

Chapter 12

Disruption in the Dynamic Balance Between Transforming Growth Factor- β and Granulocyte/Macrophage Colony-Stimulating Factor Signaling Within the Alveolar Space of the Alcoholic Lung: Impact on Epithelial and Macrophage Function

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Abstract The mammalian lung is a remarkably complex and delicate organ that has evolved to serve the principal function of exchanging expired carbon dioxide for inspired oxygen to help fuel aerobic metabolism throughout the body. The fundamental gas exchange structure within the lung is the alveolus and its surrounding capillary network. Intrauterine lung development is exquisitely regulated and progresses through stages, with the formation of alveoli occurring at the end of gestation and in the immediate postnatal period. The signaling molecules that comprise the pluripotential superfamily that includes transforming growth factor- β (TGF β) are critically involved in the branching morphogenesis and later alveolarization that are vital for normal lung development, but their expression and activity wane rapidly in the postnatal period in the healthy state. In contradistinction, the relative influence of granulocyte/macrophage colony-stimulating factor (GM-CSF) emerges in the immediate prenatal period and throughout normal lung health as the dominant regulator of alveolar functions including the maintenance of the tight epithelial barrier, the formation and recycling of surfactant, and the maturation of the alveolar macrophage, which is the unique resident host immune cell within the lower airways. There is now abundant experimental evidence that chronic alcohol ingestion disrupts the dynamic balance between TGF β 1 and GM-CSF within the lung with profound consequences for alveolar epithelial and macrophage function. In fact, the aberrant expression and activity of TGF β 1 and the consequent dampening of GM-CSF signaling within the lower airways appears to be a fundamental factor that

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drives the “alcoholic lung phenotype.” This chapter reviews the fundamental roles of these two molecules and their signaling pathways and illustrates the evolving recognition that disruption of their dynamic balance by alcohol renders the lung susceptible to a wide range of pathologies.

Keywords Transforming growth factor beta (TGF β) • Granulocyte/macrophage colony-stimulating factor (GM-CSF) • Alveolar macrophage • Alveolar epithelium • Phagocytosis • Tight junctions

Introduction

The complex structure of the lung is designed to serve its primary function of exchanging oxygen from the inspired atmospheric gas with carbon dioxide that is produced by metabolic pathways throughout the body and is eliminated with the expired gas during ventilation. The fundamental gas-exchange units within the lung are the alveoli, which are the terminal small airways lined by a unique epithelium contained within a delicate extracellular matrix and surrounded by a dense capillary network that together enable the efficient bidirectional transfer of inhaled oxygen from the airspace to the hemoglobin in circulating red blood cells and the parallel transfer of carbon dioxide from the blood to the alveoli for elimination by exhalation. Each alveolus is 200–300 μm in diameter, and their total surface has been estimated to be approximately that of a tennis court. This enormous surface area is essential for efficient gas exchange, and the structural and functional integrity of the alveoli depends on several remarkable characteristics of this unique microenvironment.

First, the alveolar epithelium provides a tight and relatively impermeable barrier that is essential to create a stable air–liquid interface even though the entire cardiac output is continuously flowing just microns away through the alveolar capillary network. The alveolar epithelium comprises two cell types. The first is a cuboidal cell named the alveolar epithelial type II (ATII or AT2) cell [1], which is a specialized cell that among its many functions produces and secretes surfactant, a complex structure of phospholipids and proteins that decreases surface tension on the alveolar surface and thereby facilitates cyclical inflation and deflation of the alveoli at very low pressures during ventilation. The ATII cell is also responsible for maintaining the local redox potential within the highly oxidizing microenvironment of the alveoli where local oxygen concentrations are the highest than anywhere in the body. A cardinal feature of this role in maintaining antioxidant defenses is the synthesis and secretion of the tripeptide glutathione into the alveolar space. In the normal healthy adult lung, the concentration of glutathione within the alveolar epithelial lining fluid is in the range of 500–1,000 μM , which is 100–200 times its concentration in plasma. The second cell type that forms the alveolar epithelial surface and barrier is the alveolar epithelial type I (ATI or AT1) cell, which is a terminally differentiated cell that is derived from the ATII cell through a process that has been termed

transdifferentiation [1]. The ATI cell is flat and provides the primary surface for gas exchange. In fact, although the ratio of ATI and ATII cells within the lung is approximately 1:1, the ATI cells comprise approximately 95 % of the total alveolar surface area. The intercellular tight junctions between these cells sharply limit the paracellular passage of water and solutes from the capillary and interstitial compartments into the alveolar space [2]. In parallel, the relatively small amounts of fluid that do leak into the alveolar space are efficiently transported back across the epithelial barrier by both ATI and ATII cells using a coordinated system involving the active transcellular pumping of sodium and the consequent passage of water back into the interstitial compartment [3–6] where it is ultimately cleared by lymphatic drainage. As a consequence, a thin layer of epithelial lining fluid with its surface covered by surfactant is continuously maintained within the alveoli and provides the unique air–liquid interface that is critical for efficient gas exchange. The earlier chapter on the effects of alcohol on the alveolar epithelium [Koval] describes these specialized barrier functions in detail and how they are perturbed by chronic alcohol ingestion.

