The role of the renin-angiotensin system in acute lung injury

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chapter 1
General introduction
Acute lung injury (ALI) and its more severe form, acute respiratory distress syndrome (ARDS), are devastating disorders. In the USA alone 200,000 individuals are affected by these disorders and result in approximately 75,000 deaths (Rubenfeld et al., 2005). ARDS was first described in 1967 by Ashbaugh and colleagues (Ashbaugh et al., 1967). They described 12 patients who showed acute respiratory distress, hypoxemia refractory to supplemental oxygen, decreased lung compliance and diffuse pulmonary infiltrates evident on a chest radiograph. Initially, the authors named the syndrome adult respiratory distress syndrome. Later, the term “adult” was replaced by “acute” as ARDS also occurs in children. Specific criteria for ARDS to identify patients were established in 1994 by the American-European Consensus Conference Committee (Bernard et al., 1994) (Figure 1A). They include acute onset of respiratory distress, bilateral pulmonary infiltrates seen on chest radiographs (Figure 1B), absence of left-sided heart failure and hypoxemia. Patients with a ratio of the partial pressure of arterial oxygen to the fraction of inspired oxygen of 300 or less are considered to have ALI. Patients with a ratio of 200 or less are considered to have ARDS. Predisposing factors for ALI/ARDS are diverse (Ware and Matthay, 2000). The commonly associated clinical disorders can be divided into those associated with direct lung injury and those that cause indirect lung injury in the setting of a systemic process (Table 1). The main direct causes are pneumonia and aspiration, while the main indirect causes include sepsis and severe trauma. Overall, the most common risk factor for ALI/ARDS is
severe sepsis with a suspected pulmonary source, followed by extra-pulmonary sepsis, aspiration and severe trauma (Rubenfeld et al., 2005).

**Pediatric versus Adult ARDS**

Although it is a cliche amongst pediatric intensivists that “children are not little adults”, medical care for children is often based on what is effective in adults. One of the main reasons for this is the small number of pediatric intensive care unit (PICU) patients afflicted by specific life-threatening diseases. Hence, pediatric intensivists have selectively adopted practices from adult critical care. This also holds true for pediatric ALI/ARDS.

As in adults, ALI/ARDS in children is responsible for high morbidity, mortality, and costs (Bernard, 2005; Dahlem et al., 2007; Erickson et al., 2007). However, there is a striking difference in the incidence of ALI/ARDS between children and adults (12.8 versus 78.9 cases per 100,000 person-years) (Zimmerman et al., 2009; Rubenfeld et al., 2005). Furthermore, mortality in adult ARDS patients has an incidence of 40% (Rubenfeld et al., 2005), whereas this is 20% in children (Zimmerman et al., 2009; Flori et al., 2005; Albuali et al., 2007; Khemani et al., 2009).

The most common cause of ALI/ARDS in children is pneumonia (Erickson et al., 2007; Flori et al., 2005). Perhaps for this reason, mortality in children is governed primarily by the severity of lung injury as measured by the oxygenation deficit rather than co-morbidities. Thus, in contrast to adults, the PaO\(_2\)/FiO\(_2\) ratio is a strong predictor of mortality in children (Dahlem et al., 2007; Flori et al., 2005; Erickson et al., 2007). A prospective cohort of 328 pediatric patients showed that multi-organ, non-central nervous system, dysfunction and the presence of central nervous system dysfunction are also associated with mortality and prolonged mechanical ventilation resulting from ARDS (Flori et al., 2005).

There are no evidence-based guidelines for the management of ALI/ARDS in children. Some children recover from ALI/ARDS just by administration of supplemental oxygen.

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**Table 1. Clinical disorders associated with the development of the Acute Respiratory Distress Syndrome**

