The extracellular matrix of the lung, comprising the interstitium and basement membrane, plays a key role in the regulation of these immune systems. The matrix consists of several hundred assembled proteins that interact to form a bioactive scaffold. This template, modified by enzymes, acts to facilitate cell function and differentiation and changes dynamically with age and lung disease. Herein, we explore relationships between innate and adaptive immunity and the lung extracellular matrix. We discuss the interactions between extracellular matrix proteins, including glycosaminoglycans, with prominent effects on innate immune signaling effectors such as toll-like receptors. We describe the relationship of extracellular matrix proteins with adaptive immunity and leukocyte migration to sites of injury within the lung. Further study of these interactions will lead to greater knowledge of the role of matrix biology in lung immunity. The development of novel therapies for acute and chronic lung disease is dependent on a comprehensive understanding of these complex matrix-immunity interactions.

## Introduction

The lungs have a critical primary function in the human body through the exchange of oxygen and carbon dioxide. The maintenance of this physiological function is dependent on lung architecture, elastic recoil and mechanical stability, all related to the pulmonary extracellular matrix (ECM) [1]. However, air not only contains oxygen but also particulate matter, pollutants, and a variety of microbes, all of which may pose a significant threat to human health. Indeed, the human lung is exposed to a high level of exogenous threats through the inhalation of several thousand liters of air everyday [2].

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A complex and highly effective lung immune system has evolved with mechanical barriers as well as both an initial first line of defense, termed innate immunity, and a second more specific adaptive immune system. These defenses include several barriers such as mucus, cilia and the mucociliary escalator, as well as proteins (e.g. collectins and defensins) with antimicrobial effects in the airway. Further physical barriers known to play key roles in immunity include the alveolar epithelium and vascular endothelium of the lung. The lung harbors a plethora of innate and adaptive immune cells, including alveolar macrophages, dendritic cells and circulating monocytes and lymphocytes, as well as a respiratory epithelium with major immune functions [3, 4]. The innate system also regulates features of the adaptive system including the activation of antigen specific immune responses in host defense [5]. The ECM of the lung plays a key role in the establishment and regulation of lung immunity. Plants and invertebrates manage solely with innate defense mechanisms [although some invertebrates have adaptive systems [6]]. The divergence of the vertebrate genome lineage from invertebrate heralded an expansion in ECM related genes [7]. The development of a complex adaptive immunity is postulated to have occurred after this expansion of ECM related genes [8]. Therefore, our complex human immune system has evolved through an intricate relationship with our tissue matrix.

In this review, we discuss the interaction between components of the pulmonary ECM and innate and adaptive lung immunity. We highlight known key interactions between innate immune receptors and molecules derived from the ECM, and the influence these interactions have on local inflammation, through cytokine/chemokine expression, and leukocyte recruitment using examples from both studies of systemic and pulmonary disease. We also discuss the role of ECM molecules in modulating the function of the adaptive immune system of the lung. This review emphasizes the important updates in recent years on the ECM's role in pulmonary immunity but also recognizes the considerable progress still required to decipher these ECM-immunity interactions and their implications for disease mechanisms within the lung.

## The ECM of the lung in health and disease

Our knowledge of ECM-immunity interactions is evolving. The composition of the pulmonary ECM had proven difficult to elucidate until recent progress in mass spectrometry and quantification algorithms [9, 10]. We know now that the ECM of the human lung consists of at least 150 different core structural proteins within 2 structures, the basement membrane and interstitial matrix [11]. The ECM proteins also engage with various enzymes, growth factors and other associated proteins which are non-structural.

In health, the interstitial matrix of the lung is a loose connective tissue meshwork comprised of both structural and non-structural content. Collagen constitutes the core protein; the composition of collagen in the lung is abnormally increased in several forms of lung disease [12]. These fibrillar collagens include several types (I, II, III, V, XI) and have considerable tensile strengths. The interstitial matrix also contains elastic fibers formed from a core of crosslinked elastin and an outer layer of microfibrils. Other components include fibrillins, glycoproteins (e.g. fibronectin), proteoglycans (e.g. versican) and glycosaminoglycans or GAGs (e.g. hyaluronan) [13]. A plethora of non-structural proteins, growth factors and