As the airways are covered with a large epithelial surface that is constantly exposed to the external environment, the protection of the delicate alveoli from inhalational injury by both biological and non-biological agents is of paramount importance. As discussed in the chapter by Sisson and Wyatt, there are exquisitely effective mechanisms within the upper and conducting airways that limit access of most of these agents to the alveolar space. However, noxious particles that are inhaled and evade these defenses in the larger airways must be cleared efficiently and rapidly before they can cause damage. In parallel, infectious agents such as bacteria that are either aspirated into the airways or enter the alveolar space from the alveolar capillaries must likewise be recognized and cleared before they can cause significant infections. Further, surfactant phospholipids and proteins that become oxidized and/or otherwise dysfunctional, even through normal “wear and tear” in the alveolar space, must be cleared and recycled into “fresh and functional” surfactant by the ATII cells. These complex tasks are served by the alveolar macrophage, a terminally differentiated cell that is derived from peripheral blood mononuclear cells and is unique to the alveolar space. This cell is among the body’s most potent phagocytes (“large eater” in Latin) and is capable of ingesting particulate matter such as inhaled dust, bacteria, and damaged surfactant; in fact, these cells were called “dust cells” by early investigators. As the sentinel that patrols the alveolar space, the alveolar macrophage acts as the principal host defense cell within the lower airways but can also activate an adaptive immune response when pathogens are recognized. Specifically, it can recruit neutrophils and lymphocytes to the alveolar space by secreting chemokines including tumor necrosis factor- α , interleukin-12, interleukin-18, and interferon- γ [7–9]. In many ways, the alveolar macrophage is not only the primary sentinel that protects the alveolar space from extrinsic and intrinsic stresses but also the primary herald that signals to cells outside of the alveolar space that their presence is needed.

Previous chapters have elucidated the incredibly complex effects of alcohol on the lung’s epithelial barrier as well as on airway immunity, including its profound inhibition of alveolar macrophage function. The focus in this chapter is to highlight

one of the fundamental mechanisms by which alcohol impairs both alveolar epithelial and macrophage function. Specifically, there is relatively recent and rapidly evolving experimental evidence that chronic alcohol ingestion causes a relative increase in the actions of transforming growth factor- β (TGF β) and a consequent dampening of the relative actions of granulocyte/macrophage colony-stimulating factor (GM-CSF) within the alveolar space. To understand how a relative shift in the influences of TGF β and GM-CSF creates the “alcoholic lung phenotype” within the alveolar space, we must first briefly review the normal actions of, and the dynamic balance between, these two pluripotent signaling molecules.

Transforming Growth Factor- β and the Lung

TGF β , named for its ability to transform the phenotype of normal fibroblasts in culture, is the titular member of a large superfamily of ~ 30 growth factors that includes diverse polypeptides such as the activins, inhibins, bone morphogenetic proteins, Mullerian inhibiting substance, and others [10]. Within this superfamily is the subfamily of TGF β , which includes three isoforms (TGF β_1 , TGF β_2 , and TGF β_3) [11]. The sequence homology for TGF β_1 among various mammalian species (human, rat, mouse, pig, and monkey) is $>97\%$, indicating remarkable evolutionary conservation. Among the three isoforms there is considerable homology as well, and in most cell systems studied the isoforms have essentially the same effects [12]. Therefore, although a distinct gene encodes each isoform and the isoforms are not expressed uniformly in all cell types, they appear to have more or less the same biological effects and are often referred to collectively as simply TGF β . Within the lung, TGF β_1 is the dominant isoform that is expressed and the one that has received the most scientific attention in studies of lung biology. TGF β_1 is a pluripotent cytokine that influences tissue injury and repair. Although first identified for its proliferative effects on mesenchymal cells, it inhibits epithelial growth and function and may even promote apoptosis in these cells.

TGF β exerts its effects by binding to specific receptors on the surface of target cells. However, its biological function is regulated at the level of activation from a latent form. Specifically, TGF β is synthesized and secreted as a 25 kDa homodimer that is non-covalently associated with a latency-associated peptide that is part of the originally synthesized pro-peptide that undergoes proteolytic cleavage [13]. The TGF β latency-associated peptide complex is called the “small latent complex.” This complex is usually bound to a larger peptide of variable length called latent TGF β -binding protein, of which four family members have been identified [13, 14]. The latent TGF β -binding protein is covalently bound to the latency-associated peptide, and the entire combination of TGF β , latency-associated peptide, and latent TGF β -binding protein forms the “large latent complex.” TGF β is inactive when associated with latency-associated peptide, either in the large or in the small latent complex, as it cannot bind to its receptors on the cell surface. The latent TGF β -binding proteins are a subfamily of the extracellular microfibrillin proteins, fibrillin 1 and fibrillin 2 [14], that target TGF β for association

with the extracellular matrix. The TGF β latency-associated peptide complex is released from the matrix by proteolytic cleavage of the latent TGF β -binding protein and subsequent release of the small latent complex. In addition, some secreted TGF β is associated only with the latency-associated peptide and may be soluble. However, free TGF β is not present in significant amounts in biological fluids, at least under normal circumstances, as it is bound by a variety of other proteins such as α_2 -macroglobulin. Therefore, multiple regulatory mechanisms maintain TGF β in an inactive form under normal healthy conditions.

