Chapter 7
Pericytes and T Cells in Lung Injury and Fibroproliferation

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Introduction

The respiratory system is essentially an external organ, constantly exposed to the external environment. As such, it is in contact with any number of antigens and chemical agents that can injure the upper (nasal cavity, pharynx, and larynx) or lower (trachea, bronchi, and lungs) respiratory tract [1]. Injuries to the lung parenchyma are particularly harmful, as the parenchyma is the site of gas (oxygen and carbon dioxide) exchange. Acute or chronic injuries to the lung result in acute or chronic hypoxemic or hypercapnic respiratory failure, respectively. While there are several structural and pathologic mechanisms that contribute to respiratory failure, some lung injuries result in a progressive fibroproliferative response that leads to respiratory failure and death.

While many lung diseases often result in some degree of fibroproliferation, two common lung disorders where fibroproliferation is the primary pathophysiological driver of disease are acute respiratory distress syndrome (ARDS) [2, 3] and idiopathic interstitial pneumonia, particularly idiopathic pulmonary fibrosis (IPF) [4]. These diseases differ completely in clinical presentation, with ARDS resulting from acute lung injury (hours to days) and respiratory failure, whereas idiopathic

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pulmonary fibrosis occurs over months to years as a result from either chronic injury or an abnormal and progressive aberrant host response to an acute injury. While the clinical presentation and treatment of these diseases differ, lung fibroproliferation is the common pathophysiology that mediates respiratory failure in both of these conditions.

Acute Respiratory Distress Syndrome

ARDS affects at least 200,000 persons per year in the United States alone and carries an acute mortality risk of 30–40% [5]. ARDS can occur via direct or indirect injuries to the lung. Examples of direct injuries include aspiration of gastric fluids, pneumonia, toxic inhalations, drowning, or burns [6, 7], whereas indirect lung injuries can occur secondary to severe trauma, sepsis, blood transfusions, and pancreatitis [8]. Direct or indirect injuries result in damage to the lung epithelium or endothelium resulting in a cascade of events leading to acute respiratory failure [2].

An acute and a resolution phase characterizes ARDS pathophysiology following the inciting injury. In the acute phase, a protein-rich exudate floods the alveoli mediating damage to both the alveolar epithelial and the vascular endothelium, compromising the integrity of the functional lung unit [2]. The major purpose of the resolution phase is to re-establish lung homeostasis by removing proteins, fluids, and dead cell debris from the alveolar airspace, activating type II pneumocytes to regenerate type I pneumocytes, and restoring the architecture of the lung unit [2]. Patients that survive ARDS have ongoing problems, including skeletal muscle weakness and neurocognitive and psychiatric impairment leading to reduced quality of life, hospital readmissions, and increased mortality risk [9].

In patients that fail to resolve lung injury, a dangerous fibroproliferative phase ensues [10, 11]. Fibrosis is initiated if the immune response fails to remove the trigger or if lung damage progresses faster than the body can repair it [12]. In ARDS, extracellular matrix proteins accumulate, predominantly collagen, and lead to reduced pulmonary compliance and ongoing hypoxemia [13]. These patients often fail to liberate from mechanical ventilation and die from chronic respiratory failure and multi-organ failure. Mechanical ventilation with higher tidal volumes can independently contribute to ongoing lung injury, known as ventilator-induced lung injury (VILI), where fibroproliferation in the lung was also observed [14].

Idiopathic Pulmonary Fibrosis

IPF is the most common and severe form of the idiopathic interstitial pneumonia, with an incidence of 5–16 cases per 100,000 persons or up to 50,000 patients per year in the United States [15]. Similar to ARDS, IPF incidence increases with age [5, 16]. However, in contrast to ARDS, the clinical presentation of IPF is insidious over a period of months to years, as patients present with chronic hypoxemic

respiratory failure. Most patients diagnosed with IPF die within 2–5 years of diagnosis, although there are variable rates of disease progression. Recently approved new treatments hold promise for improving outcomes of these patients [17–19].

The pathophysiology of IPF is thought to result from varying combinations of environmental, aging, and/or genetic factors that lead to alveolar injury susceptibility. Endogenous or exogenous irritants may injure the lung chronically, or an abnormal host repair ensues, leading to lung fibroproliferation, ultimately leading to chronic hypoxemic respiratory failure and death.

A better understanding of the cells and mechanisms that underlie lung fibroproliferation in ARDS and IPF will open new avenues for treatment for these debilitating disorders. Here, we consider the role of pericytes in the lungs as possible cellular targets for more effective treatments for these debilitating disorders. The location of pericytes in the lung and their known role in vascular structural integrity and fibroproliferation make pericytes interesting and relatively unexplored cellular targets for fibrotic lung diseases.