cytokines are harbored within the interstitial matrix of the lung. Within the alveoli, the interstitial matrix is composed mainly of type I and type III collagen and elastin, with several other ECM proteins interspersed in the collagen and elastic fibers. The main cell types lining the alveolar spaces include type 1 and type 2 pneumocytes (see Fig. 1). This meshwork of ECM proteins and reservoir of bioactive molecules is built around a range of cell types including fibroblasts, pericytes, epithelial cells and resident leukocytes. The pulmonary blood vessels, capillary network and lymphatics further expose the matrix to large volumes of circulating leukocytes. The meshwork of the interstitial matrix is anchored to the basement membrane layer of the epithelium and endothelium in the alveoli, and is a relatively thin organized layer. Components of the basement membrane include type IV collagen, laminin, nidogen/entactin, and perlecan. While the matrix is minimal within the normal lung parenchyma, disease of the lung often results in gross abnormality with the aberrant deposition of ECM components that leads to significant physiological malfunction (See Fig. 1). In recent years, many interactions between specific components of pulmonary immunity and the lung ECM have been identified [14]. Matrix components such as hyaluronan and versican can act as ligands for innate receptors on resident and recruited leukocytes to regulate injury and inflammation, thereby promoting acute and chronic lung injury. To date most work describing ECM-immunity interactions has focused on interstitial matrix proteins and other components including proteoglycans; thus, this will be our focus. We do not describe in detail the role of the basement membrane components in regulating pulmonary immunity in this review; however, it should be noted that components of the basement membrane can become targets of immune responses to cause important lung diseases. For example, an autoimmune antibody response against type IV collagen α3 causes Goodpasture's Syndrome[15]. Another example is the ability of type IV collagen and laminin to cause lymphocyte activation in Scleroderma patients[16].

## The pulmonary innate immune system and the ECM of the lung

A family of proteins termed pathogen recognition receptors (PRRs) can be expressed both by hematopoietic and non-hematopoietic cells and are important sensors to trigger innate immunity. These proteins function to sense and initiate a first line host defense response to invading pathogens and initial injury [17]. PRRs are germline encoded and at this time, the family of PRRs includes Toll-like receptors (TLRs), nucleotide-binding oligomerization domain receptors (NOD-like receptors or NLRs), C-type lectin receptors, retinoic acid inducible gene 1(RIG-1)-like receptors (RLRs) and cytosolic DNA receptors [18]. PRRs recognize highly conserved molecular motifs derived from microbes (pathogen associated molecular patterns – PAMPs). PAMPs include a broad range of microorganism-derived molecular patterns that are invariant, difficult to alter and vital for microbial physiology and lifecycle. They include lipopolysaccharide (LPS), peptidoglycan, flagellin and nucleic acids such as ssRNA, dsRNA, and CpG DNA. The full scope of engagement between PRRs and microbial ligands has not yet been fully determined. We now know that the innate immune response is not simply limited to the recognition of invasive pathogens but also acts in the sensing of danger signals derived from endogenous molecules. These molecules arise from cell death, stress and/or injury, and are so called damage associated molecular patterns (DAMPs). One of the first studies to denote immunostimulatory properties for endogenous

mammalian derived products examined the effect of heat shock protein 60 (hsp60), a chaperone protein involved in protein synthesis. Treatment of human and murine macrophages with endogenous hsp60 resulted in the generation of pro-inflammatory cytokines [19]. Further studies have confirmed these findings and characterized interactions between DAMPs and innate immune receptors in disease (and are reviewed elsewhere [20, 21]). The ECM provides a reservoir of potential DAMPs for the innate immune system. These molecules are sequestered in the ECM in normal physiological conditions where interactions with PRR expressing innate immune cells are limited. However, changes to normal homeostasis that occur in disease, injury and inflammation alter these conditions, promote the recruitment of leukocytes into the matrix and facilitate engagements between ECM DAMPs and PRR-expressing leukocytes. In the next section, we describe relevant components of innate immunity in the lung.

## Innate Immunity and the key role of PRRs

TLRs are a family of transmembrane proteins that play a key role in shaping the innate immune response to acute lung injury and inflammation. The receptor family includes TLR1-13 in mammals. First discovered in 1994 by Nomura *et al*, the name "toll" was derived from the protein's similarity to the "toll" protein in the fruit fly (*Drosophila melanogaster*) [22, 23]. TLRs1-11 are conserved in humans and mice [24]. TLR3, TLR7, TLR8, TLR9, TLR11, TLR12 and TLR13 are expressed in intracellular compartments. TLR1, TLR2, TLR4, TLR5, TLR6 are all expressed at the cellular surface where they interact with different categories of ligands compared to intracellular receptors (see Fig. 2). TLR4 can demonstrate both cell surface and intracellular expression which allows alterations in receptor-ligand interactions and downstream signaling targets [25, 26]. There is an extensive range of TLR-PAMP/DAMP interactions studied to date (Fig. 2). Adaptor molecules, co-receptors and co-factors that interact with or modulate a specific subset of TLRs are reviewed elsewhere [27, 28]. TLR ligation ultimately results in translocation of transcription factors to the nucleus with subsequent changes in inflammatory profiles at cellular and tissue levels.

