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Review:

Acute lung injury/acute respiratory distress syndrome (ALI/ARDS): the mechanism, present strategies and future perspectives of therapies

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Abstract: Acute lung injury/acute respiratory distress syndrome (ALI/ARDS), which manifests as non-cardiogenic pulmonary edema, respiratory distress and hypoxemia, could be resulted from various processes that directly or indirectly injure the lung. Extensive investigations in experimental models and humans with ALI/ARDS have revealed many molecular mechanisms that offer therapeutic opportunities for cell or gene therapy. Herein the present strategies and future perspectives of the treatment for ALI/ARDS, include the ventilatory, pharmacological, as well as cell therapies.

Key words: Acute lung injury, Acute respiratory distress syndrome, Ventilator, Cell therapy
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INTRODUCTION OF ACUTE LUNG INJURY/ACUTE RESPIRATORY DISTRESS SYNDROME (ALI/ARDS)

Acute lung injury (ALI) and its more severe form, the acute respiratory distress syndrome (ARDS) are syndromes of acute respiratory failure, which was defined by radiological (bilateral lung field infiltrates) and physiological (the ratio of arterial oxygen pressure and the inspiratory oxygen concentration, P_{aO_2}/F_{iO_2} ≤300 mmHg for ALI and ≤200 mmHg for ARDS) criteria in which widespread damage to cells and structures of the alveolar capillary membrane occurs within hours to days (Bernard *et al*., 1994). The ALI/ARDS may occur as a consequence of critical illness of diverse etiologies, including direct injury to lung, such as pneumonia, aspiration, toxic inhalation, near-drowning, or lung contusion; as well as indirect mechanisms, such as sepsis, burn, pancreatitis, gynecological insults (abruption of placenta, amniotic embolism, eclampsia), or massive blood transfusion (Ware and Matthay, 2000). The incidence of ALI/ARDS was still unknown, but its annual mortality was estimated to be 36000 patients in the country the size of the US (Luhr *et al*., 1999). The mortality rate associated with ARDS has declined from 90% about twenty years ago to 30%~40% at present (Milberg *et al*., 1995). However, it is still one of the major causes of pulmonary and nonpulmonary morbidity in patients after discharge (Davidson *et al*., 1999).

The pathophysiological consequences of ALI/ARDS are related to the altered pulmonary capillary permeability and alveolar diffusion capacity, as well as the increased intrapulmonary shunt. Endothelial injury and increased vascular permeability as a central feature of ALI/ARDS is well established, and some studies have supported the neutrophils as a defense, instead of an injurious mechanism (Ware and Matthay, 2000; Sivan *et al*., 1990). Epithelial injury is also important not only in the development but also the repair of the ALI/ARDS (Matthay and Wierner-

Kronish, 1990). The degree of epithelial injury can predict the outcome (Sanajder, 1999). Loss of epithelial integrity and injury to type II alveolar cells can disrupt the normal fluid transport, thereby impairing the removal of fluid from the alveolar space. Injury to the type II pneumocytes can reduce the production of surfactant, which contributes to the clinical course of worsening atelectasis and gas exchange. The process of epithelial repair is usually inadequate, leading to fibrosis (Zapol *et al*., 1979a).

ALI/ARDS can be divided into two histopathological phases. Exudative phase (1 to 3 d) is characterized by diffuse alveolar damage (DAD) with the majority of type I pneumocytes necrosis, diffuse microvascular injury and influx of inflammatory cells and proteinaceous fluid into the interstitium (Tomashefsk, 1990). Fibroproliferative phase (3 to 7 d) is a process of lung repair manifested as type II pneumocytes hyperplasia and proliferation of fibroblasts (Tomashefsk, 1990). There are complex autocrine and paracrine inter-relationships of cytokines, as well as pro-inflammatory mediators that initiate and amplify the inflammatory response in ALI/ARDS. The cellular responses include the endothelial adhesion molecules expression, as well as the margination and migration of PMNs. There are also humoral responses dependent or independent of the cells such as cytokines, lipid mediators, proteases, oxidants, growth factors (eg. TGFs), nitric oxide (NO), neuropeptides, and nuclear factor-κB (NF-κB) (Windsor *et al*., 1993).