Pericytes

In 1923, Karl Zimmermann noted the contractile properties of a population of cells he named *pericytes* because they were located around blood vessels [20]. Morphological identification of pericytes has been the mainstay in the past, which is still a useful approach. Electron and early light microscopy identified elongated cell bodies with a prominent nucleus and cytoplasmic processes that embrace endothelial cells [21, 22]. Typically, pericytes are found surrounding nonmuscular microvessels, capillaries, and postcapillary venules, embedded in the basement membrane, where they partially envelop and attach to endothelial cells by focal contacts named *peg-to-socket junctions* [23]. Pericytes are widely distributed, since microvessels are present in most organs, and are responsible for regulating blood flow, supporting angiogenesis, and serving as cell progenitors, depending on the organ [24].

Although morphology is important in identifying pericytes, several other cell types have similar morphological characteristics. To avoid confusion, scientists started to consider both anatomical location and biochemical markers, such as cell membrane and intracellular proteins. These molecular markers are also useful in tracing genetic lineage, using methodologies that identify the cells that give rise to pericytes and those into which pericytes differentiate [25]. However, identifying pericytes by their markers is quite tricky. Depending on their embryonic origin and the host organ, pericytes may display a vast number of markers [26], possibly related to their specific functions. For example, pericytes that help to regulate the blood flow in, and contractility of, microvessels express contractile proteins like alpha-smooth muscle actin (α-SMA) [27] and, as mural cells (pericytes and vascular smooth muscle cells), provide the physical integrity microvessels require [28–30], while pericytes that serve as nervous cell progenitors express neural cell markers like NG2 [31]. Other markers used to identify pericytes include platelet-derived

growth factor receptor-β (PDGFR-β) [32, 33], aminopeptidase N (CD13) [34], and nerve/glial antigen-2 (NG2) proteoglycan (CSPG4) [35, 36].

The most well-known functions of pericytes are related to maintaining organ homeostasis. During angiogenesis, pericytes dissociate from endothelial cells, momentarily destabilizing the blood vessel and allowing endothelial sprouting into the tissue. Growth factor signaling (PDGF, VEGF, and TGF) then recruits the pericytes to stabilize the newly formed vessel [32, 37, 38]. Pericytes have antifibrinolytic properties and play a role in coagulation [39, 40]. Pericytes also affect immune responses in the central nervous system [41–43] and promote T-cell proliferation and activation [44–46]. Pathologically, pericytes may respond to angiogenic signals from tumors, thus supporting tumor progression and sometimes facilitating metastasis [47]. Pericytes also participate in adipogenesis, converting to adipocytes in response to injury, and fibrotic scar tissue through several mechanisms that lead to collagen deposition and fibroblast proliferation [29].

Pericytes are heterogeneous with regard to phenotype, distribution, and origin [30, 48, 49]. Zimmerman distinguished three types based on their location in the blood vessels: precapillary, true-capillary, and postcapillary [29, 50]. Precapillary pericytes have circular branches that tend to wrap around the vessel and express varying amounts of α-smooth muscle actin [51]. True-capillary pericytes are spindle-shaped with many short secondary processes and extend mainly along the vessels' long axis. They do not express α-smooth muscle actin [51]. Postcapillary pericytes are shorter and stellate and cover the abluminal surface of postcapillaries [29].

The number and density of pericytes vary by organ, possibly linked to the organ's blood pressure and the number of large vessels that serve it. These variations contribute to different pericyte-endothelial cell ratios. In the lungs, it is 1:10; in the central nervous system, 1:1; and in skeletal muscle, 1:100. This heterogeneous distribution has raised questions about whether lung pericytes have functions distinct from those in other organs, and several groups are studying the specific roles they play in normal and injured lungs [28, 52]. Some suggest that the more pericytes in a tissue, the higher its blood pressure and the more controlled are its vessels [28], which may explain why there are more pericytes identified on vessels of larger diameter [30].

Pericytes also differ in their origins [53]. Lineage-tracing studies indicate that forebrain pericytes have a neuroectodermal origin [54]. In sharp contrast, in most other organs explored, pericytes are derived from the mesoderm, more specifically from the sclerotomal compartment [55–62]. The exact origins of pericytes in the lung remain unknown.