In addition, several other PRRs exist including NLRs and the RNA-helicase-family of proteins which includes retinoic acid inducible gene 1 (RIG-1) and melanoma differentiation-associated gene 5 (MDA5) [29, 30]. NOD signaling is implicated in a number of pulmonary disorders including asthma and the systemic granulomatous disease, early onset sarcoidosis [31–33]. The cytokines elicited by innate receptor activation are also known to tune the resultant adaptive immune response.

As a biologically active scaffold the ECM has a key role to play in a wide variety of biological processes including host defense and tissue repair. Small leucine-rich proteoglycans (SLRPs) can exist in a sequestered form bound within the tissue matrix serving as master regulators of ECM assembly in general by binding collagen type I, II, III [34] and type VI [35]. They act to inactivate or in some cases localize certain biological processes by binding and sequestering molecules such as growth factors (e.g. biglycan, decorin and fibromodulin can all bind transforming growth factor (TGF)β within the matrix), thus limiting the abilities of these sequestered growth factors to interact with

molecular targets [36, 37]. SLRPs may also exist in a soluble form in which they may display affinities for a wide variety of receptors [38–41]. SLRPs consist of a small protein core and tandem leucine-rich repeats (LRRs) flanked by cysteine rich clusters [42, 43]. Importantly, alterations in the composition of the ECM proteins including SLRPs but also other proteoglycans may arise during injury and inflammation and can result in activation of PRRs. ECM proteins have been implicated in the pathogenesis of pulmonary disorders including asthma, chronic pulmonary obstructive disease, pulmonary fibrosis, sepsis and acute lung injury [44–48]. Next, we discuss the current evidence supporting the key role innate immunity occupies in this context using examples of matrix components including proteoglycans, glycoproteins and GAGs as innate "danger" signals (see Fig. 2).

## PRRs and Proteoglycans

Proteoglycans are composed of GAGs covalently linked to specific core proteins, and are major ECM components of most tissues including the lung. They form the hydrated gel in which other core components of the ECM (i.e collagen, elastin fibers) are embedded [49]. Proteoglycans are involved in the maintenance of the mechanical stability of the collagen-elastin network. Examples of proteoglycans include versican, biglycan and decorin but there are many proteoglycans within the lung and other tissues.

Versican is a chondroitin sulfate proteoglycan that exists in several different isoforms and plays a role in matrix assembly, tissue hydration and collagen fibrillation. While other proteoglycans contain LRRs, and interactions with LRR-containing TLRs may be expected, versican does not possess LRR motifs. Studies have shown that versican may act through ligation with TLR2/TLR6 heterodimers and the CD14 co-receptor on myeloid cells with downstream TNF-α and IL-6 production [50]. Versican is synthesized and released in inflammatory disorders by activated macrophages, where it may act as an endogenous ligand for TLRs expressed on the surface of innate immune cells [51]. Versican is also known to bind and interact with hyaluronan, and since hyaluronan can bind to the cell surface of leukocytes and smooth muscle cells, this interaction can lead to cellular adhesion during inflammation (e.g. in vascular walls) [52]. Furthermore, treatment with Poly(I:C), a synthetic TLR3 ligand, has previously been shown *in vivo* to stimulate hyaluronan and versican expression in lung fibroblasts[53]. Subsequent studies determined that versican was responsible for mediating inflammatory responses through the generation of hyaluronan-containing ECM cable structures that provided a substrate for monocytic cell infiltration into the inflamed lung [54]. Versican$^{-/-}$ mice had markedly reduced inflammatory cell numbers in bronchoalveolar fluid (BALF), reduced inflammatory cytokine expression and an absence of these hyaluronan-enriched ECM cable structures. Therefore, the evidence supports a key role for versican in regulating inflammatory signaling within the lung interstitial matrix through interactions with PRRs. Versican has been implicated in several forms of human lung disease. Versican deposition has been reported in hallmark fibrogenic lesions of idiopathic pulmonary fibrosis (IPF) patients [55]. Several studies have demonstrated a role for versican in pulmonary disorders and have been reviewed elsewhere [56].

Biglycan is a widely expressed proteoglycan that maps to the X chromosome [57]. It has an assembly role in the ECM, and disruption of the biglycan gene results in abnormal collagen

fibril morphology [58]. Degradation of the ECM by proteolytic enzymes occurs in injury and stress, releasing soluble biglycan which is then capable of engaging TLRs with resultant inflammatory signaling. When bound to ECM, biglycan is unable to ligate TLRs. Schaeffer *et al.* described a novel role for biglycan as an endogenous ligand for TLR2 and TLR4 when expressed on the surface of macrophages leading to activation of transcription factors and TNF-α release [48]. Biglycan$^{-/-}$ (null) mice demonstrate a survival benefit after LPS challenge as a result of reduced inflammatory cytokine production including TNF-α and the stimulatory properties of biglycan are abolished in TLR4$^{-/-}$/TLR2$^{-/-}$ murine macrophages [48]. Biglycan has an important role in IL-1β generation during the innate immune response as well [59]. In summary, biglycan plays a crucial role in orchestrating TLR2 and TLR4 innate responses to injury and the release of biglycan from the ECM and its *de novo* synthesis are key steps in this process. Biglycan has recently been identified as a potential biomarker of mortality in chronic obstructive pulmonary disease [60]. Higher levels of biglycan, as well as collagen and elastin were associated with a greater risk of mortality. The authors concluded that ECM turnover in the airways and lung parenchyma may be an important marker of disease activity in COPD. Greater biglycan deposition has also been reported in the smooth muscle layer of moderate and severe asthma patients over healthy control biopsy samples, suggesting that biglycan may play a role in the severity of airway hyper-responsiveness and asthma phenotype[44].