The study of TGF β in complex biological systems has been somewhat hampered by difficulties in distinguishing the latent from the active form, particularly in tissues. Rifkin and colleagues developed a bioassay for active TGF β that uses a mink lung epithelial cell line in which the promoter for the plasminogen activator inhibitor-1 (PAI-1) gene is linked to a luciferase reporter [15]. Biological samples are co-incubated with these cells, and luciferase activity correlates with the amount of biologically active TGF β present. Although this assay has been used to assess the amount of active TGF β in fluids such as lung lavage fluid, the detection of active TGF β in tissues has proven to be problematic, as the extraction of tissue lysates activates the latent complex. Brunner and colleagues modified the original Rifkin assay by placing frozen sections of rat tissue (i.e., no extraction methods used) directly over the mink lung cells in culture [16]. This assay is only semiquantitative but can be used to provide some evidence as to whether tissue-bound TGF β is bioactive. However, the study of TGF β and its role in complex processes such as tissue injury and repair has been problematic because of this fundamental problem in distinguishing the latent from the active form within tissues. However, there is strong experimental evidence that only the active form of TGF β is released into the alveolar space during acute inflammatory insults such as endotoxemia, which enables its levels within this space to be quantified using more sensitive and accurate assays such as ELISA [17].

TGF β can be released and activated from the latency-associated peptide by a myriad of factors identified thus far, including oxidants [18, 19], nitric oxide and/or reactive nitrogen species [19, 20], at cell surfaces by thrombospondin-1 from platelets or by cell-associated plasmin [11], and via interactions with specific integrins such as $\alpha v \beta 6$ [21] or matrix glycoproteins [22, 23]. Upon activation, TGF β can interact with specific receptors on the surface of its target cells [12, 13, 24]. There are three classes of TGF β receptors, RI, RII, and RIII [11]. The RIII receptors appear to serve only to facilitate the association of TGF β with the RII receptors, which upon activation by binding to TGF β recruit, bind, and transphosphorylate the RI receptor [11]. The subsequent intracellular signaling cascade involves phosphorylation of a unique family of intracellular molecules known as Smads [11, 12], which ultimately affect transcription of a wide array of TGF β -responsive genes. The intracellular signaling is complex, including in epithelial cells where it may involve pathways other than the Smads, such as Ras-dependent and Ras-independent pathways that go through MAP kinase signaling [12]. The end result of TGF β signaling on a target cell likely depends on a myriad of factors as TGF β can act as a proliferative or an antiproliferative factor depending on the target cell and the local

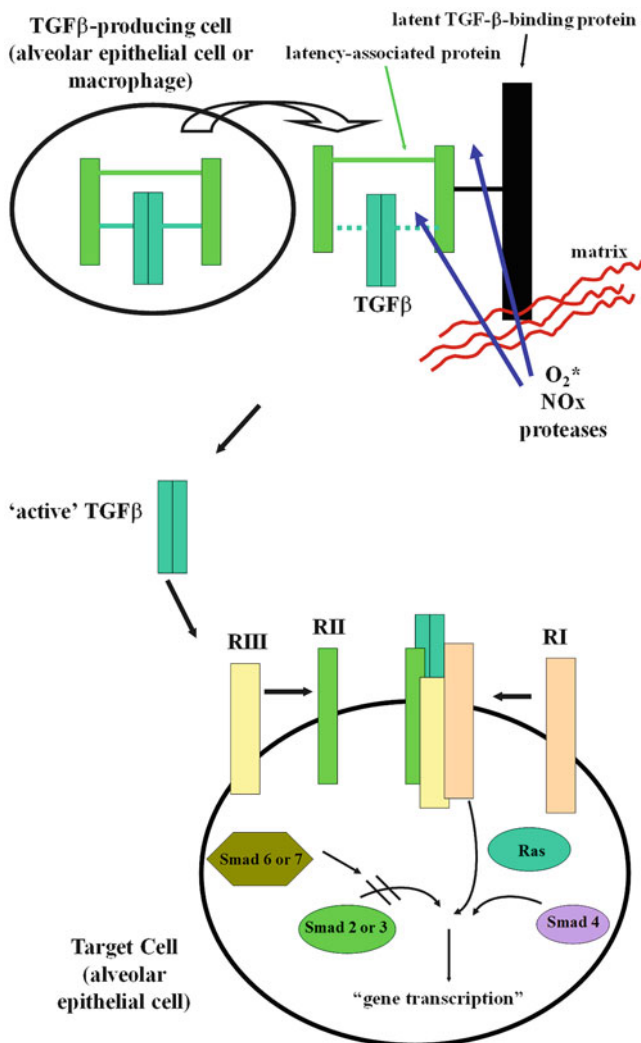


Fig. 12.1 Simplified schema for the expression, activation, and signaling of transforming growth factor beta (TGFβ). Cells synthesize and secrete TGFβ in a complex with latency-associated peptide, and this complex is bound to a latent TGFβ-binding protein that localizes the complex to the extracellular matrix. The TGFβ can be released from the complex by oxidants, reactive nitrogen species, proteases, and other mechanisms, allowing it to act on target cells by binding membrane receptors. TGFβ signal transduction uses a unique family of proteins known as Smads as well as other factors such as Ras to alter transcription of a variety of genes in a cell-specific and condition-specific manner

conditions. Although much has been learned about TGFβ in the past decade, it remains largely unknown how specificity in signal transduction and responses are governed in a cell-specific and condition-specific manner. A simplified schema of TGFβ expression, activation, and signaling is depicted in Fig. 12.1.