<table>
<thead>
<tr>
<th>Common causes</th>
<th>Indirect Lung Injury</th>
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<tbody>
<tr>
<td>Pneumonia</td>
<td>Sepsis</td>
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<tr>
<td>Aspiration of gastric contents</td>
<td>Severe trauma with shock and multiple transfusions</td>
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<td>Less common causes</td>
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<tr>
<td>Pulmonary contusion</td>
<td>Cardiopulmonary bypass</td>
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<tr>
<td>Fat emboli</td>
<td>Drug overdose</td>
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<tr>
<td>Near-drowning</td>
<td>Acute pancreatitis</td>
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<tr>
<td>Inhalational injury</td>
<td>Transfusions of blood products</td>
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<tr>
<td>Reperefusion pulmonary edema after lung transplantation or pulmonary embolostomy</td>
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However, most patients require assisted ventilatory support (Flori et al., 2005). Maintenance of a $\text{PaO}_2$ of 8.0 to 10.7 kPa (or $\text{SpO}_2 \geq 90\%$) and an arterial pH level above 7.30 are usually well tolerated in children with ALI/ARDS (Randolph, 2009; Kavanagh and Laffey, 2006). However, long-term outcome studies in children with ALI/ARDS have not been performed. In the landmark ARDS-Net trial it was shown that reduced tidal volumes (i.e. from 12 ml/kg to ≤ 6 ml/kg) decreased mortality in adults with ALI/ARDS from 40 to 31% (The ARDS Network, 2000). Such a study is still lacking in children. The low tidal volumes have been adopted in pediatric critical care (Randolph, 2009; Santschi et al., 2010) without a trial because of ethical constraints associated with exposing children to a strategy of mechanical ventilation that has been found to be harmful in adults (Hanson and Flori, 2006).

**Pathogenesis of ALI/ARDS**

Although the causative events for ALI/ARDS are diverse, the histological changes are identical and represent a common end point. The pathophysiological mechanisms that play a central role in the acute phase of ALI/ARDS are diffuse alveolar damage, an acute inflammatory mediator response and alveolar epithelial cell apoptosis (Figure 2). These pathological changes lead to diffusion abnormalities, ventilation-perfusion mismatch and a decrease in lung compliance resulting in hampered gas exchange.

**Diffuse alveolar damage**

The alveolar capillary membrane is formed by micro-vascular endothelium and alveolar epithelium. Alveolar epithelium is composed of type I and II alveolar epithelial cells (Figure 2). The type I cells result from differentiation of type II cells. These cells are flat, making up 90% of the alveolar surface area. Their thin morphology allows for rapid diffusion and exchange of gases. Type II cells are cuboidal and account for the remaining 10% of the surface area. They produce surfactant and regulate fluid balance across the epithelium (Mason, 2006). Both cell types have also a role in host defense and immunity (see below). Type I cells are more susceptible to injury and cell death than type II cells. Damage of these cells and of endothelial cells leads to influx of protein-rich edema fluid into the alveolar spaces. In animal experiments, it has been shown that endothelial injury is prominent within minutes to hours after an injurious insult to the lung. It is characterized by the formation of intercellular gaps between endothelial cells along with variable degrees of necrosis, fragmentation and sloughing of the endothelium (Ware, 2006). This gap formation forms the pathophysiological background for increased micro-vascular permeability leading to the formation of pulmonary edema as observed in ALI/ARDS. In addition, injury and loss of type II cells impair the removal of edema fluid from the
Figure 2. The Normal Alveolus (Left-Hand Side) and the Injured Alveolus in the Acute Phase of Acute Lung Injury and the Acute Respiratory Distress Syndrome (Right-Hand Side). In the acute phase of the syndrome (right-hand side), there is sloughing of both the bronchial and alveolar epithelial cells, with the formation of protein-rich hyaline membranes on the denuded basement membrane. Neutrophils are shown adhering to the injured capillary endothelium and marginating through the interstitium into the air space, which is filled with protein-rich edema fluid. In the air space, an alveolar macrophage is secreting cytokines, interleukin-1, 6, 8, and 10, (IL-1, 6, 8, and 10) and tumor necrosis factor α (TNF-α), which act locally to stimulate chemotaxis and activate neutrophils. Macrophages also secrete other cytokines, including interleukin-1, 6, and 10. Interleukin-1 can also stimulate the production of extracellular matrix by fibroblasts. Neutrophils can release oxidants, proteases, leukotrienes, and other proinflammatory molecules, such as platelet-activating factor (PAF). A number of antiinflammatory mediators are also present in the alveolar milieu, including interleukin-1–receptor antagonist, soluble tumor necrosis factor receptor, autoantibodies against interleukin-8, and cytokines such as interleukin-10 and 11 (not shown). The influx of protein-rich edema fluid into the alveolus has led to the inactivation of surfactant. MIF denotes macrophage inhibitory factor. Adopted from Ware and Matthay (Ware and Matthay, 2000) with permission.

alveolar space (Matthay et al., 2002). This further exacerbates pulmonary edema. Injury to type II cells also impairs surfactant production and turnover, resulting in alveolar collapse and decreased lung compliance.

