EPITHELIAL INJURY AND REPAIR 12

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Since the mid-1970s, increasing attention has been paid to the biological responses of alveolar epithelial type II cells following acute lung injury and their contribution to the syndrome of acute respiratory distress in adults (ARDS) [1]. Research has been greatly aided by techniques developed to isolate and culture type II cells in an environment separated from the complex milieu of the whole lung. In addition, application of molecular biological techniques to the study of these cells has rapidly expanded our understanding of the type II cell's role in the structure and function of the alveolus, which will improve our knowledge of the mechanism by which a diverse group of risk factors can lead to the common final endpoint which is called ARDS, and may result in the development of specific therapies and interventions by which it can be treated [2,3].

This chapter reviews current knowledge of alveolar type II cell biology, particularly relating to acute lung injury from a variety of causes. An overview will outline the pathology of the type II cell in the normal and injured alveolus. Subsequent sections will review type II cell functions: (1) in ion transport, (2) in surfactant synthesis and secretion, and (3) as the type I cell progenitor in repair of the injured alveolar epithelium. We attempt to relate observations at the alveolar epithelial cellular level to events occurring in the clinical syndrome.

Specifically, ARDS can be divided into three major clinicopathological phases (Chapters 4 and 16):

1. an acute exudative phase in which ion transport and surfactant abnormalities may predominate;
2. a proliferative phase in which cytokines and growth factors are likely to play an important role;
3. a resolution phase that involves type II cell differentiation into type I cells with restoration of normal alveolar architecture and function.

A fourth phase, mentioned briefly in this chapter, is the fibrotic phase, with failure of normal alveolar restoration, that occurs in patients with recurrent lung insults [4]. Death can occur during any of these clinical/pathological phases. Each phase will be reviewed with specific reference to type II cell biology.

PATHOLOGY

In the normal adult human lung, type II cells comprise approximately 15% of the total lung cell population. These cuboidal cells are found in the corners of the alveolus and are polarized, with their microvilli projecting into the alveolar lumen and the basement membrane adjacent to the basement membrane of capillary endothelial cells and interstitial fibroblasts. By electron microscopy characteristic lamellar bodies, the organelles of surfactant storage, can be identified. Besides

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surfactant synthesis and secretion, type II cells function in vectorial solute transport and are the progenitor cells of alveolar type I cells [5]. As type II cells proliferate and differentiate into type I cells, they attenuate their metabolic machinery (mitochondria, endoplasmic reticulum, Golgi), lose their storage granules (lysosomal bodies, multivesicular bodies, lamellar bodies) and become flattened. Although representing less than 10% of the total lung cell number, type I cells cover more than 90% of the apical alveolar surface. The very thin type I cell surface is the site of alveolar gas exchange. Morphometry estimates reveal that potential oxygen delivery through this barrier is proportional to the oxygen requirements of the animal [6].

An important concept is that while there are many causes of ARDS, the response of the lung to injury is limited, resulting in a similar pathologic picture irrespective of etiology [7–12]. In histological sections taken from patients who have developed ARDS and died during the acute exudative phase (Chapter 4), the earliest lesion appears to be interstitial edema [11]. There is a twofold increase in septal width and a two- to threefold increase in the volume of the alveolar epithelial cells [5]. Intercellular tight junctions show a decrease in the number of junctional strands and an irregularity in distribution [12] as type I epithelial cells swell. There are areas of focal alveolar collapse and flooding. The pulmonary edema fluid is composed of proteinaceous material, inflammatory cells, cell debris, blood and surfactant remnants. Amorphous, fibrinous hyaline membranes line the alveolar septae, particularly near the distal terminal bronchioles where they form rings at the entrances to the alveolar spaces. Soon after, gross alveolar epithelial cell damage is visible. The swollen type I cells necrose, leaving intercellular gaps and a denuded, but often intact, basement membrane with overlying hyaline membranes. The type I alveolar epithelial cell is highly vulnerable to injury for at least two reasons. Firstly, there is a very large surface to volume ratio of the cell, thus leaving a large surface area exposed to injurious agents. Secondly, compared to other lung cell types, particularly the progenitor type II cell, the type I cell has relatively few intracellular organelles associated with energy production or the synthesis of macromolecules that might be associated with cellular defense against injury. By inference from cell culture results, type I cells have a significant decrease in catalase, glutathione reductase, and glutathione peroxidase activities compared with type II cells [14] (chapter 11). By contrast, type II cells have a high endogenous content of antioxidant enzymes such as manganese superoxide dismutase [15] that may protect them from the injury seen in type I cells.