The aforementioned “dual roles” for TGFβ, specifically both proliferative and anti-proliferative effects, have made studies of its biological actions fascinating as well as daunting. Specifically, TGFβ has such diverse effects and in so many contexts that it has been challenging to elucidate its discrete roles in both physiology and pathophysiology. Within the lung it is well established that TGFβ is absolutely critical for lung development. However, even in this context it has apparently contradictory roles at different stages. For example, whereas there is consistent experimental evidence that TGFβ regulates branching morphogenesis in early lung development, it appears that TGFβ acts as both a positive and a negative regulator of alveolarization in late lung development. One essential function for TGFβ in alveolarization is in stimulating the transdifferentiation of AT2 epithelial cells into AT1 epithelial cells. As discussed previously, the AT2 cell is the progenitor of the AT1 cell, and its transdifferentiation is essential to develop the enormous surface area of the alveoli required to make the transition to air breathing at birth. However, TGFβ can also stimulate alveolar epithelial cells to undergo epithelial-to-mesenchymal transition or “EMT,” which may be necessary in the context of lung repair following injury (although this is at present poorly understood) but is clearly detrimental to normal alveolarization in the prenatal and postnatal period. In parallel, although TGFβ may have a role in alveolarization during late lung development and perhaps even in the early postnatal period, there is virtually no significant TGFβ expression in the healthy mature lung, and at least experimentally it is clear that TGFβ impairs alveolar epithelial barrier function [17, 21, 25]. During the acute response to an injury such as pneumonia or trauma, TGFβ expression is robust at the local site of the inflammatory response and its ability to loosen the epithelial barrier and even promote EMT is likely important to an appropriate injury response. However, once the acute inflammation has resolved and the lung undergoes normal repair, TGFβ expression wanes once again. Therefore, it appears that there is an exquisite balance that governs its functions in a particular context, and, while crucial for tissue development and function, TGFβ has also been implicated as a pathophysiological agent in a wide range of diseases. In particular, aberrant signaling by TGFβ has been identified as a likely mediator of bronchopulmonary dysplasia (BPD) in the premature newborn as well as chronic obstructive pulmonary disease (COPD) and pulmonary fibrosis which are primarily seen in much later stages of life [26].

Granulocyte/Macrophage Colony-Stimulating Factor and the Lung

Granulocyte/macrophage colony-stimulating factor or GM-CSF is a 23-kDa glycosylated monomeric peptide that is secreted by multiple cell types, including the alveolar epithelial type II cell [9]. It was first identified in mouse lung cell-conditioned medium and was named for its ability to stimulate the growth of granulocytes and macrophages from cultured hematopoietic progenitor cells. The cloning of this protein permitted a variety of studies *in vitro* and *in vivo* to characterize its functions, and it was subsequently found to stimulate the production of

eosinophils, erythrocytes, megakaryocytes, and dendritic cells in addition to granulocytes and macrophages. GM-CSF has been widely used clinically to improve bone marrow recovery following chemotherapy. Therefore, although it had first been isolated from lung tissue the prevailing view evolved that its functions were largely restricted to bone marrow lineage development.

The construction of GM-CSF knockout mice produced startling findings that had not been predicted based on the initial studies of its functional roles. Specifically, mice with targeted ablation of the GM-CSF gene had unexpectedly normal bone marrow maturation and normal circulating levels of all blood cell lines [27]. However, these mice were found to develop a lung disease similar to human pulmonary alveolar proteinosis (PAP) [27], which is a relatively rare disorder characterized by massive accumulation of surfactant phospholipids and proteins in the airspaces, leading to progressive respiratory failure and death in most cases if untreated. It is interesting that alveolar macrophages from patients with PAP are dysfunctional when examined *in vitro*, and in parallel, macrophages from GM-CSF-deficient mice are deficient in TNF α secretion, respiratory burst, and bactericidal activity [28]. Importantly, site-directed expression of GM-CSF in the alveolar type II cells of GM-CSF-deficient mice (by reinserting the gene and coupling it to the surfactant protein C promoter) completely eliminated the pulmonary defect [29]. Further, GM-CSF treatment by inhalation corrects the PAP defect in GM-CSF-deficient mice [30]. Taken together, these and related studies demonstrate that the most important site of GM-CSF activity is within the alveolar airspace, where it induces maturation of alveolar macrophages via a process that has been termed “priming.” Although patients with the acquired or the idiopathic form of PAP do not have an apparent genetic defect in GM-CSF, they have autoantibodies directed against GM-CSF and therefore have a functional deficiency of GM-CSF signaling in the lung. For additional information the reader is referred to an excellent review on this topic [8]. In parallel, GM-CSF acts in an autocrine and/or a paracrine manner within the airway and induces alveolar epithelial barrier integrity and surfactant secretion. In fact, there are receptors for GM-CSF on the epithelial surface of conducting airways including the trachea and the bronchi, suggesting that it may be critical for epithelial barrier integrity throughout the entire airway and not just within the alveolar space [31]. Not surprisingly, whereas TGF β is expressed abundantly in the developing lung *in utero*, including at the earliest stages of development, GM-CSF is expressed only late in lung development. It is just prior to birth and thereafter when it assumes a critical role in maintaining the postnatal “air breathing” lung in which a tight alveolar epithelial barrier and a dynamic surfactant layer at the air–liquid interface within the alveolar space are absolutely essential for normal lung function.