Pericyte heterogeneity is exemplified by their marker expression profiles [29]. For example, pericytes localized on venules express desmin and αSMA, while those on capillaries express desmin but not usually αSMA [36, 63]. ATP-sensitive potassium channel Kir6.1 is undetectable in pericytes in the skin and heart but highly expressed in brain pericytes [64]. In the spinal cord, pericytes that express the glutamate-aspartate transporter (GLAST) differ from those that express desmin and αSMA [65]. Bone marrow has both sinusoid-associated leptin receptor (LEPR)+ and LEPR- pericytes [66]. In the skin, NG2- and NG2+ pericytes have been

described. Additionally, two populations of pericytes were identified in several organs, including the lungs, based on nestin-GFP expression [67, 68]. Using a nestin-GFP/NG2-DsRed bi-genic mouse, nestin-GFP-/NG2-DsRed+ (type I) and nestin-GFP+/NG2-DsRed+ (type II) pericytes were found in close proximity to endothelial cells [68]. Discovering the functions of specific pericyte subtypes in the lungs will help target the most appropriate cells for treating various pulmonary diseases.

Physiology of Pericytes in the Lungs

In the gas exchange process, carbon dioxide and oxygen diffuse into the microvasculature from the alveolar airspace at the terminations of ducts, or sacs, whose walls contain elastic fibers coated with types I and II pneumocytes [69, 70]. Type I pneumocytes have a squamous (flat) morphology and cover 90–95% of the alveolar surface, so they are responsible for maintaining the equilibrium of fluids in the airspace; their occlusive junctions prevent tissue fluids from leaking into it [71]. However, pneumocytes cannot replicate and are very susceptible to the toxic agents that compromise alveolar functions in cases of injury, and type II pneumocytes must form type I pneumocytes to re-establish homeostasis [72, 73]. Although crucial for the recovery of type I pneumocytes, type II pneumocytes cover less than 5% of the alveolar surface due to their cuboidal morphology [74]. Type II pneumocytes play an important physiological role in the secretion of pulmonary surfactant, a substance that decreases alveolar surface tension. Surfactant prevents the alveolar sacs from collapsing at the moment of expiration and maintains the optimal fluidity of the surface that facilitates gas diffusion to and from the alveolar airspace [75–77] (Fig. 7.1).

Efficient gas exchange requires alveolar fluid balance but also control of the pulmonary blood flow [78] mediated by cardiac output and several other factors, including body position (i.e., upright or supine) [79]. Within the microvasculature of the alveolar sacs, more refined regulation is required. For example, the resistance and permeability of pulmonary arterioles vary with the integrity of the endothelial and subjacent cell layers [80]. Pulmonary arterioles and small-diameter vessels, including venules and capillaries, integrate the vascular bed of the alveolar sacs, where controlling blood flow is crucial because it is the region where the major function of the organ is carried out. The vascular bed that surrounds the alveolar sacs serves two main purposes: structural support and gas exchange [81–83].

Previous studies demonstrated that pericytes are critical for vascular development and support. Boström and colleagues used animal models to show that the formation of functional alveoli depends on platelet-derived growth factor alpha (PDGFα) signaling. Without the action of this growth factor during embryonic development, vascular bed formation compromise ensues, and newborn knockout mice had smaller lungs with malformed alveoli [84]. Pericytes express PDGF receptors – in fact, they are biochemical markers used to identify them – and PDGF singling contributes to angiogenesis both in organ development and homeostasis.