Hyperplastic and hypertrophic type II cells characterize the proliferative phase of ARDS. Normal type II cell turnover *in vivo* is about 4–5 weeks, but following acute lung injury there is a dramatic increase in type II cell labeling index of up to 23% [16]. These cells can be demonstrated to be metabolically active in the synthesis of type IV collagen matrix and surfactant protein A [17]. In the proliferative phase of ARDS there are two competing phenomena: type II cell proliferation with restoration of the normal alveolar architecture, and the fibroproliferative response with organization of the intra-alveolar space exudate. If type II cell restoration prevails, clinical and pathologic resolution occurs. If the fibroproliferative response dominates, the fibrotic phase (also termed sclerosing alveolitis) results.

In the resolution phase of ARDS, type II cells differentiate into type I cells and restore the normal alveolar epithelial barrier. Type II cells that do not differentiate into type I cells synthesize and secrete surfactant to line the alveolus. Epithelial polarity is re-established with the return of vectorial solute transport (Chapter 20). There is restoration of the normal alveolar architecture histologically, with corresponding clinical improvement in gas exchange, increased pulmonary compliance,

decreased physiological shunt and clearing of the chest radiograph.

Although the pathological lesion of acute lung injury is diffuse alveolar damage, it is important to recognize that different areas of the lung parenchyma are variably affected, resulting in a patchy injury and response [18]. This patchy response has also been suggested clinically by regions of uneven nitrogen washout seen acutely in trauma patients [19] (Chapter 15). One mechanism may be that irregularly distributed corner alveolar capillaries become obstructed with cellular-fibrin debris, regional hypoxia occurs and nearby epithelial cells are more severely injured. Regional alveolar capillary plugging has been demonstrated in rabbits injured by intravenous administration of ethchlorvynol [20]. Enhanced coagulation and suppressed fibrinolysis activity have been demonstrated in bronchoalveolar lavage (BAL) fluid from patients with ARDS [21,22]. Since alveolar injury results in surfactant abnormalities and decreased alveolar compliance, mechanical ventilation, itself a necessary therapy for ARDS, may result in the more compliant normal alveolus becoming overdistended (Chapter 23).

ARDS-like lesions can be produced experimentally in a number of ways:

- Mechanical
  - serial lung lavage [23]
- Chemical
  - high F_{IO_2} [24]
  - hydrochloric acid [25]
  - endotoxin [R. Deterding and R. Mason, personal communication]
  - oleic acid [26]
  - ethchlorvynol [20]
  - *N*-nitroso-*N*-methylurethane (NNNMU) [27,28]
- Radiation [29]
- Infectious
  - viral [30]
  - bacterial [31].

These are only a few of the methods that have been used to induce acute lung injury. In animals given NNNMU subcutaneously, the alveolar epithelium is preferentially injured with relative sparing of the endothelium [27]. Lung compliance decreases as epithelial cell necrosis occurs and is restored with epithelial regeneration. No experimental animal model exactly duplicates the conditions and response of ARDS in humans. Furthermore, the mechanisms of injury can probably involve either a direct or indirect toxic effect on the alveolar epithelial cell, since risk factors for the syndrome include both inhalation and intravenous (systemic) routes of injury.

Intense investigations are in progress to find the causative factor(s) in the propagation of lung injury in ARDS. One hypothesis is that the influx of inflammatory cells, particularly neutrophils, is injurious to the alveolar epithelial cells, either by direct toxic effects or indirectly by the secretion of products detrimental to the bystanding alveolus. Parsons and colleagues [32] demonstrated neutrophil chemotactic activity present in BAL from 14 of 16 patients with ARDS but no activity in normal controls (Chapter 6). Patients with ARDS have alveolar macrophages that release more interleukin (IL)-1 than normal subjects [33]. Eosinophil cationic protein and myeloperoxidase, markers of eosinophil and neutrophil activation, respectively, are increased in the BAL of patients with ARDS [34,35]. C5a and C3, products of complement activation and neutrophil chemoattractants, are elevated in the BAL of patients with ARDS [36]. These patients have higher levels of BAL neutrophil elastase [37,38]. Although neutrophils have been intensely studied as the agents which propagate acute lung injury, they are not absolutely required to produce ARDS, which may develop in neutropenic patients [39,40] (Chapter 5).