GM-CSF receptors share a common beta subunit chain with other cytokine receptors but also have a unique alpha subunit chain. The GM-CSF receptor beta chain (GM-CSF R β) is common to the interleukin-3 and interleukin-5 receptors [9, 32], whereas the alpha chain (GM-CSF R α) is unique to the GM-CSF receptor. Neither chain has any catalytic activity, but the β chain is constitutively associated with the tyrosine kinase JAK2. The α chain binds with low affinity to GM-CSF, and this

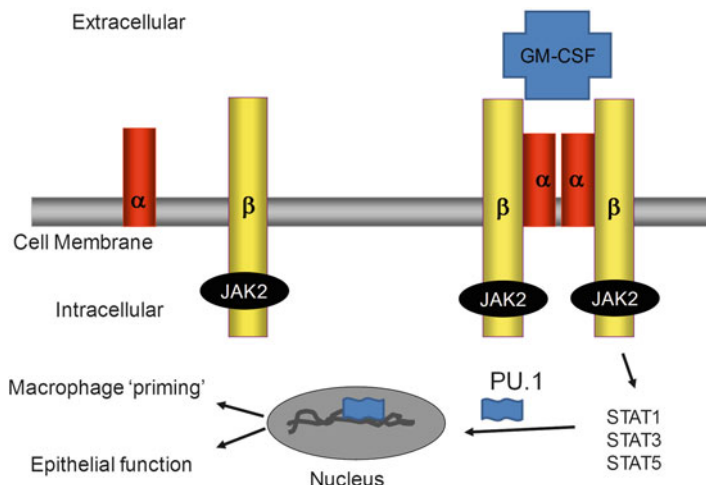


Fig. 12.2 Schematic illustration of GM-CSF signaling within the alveolar compartment. GM-CSF is synthesized by alveolar epithelial cells and secreted into the alveolar space where it can prime macrophages in a paracrine manner as well as stimulate epithelial function in an autocrine manner. The active GM-CSF receptor requires the coordinate clustering of two alpha chains (GM-CSFR α) that bind the soluble GM-CSF on the cell surface and two beta chains (GM-CSFR β) that are associated with JAK2, a kinase necessary to initiate a series of intracellular signaling steps involving the STAT family that terminate in the translocation of the master transcription factor, PU.1, into the nucleus. PU.1 then activates a program of gene expression that either primes and then activates the alveolar macrophage or induces a range of alveolar epithelial cell functions including the formation of tight junctions that are critical to maintaining a tight alveolar barrier

facilitates formation of a high-affinity six-polypeptide complex composed of two α chains, two β chains, and two JAK2 chains. Activation of JAK2 following GM-CSF binding initiates a series of intracellular signaling pathways that are both in parallel and in series [32] and that ultimately leads to activation of the nuclear transcription factor PU.1. PU.1 is in the ets family of transcription factors and is a “master” transcription factor in the proliferation and differentiation of myeloid cells. PU.1 is expressed in alveolar macrophages of normal mice, but its expression is lost in GM-CSF-deficient mice [9]. Interestingly, restoration of GM-CSF expression in the type II cells of these mice also restores PU.1 expression in the alveolar macrophage [9]. GM-CSF-mediated nuclear binding of PU.1 activates an array of genes that are required to “prime” monocytes to mature into functional alveolar macrophages that are proficient in the phagocytosis of pathogens, surfactant phospholipid clearance, cell adhesion, and inflammatory signaling [9, 33, 34]. In fact, the constitutive expression of PU.1 (using a retroviral vector) completely reverses the PAP defect in alveolar macrophages of GM-CSF-deficient mice [34]. Therefore, GM-CSF-dependent expression of PU.1 appears to be absolutely required for terminal maturation and function of the alveolar macrophage in the normal host. A schema illustrating the signaling mechanisms of GM-CSF is shown in Fig. 12.2.