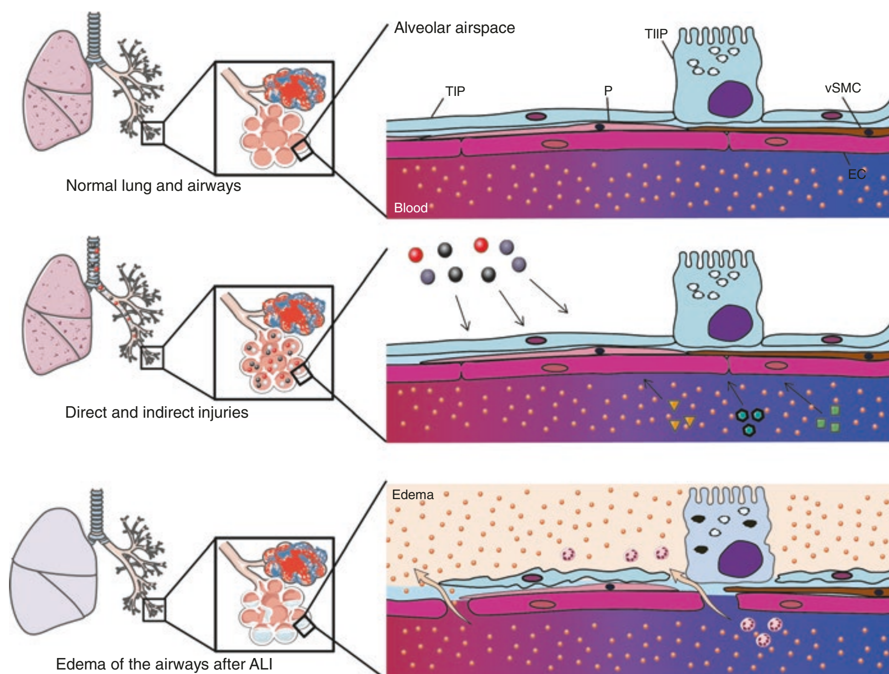

Fig. 7.1 Direct and indirect injuries to the lung. Types I and II pneumocytes (TIP and TIIP) compose the lung epithelium and maintain alveolar structure. On the other side of the blood-air interface, the microvasculature is composed of endothelial cells (EC) and perivascular cells, such as pericytes (P) and vascular smooth muscle cells (vSMC). Lung injury begins with direct damage to the lung epithelium that causes inflammatory components to destabilize vascular endothelial cells and indirectly damage the lungs. As a result of either direct or indirect injuries, vascular and perivascular cells become loosely attached, allowing proteins, fluids, and immune cells to leak into the alveolar airspace and damage type I and II pneumocytes

Therefore, pericyte recruitment via PDGF signaling influences the formation of a stable vascular bed that, in the lungs, leads to the development of functional alveolar sacs [24, 85]. Although staining shows that both pericyte subtypes co-localize with two classical pericyte markers, PDGFRβ and CD146, at least in the skeletal muscle, only type I expresses the adipogenic progenitor marker PDGFRα [68, 85, 86]. Whether the same is true in the lungs remains unknown, but if so, type I pericytes may exclusively control the functions related to this receptor.

The gas exchange also depends on a stable vascular bed through which pulmonary capillaries transport venous blood from the right heart to the alveolar sacs [87] and then oxygenated blood to the left atrium and systemic circulation [88]. Pericytes help to control blood flow in two ways: (1) regulating vessel permeability to optimize gas exchange and (2) providing contractile force to the vascular bed, assuring that flow decreases and subsequently increases before and after gas exchange, respectively [89, 90]. Type I pneumocytes control permeability within the airspace; the pulmonary surfactant controls fluidity. However, vascular and perivascular cells also participate by preventing leaks from the bloodstream into the airspace and

ensuring proper gas diffusion both ways [91, 92]. Endothelial cells lining the vascular-facing surface of vessel walls primarily control blood vessel permeability [93]. Small-diameter vessels have fewer endothelial cells, resulting in the formation of small spaces (termed *fenestrations*) [94], possibly allowing fluid to leak out. Endothelial cell fenestrations are involved with cell migration into the tissue during inflammatory responses [95]. Pericytes provide a remedy. Because of their characteristic morphology and increased localization around small vessels, pericytes provide an extra layer of cellular support, which if injured could theoretically lead to endovascular leak [96]. Also, perivascular cells control the contractility of pulmonary capillaries, which have no or only a thin muscular layer [97]. Another perivascular-like cell type with contractile properties are vascular smooth muscle cells that surround small-diameter vessels [98], but Edelman and colleagues established that pericytes could express contractile proteins, such as desmin, α-smooth muscle actin, and myosin, and thereby potentially regulate blood flow in pulmonary capillaries by vasoactive signaling [99]. Future studies are needed to determine whether regulation of blood flow and vascular permeability and contraction is unique to pericyte subtypes.

Although pericytes can function as mural cells in pulmonary capillaries, some researchers point to other cell populations that reside in the lungs and may stabilize alveolar sac structure [104], such as fibroblasts [100], mesenchymal stem cells, smooth muscle cells, adventitial cells [101], and macrophages [102, 103]. Like pericytes, resident fibroblasts and myofibroblasts surround vessels, and these cell types are difficult to distinguish based on morphology alone [105, 106]. Biochemical markers are also problematic since other cell types express pericyte markers, and even in pericytes, their expression varies with developmental stage [49]. For instance, PDGFRβ is a known marker of such cell types as fibroblasts [100, 107], while NG2 proteoglycan can be expressed in macrophages [108]. Pericytes that do not express NG2 were also recently described [109]. Note that sometimes, perivascular cells can be distinguished by the circumstances under which they appear, and classical electron microscopy studies reveal that in contrast to other perivascular cells, pericytes localize under the vascular basal lamina [110]. Whether all functions attributed to perivascular cells in the lungs correspond to pericytes remains unclear. Combining pericyte molecular markers with immunolabeling of the basal lamina in genetic lineage-tracing models in the lungs will clarify several open questions in pulmonary biology.