There is evidence of oxygen free radicals (OFRs) in acute lung injury [41] (Chapter 11). These can inactivate proteinase inhibitors such as α_1-protease inhibitor, which are then more susceptible to digestion by proteolytic

cleavage by other enzymes such as neutrophil elastase. In addition, OFRs inhibit type II cell surfactant synthesis *in vitro* [42]. Xanthine oxidase instilled intratracheally into guinea pigs results in decreased BAL surfactant and decreased lung compliance [43]. *Streptococcus pneumoniae* strains deficient in pneumolysin produce hydrogen peroxide in concentrations that are directly toxic to rat alveolar epithelial cells [44]. BAL glutathione, an important antioxidant, is reduced in patients with ARDS [45]. Guinea pig type II cells cultured under normoxic conditions have significant amounts of glutathione. When exposed to hyperoxic conditions, cell injury is significantly correlated with the amounts of reduced glutathione and inversely correlated with the amounts of total intracellular glutathione; inhibition of new glutathione synthesis is associated with increased susceptibility to hyperoxic injury [19].

The type II cell may have a role in the defense against oxygen toxicity and the generation of OFRs by inflammatory cells. For example, surfactant protein (SP)-A preincubation significantly decreases alveolar macrophage superoxide production [46]. Exposure to hyperoxia produces a twofold stimulus in SP-A and SP-B mRNA synthesis by type II cells [47]. Tumor necrosis factor (TNF)α is abundant in type II cells at all stages of ARDS [48]. Cytokines such as TNF and IL-1 protect against hyperoxic lung injury [24], possibly via an increase in alveolar cell antioxidant enzymes such as manganese superoxide dismutase [49] (Chapter 7).

ION TRANSPORT AND THE ALVEOLAR EPITHELIAL BARRIER

The polarized alveolar epithelial type II cell functions in active transepithelial solute transport of sodium from the alveolar space to the interstitium, allowing resorption of alveolar fluid. In addition, tight junctions between type II cells and neighboring type I cells form the basis for an alveolar epithelial barrier that is 15 times tighter than the adjacent endothelial barrier and thus provides the major defense against noncardiogenic pulmonary leak that is characteristic of ARDS (Chapter 20).

Several methods have been employed to demonstrate and measure alveolar barrier dysfunction in humans [50–52]. In general, there is agreement that there is an increase in permeability of the air–capillary barrier in acute lung injury, whether measured as a function of extravascular lung water or in terms of solute or serum protein leakage into the alveolar space [34]. Radiolabeled aerosol clearance is also increased in patients at risk for and with ARDS [50,53,54], demonstrating that the increased barrier permeability is not due to an increase in vectoral transport by the alveolar epithelial cells. In a study of 34 mechanically ventilated patients, clinical improvement in gas exchange and radiographic abnormalities were associated with restoration of active ion transport accompanied by alveolar fluid resorption and an increase in alveolar protein concentration [55].

There is a significant increase in extravascular lung water as early as 1 hour after intravenous oleic acid injection into rats, preceding the influx of inflammatory cells in the BAL at 4 hours, suggesting that the initial mechanism of increased barrier permeability is not due to mediator release from neutrophils [56]. Neutrophil depletion prior to injury does decrease the permeability changes, suggesting that these cells may have some effect on the type II cell's ability to restore the alveolar barrier.

In patients with increased capillary permeability, protein leakage results in BAL protein contents 12–35 times that of normal controls [57,58]. Most studies agree that there is loss of size selectivity of the alveolar epithelium, as large molecular weight proteins such as albumin, IgM, and α_2-macrogobulin can be recovered in BAL from affected patients. The loss of size selectivity could be secondary to

loss of epithelial intercellullar junctional integrity or to epithelial cell death with resultant basement membrane denudation. The degree of injury probably affects the degree of protein leakage and size selectivity. Jenkins and colleagues found that the increase in BAL total protein content in septic animals was primarily due to low molecular weight plasma proteins, suggesting a relative preservation of the size selectivity of the alveolar-capillary barrier in this model of acute lung injury [31]; alveolar protein leak was completely blocked by pretreatment with ibuprofen. Byrne and colleagues [59] also demonstrated that ibuprofen could block protein leak as well as maintain arterial oxygen content and lung compliance in a sepsis model of acute lung injury. Nonsteroidal anti-inflammatory agents such as ibuprofen inhibit prostaglandin synthetase, suggesting that arachidonic acid metabolites may be important in the pathogenesis of ARDS, particularly in disrupting the alveolar epithelial barrier function of type I and type II cells. Related to this concept is the demonstration of elevated BAL leukotriene D_4, but not B_4 or C_4 or prostaglandin E_2, in patients with ARDS compared with controls [60]. Adult type II cells produce prostaglandins *in vitro* [61]. Prostaglandins in turn affect fibroblast proliferation, collagen synthesis, and collagenase secretion. These observations also suggest a possible therapeutic intervention in the early, exudative phase of ARDS and perhaps in the later proliferative phase. However, these agents could also exert detrimental effects through other mechanisms. It is important to note that aspirin, whose mechanism of action is similar to that of the nonsteroidal anti-inflammatory agents such as ibuprofen, has, with overdose, itself been reported to induce ARDS [62].