ALI/ARDS frequently has systemic manifestations because its triggering conditions (sepsis or shock) are systemic syndromes and injury or infection of the lung can cause systemic sepsis or inflammatory response syndrome (St. John *et al*., 1991; Brun-Buisson *et al*., 1995). Many patients with refractory ALI/ARDS die of multiple organ dysfunction syndrome (MODS), rather than isolated respiratory failure (Crouser and Dorinsky, 1994; Matuschak, 1994). There have been many studies to investigate the cellular or molecular mechanisms that mediate pulmonary injury responses to the systemic insults, the roles of blood cells and humoral factors that mediate either the pulmonary or systemic injury, and the mechanisms governing lung injury to spread to systemic manifestations (Munford and Pugin, 2001). Many steps participated in the formation from ALI/ARDS to MODS, including the activation of inflammation, chemoattractants which result in endothelial changes and the release of pro-inflammatory cytokines such as tumor necrosis factor (TNF), interleukin-1 and -6, as well as release of counter-inflammatory cytokines with immunosuppressive effects, the margination of modulating neutrophils (polymorph-nuclear cells, PMNs) and systemic activation of monocytes, and the microcirculatory injury which causes tissue necrosis (Goris *et al*., 1985; Deitch, 1992).

The clinical manifestations of ALI/ARDS can develop either insidiously or acutely, after various initiating events mentioned above. Typical symptoms are dyspnea, tachypnea, dry cough, retrosternal discomfort, and agitation. Cyanosis might be present (Fowler *et al*., 1983). Patients might expectorate blood tinged sputum, with auscultation of their chest revealing coarse crackles or bronchial breath sounds. Arterial blood gas analysis shows severe hypoxemia with a normal or decreased arterial carbon-dioxide pressure (P_{aCO_2}). Measuring the left ventricular filling pressure by pulmonary arterial catheter can differentiate whether the pulmonary infiltrate is caused by elevated hydrostatic pressure (cardiogenic edema) or altered alveolar-capillary membrane permeability [ALI/ARDS, with normal pulmonary arterial wedge (PAW) pressure]. Sampling of edema fluid from the endotracheal tube reveals higher protein concentration in patients with ALI/ARDS than in those with cardiogenic edema (Sprung *et al*., 1987). The major pathophysiologic consequence of edema in patients with ALI/ARDS is impaired gas exchange with intrapulmonary shunt, which is manifested as profound hypoxemia, rather than ventilatory failure, which is presented as hypercapnia (Ralph *et al*., 1985; Breen *et al*., 1982). ALI/ARDS might also be accompanied by pulmonary arterial hypertension, which result in elevated right ventricular strain and can cause subsequent right heart failure. Radiological features of ALI/ARDS include bilateral pulmonary infiltrates with peripheral distribution, normal heart silhouette, central pulmonary vasculature and pulmonary blood flow distribution. In contrast to the lung edema with cardiac origin, the appearance of air-bronchogram is more common but the peribronchial cuffs and the septal lines are relatively rare (Milne *et al*., 1985; Pistolesi *et al*., 1984).

PRESENT THERAPEUTIC APPROACHES FOR ALI/ARDS

The present therapeutic approaches for ALI/ARDS include supportive care, ventilator support, and pharmacological treatments.

General principles of the supportive care for ALI/ARDS patients with or without MODS include identifying and treating underlying causes of ALI/ARDS; avoiding secondary lung injury such as aspiration, barotraumas, nosocomial infections or oxygen toxicity; maintaining adequate oxygen delivery to end-organs by minimizing metabolic rate and optimizing cardiovascular function and body fluid balance; and nutritional support (Schumacker and Samsel, 1990; Tuchschmidt *et al*., 1992).

The principles of ventilator settings for patients with ALI/ARDS include lower tidal volume (5~7 ml/kg), adequate positive end-expiratory pressure (PEEP, usually higher than the lower reflection point of the pressure-volume curve) to provide adequate oxygenization (P_{aO_2} >60 mmHg) with safe level of F_{iO_2} (<0.6), avoiding barotraumas (with mean airway pressure less than 35 cmH_2O or below the upper reflection point of the pressure-volume curve) and adjusting ventilatory rate [higher or reverse inspiratory to expiratory (*I*:*E*) time ratio and permissive hypercapnia] (Roupie *et al*., 1995; Lessard, 1996).