Inflammatory mediator response
A complex network of inflammatory mediators initiates and amplifies the inflammatory response in ALI and ARDS. Polymorphonuclear leukocytes are the paradigmatic inflammatory cell type. These neutrophils are critical for innate immunity and pathogen clearance. In addition, they also cause tissue destruction (and repair) by releasing proteases, reactive oxygen intermediates, cytokines and growth factors (Nathan, 2006). Alveolar epithelial cells and alveolar macrophages also play a pivotal role in the
inflammatory mediator response in the acute phase of ALI/ARDS (Goodman et al., 1998; Standiford et al., 1991; Thorley et al., 2007; Puneet et al., 2005). By producing pro-inflammatory cytokines, chemokines and other factors, these cells induce the migration of neutrophils to sites of injury or infection. It seems likely that interactions between migrating neutrophils, pro-inflammatory cytokines and chemokines in the microvascular and tissue environments act in concert to produce the lung dysfunction that is characteristic for ALI/ARDS.

Several inflammatory mediators are considered key regulators in ALI/ARDS (Table 2). Tumor necrosis factor-α (TNF-α) and interleukin (IL)-1β are derived predominantly from activated macrophages and act via specific cell membrane-bound receptors (Cohen, 2002; Norman et al., 1995; Putensen and Wrigge, 2000). IL-1β impairs fluid clearance via down-regulation of the epithelial sodium channel (Roux et al., 2005). Moreover, like TNF-α, it binds locally on macrophages, fibroblasts and endothelial and epithelial cells. This activates a second level of inflammatory cascades. As a result, cytokines, lipid mediators, and reactive oxygen species are released. Moreover, production of cell adhesion molecules is increased, leading to the initiation of inflammatory cell migration into tissues (Cohen, 2002). Furthermore, both TNF-α and IL-1β are present in the bronchoalveolar lavage fluid (BALF) of patients at risk for ARDS and with established ARDS (Park et al., 2001; Siler et al., 1989).

IL-6 is produced by a wide range of cells including monocytes/macrophages, endothelial and epithelial cells, fibroblasts, and smooth muscle cells. Its production is induced by endotoxin, TNF-α and IL-1β (Cohen, 2002; Bhatia et al., 2000; 2001). IL-6 is one of the most important mediators of fever and of the acute phase response. The circulating level of IL-6 is a predictor of the severity of ARDS of different etiologies, such as sepsis (Leser et al., 1991) and acute pancreatitis (Remick et al., 2002). IL-10 is an anti-inflammatory cytokine (Kasama et al., 1994; Fiorentino et al., 1991). It has been shown to inhibit alveolar production of TNF-α, IL-1β and IL-6 by macrophages, thus preventing tissue damage (Howard et al., 1992; 1993; Smith et al. 1994; van der Poll et al. 1997). IL-10 also inhibits neutrophil function (van der Sluijs et al., 2004) and stimulates

<table>
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<th>Inflammatory mediator</th>
<th>Function</th>
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<tr>
<td>TNF-α</td>
<td>Pro-inflammatory: neutrophil activation in ARDS</td>
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<tr>
<td>IL-1β</td>
<td>Pro-inflammatory: neutrophil activation in ARDS</td>
</tr>
<tr>
<td>IL-6</td>
<td>Leukocyte growth/activation; proliferation of myeloid progenitor cells; acute phase response; pyrexia</td>
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<tr>
<td>IL-10</td>
<td>Anti-inflammatory; inhibits release of pro-inflammatory cytokines</td>
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<tr>
<td>TGF-β</td>
<td>Resolution of tissue injury; pro-inflammatory</td>
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<tr>
<td>Chemokines</td>
<td>Leukocyte activation and chemotaxis</td>
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Chapter 1

the production of the receptors of the pro-inflammatory cytokines, thereby neutralizing the pro-inflammatory action of these cytokines (Seitz et al., 1995).

Transforming growth factor (TGF)-β regulates diverse biological activities including cell growth, cell differentiation, cell death or apoptosis, and extracellular matrix synthesis. TGF-β is believed to be one of the most potent inducers of fibrosis (i.e., collagen synthesis, fibroblast proliferation, and chemotaxis) (Penttinen et al., 1988; Roberts et al., 1986).

TGF-β plays a critical role in the resolution of tissue injury in multiple organs, including the lung (Shull et al., 1992).