An interesting autosomal recessive clinical disorder, lysinuric protein intolerance, is characterized by a basolateral epithelial diamino acid transport defect. These patients are predisposed to ARDS and the concentration of diamino acids in the BAL of asymptomatic patients is increased [63], suggesting that epithelial transport functions of type II cells may be very important in the cellular mechanisms of ARDS.

SURFACTANT FUNCTION (CHAPTER 16)

Type II alveolar epithelial cells synthesize surfactant in microsomes and store it in lamellar bodies. Surfactant is normally composed of a complex of phospholipids, proteins and neutral lipids. Proteins comprise approximately 4% by weight of surfactant obtained in normal BAL fluid. There have been four major surfactant apoproteins identified, including SP-A, SP-B, SP-C and SP-D [for review see [64]. cDNAs for these proteins have been cloned and sequenced for several species and their secondary structure is largely known. SP-A is a glycosylated protein with a collagen-rich region that forms large oligomers under native conditions. Its molecular weight under reducing conditions ranges from 26 to 36 kDa, depending on the degree of post-translational modifications. In the normal lung, surfactant synthesis and reuptake is an active, temperature dependent process highly regulated by type II cells. The activity of these cells in this function depends on feedback loops that monitor the amount of surfactant present in the surrounding milieu. Regulation of surfactant synthesis and reuptake is a major function of SP-A, which acts via a receptor on the type II cell plasma membrane. SP-B and SP-C are small (reduced molecular weights of 8 kDa and 4 kDa, respectively) proteolipids that are very hydrophobic and whose major function is to optimize the biophysical properties of alveolar surfactant in reducing surface tension. SP-D is more recently described and its functions are largely unknown, although its lectin properties make it an attractive candidate for local defense against invading microbes [65].

Phospholipids comprise the greatest proportion of surfactant, 90% by weight. The

most abundant surfactant phospholipid by far is the disaturated form of phosphatidylcholine (PC), dipalmitoyl-phosphatidylcholine (DPPC). Type II cells utilize the cytidine diphosphate (CDP)-choline pathway for *de novo* synthesis of PC. Lesser quantities of phosphatidylglycerol (PG), phosphatidylethanolamine (PE), phosphatidylinositol (PI), sphingomyelin (SM) and others are also present. Normally, there is less than 1% lysophosphatidylcholine present in BAL [42]. Neutral lipids comprise approximately 6% of total surfactant by weight. The majority of this is cholesterol.

There are several factors known to regulate type II cell surfactant secretion *in vivo* or *in vitro* (for review see [66], including β-adrenergic and cholinergic agonists [67,68], vasopressin [69], hyperventilation [70,71], changes in pH [72], diglycerides, lipid mediators [73], phorbol esters, calcium ionophores, arachidonic acid metabolites [74], adenosine, ATP, cAMP [75] and even cell shape and extracellular matrix (ECM) interactions [76–78]. ATP and cAMP also stimulate SP-A synthesis which in turn, through a receptor-mediated mechanism, inhibits type II cell surfactant secretion [79]. Stilbene disulfonates also inhibit *in vitro* type II cell surfactant secretion [80].

There are at least six mechanisms proposed [81,82] by which surfactant abnormalities in acute lung injury can result in ARDS:

1. inadequate surfactant synthesis leading to a surfactant lining layer insufficient to maintain alveolar patency;
2. altered surfactant composition resulting in decreased or altered surfactant function;
3. abnormal surfactant metabolism;
4. inhibition of surfactant synthesis and/or function by serum proteins exuded into the alveolar space;
5. direct surfactant biochemical toxicity or structural alteration by proteases or free oxygen radicals;
6. compartmentalization of surfactant into intra-alveolar hyaline membranes rendering it unavailable to function.