Alcohol Perturbs the Normal Dynamic Balance Between TGF β and GM-CSF in the Lung

The recognition that the relative influences of TGF β and GM-CSF are in a dynamic equilibrium has evolved as experimental models of the “alcoholic lung” phenotype have elucidated the mechanisms by which alcohol alters alveolar epithelial and macrophage function. As discussed above, in many ways these growth factors have somewhat diametrically opposed effects on these cellular constituents within the alveolar space. Within the healthy mature lung, the expression of TGF β is very low and there is no detectable active TGF β within the alveolar space. In contrast, GM-CSF is readily detectable within the alveolar space where it is synthesized and secreted in continuous fashion by the alveolar epithelium.

Although TGF β plays a relatively lesser role in the healthy adult lung, it is rapidly activated in response to a wide variety of stresses. Its physiological roles in the context of acute lung inflammation are likely to increase epithelial permeability at the local site, modulate the immune response in a time- and context-dependent manner, and transform fibroblasts into myofibroblasts and thereby promote a scar formation where necessary. However, aberrant expression and activation of TGF β has been implicated as a pathophysiological mechanism involved in diverse lung diseases from bronchopulmonary dysplasia in the premature neonate to pulmonary fibrosis in adults.

Soon after the landmark epidemiological study showing an association between alcohol abuse and the acute respiratory distress syndrome [35], our research group identified that in experimental animal models chronic alcohol ingestion dramatically induced the expression of latent TGF β in the lung and markedly increased the release of activated TGF β into the alveolar space [17, 25] where it increases alveolar epithelial permeability and promotes diffuse lung edema. In fact, the ability of soluble factors within the alveolar space to alter epithelial permeability in the inflamed alcoholic lung could be completely attributed to the actions of TGF β [17].

The mechanisms by which chronic alcohol ingestion induces the expression of TGF β within the lung and in turn promotes its activation during acute inflammatory stresses such as sepsis are not entirely clear, but several interdependent mechanisms have been identified in experimental modes. It had been recognized previously that alcohol abuse in humans is associated with hypertension, and activation of the renin–angiotensin system has been postulated as the underlying mechanism. Interestingly, blockade of the renin–angiotensin system in alcohol-fed animals with either an angiotensin-converting enzyme inhibitor (that complexes the conversion of angiotensin I to angiotensin II) or an angiotensin II receptor blocker completely inhibits the induction of TGF β expression in the lung and decreases the release of active TGF β into the alveolar space during endotoxemia [25]. In fact, blocking the renin–angiotensin system prevented alcohol-induced glutathione depletion within the alveolar space [25], possibly by inhibiting alcohol-induced expression and/or activation

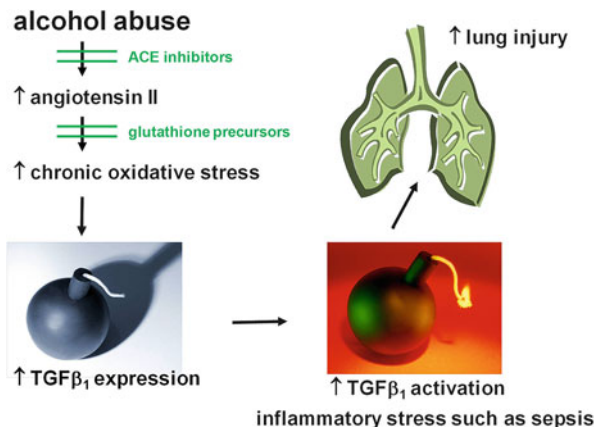


Fig. 12.3 Pathophysiological mechanisms by which alcohol induces TGF β and promotes lung injury. Experimental evidence indicates that chronic alcohol ingestion activates the renin–angiotensin system. As the lung is a primary source of angiotensin-converting enzyme (ACE), this leads to increased production of angiotensin II which in turn increases the local production of reactive oxygen species that contribute to the oxidative stress and glutathione depletion in the alcoholic lung. As a consequence, there is an aberrant and robust induction of TGF β within the lung. Most of this alcohol-induced TGF β remains in the latent or inactive form, and therefore the otherwise healthy alcoholic may have no apparent lung dysfunction. However, in the event of an acute inflammatory stress such as sepsis, this TGF β becomes activated and released into the alveolar space where it causes epithelial barrier disruption and increases the severity of acute lung injury

of NADPH oxidase within the lung [36]. Consistent with these observations, alcohol-induced expression of TGF β could also be blocked by supplementing the diets of alcohol-fed animals with glutathione precursors [25]. Taken together, these experimental observations suggest the pathophysiological scheme depicted in Fig. 12.3.

Although confirmation of this pathophysiological pathway in humans will require further studies, at present there is preliminary evidence that TGF β levels are increased in the airways of critically ill alcoholics as compared to nonalcoholics and that the alveolar macrophages of even otherwise healthy alcoholics have increased expression of TGF β (Lou Ann Brown, personal communication). Consistent with the latter observation, more recent experimental evidence suggests that alveolar macrophage-derived TGF β can degrade the alveolar epithelial barrier via cell-to-cell interactions in which the TGF β is activated at the epithelial surface (Tiana and Pratibha paper). This pathway therefore raises the intriguing possibility that chronic treatment with angiotensin-converting enzyme inhibitors or angiotensin receptor blockers (widely used to treat cardiovascular disease), alone or in combination with glutathione precursors (such as the widely available supplement *S*-adenosylmethionine), could mitigate the effects of chronic alcohol abuse on the lung.