Chemokines are grouped on the basis of their chemical structure. They have historically been divided into two major groups based on the positioning of the cysteine-residues. CXC chemokines (such as IL-8, epithelial-derived neutrophil-activating peptide(ENA)-78, and growth-related oncogene(GRO)-α) are produced by alveolar macrophages. They recruit leukocytes and are potent regulators of vascular remodeling (Bleul et al., 1996; Loetscher et al., 1996; Keane et al., 1999; Moore et al., 1998). High levels of IL-8 have been reported in the pulmonary edema fluid and BALF of patients at risk for and with ALI/ARDS (Miller et al., 1996; Donnelly et al., 1993; Wiedermann et al., 2004). Recently, it was shown that plasma IL-8 levels have major prognostic value in patients with ALI/ARDS. This underscores the importance of acute inflammation in the pathogenesis of human ALI/ARDS (Ware et al., 2010). CC chemokines (such as macrophage inflammatory protein (MIP)-1α and 1β, and regulated upon activation, normal T cell expressed and secreted (RANTES)) predominantly recruit mononuclear cells, including lymphocytes, natural killer (NK) cells, mononuclear phagocytes, eosinophils, and basophils (Rollins, 1997; Baggioioli et al., 1997). Both alveolar macrophages and alveolar type II cells can highly express both groups of chemokines in response to pro-inflammatory cytokines or bacterial products (Puneet et al., 2005).

Alveolar epithelial cell apoptosis

The loss of epithelial cells and other cells in the lungs can occur by regulated (apoptosis) or non-regulated mechanisms (necrosis). Both are likely to be important components in the pathophysiology of ALI/ARDS (Martin et al., 2003). Apoptosis, also known as programmed cell death, is mediated by a family of receptors, which activate a series of intracellular caspases. This activation leads to DNA fragmentation and cellular involution, thereby preventing release of the intracellular products into the extracellular space. Apoptosis can also be mediated by a separate internal (or mitochondrial) pathway. This pathway causes cell death when oxidants or other stresses damage mitochondrial membrane proteins, thereby releasing cytochrome c into the cytoplasm. Necrosis is characterized by a progressive loss of cytoplasmatic membrane integrity. In contrast to apoptosis, this results in the release of lysosomal and granular contents into the
extracellular space. As a consequence, injury and inflammation of the surrounding tissue is increased. Alveolar epithelial cell apoptosis can be induced by several pathways including the Fas/Fas ligand system, Toll-like receptor signaling and oxidative stress pathways (Matute-Bello et al., 1999; Kaiser and Offermann, 2005; Tasaka et al., 2008; Syrkind et al., 2008). It seems that the Fas/FasL system plays a role in the pathogenesis of lung injury (Albertine et al. 2002; Matute-Bello et al., 1999; Imai et al., 2003; Hashimoto et al., 2000). Recombinant FasL induces apoptosis of alveolar epithelial cells in vitro and caused lung injury in rabbits when administered via bronchoscopy (Matute-Bello et al., 2001). Moreover, the concentration of FasL is elevated in BALF of patients with ALI or ARDS (Albertine et al., 2002; Matute-Bello et al., 1999; Hashimoto et al., 2000). Such BALF induces apoptosis of human distal lung epithelial cells in vitro that express Fas (Matute-Bello et al., 1999). Specific antibodies raised against Fas or FasL block this effect. The involvement of the Fas/FasL pathway in lung injury was also indicated by immuno-localization (Albertine et al., 2002). Signals for both the receptor and the ligand were increased in patients who died from ALI or ARDS compared to patients who died without pulmonary disease. Both proteins were formed by epithelial and endothelial cells of the alveolar walls and by inflammatory cells in the air spaces. These findings indicate that local up-regulation of the Fas/FasL system occurs in the alveoli and is associated with increased apoptosis and worse clinical outcome.

**Interactions between mechanical and biological processes in acute lung injury**

Over the last 30-40 years, mechanical ventilation has taken an indispensable place in the treatment of patients with ALI/ARDS. However, mechanical ventilation can cause or increase lung damage, often referred to as ventilator-induced lung injury (VILI) (Parker et al., 1993; Ricard et al., 2003; Dreyfuss and Saumon, 1998). VILI is typified by the same characteristics found in patients with ALI/ARDS, namely increased alveolar-capillary membrane permeability, pulmonary edema, production of inflammatory mediators and impaired gas exchange (Parker et al., 1993). Over-stretching of alveoli and repeated collapse and re-opening of the alveoli are the two mechanisms underlying VILI. The pattern of lung injury in patients with ALI/ARDS is heterogeneous. The effective alveolar volume in many patients with ALI/ARDS is significantly reduced because of extensive areas of alveolar filling and collapse (Gattinoni et al., 1986; Maunder et al., 1986). This results in an over-distension of those alveoli that are relatively uninjured. Alveolar over-distension causes endothelial and epithelial injury leading to increased permeability pulmonary edema. High tidal volume ventilation can also cause activation...
of stretch-responsive signaling pathways in the alveolar walls and airspace leukocytes. As a consequence, an inflammatory response is induced. In addition to the injurious effects of stretching of alveoli, the repeated collapse and reopening of alveoli can also initiate a cascade of production of pro-inflammatory cytokines (Slutsky and Tremblay, 1998). These mechanisms of VILI are likely to occur during mechanical ventilation of ALI/ARDS patients, particularly in areas where function of surfactant is impaired.