In a recent article, Lefrançais and colleagues demonstrated that the lung is a reservoir for hematopoietic stem cells (HSCs). These progenitors can migrate out of the lungs, repopulate the bone marrow, and contribute to many hematopoietic cells [111]. In adult bone marrow, pericytes form a special niche for quiescent HSCs, promoting the dormancy essential for their maintenance [66, 112]. Whether pericytes contribute to HSC maintenance in the lungs remains unknown.

Rock and colleagues pointed out that pericytes and resident fibroblasts comprise 10–20% of all lung cells [113]. They both contribute to the structure of the lung parenchyma, but their participation differs [113]. Fibroblasts are the most common cell in all connective tissues, mainly producing extracellular matrix and collagen [114]. They are critical to wound healing and the formation of an organ's stroma

[115] but are strongly correlated with pathological processes. Fibroblasts initiate inflammatory responses, play roles in tumor growth and resistance, and may compromise function in various organs, including the lungs, by supporting the substitution of scar-like connective tissue for functional tissue [114, 116]. Pericytes, on the other hand, are quite plastic. Because their distribution around the body is so heterogeneous, their origin, even within the same tissue, may also be heterogeneous, which may explain their variable markers and functions [53]. Marriott and colleagues determined that pericytes in the lungs are part of a large population of mesenchymal stem cells and expressed, among a vast number of markers, NG2 and αSMA markers, which are related to neural and contractile cells, respectively. These cells played different roles in the lungs than other cells derived from the same embryonic origin [49, 117, 118].

Pericyte Involvement in Fibrosis

Fibrosis is characterized by the excessive deposition of fibrous connective tissue (*fibroproliferation* or *fibrodeposition*) as a reparative or healing process. Without removal of the injurious agent, fibrosis may become pathological. If the tissue damage is too extensive, the injury may result in the remodeling of the organ's architecture [119–121]. Sometimes this remodeling is harmless; for example, after a cut or bruise, scar tissue, the result of fibrodeposition, can replace the epithelium without compromising homeostasis in any body functions. At other times, the scar tissue formation can compromise the organ's entire function, leading to systemic impairment or death, depending on the extent of the scar and the organ, for instance, the lungs or kidneys.

Fibrodeposition is not a simple process, requiring well-organized cell recruitment, differentiation, signaling, and protein deposition. The resulting mass of scar tissue is composed of various cell types and proteins that replace the organ's functional units [122]. Key among them are myofibroblasts, well-known producers of extracellular matrix proteins, such as collagen, glycoproteins, and proteoglycans [116, 123, 124], that primarily produce collagen when activated by autocrine and/or paracrine signals, such as transforming growth factor beta (TGF-β) [125]. In the aberrant wound healing observed in IPF, myofibroblast proliferation increases, and they express high levels of actin, especially α-SMA, and myosin, which enhances the connection of myofibroblast with the extracellular matrix, contributing to the contractile properties that characterize scar tissue [114, 126, 127]. Identifying the cells that originate myofibroblasts might allow the arrest of fibrosis, or even reverse fibrosis, in certain disease conditions [128]; however, recent findings demonstrate that the origins of myofibroblasts may be heterogeneous, even within a single tissue [129].

Studies of antifibrotic drugs have tested their effects on endothelial cells [130], epithelial cells [131–133], circulating progenitor cells [134–139], resident fibroblasts [140], and pericytes [29] from multiple tissues. Our knowledge of cellular complexity in the lungs has improved, but the biological processes of fibrous tissue deposition here are not fully understood. The inflammatory response in ARDS and

IPF may stimulate pericytes to differentiate into myofibroblasts and fibroblasts; pericyte differentiation is known to increase the number of cells producing extracellular matrix and collagen deposition [141]. Moreover, when pericytes differentiate, their morphology changes, and they detach from the endothelial cell layer [142]. Therefore, during fibrodeposition in the lungs, pericytes not only cause remodeling in the parenchyma but also destabilize the pulmonary capillaries, compromising gas exchange and the stability of the alveolar sacs [143, 144].

Data in a murine model of ARDS suggests that some degree of fibroproliferation is part of normal lung injury repair [145]. Several researchers define pericyte participation in fibrodeposition as an "organ-dependent" process, and its role in some organs, like the lungs, is still under debate. Using different animal models of cholestatic, toxic, and fatty liver diseases, researchers demonstrated that liver-resident pericytes (called *hepatic stellate cells*) are the main source of collagen and play an important role in fibrodeposition [146–148]. Dulauroy and colleagues showed that after acute injury, skeletal muscles generated scar tissue with the active participation of collagen-producing pericytes [149]. In kidney fibrosis, the contribution of pericytes is not well stablished. Using a model of angiotensin-II-induced renal fibrosis, Faulkner and colleagues showed that fibrogenic cells derive from perivascular cells later identified as pericytes by Humphreys and colleagues [150, 151]. In contrast, LeBleu and colleagues found that ablating pericytes did not alter the level of kidney fibrosis [152].