This pathological alcohol-induced expression and activation of TGF β in the lung are paralleled by profound dampening of the GM-CSF signaling within the alveolar space. Our laboratory first identified the impact of chronic alcohol ingestion on GM-CSF-dependent alveolar epithelial cell and macrophage function in a relatively

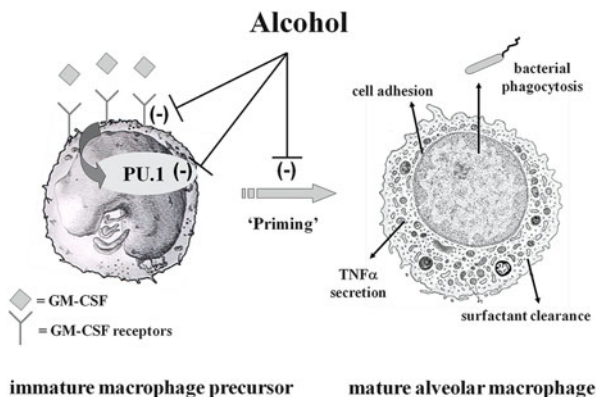


Fig. 12.4 Proposed mechanisms by which alcohol abuse inhibits alveolar macrophage maturation and innate immune functions that are critical to respond to pathogens within the alveolar space. In an experimental model, although chronic alcohol ingestion has no effect on GM-CSF protein levels in the alveolar space, it decreases the cell surface expression of the GM-CSF receptor in alveolar macrophages. In parallel, chronic alcohol ingestion decreases the expression and nuclear binding of the GM-CSF master transcription factor, PU.1. As a consequence, GM-CSF priming and maturation of the precursor cell into a fully functional alveolar macrophage is inhibited. Importantly, recombinant GM-CSF treatment delivered to the airway in alcohol-fed rats rapidly (within 48 h) restores GM-CSF receptor expression, PU.1 expression and nuclear binding, and innate immune function in the alveolar macrophage [39]. This same pathophysiological sequence, including rapid resolution in response to recombinant GM-CSF treatment, also underlies alcohol-mediated alveolar epithelial barrier dysfunction in the same experimental model [37]. Reprinted from the author's review paper in the *American Journal of Physiology: Lung Cellular and Molecular Physiology* (292: L813–L823, 2007) and reprinted here with permission

indirect fashion. Specifically, we first determined that recombinant GM-CSF delivered via the upper airway restored alveolar epithelial barrier function and fluid transport in alcohol-fed rats, even during endotoxemia [37]. Importantly, although that study showed that GM-CSF treatment decreased endotoxin-mediated lung injury even in control-fed rats, the magnitude of the efficacious response was clearly greater in the alcohol-fed rats. The efficacy of recombinant GM-CSF turned out to be more than a serendipitous finding, as these initial observations led to the discovery that chronic alcohol ingestion decreases the expression of GM-CSF receptors in the airway epithelium and macrophages and in turn dampens intracellular signaling to the GM-CSF master transcription factor, PU.1. As a consequence, GM-CSF-dependent functions in each cell type are impaired. These observations in experimental models have recently been confirmed in the human condition. Specifically, alveolar macrophages isolated from young and otherwise healthy alcoholics have significantly decreased expression of GM-CSF receptors [38]. Remarkably and to date via unknown mechanisms, recombinant GM-CSF treatment restores GM-CSF receptor expression and signaling and normalizes both alveolar epithelial barrier function [31] and alveolar macrophage immune function in experimental models [39]. These experimental findings are summarized in schematic form in Fig. 12.4.

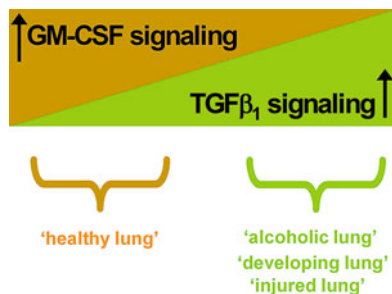


Fig. 12.5 The relative shift from the influence of GM-CSF to TGF β signaling in the alcoholic lung. In the healthy adult lung the relative influence of GM-CSF, which through its discrete signaling pathway promotes alveolar macrophage activation and alveolar epithelial barrier formation, is dominant over the influence of TGF β . However, in the alcoholic lung the shift towards the relative influence of TGF β signals a decrease in epithelial barrier and changes the activation of the alveolar macrophage. Therefore, the alcoholic lung in some respects recapitulates the developing lung in utero and the normal lung responding to a localized acute injury such as pneumonia. Specifically, a relative shift in this balance is physiological in the correct context, but in the adult lung the aberrant and diffuse expression of TGF β creates a dangerous vulnerability to infection and/or acute lung injury