The renin-angiotensin system and acute lung injury

Angiotensin-converting enzyme (ACE) is the key enzyme of the renin-angiotensin system (RAS). The human ACE gene contains a restriction fragment length polymorphism consisting of the presence (insertion, I) or absence (deletion, D) of a 287-bp alu repeat sequence in intron 16 (Rigat et al., 1990). The I/D polymorphism has been reported to account for approximately half of the variance in plasma ACE levels, where the DD genotype is associated with the highest activity in most of the ethnic groups (Rigat et al., 1990; Tiret et al., 1992). The serum ACE activity therefore corresponds to ACE insertion/deletion (I/D) genotypes in the order: II<DI<DD (Tiret et al., 1992). In 2002, an association was described between the ACE insertion-deletion polymorphism and the susceptibility and outcome of ARDS in adults (Marshall et al., 2002). The DD genotype and the D allele frequencies were enriched in patients with ARDS compared to control groups and were significantly associated with mortality in the ARDS group. This finding triggered research into the role of RAS in ARDS.

In the first step of RAS, renin cuts the glycoprotein angiotensinogen (Figure 3). The resulting decapeptide angiotensin I (Ang I) is subsequently converted by the zinc-metallopeptidase ACE to the octapeptide angiotensin II (Ang II). ACE is a type-I transmembrane glycoprotein. It has an extracellular amino-terminal domain and a short intra-cellular carboxy-terminal cytoplasmic tail (Figure 4). The topology of the enzyme makes that it can hydrolyse a wide variety of peptides in the extracellular milieu. ACE can act either as a carboxydipeptidase (e.g. in the case of Ang I and bradykinin), or as an endopeptidase (i.e. in the case of substance P or luteinizing hormone-releasing hormone). The two homologus catalytic domains of ACE, termed the N- and C-domains, have somewhat different substrate and inhibitor profiles and can be distinguished by selective inhibitors.

The effector peptide of ACE, Ang II, binds to two G protein-coupled receptors, AT1 and AT2. By binding to the former receptor blood pressure homeostasis and fluid and salt balance are maintained (Baudin, 2002). Moreover, binding to the AT1 receptor mediates key events in inflammation, fibrosis and apoptosis (Ruiz-Ortega et al., 2001; Suzuki et
The effects of Ang II binding to the AT2 receptor are less well-characterized but are assumed to oppose those mediated by the AT1 receptor. Besides its key role in the RAS, ACE is also an important enzyme in the kallikrein-kinin system (Baudin, 2002). Herein, ACE degrades the bioactive nonapeptide bradykinin. Bradykinin is a potent vasodilator. It also influences inflammation and apoptosis. Bradykinin has pro-inflammatory actions, including increased vascular leakage and induction of a variety of cytokines (Hayashi et al., 2000). The anti-apoptotic action of bradykinin in decreasing caspase-3 activation is well established (Feng et al., 2005). Until recently, ACE was considered to be the key enzyme in the RAS, but this classical view was challenged with the discovery of the enzyme ACE2 (Donoghue et al., 2000; Tipnis et al., 2000). ACE2, like ACE, is a zinc-metallopeptidase, displaying approximately 42% identity with ACE in its catalytic domain (Figure 4). However, ACE2 only contains a single catalytic site and functions as a carboxysonopeptidase by cleaving a single
C-terminal residue from peptide substrates. Notably, both ACE and ACE2 are able to cleave Ang I. The affinity of ACE2 for Ang I is poor in comparison with ACE. Therefore, Ang I is probably not a physiological substrate of ACE2 (except under conditions in which ACE activity is inhibited, which would result in the formation of Ang-(1-9)). Unlike ACE, however, ACE2 efficiently cleaves Ang II to Ang-(1-7) (Guy et al., 2003). As a result, the effects of ACE are counter-acted.