Pericyte contribution to fibrous tissue in the lungs is also controversial. Rock and colleagues found that pericytes proliferated after lung lesion but did not produce fibrogenic cells after injuries induced by bleomycin [111]. In contrast, a recent study using lineage-tracing mapping showed that FoxD1+ pericytes do contribute to pulmonary fibrogenesis [105]. The use of different transgenic mouse models might explain this discrepancy. Rock et al. [113] used the inducible NG2-CreER transgenic mouse, which has a very low recombination efficiency; thus, the labeling does not include the whole pericyte population. As lung pericytes are heterogeneous, and at least two subtypes have been described, perhaps only a fraction of these cells contribute to pulmonary fibrosis. Indeed, using bi-genic nestin-GFP/NG2-DsRed mice, type I (nestin-GFP^-/NG2-$DsRed^+$/$PDGFR\beta^+$), but not type II (nestin-GFP^+/NG2-$DsRed^+$/$PDGFR\beta^+$), pericytes were found to contribute to collagen production in the lungs after bleomycin-induced injury [68]. Fibrous tissue formation depends on several molecular processes, including TGFβ signaling, supporting the idea that growth factors play an essential role [153]. Strikingly, a recent study demonstrated that perivascular Gli1+ cells in a pericyte niche adjacent to endothelial cells in the lungs expand and significantly contribute to αSMA+ myofibroblasts after pulmonary injury [117]. The overlap between Gli1+ cells and previously described pericyte populations in the lungs remains unclear.

Although several studies indicate that pericytes do participate in lung, kidney, liver, and spinal cord fibrodeposition after injury [65, 100, 113], the exact cellular and molecular mechanisms remain unknown. Whether pericytes influence fibroproliferation only directly by producing extracellular matrix proteins or also stimulate other cells to differentiate into myofibroblasts that act as immune regulators, modulating cells that favor the proliferative process, is an open question.

Interaction Between Pericytes and the Immune System

The vascular events of inflammation mark the lung parenchyma's first response to injury. Due to increased permeability of the microvessels in the blood-air barrier, a protein-rich fluid floods the alveolar airspace that damages both types I and II pneumocytes after injury [154, 155]. Damage to type I pneumocytes disrupts the integrity of the blood-air barrier and allows interstitial fluids, proteins, immune cells especially neutrophils, and fibroblasts to leak into the airspace [156]. Damage to type II pneumocytes decreases pulmonary surfactant production, possibly inactivated by the amount of fluids in the airspace, and compromises regeneration of type I [155, 157]. These are the first events of vascular and blood-air barrier destabilization leading to clinical ARDS onset. Inflammation is not triggered to increase organ damage [158]; rather, inflammation is an attempt to restore normal function in the lung and to heal wounds. The immune system controls the main events of inflammation – vascular alterations, extravasation of plasma proteins, and cell migration – that lead to organ remodeling [159]. The remodeling may be irreversible if the initial insult is not removed or if inflammation cannot resolve; the tissue then becomes compromised and is replaced by fibrotic scar tissue [160].

Pericytes in the pulmonary capillaries usually regulate the endothelial cell layer via paracrine signaling, controlling the diffusion of proteins and cells through the vessel walls. During the inflammatory response, endothelial cells retract, and pericytes cover the resulting gaps, preventing proteins and cells in the bloodstream from escaping [95]. Recent studies demonstrated that the presence of pro-inflammatory cytokines, such as IL-2, mediates a conformation change to pericytes around the leaky vessel [161]. Pericytes re-establish their junctions with endothelial cells, allowing plasma components with a high protein concentration [162] to spill into the airspace and interact with collagen and other extracellular matrix components, leading to the release of more cytokines, growth factors, and chemoattractant factors [163, 164].