In the alveolar microenvironment all of these mechanisms may play some role in acute lung injury.

INADEQUATE SURFACTANT SYNTHESIS

There is some disagreement as to the total BAL phospholipid content in patients with ARDS. Some have found an increase in total phospholipid [83], while others demonstrated no change [84,85], and still others found a decrease [86]. In rats with experimental peritonitis, type II cells have a marked decrease in their ability to synthesize surfactant phospholipids, as measured by their reduced uptake of choline and fatty acids [87]. Ambroxol, an experimental agent that acts as a secretagogue, increases surfactant choline but not fatty acid incorporation in rabbits with peritonitis and produces no marked histologic difference in these animals as compared with uninjured controls [88].

ALTERED SURFACTANT COMPOSITION

After describing ARDS, Petty and colleagues went on to demonstrate that the syndrome was associated with fundamental abnormalities in surfactant composition and properties [89]. They were able to isolate three lipid–protein aggregates from the lungs of patients with ARDS rather than one major aggregate from normal lavage. *Ex vivo*, the abnormal surfactant was much more compressible, suggesting a mechanism for alveolar instability in the syndrome. Autopsied lungs from these patients demonstrated a significant loss of volume and reduced compliance similar to clinical premortem findings. More recently, investigations have focused on BAL content in ARDS patients [90].

Many studies indicate alterations in BAL surfactant composition in humans (reviewed in [81,82,91–93]). In general, BAL phos-

pholipid composition in patients with ARDS is altered so that the relative amounts of phosphatidylcholine (especially DPPC) and phosphatidylglycerol decrease, while phosphatidylinositol, phosphatidylethanolamine, and sphingomyelin increase. Similar alterations in surfactant composition have been reported in a variety of animal models [28,94,95]. The observation that lysophosphatidylcholine increases as well [28] is intriguing because this compound indirectly inhibits surfactant function (see below). One study [96] also demonstrated a 20-fold increase in BAL glycolipids in ARDS patients, a potentially important observation because glycolipids decrease surfactant activity *in vitro*. SP-D binds glucosylceramide, a glycolipid found in BAL [97], suggesting a mechanism for glycolipid regulation. Pison and colleagues demonstrated a decrease in BAL SP-A in 19 patients with multiple trauma and correlated this decrease with the severity of lung injury [98]. Gregory *et al.* [86] showed that SP-A is decreased in both patients at risk for, or with ARDS.

ABNORMAL SURFACTANT METABOLISM

In NNNMU-treated animals, Lewis and colleagues [28] demonstrated a decrease in the BAL large aggregate surfactant and an increase in DPPC in lamellar bodies. This was followed by increased DPPC secretion and an increase in BAL inactive small aggregate surfactant. The small aggregate fraction is believed to be a surfactant form that has been metabolized prior to reuptake and recycling by type II cells. Others have also found an increase in the less active small aggregate fraction in BAL from ARDS patients [84]. A chronic form of ARDS induced experimentally in mice by radiation treatment is associated with an increase in BAL total phospholipid and protein content, an increase in the high density large aggregate surfactant subtype, and a decrease in the nonactive low density small aggregate surfactant subtype [29], the latter apparently due to a protein inhibitor of the conversion from high to low density surfactant present in the alveolar fluid [99]. These studies taken together indicate that type II cells may have very different acute and chronic responses to lung injury.

There is also evidence that the surfactant proteins and phospholipids may have altered patterns of metabolism during hyperoxic injury [100]. In this hamster model, SP-A, -B and -C declined, while DPPC increased, during exposure to hyperoxia.

Mechanical stimulation (stretch) of the alveolus stimulates surfactant secretion as well. Conversely, one might suppose that conditions of less stretch (e.g. shallow breathing) might have the opposite effect; that is, the aggregation of surfactant into less functional forms, with increased surface tension and atelectasis [101].

INHIBITION OF SURFACTANT SYNTHESIS AND/OR FUNCTION BY PLASMA PROTEINS

Plasma proteins inhibit the active, temperature-dependent surfactant synthesis and endocytosis by type II cells [102] *in vitro*. Fibrinogen degradation products and laminin also inhibit surfactant surface tension activity (103). Fibrinogen and its degradation products are found in the vast majority of patients with ARDS [92]. In oleic acid-treated rabbits there is a rapid decrease in compliance and lung volume, associated with a marked increase in cell-free BAL protein content but no significant change in phospholipid content or composition [57]. The alteration in BAL protein content is associated with a significant decrease in surface activity. Thus, plasma proteins in the alveolus can have profound effects on surfactant synthesis and function, suggesting this is an important mechanism of lung dysfunction in ARDS.