It has been shown that Ang-(1-7), via its recently discovered Mas receptor (Santos et al., 2003), has a protective role in cardiovascular, renal and liver function. Ang-(1-7) prevents hypertension-induced cardiac hypertrophy and fibrosis in chronic Ang II infusion animal models (Grobe et al., 2007; Mercure et al., 2008). Furthermore, pharmacological blockade of the Mas receptor accelerates liver fibrosis in bile-duct-ligated rats by an increase of collagen and transforming growth factor-β1 in the liver content (Pereira et al., 2007). The role of Ang-(1-7) in lung injury, however, has not yet been established.

The RAS and lung injury

The RAS system has a well-described role in systemic circulatory homeostasis. Moreover, it seems that local renin-angiotensin systems occur in a number of human tissues, including the lung (Campbell et al., 1995). In these local systems components of RAS are produced independent of circulating precursors. Activation of the pulmonary RAS would result in increased ACE and as a consequence increased levels of Ang II. This
peptide may influence the pathogenesis of lung injury via a number of cellular effects. Ang II can increase vascular permeability by stimulating the synthesis of prostaglandins and vascular endothelial cell growth factor. This would result in pulmonary edema (Suzuki et al., 2003). In addition, Ang II is involved in key events in the inflammatory process. It stimulates leukocyte adhesion and migration by increasing expression of selectins and endothelial adhesion molecules (Piqueras et al., 2000; Pastore et al., 1999; Tummala et al., 1999; Pueyo et al., 2000; Gräfe et al., 1997; Tayeh and Scicli, 1998). Besides its direct effect on inflammatory cells, Ang II also influences the inflammatory response indirectly by stimulation of the production of pro-inflammatory mediators like IL-8, IL-6 and transforming growth factor β (TGF-β) (Dol et al., 2001; Hernández-Presa et al., 1998; Han et al., 1999; Kataoka et al., 2002). Finally, Ang II may exert its effect on pathogenesis of lung injury by induction of apoptosis (Wang et al., 1999; Wang et al., 1999). Fas-mediated apoptosis of alveolar epithelial cells in vitro depends on the interaction of the Ang II receptor with Ang II (Wang et al., 1999). Ang II also induces apoptosis of endothelial cells as was shown in in vitro experiments by blocking the angiotensin receptor of human umbilical vein and coronary artery endothelial cells (Dimmeler et al., 1997; Li et al., 1999).

Elevated levels of ACE were found in BALF, while those in serum were reduced in the early stages of ARDS (Idell et al., 1987; Fourrier et al., 1985; Cookson et al., 1985). The functional significance of this distribution in respiratory disease is not yet clear but may contribute to the pathology of ARDS. It is also unknown whether the increased BALF ACE activity is the result of leakage of the peptidase from the vascular to the alveolar compartment or is due to a local pulmonary production.

**Outline of this Thesis**

The aim of this Thesis was to assess the role of the RAS system in acute lung injury. For this, material from patients with RSV induced acute lung injury was used as well as an experimental lung injury model in rats. In Chapter 2 it is shown that injurious mechanical ventilation increases ACE activity in BALF of rats. This increased BALF ACE activity mediates the inflammatory mediator response as well as apoptosis that are observed during injurious mechanical ventilation. ACE exerts its effect via Ang II and not via bradykinin.

In Chapter 3 it is described that ACE activity is increased by mechanical ventilation of LPS-exposed animals. Levels of Ang II and inflammatory mediators in BALF were also higher. Inhibition of ACE or blocking the Ang II receptor attenuated the lung inflammatory response and decreased lung injury. These results underscore the important role of the RAS in ALI/ARDS.
In Chapter 4 the role of ACE and ACE2 and their effector peptides Ang II and Ang-(1-7) in ALI/ARDS are assessed. In an experimental ARDS model, it was shown that the balance of the levels of the two enzymes is critical for disease development. In an unbalanced situation ARDS would develop due to reduced pulmonary levels of Ang-(1-7). Repletion of this peptide is shown to halt the development of ARDS.

Chapter 5 describes the lack of association between the insertion/deletion polymorphism of the ACE gene and the severity and outcome of respiratory syncytial virus-induced acute lung injury in children. Moreover, it is described that ACE activity and levels of inflammatory mediators in BALF of RSV patients are similar for the different genotypes. Chapter 6 summarizes and discusses the results of this Thesis.