In ARDS, the cells that respond first to injuries are neutrophils, rapidly invading the lung parenchyma, and once inside the alveolar airspace, neutrophils produce cytokines and pro-inflammatory mediators that affect the integrity of the alveolar sacs, compromising type I and II alveolar epithelial cells [165]. In addition to vascular permeability, pericytes contribute directly to neutrophil migration from the vessels into the tissue. Once neutrophils have penetrated the endothelial cell layer, direct contact with the pericytes' basement membrane relaxes the perivascular cytoskeleton via inhibition of intracellular signaling RhoA/ROCK, changing the conformation of pericytes that direct neutrophils to regions that express low quantities of extracellular matrix proteins [165, 166]. The expression of intercellular adhesion molecule-1 (ICAM-1), macrophage antigen-1 (Mac-1), and leukocyte function-associated antigen-1 (LFA-1) on pericytes facilitates the transmigration of immune cells. Pericytes expressing ICAM-1 and the chemoattractant MIF were shown to attract and activate neutrophils and macrophages as well as facilitate their trafficking. Pericytes also participate in the immune response by enhancing the functions of neutrophils and macrophages in the interstitial space [167] (Fig. 7.2).

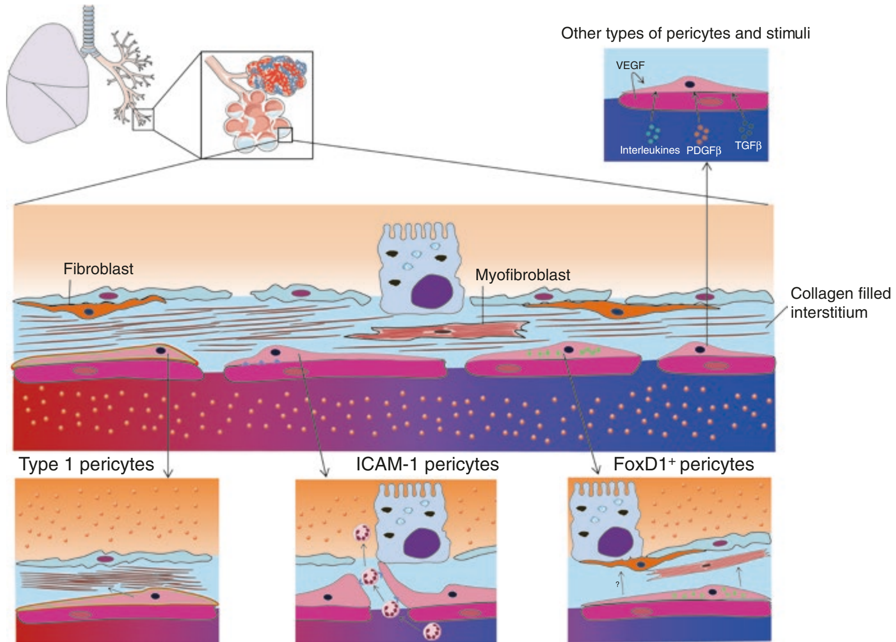

Fig. 7.2 Heterogeneity of pericytes in the blood-air interface and their role in lung fibrodeposition. At least three types of pericytes influence fibrodeposition in the lungs. Type I is the source of type I collagen, which, when deposited in the interstitium, remodels the tissue's architecture. Pericytes expressing the adhesion molecule ICAM-1 direct and support neutrophil migration from the bloodstream to the alveolar airspace. FoxD1+ pericytes can differentiate into myofibroblasts that express αSMA. The overlap between the two distinct pericyte populations described so far remains unknown. The hypotheses that there might be other pericyte subtypes and that pericytes might be stimulated by TGFβ, PDGF, interleukins, and VEGF to support fibrodeposition have not been confirmed

Pericytes and T Cells in Lung Fibrosis

In ARDS, multiple variables, including the nature, duration, and intensity of the aggression and the individual patient's response, influence the development of the inflammation, driving the resolution process along different pathways [168]. The ideal outcome after injury is complete resolution, the restoration of the organ's normal architecture with no or little compromise of lung function [169]. When an injury ends rapidly, or if the tissue sustains little damage, macrophages mediated the removal of cell debris, and the lymphatic system reabsorbs edema fluid, resulting in complete restoration [169, 170].

Persistent injury leads to extensive tissue damage [171] in organs like the lungs where the regeneration rate is low, and in conditions such as fibroproliferative ARDS and IPF associated with substantial exudate and fibrin deposition, the inflammatory and late immune system responses cannot remove or resolve the injury, perpetuating fibrosis [172]. In both ARDS and IPF, we do not fully know what

causes tissue fibrosis [173]. Certain cell types seem pivotal. Researchers identified many cell types, including, but not limited to, fibroblasts and myofibroblasts, which participate in the direct deposition of matrix proteins or differentiate into matrix-secreting cells by either inhibiting or stimulating signaling [174]. Pericytes are recruited to wounded tissue by PDGF signaling and can produce collagen. Several groups suggested, and fate-mapping studies confirmed, that in cases of acute tissue injury, subsets of pericytes detach from the perivascular space and differentiate into myofibroblasts, making a key contribution to the formation of scar tissue [149]. Note that all groups agree that pericytes alone are not responsible for the resultant fibrosis in these conditions [153].