Decorin is a SLRP with a protein core consisting of 12 LRRs and one GAG side chain, similar in structure to biglycan[37]. As an ECM protein it fulfills two roles, one as a contributor to ECM structure and the other as a signaling molecule. Decorin displays multiple functions as a signaling molecule including its ability to interact with various tyrosine receptor kinases and low density lipoprotein receptor related protein [39, 61, 62]. Decorin has reported association with innate immune receptors, namely its ability to ligate TLR2 and TLR4 as an endogenous activator [63]. Decorin binds to TLR2 and TLR4 on macrophages with increased production of programed cell death 4 (PDCD4) with subsequent attenuated release of the anti-inflammatory cytokine IL-10 [63]. Thus, decorin may have important roles to play in regulating TLR-mediated inflammation in the lung, however specific lung decorin and innate immune receptor interactions have not been extensively studied. Decorin is present at attenuated levels in the peri-bronchiolar airways in patients with severe emphysema [64]. Fluticasone, an inhaled corticosteroid employed in the treatment of emphysema has been reported to induce decorin expression in airway fibroblasts from obstructive airway disease patients [65].

## PRRs and Glycoproteins

Tenascin-C is an ECM glycoprotein which is upregulated in injury and repair, and persistently upregulated in the chronic inflammatory environment [66]. In studies of transgenic mice, tenascin C$^{-/-}$ mice were protected from persistent erosive joint inflammation, through reduced TLR4-mediated pro-inflammatory cytokine signaling. The ligation of tenascin-C to TLR4 was independent of CD14, a TLR4 co-receptor [67]. This innate immune effect was postulated to occupy a key role in the persistent inflammation that is observed in Rheumatoid Arthritis, which can have pulmonary disease manifestations [67]. Furthermore, the activation of TLR4 by endogenous tenascin-C promotes TLR4-dependent

collagen gene expression and myofibroblast transformation, both of which are central components of progressive pulmonary fibrosis [68]. Transgenic mice lacking tenascin-C demonstrated attenuated skin and lung fibrosis in models of Systemic Sclerosis [68]. Estany *et al.* reported that primary pulmonary fibroblasts from idiopathic pulmonary fibrosis (IPF) patients produced higher levels of tenascin C [55]. In addition, tenascin C was found in fibroblastic foci in congregation with versican and fibronectin. Finally, in a deep proteomic study of fibrotic tissue from interstitial lung disease (ILD) patients, tenascin C was reported as significantly upregulated [69]. Although, not studied conclusively, tenascin C may play a key role as a danger signal within the lung, promoting chronic inflammation.

Fibronectin is a glycoprotein involved in cell matrix adhesions. It binds ECM proteins within the matrix such as collagen and others as well as binding to integrins. As a result of alternative splicing of its transcript, there are some 20 different variants of fibronectin in humans [70]. Fibronectin variants can initiate TLR4-dependent NFκB-dependent release of multiple inflammatory cytokines from fibroblasts [71]. The potential impact of TLR4-mediated signaling by danger signals derived from matrix, including fibronectin in pulmonary fibrosis and systemic sclerosis has been reviewed elsewhere [72].