In parallel, more recent experimental studies have identified that alcohol ingestion interferes with the absorption of dietary zinc in the intestine and its transport into the alveolar space and that dietary zinc supplements restore GM-CSF receptor expression and phagocytic function in the alveolar macrophage [40]. Although the precise mechanism(s) by which chronic alcohol ingestion decreases GM-CSF receptor expression and signaling within the alveolar space are unknown, unpublished observations suggest that TGF β causes internalization of the GM-CSF receptor in alveolar macrophages (Pratibha Joshi, personal communication). Whether alcohol-induced dampening of GM-CSF signaling is mediated by this mechanism alone or in combination with zinc deficiency or other as yet unidentified mechanisms, there is rapidly growing evidence that the alcoholic lung undergoes an insidious shift from the normal dynamic balance in which the influence of GM-CSF greatly dominates that of TGF β to one in which TGF β assumes a pathological role (and may even be directly responsible for inhibiting GM-CSF signaling). In fact, such a shift in the balance between TGF β and GM-CSF is part of the program of lung development in utero and is likely a critical facet of the normal response to localized lung injury and repair in the postnatal lung. In this context, chronic alcohol abuse may in a perverse manner recapitulate the developing lung and/or the injured lung by shifting the balance away from GM-CSF signaling to that of TGF β , as depicted in Fig. 12.5.

The alcoholic lung is proving to be a remarkably complex perturbation of normal lung function, and every new experimental observation makes the overall pathophysiological scheme impossible to lay out in a linear fashion. In contrast, multiple molecular, organellar, and cellular derangements appear to interact in a matrix fashion in which seemingly disparate factors conspire to change lung function in ways that render it susceptible to infection and injury. Therefore, there is no one “master switch” or discrete derangement that can explain the complexities of the alcoholic lung.

However, the dynamic imbalance between TGF β and GM-CSF can certainly be implicated as playing an important role in the phenotypic and functional changes within the alveolar space that confer this remarkable vulnerability. Perhaps more importantly, there is growing experimental evidence as well as intriguing observations from clinical studies that this dynamic imbalance can be manipulated and therefore may be a target for novel therapeutic interventions. This may be of particular value in the chronic setting before pneumonia and/or acute lung injury occurs. For example, as discussed previously angiotensin-converting enzyme inhibitors and angiotensin receptor blockers are already widely used to treat cardiovascular diseases including hypertension and congestive heart failure. As they completely inhibit the aberrant expression of TGF β and the development of the “alcoholic lung phenotype” in experimental animal models [25], it is reasonable to predict that they could mitigate the pathophysiological effects of alcohol abuse on the lung in humans. Even if their use were limited to individuals with alcohol-use disorders and other medical indications (such as hypertension), they might decrease the risk of serious lung complications in many individuals. In parallel, dietary supplementation with glutathione precursors and/or zinc, which again are remarkably effective in experimental models including decreasing TGF β expression [17] and restoring GM-CSF receptor expression and signaling [40, 41], is a potentially simple and inexpensive therapy that could enhance lung health in individuals who suffer from alcohol-use disorders. In fact, clinical studies are already under way to determine whether or not interventions such as dietary supplementation with *S*-adenosylmethionine and/or zinc can enhance alveolar macrophage function in subjects seeking treatment for alcohol addiction.

Clearly the perturbations in cellular and organ function that characterize the alcoholic lung are complex and likely cannot be explained entirely by the pathophysiological shift in the relative influences of TGF β and GM-CSF. However, it is remarkable how many of the experimental features are intimately related to, if not directly caused by, this disequilibrium between these two cardinal signaling molecules within the alveolar space. Therefore, the elucidation of this alcohol-mediated shift towards a TGF β -driven alveolar microenvironment has not only shed light on the mechanisms by which alcohol abuse renders individuals susceptible to pneumonia, acute lung injury, and other lung diseases, but it has also identified novel therapeutic targets that can now be tested in clinical studies. The goal is clearly not to make it safer to abuse alcohol but rather to limit the devastating pulmonary and perhaps even the systemic consequences of chronic alcohol abuse in individuals who are seeking treatment for their addiction. In this context, it is imperative that we identify mechanisms to mitigate the devastating effects of alcohol abuse on the lung and other target organs as history reminds us that many of us will use and abuse alcohol regardless of any social, cultural, or religious prohibitions. This is in fact entirely consistent with the approach to many lifestyle-related health problems. For example, we treat hypertension, dyslipidemias, and diabetes mellitus in obese individuals with the metabolic syndrome because we recognize that weight loss through diet and exercise may be the fundamental treatment but for many is difficult if not impossible to attain. Therefore, we have an obligation to develop therapies to mitigate the medical complications of alcohol abuse that can be applied in parallel with cognitive and behavioral therapy for their underlying addiction.

Summary

Although the mechanisms by which chronic and excessive alcohol consumption render the lung susceptible to a wide range of infectious and inflammatory injuries are complex and we have only recently begun to elucidate them, there is growing evidence that alcohol disrupts the normal dynamic balance between GM-CSF and TGF β signaling in the lung. The relative shift towards the influences of TGF β over GM-CSF in the alcoholic lung has profound consequences for epithelial function as well as for host defense responses by the macrophage. In this context, therapeutic strategies to restore a “healthier” balance in which GM-CSF signaling resumes its dominant influence may limit the detrimental expression of the “alcoholic lung phenotype” and decrease the risk of pneumonia and lung injury in these vulnerable individuals.

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