DIRECT SURFACTANT STRUCTURAL ALTERATION

One degradation product of surfactant PC and DPPC is lysophosphatidylcholine (LPC), formed by plasma phospholipase A_2 (PLA) digestion. PLA activity is increased in patients with septic shock [104], especially in those with ARDS. Intravenous administration of PLA into rats results in intra-alveolar pulmonary edema that can be blocked by pretreatment with indomethacin [105]. In rabbits injured by oleic acid, Casals and colleagues [94] found that although BAL PLA activity was decreased after acute lung injury, whole lung microsomal PLA activity was increased. They also reported an increase in BAL LPC in these animals at 2.5 hours after injury. LPC increases fibrinogen inhibition of surfactant function *in vitro* [106]. Type II cells, as well as Clara cells, synthesize uteroglobin, a PLA inhibitory and anti-inflammatory protein [107]. These data, taken together, demonstrate not only direct surfactant structural alteration but suggest that the alveolar epithelium may have mechanisms to counteract these alterations.

SURFACTANT COMPARTMENTALIZATION

There is currently no direct evidence for compartmentalization of surfactant into intra-alveolar hyaline membranes rendering it unavailable as a mechanism of abnormal surfactant function in acute lung injury. However, as hyaline membranes are a significant finding in ARDS lesions, and as their physicochemical properties are such that they might be expected to neutralize surfactant function *in vitro* and *in vivo*, we include this possibility here for completeness.

SURFACTANT REPLACEMENT THERAPY

Recognition of surfactant abnormalities and the successful use of exogenous surfactant in the therapy of neonatal respiratory distress syndrome has led to similar interest in the use of surfactant replacement as therapy for ARDS [43,91,108–112]. A number of investigations have studied the effects of exogenous surfactant in healthy and injured animal models.

In guinea pigs, lung injury induced by repeated lung lavage could be reversed by surfactant replacement. The treated animals had significant improvement in gas exchange as well as histologically demonstrated improvement in alveolar air expansion [23]. Surfactant replacement in rabbits injured by intratracheal instillation of hydrochloric acid restored normal recoil, but these authors found no effect on gas exchange as measured by arterial blood gases [25]. Gas exchange was significantly improved in acute lung injury produced in rats by Sendai virus infection after intratracheal surfactant administration [113]. Possible explanations for these apparent discrepancies in the effects on gas exchange include the mechanism of lung injury, or the time, dose or specific differences in the composition of surfactant administered. Exogenous surfactant administered to healthy rabbits appears to stimulate both synthesis and secretion of endogenous surfactant [114] by type II cells. It does not appear to normalize either the decrease in DPPC nor the decrease in small aggregates (i.e. normalize the altered surfactant metabolism) in NNNMU-injured rabbits [115].

Surfactant therapy must be approached with caution. Human surfactant is immunogenic, as can be shown by demonstrating circulating immune complexes in ARDS patients [116]. Mice inoculated intraperitoneally with hybridomas producing antibodies to surfactant apoproteins develop acute respiratory failure with histologic lesions similar to those seen in ARDS [117].

Intratracheal administration of antibodies which recognize SP-A actually improved gas exchange in rats depleted of surfactant by repeated lung lavage [118]. SP-A accelerates generation of thromboplastin and inhibits fibrinolysis, inhibits surfactant release and

facilitates surfactant reuptake. This suggests that there are very complex interactions among the various surfactant components that must be carefully considered while instituting clinical trials of surfactant therapy.

PROLIFERATION AND REPAIR

Several processes contribute to lung remodeling after acute lung injury; these include alveolar interstitial thickening, intra-alveolar connective tissue matrix deposition, alveolar collapse and alveolar contraction [119]. Appropriate remodeling and resolution of injury is likely to result from a highly synchronized spatiotemporal expression of autocrine and paracrine factors. Failure of these checks and balances could result in an overabundant fibroproliferative response in the alveolar space and propagation of chronic scarring [120, 121]. In the proliferative phase of diffuse alveolar damage, there is rapid proliferation of fibroblasts in the interstitium and obliteration of the air spaces [122]. Fibroblasts deposit type III collagen and EDIIIa-containing fibronectin which can, in turn, serve as receptors for cell adhesion molecules of the integrin type. Cell adhesion molecules are found on all types of interstitial and alveolar cells, thus cellular proliferation and migration may depend on ECM signals from specific types of collagen or fibronectin.