## PRRs and GAGs

Hyaluronan is a high molecular weight non-sulfated GAG with a multitude of effects including the ability to ligate several different receptors, promoting various features of cell growth, adhesion and inflammation [73]. Interestingly, alterations in the molecular weight of hyaluronan regulate the anti- vs. pro-inflammatory innate immune signaling outcomes[74]. Hyaluronan is generated in lung injury, correlates with extent of injury and its clearance is crucial for resolution of inflammation [73]. Hyaluronan can be dismantled into low molecular weight (LMW) fragments during the inflammatory process by reactive oxygen species and hyaluronidase, subsequently engaging TLR2 to cause activation of the downstream NF-κB pathway [75]. Hyaluronan oligosaccharides, also generated during inflammation, have reported associations with TLR4 signaling in dendritic cells with downstream activation of transcription factors [76]. However, high molecular weight (HMW) hyaluronan may occupy a key anti-inflammatory role within the matrix. TLR2 signaling by LMW hyaluronan fragments can be inhibited by intact HMW hyaluronan [75]. HMW hyaluronan can serve to actively suppress inflammation through activation of regulatory CD4(+) CD25(+) T cells which in turn suppress effector T cells [77]. LMW hyaluronan molecules are unable to achieve this anti-inflammatory effect. Hyaluronan is an important mediator of the initial innate immune response and has known roles in acute and chronic lung disease. Higher levels of hyaluronan in sputum from COPD patients correlated with lower pulmonary function, higher neutrophil influx and inflammatory cytokine production [78]. Hyaluronan levels are elevated in patients with persistent asthma and primary airway fibroblasts from asthma patients generate higher baseline levels of hyaluronan than normal [79]. Furthermore, TNF-stimulated gene 6 (TSG-6) facilitates transfer of inter-α-inhibitor heavy chains to hyaluronan to facilitate hyaluronan receptor binding, and this pathologic hyaluronan-heavy chain matrix is necessary for the development of allergic airway responsiveness. In the presence of TSG-6, soluble hyaluronan or environmental agents such as ozone can induce Rho A, PI3-K/AKT and ERK

signaling in smooth muscle cells to promote airway hyper-responsiveness and TSG6−/− mice are protected from these outcomes[80].

In IPF, elevated levels of hyaluronan have been found in BAL fluid and lung tissue [81, 82]. Hyaluronan was found to be highly sulfated in areas of fibrotic lung bordering normal lung parenchyma. Furthermore, recent work has proposed a pivotal role for hyaluronan in the maintenance of alveolar homeostasis through the promotion of alveolar stem cell renewal [83]. Surfactant-protein-C (SPC) positive type 2 alveolar epithelial cell (AEC) renewal is regulated by interactions between TLR4 and hyaluronan. This results in the renewal of type 2 AECs and the prevention of severe pulmonary fibrosis in animal models [83]. Taken together, hyaluronan in its variable forms is an important regulator of the innate immune response to injury in the lung.

## ECM interactions in leukocyte migration

The elaboration of an effective innate and adaptive host defense is mediated in part via the release of chemotactic cytokines known as chemokines. With regards to the subject matter of this review, the composition of the ECM is crucially intertwined with the function of chemokines. Chemokines bind to GAGs present within the ECM to generate chemotactic gradients which leukocytes sense via their expression of corresponding chemokine receptors to navigate to sites of insult. In order to extravasate from vascular beds into most inflamed tissues, leukocytes use cell surface receptors including CD44, P-selectin glycoprotein ligand-1 (PSGL-1) and E selectin ligand-1 (ESL-1) to first bind P-selectin and/or E-selectin to initiate rolling or adherence on activated endothelium [84]. This leucocyte adhesion cascade, mediated by selectin and ligand interactions, results in progressive slowing of leukocytes, facilitating leukocytes ability to derive signals from chemokines at the endothelial surface. This chemokine signal activates integrins and firm adhesion of leukocytes occurs. Although not extensively studied, this may be the case for leukocyte migration within the bronchial vasculature, an extension of the systemic vasculature supplying a small proportion of the lung, in particular the large airways [85]. However, in contrast to the systemic circulation, neutrophil trans-endothelial migration in the lung occurs in the pulmonary capillaries, an enormous complex network of branching vessels. The spatial limitation within the capillary vessels obviates the need for rolling and here neutrophil accumulation is regarded as selectin independent [86]. Rolling may occur in the pulmonary venules which is L-selectin dependent. Neutrophils undergo morphological changes to migrate into the lung [87]. While the sequestration of neutrophils in lung injury has been extensively studied, the migration mechanisms of other leukocytes is less clear, but studies have identified a role for L-selectin and endothelial intercellular adhesion molecule 1 (ICAM-1) in promoting T-cell migration to the inflamed lung [88].

## Chemokines, Cytokines, Growth Factors and ECM

As mentioned, chemokines are responsible for the activation of leukocyte firm adhesion to allow transmigration to areas of injury or infection within the body, and they do this in part by binding to GAGs found within the ECM to create a chemotactic gradient [89]. For instance, interleukin-8 (IL-8, CXCL8) is an important recruitment molecule for neutrophils

and IL-8 binds to heparan sulfate and chondroitin sulfate in the ECM, a feature which helps to localize and facilitate the dimerization, retention and compartmentalization of this chemokine in the lung [90, 91]. For many chemokines and cytokines, binding to the GAGs in the ECM can protect the chemokine or cytokine from degradation by proteases in the tissue environment thus prolonging the biological action of the molecule to recruit leukocytes or in some cases inducing a confirmation that alters receptor binding (reviewed in [89, 92]). Sulfation status of the ECM has also been suggested to impact the ability of the ECM to sequester growth factors in the lung to mediate cellular responses. *In vitro* studies of isolated type II AECs have shown that the degree of sulfation of the basement membrane substrata the AECs are grown on can impact their response to heparin-binding growth factors. For example, AECs grown in the presence of de-sulfated chondroitin sulfate or de-sulfated heparin along with laminin proliferate in response to FGF-2 whereas sulfated heparin can inhibit this proliferation [93, 94] suggesting that the sulfation status of the basement membrane proteoglycans may be an important determinant of AEC responses to injury, repair and differentiation. Not surprisingly, the well-known anti-inflammatory effects of heparin have recently been determined to be mediated by the sulfation status of the molecule [95].