Pericytes may contribute to fibrosis in other ways [141]. Lung injury resolution requires a microenvironment that enables cells, such as fibroblasts and pericytes, to develop and expand [175]. Pericytes physically help immune cell migration into the injured tissue through the endothelial cell layer, but whether immune cells communicate with type I pericytes to maintain the microenvironmental conditions that support fibrosis remains unknown.

The first cells to invade the lung parenchyma in ARDS are neutrophils. These cells release cytokines that attract, and possibly activate, pericytes. Once the inflammatory response is established, T cells (both Th1 and Th2) are recruited to the damaged site [176]. Th1 cells secrete IFN-γ to directly suppress fibroblasts' synthesis of collagen and control the rates of collagen degradation by regulating metalloproteinases (MMP) in the extracellular matrix [177]. While T cells clearly play an important role in controlling collagen deposition in the injured lung, they do not contribute to the resolution process [176, 178]. No one has examined whether they inhibit pericyte-dependent fibrogenic responses.

In the later stages of ARDS, lymphocytes mobilize to the damaged tissue. Inflammatory mediators secreted by CD4+ T cells strongly influence extracellular matrix deposition and tissue remodeling [179, 180]. The cytokine secretion profile of CD4+ T cells determines their classification [181], with T helper 1 (Th1) cells secreting relatively large amounts of interferon-γ (IFN-γ) and other pro-inflammatory cytokines, such as IL-2 and tumor necrosis factor-α (TNF-α), as defense mechanisms associated with infectious diseases and phagocytosis [182]. In contrast, T helper 2 (Th2) cells secrete IL-4, IL-5, and IL-13 and are associated with immunoglobulin E (IgE) production and immune reactions mediated by mononuclear cells, as observed in the initial stages of ARDS [183]. These two subpopulations of CD4+ cells are mutually antagonistic: IFN-γ inhibits Th2 cells, while IL-10 inhibits Th1 cells [176, 184, 185]. Th2 cells also play an important role in collagen deposition [186]. They control collagen synthesis by regulating the expression of tissue inhibitors of matrix metalloproteinase (TIMP). The main cytokines Th2 cells secrete (IL-4, IL-5, IL-13) enhance collagen deposition [187]. IL-10, a cytokine related to the Th2 response, is crucial to the fibrosis process. Secreted by T regulatory cells, macrophages, and dendritic cells, it inhibits Th1 cells and cells to secrete IL-13, which activates fibrogenic cells, leading to collagen deposition and fibrosis [176, 188]. T regulatory cells have been shown in animal models of ARDS to mediate active lung injury resolution and regulate collagen removal in the late phase [145, 189]. Future studies should explore pericyte and immune cell interactions in lung injury and resolution.

Therapeutic Options

Mortality from ARDS has decreased significantly since the original description of this syndrome in the 1970s [2, 190]. Much of this decrease can be attributed to overall improvements in the care of critically ill patients and the use of low tidal volume ventilation strategies [191]. Other emerging treatments include the use of early neuromuscular blockade and prone positioning [192, 193]. Despite these improvements, there are no specific lung-targeted pharmacologic therapies to facilitate lung injury resolution in ARDS. Additional cellular and molecular pathways that might be involved in the development of lung fibrosis must be identified as possible therapeutic targets.

Based on the results of promising randomized controlled trials showing reduced lung function decline, the FDA approved two pharmaceuticals nintedanib and pirfenidone for the treatment of IPF in 2014, opening new potential avenues of treatment for this debilitating disease [194, 195]. While the mechanism of action of these drugs is incompletely understood, nintedanib is a tyrosine kinase inhibitor and targets PDGFRα, which is expressed by pericytes as mentioned above [196]. It is unknown if one of the mechanisms of benefit of nintedanib in IPF occurs via pericyte involvement. Another recent study showed that the small-molecule Gli inhibitor GANT61 reversed the fibrosis phenotype in bone marrow by impairing the expansion of Gli1+ myofibroblasts [197]. Since Gli1 labels a significant portion of lung pericytes, a GANT61 drug might be able to inhibit pulmonary pericyte-derived fibrosis.

Conclusion

Although a subpopulation of pericytes may play a central role in lung disease, their contribution under physiologic conditions remains unknown. From a drug development perspective, pericytes provide a cellular target with a consistent molecular repertoire and response to signals. The challenge will lie in limiting the deleterious functions of pericytes while preserving the healthy ones.

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