Remodeling of the ECM by fibroblasts and type II epithelial cells probably involves a balance between collagen deposition and degradation. Christner and colleagues [123] measured BAL collagenase activity in ARDS patients and found evidence of both type I and type III collagenase activity in the majority of these subjects. Similar activity was not detectable in their controls. Interestingly, in one patient they were able to follow sequentially, type III collagenase activity peaked before type I activity. In the normal lung, type IV collagen is deposited by type II epithelial cells and found in a uniform distribution along the basement membrane [124]. Type III collagen, produced by fibroblasts, is found at alveolar entrance rings, in the alveolar septae and within the interstitium. Importantly, in acute lung injury, type III and type I collagens have increased deposition in the interstitium, alveolar septae and alveolar spaces, with type III collagen deposition preceding type I collagen deposition. It appears that in ARDS a remodeling of the alveolar structure occurs, and that this takes place in an ordered fashion and depends on the balance between type II cells, fibroblasts, probably other lung cell types, and the extracellular matrix. Alveolar type II cell–fibroblast interactions may play an important role in repair. Through gaps in the basement membrane, close contacts are established and fibroblast type I collagen secretion is stimulated by a factor released by type II cells; fibroblasts reciprocally stimulate SP-A mRNA synthesis by type II cells [125].

A number of soluble factors have been demonstrated to stimulate type II cell DNA synthesis *in vitro*:

- epidermal growth factor (EGF) [126]
- hepatocyte growth factor/scatter factor (HGF/SF)
- keratinocyte growth factor (KGF)
- acidic fibroblast growth factor (aFGF)
- insulin [127]
- cholera toxin [128]
- serum
- BAL fluid [129]
- pituitary extract [130]
- gastrin releasing peptide (GRP) [130].

While HGF is probably produced by alveolar macrophages in the microenvironment, HGF receptor mRNA can be demonstrated in isolated type II cells [131]. Both KGF and KGF receptor mRNA are found early in lung development and in adult lung (J. Shannon, unpublished observations). Insulin-like growth factors IGF-1 and IGF-2 stimulate fibroblast but not type II cell proliferation [130]. There has been a great deal of interest in the use of various cytokines to stimulate type II cell specific proliferation in injury