Migration of cells can also be influenced by the expression of proteoglycans which can encourage the binding of chemokines to cells to promote cellular movement. An example of this is binding of the somewhat misnamed, macrophage migration inhibitory factor (MIF) to syndecan-1 on lung epithelial tumors to promote cellular migration [96]. Alternatively, the binding of CXCL10, an anti-fibrotic chemokine to syndecan-4 can inhibit lung fibroblast migration [97]. The ECM can also serve as a reservoir to regulate the function of cytokines produced by the recruited immune cells. For instance, binding of interferon-gamma to heparin sulfate can limit its degradation and increase its bioavailability significantly (up to 600-fold) [98].

Not only can the ECM sequester chemokines and cytokines to activate leukocytes, but activated leukocytes can also remodel the ECM through elaboration of reactive oxygen species and proteases which can serve to stimulate, perpetuate or limit inflammation. Examples of this include degradation of collagen XVIII by proteases which can produce endostatin, a potent anti-angiogenic factor formed from cleavage of the C-terminal end of the protein [99]. In contrast, neutrophil-mediated release of matrix metalloproteinase (MMP)-9 can degrade type I collagen to produce N-acetyl Pro-Gly-Pro (Ac-PGP), a peptide which serves as an important neutrophil chemoattractant in chronic inflammation in diseases such as COPD [100] [101]. The role of MMPs in general and MMP-9 in particular however are controversial and likely context-dependent as overexpression of human MMP-9 in murine macrophages reduced inflammatory cell recruitment in a bleomycin model of lung fibrosis, an effect attributed to diminished cleavage of insulin-like growth factor binding protein-3 [102]. The varied impact that MMPs have on leukocyte activation has recently been reviewed [103]. Likewise, previous reviews have also explored the impacts that neutrophil elastases, which can be released from activated neutrophils during inflammation, can have in both lung destruction and repair [104].

## Pulmonary inflammation and matricellular proteins

Another way in which the inflammatory response can impact the ECM is through the secretion of matricellular proteins. Matricellular proteins serve as bridges to mediate interactions between ECM and cell surface integrin receptors. There are many matricellular proteins known to impact lung physiology including periostin, osteopontin, thrombospondin and secreted protein acidic and rich in cysteine (SPARC)[105–108]. Matricellular proteins are known to be upregulated in activated cells including leukocytes that are recruited to sites of injury or infection and includes molecules previously discussed e.g Tenascin-C. In our own laboratory, we have shown that CC chemokine receptor (CCR)2-binding chemokines can recruit fibrocytes to the lung in response to fibrotic insults or viral infections [109, 110]. Fibrocytes are circulating inflammatory cells defined by having characteristics of both leukocytes (e.g. expression of CD45 and chemokine receptors) but also mesenchymal cells (e.g. expression of type I and type II collagen)[111]. Many studies have shown that fibrocytes augment or correlate with the development of fibrotic lung diseases [112, 113]. Recently, we suggested that the ability of fibrocytes to increase lung fibrosis in the murine bleomycin model was due to their paracrine functions such as secretion of pro-fibrotic cytokines [114], but also by their ability to secrete periostin [115], rather than their ability to secrete type I collagen [116] or to differentiate into myofibroblasts *in vivo* [115], a result also seen by Madala et al. using a transgenic TGFα fibrosis model [117]. This was attributed to the ability of periostin to interact with bone morphogenic protein (BMP)1 to localize its ability to activate lysl-oxidase to stimulate collagen type I crosslinking[118]. It should also be noted that fibrocytes themselves are potent secretors of MMPs [119] which may also serve to remodel the ECM as noted above.