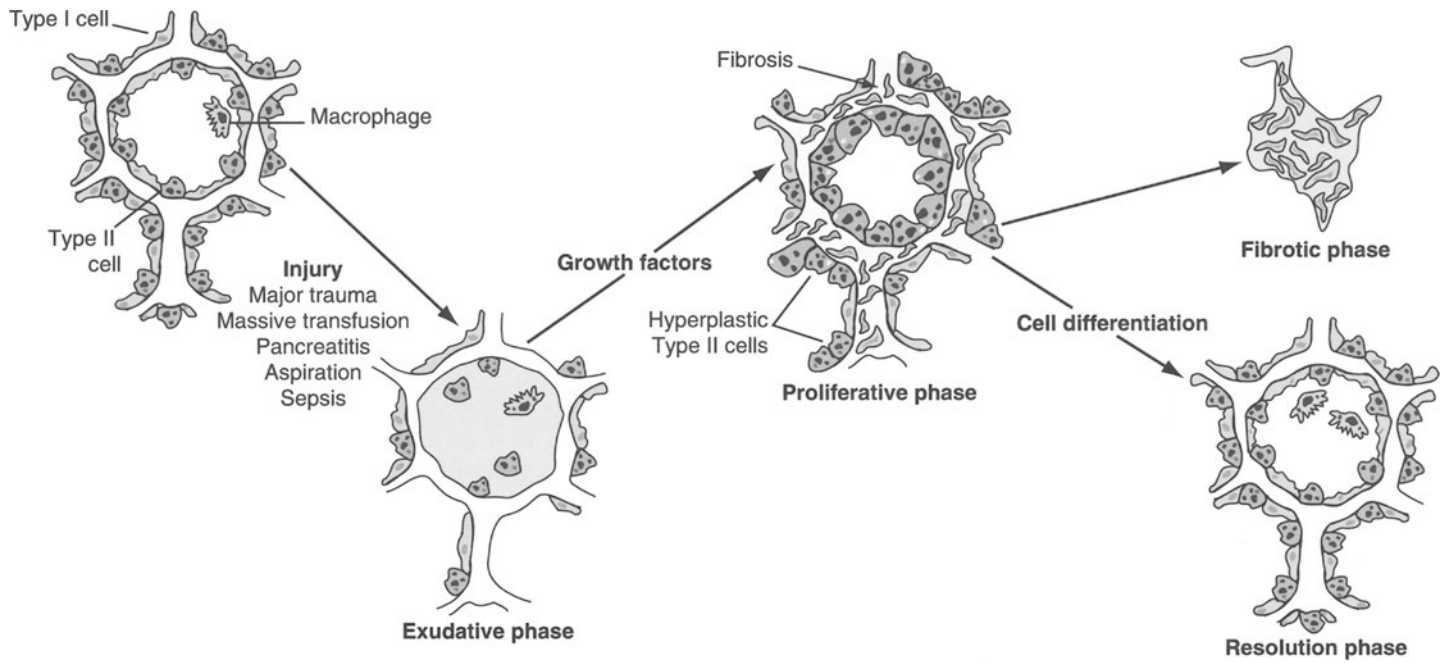

Figure 12.1 Lung injury and repair in ARDS. Following various systemic or inhalational insults, the alveolus characteristically undergoes four major clinicopathological phases. These are (1) the exudative phase, distinguished by interstitial edema, type I epithelial cell death, and alveolar flooding; (2) the proliferative phase, charaterized by increases in growth factor and cytokine expression as well as both type II epithelial cell and fibroblast proliferation; and (3) the resolution phase during which type II cells differentiate into type I cells and migrate via cell–cell and cell–extracellular matrix adhesion molecule regulation to restore normal alveolar architecture and function. The fourth, fibrotic, phase results from repeated insults and/or excessive fibroblast compared to epithelial cell proliferation. (Courtesy of Leigh Landskronen.)

models as well. It is likely that in the near future clinical trials will begin.

The concept that the ECM is more than simply an inert framework is now firmly established. The normal ECM is a complex of collagens, glycosaminoglycans, proteoglycans and cell adhesion molecules (such as laminin and fibronectin) that are involved in cell–ECM adhesive interactions. Through transmembrane signal transduction and tyrosine kinase activation, cell adhesion molecules appear to trigger cellular proliferation and differentiation [132]. During development, inflammation and repair, integrin-type fibronectin–fibrinogen receptors are upregulated [133]. Isolated type II cells cultured on a fibronectin substratum develop a significantly higher transepithelial resistance as compared with those cultured on collagen, indicating that ECM has a significant effect on the formation of tight junctions and a polarized epithelium [134]. Neutrophil products, such as metalloproteases, elastase and cathepsin G, solubilize ECM *in vitro* [135] and likely affect the balance as well.

Other families of adhesion molecules are found in the alveolus as well. The immunoglobulin type adhesion molecule ICAM-1 is normally expressed primarily on type I cells, especially near junctions. In acute lung injury induced by hyperoxia, ICAM-1 is found more dispersed on the surface of the type I cells and its expression is markedly induced on type II epithelial cells [136]. ICAM-1 is not detectable on freshly isolated type II cells but is upregulated when these cells are cultured on substrata that promote spreading [137]. The counterreceptors for this molecule include LFA-1 and Mac-1, which are found on leukocytes, and may contribute to transmigration across the alveolar epithelium and pulmonary sequestration of inflammatory cells during acute lung injury.

Transmembrane glycoprotein cell adhesion molecules of the cadherin type mediate homotypic intercellular adhesion. Although epithelial (E)-cadherin appears to be the major cadherin in the normal adult lung, isolated type II cells contain mRNA for both E-cadherin and another cadherin, placental (P)-cadherin (E. Aronsen, unpublished observations). It is not known whether P-cadherin may be important in type II cell proliferation or differentiation into type I cells although P-cadherin expression in fetal lung development makes this an attractive hypothesis. It is obvious that disruption of homotypic intercellular adhesion can be a mechanism leading to the alveolar leak syndrome seen early in acute lung injury and that re-establishment of normal cell–cell contacts is critical to restoring the integrity of the alveolar epithelium.

CONCLUSION

ARDS is a devastating illness with high mortality (Chapter 2). Many of the clinical manifestations can be understood in the context of type II cell biology (Figure 12.1). Understanding how the type II cell responds in both its proliferative and differentiated capacities may result in specific therapeutic interventions.

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