Periostin can upregulate expression of TGFβ1 [115] and this cytokine is the best-described regulator of fibrosis. The activation of TGFβ1 is very complex and highly orchestrated by the ECM. This cytokine is held in a latent state complexed with the latency associated protein (LAP) and sequestered to the ECM by latent TGFβ binding protein (LTBP). In this conformation, the growth factor is not bioactive until acted on by proteases, pH or mechanical stress to facilitate the dissociation of active TGFβ as recently reviewed [120–122]. Once TGFβ1 promotes the deposition and stiffening of the lung ECM, this stiffened matrix can serve to activate fibroblasts [123, 124], potentiating the fibrotic reaction. Importantly, this thickening of the ECM, especially in the context of lung cancer can serve to impede the ability of activated T cells to migrate through the stiffened matrix, lessening the chance of an effective anti-tumor CTL response [125]. Similar impediment of CTLs is likely to occur in other granulomatous lung disease as well. When faced with a pathogen that cannot be easily cleared (e.g. mycobacteria or parasites), activated T cell cytokines are believed to modulate granuloma formation [126], with Th2 cytokines such as IL-13 being particularly noted for the ability to activate fibroblasts to promote the development of thickened granuloma walls of ECM by stimulating production of type I collagen [127]. Thus, immune responses can serve to initiate secretion of ECM, ECM can modulate leukocyte inflammation and ultimately, thickened ECM can exclude leukocytes from performing their effector functions at the needed site of infection or tumor niches.

## Conclusion

In this review, we have summarized some of the important interactions that have been characterized between the pulmonary ECM and the immune system within the lung. We know the ECM plays an important role in the regulation of innate and adaptive immunity. Recognition of ECM components by PRRs on innate immune cells may be the earliest indication of injury and infection within the lung. The ECM acts as a reservoir where components can interact with a variety of cytokines, chemokine and growth factors thereby modulating inflammation and repair. The release of ECM-bound cytokines can act to shape the effector T cell responses to promote development of variable T cell responses and ultimately disease pathogenesis. However, considerable work remains to understand these pulmonary ECM-immunity interactions in more detail. Furthermore, little is known about how immune cells may sense stiffened lung ECM and if so, whether such mechanosensing alters immune cell functions. A greater understanding of how the matrix promotes innate and adaptive immunity may lead to tailored therapies in both infectious and non-infectious lung disease. These therapies may limit the leukocyte and inflammatory cytokine cascade, may attenuate cell and tissue damage or may be tailored to provoke immune-mediated responses needed for appropriate repair. The next decade should herald a greater understanding of how to harness ECM-immune interactions in these ways.

## Acknowledgments

Grant support: NIH grants AI117229, HL127805, HL115618, HL119682 (BBM) and K99HL139996 (DOD)

We would like to thank Dr. Rachel Zemans (University of Michigan) for her image of the type 2 human pneumocytes and Dr. Jeffrey Myers (University of Michigan) for his image of the interstitial matrix in IPF.

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## Highlights

- Pulmonary immunity includes physical barriers including mucus and the mucocilary escalator of the airway, the alveolar epithelium and the capillary endothelium
- These physical barriers work in tandem with a well-developed innate and adaptive immune system to protect the lung from injurious agents and pathogens
- The lung extracellular matrix is a bioactive scaffold lying beneath these physical barriers that has important roles in the regulation of pulmonary innate and adaptive immunity
- Lung pathology promotes abnormalities within the composition and structure of the extracellular matrix and generates matrix molecules that act as "danger signals" for innate immune receptors
- The release of extracellular matrix bound cytokines can affect effector T cell responses and disease pathogenesis
- Leukocyte recruitment to inflamed pulmonary sites relies on morphological alterations of cells to pass through the confined spaces of the pulmonary capillary network
- A greater understanding of these important extracellular-immunity interactions will lead to improved therapies for acute and chronic lung disease

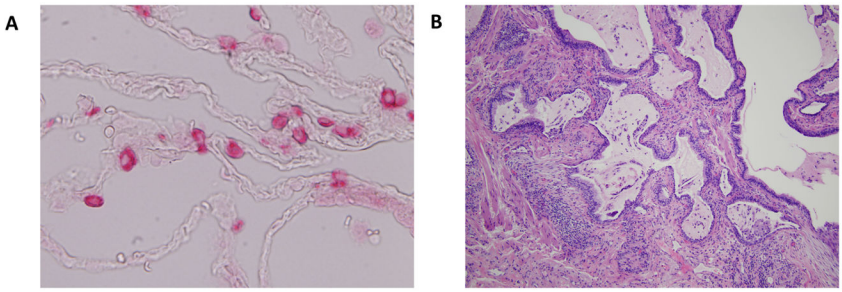


**Fig. 1. The pulmonary ECM in health and disease**

Image **A** depicts the normal alveolar space at high power. The type 2 pneumocytes are stained pink. There is minimal matrix present and the alveolar epithelium is very thin allowing for ease of gas exchange. Image **B** depicts fibroblastic foci of IPF, with layering down of aberrant ECM and the loss of the normal alveolar epithelium leading to impaired gas exchange.

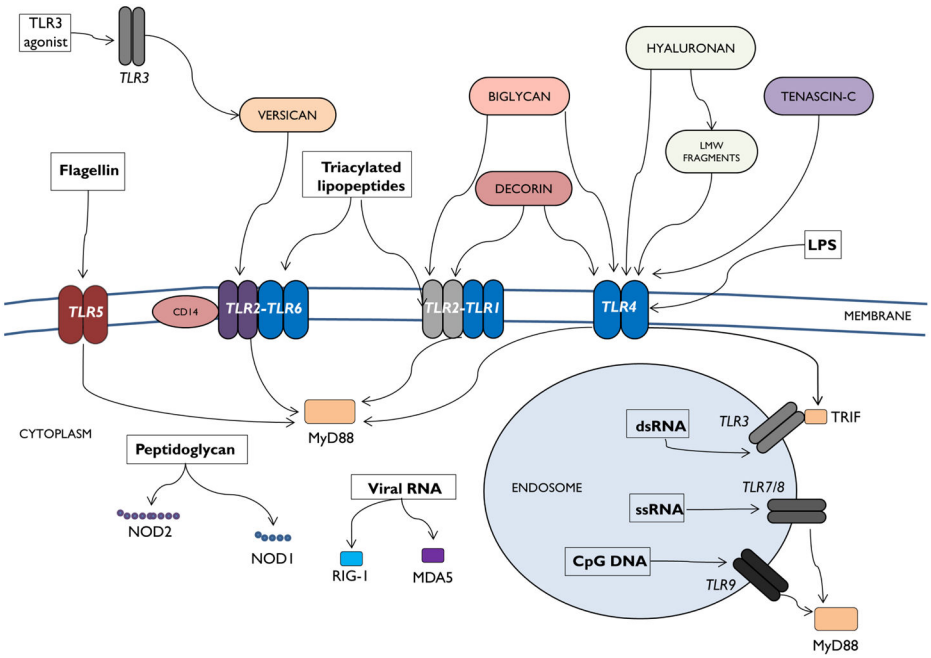


**Fig. 2. Innate immune receptors and ECM molecule interactions**

Canonical ligand-pathogen recognition receptor (PRR) interactions in the lung are depicted in this schematic. TLRs, NLRs, cytosolic sensors and their canonical ligands are shown. In addition, known interactions between several ECM molecules and PRRs are also displayed.

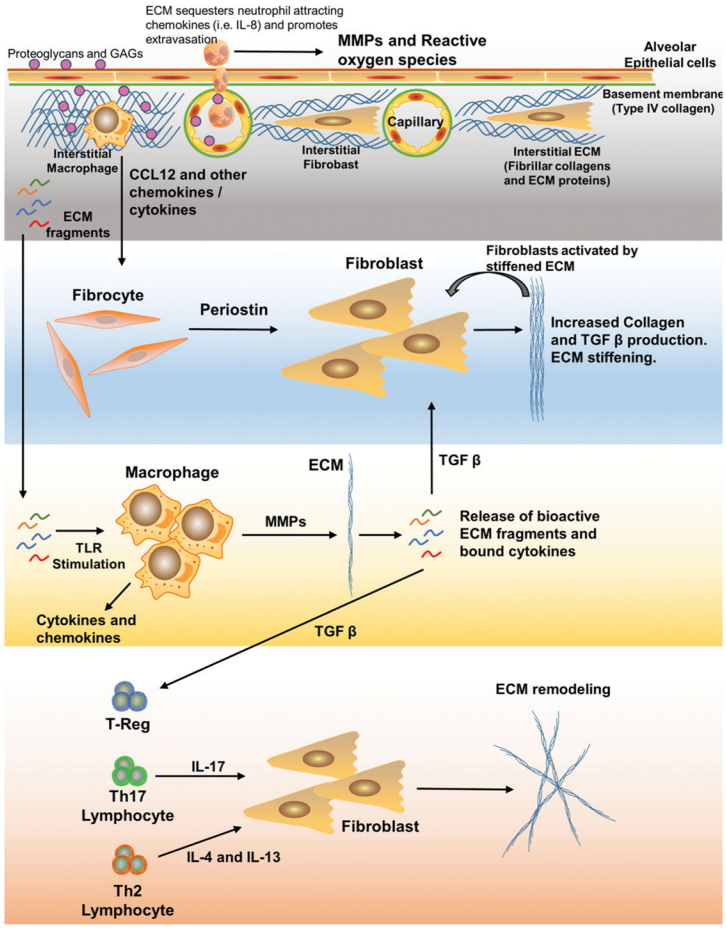


**Fig. 3. Features of ECM and adaptive pulmonary immunity**

ECM fragments can stimulate TLRs to generate inflammatory cytokines and matrix metalloproteinases that may alter the ECM resulting in the release of TGF-β which in turn results in ECM remodeling through direct effects on pulmonary fibroblasts. TGF-β also modulates T cell populations to generate cytokines that influence the ECM. Finally, fibrocyte recruitment to the inflamed lung can lead to increased expression of periostin, which alters fibroblasts leading to increased collagen deposition and ECM stiffening.