Innovative ventilatory strategies for ALI/ARDS include high-frequency ventilation (HFV), inverse ratio ventilation (IRV), airway pressure release ventilation (APRV), positioning of the patients, exogenous surfactant administration, liquid mechanical ventilation and extracorporeal membrane oxygenation (ECMO) and carbon dioxide removal ($ECCO_2R$) (Paulson *et al*., 1996; Tharratt *et al*., 1988; Sydow *et al*., 1994; Gattinoni *et al*., 1991; Zapol *et al*., 1979b; Walmrath *et al*., 1996; Leach *et al*., 1993). HFV can maintain adequate ventilation and prevent the alveoli from collapse by high frequency (300 breaths/min) and small tidal volumes (3~5 ml/kg). This technique can be successfully applied in neonates with hyaline membrane disease. However, the benefit of HFV in adult ALI/ARDS remains unverified. IRV is designed to prolong the inspiratory phase of the ventilatory cycle, thereby improving oxygenation. The normal inspiratory to expiratory (*I*:*E*) ratio is 1:2, and the IRV can prolong the inspiratory phase to make the *I*:*E* ratio exceed 1:1. The benefit of IRV in patients with ALI/ARDS is still controversial, and the discomfort associated with this mode often requires heavy sedation and muscle paralysis to patients. APRV is designed to deliver the tidal volume during transient decreases in intrathoracic pressures, and this can maintain a constant inspiratory pressure and increase the internal PEEP (PEEPi), thereby improving the oxygenation of patients with ALI/ARDS. The strategies of positioning patients with ALI/ARDS include prone position and worse lung positioning on the non-dependent side, which can improve oxygenation by diminishing ventilation-perfusion (V/Q) mismatch. Alveolar surfactant deficiency, a common manifestation in patients with ALI/ARDS, may aggravate the pulmonary dysfunction by promoting instability of the alveolar units and losing barriers to invasion of the inflammatory cells and mediators. ECMO is designed to establish an extracorporeal circuit, either venous to arterial (V-A ECMO) or venous to venous (V-V ECMO) pattern. V-A ECMO can improve both the oxygenation by extracorporeal membrane oxygenator and cardiac output by pumping system. However, V-V ECMO can only improve the tissue oxygenation. $ECCO_2R$ employs a venovenous circuit and the blood CO_2 can be removed by an extracorporeal machine. Although some studies have revealed the beneficial effects of ECMO or $ECCO_2R$, these treatments are still not recommended for routine managements in patients with ALI/ARDS. Liquid mechanical ventilation with perfluorocarbon, which can dissolve more oxygen and consume less surfactant than conventional ventilation as well as possess lower surface tension and reduce inflammatory responses, is a promising new alternative therapy for patients with ALI/ARDS. The lungs will be partially filled with the liquid while this mode of ventilation is used. The clinical recommendation levels based on quality of evidences for all the above therapies for ALI/ARDS are summarized in Table 1 (Kollef and Schuster, 1996).

Pharmacologic treatments for ALI/ARDS include inhaled NO and corticosteroid therapy (Rossaint *et al*., 1995; Dupont *et al*., 1999; Hooper, 1991; Meduri *et al*., 1994). NO is a potent smooth muscle relaxant that plays a cornerstone role in the regulation of blood flow within the lungs. Inhaled NO can reduce vascular and airway smooth muscle contraction,

Table 1 Recommendations for the nonpharmacologic management of ALI/ARDS

Treatment	Recommended	Grade
Mechanical ventilation	–	Ungraded
Initial settings: assist-control mode; F_{iO_2} =1.0; *PEEP*≤5 cmH_2O; inspiratory flow=60 L/min	Yes	–
Tidal volume=6~10 ml/kg	Yes	C
Prophylactic PEEP (≤5 cmH_2O)	No	B
Least PEEP with S_{aO_2} ≥0.9 and F_{iO_2} <0.6 (pressure-targeted ventilation) to maintain peak *AWP*<40~45 cmH_2O and plateau pressures<35 cmH_2O	Yes	Ungraded
Routine use of IRV	No	C
IRV for persistent hypoxemia or elevated AWP	Yes	C
AWP-release ventilation	No*	
High-frequency ventilation	No	B
Tracheal gas insufflation	–	–
Partial liquid ventilation	–	–
ECMO	No	B
$ECCO_2R$	No	B
Intravenous gas exchange catheter	–	–
Patient repositioning (including prone position)	Yes	C
Hypothermia (32~35 °C)	–	B
Early fluid restriction or diuresis	Yes	B
Supranormal oxygen delivery goals	No	D

Refer to Kollef and Schuster (1996); F_{iO_2} : Inspired concentration of oxygen; PEEP: Positive end-expiratory pressure; S_{aO_2} : Arterial oxygen saturation; AWP: Airway pressure; IRV: Inverse ratio ventilation; * Pending results of clinical trials

thereby reducing pulmonary hypertension, pulmonary edema and V/Q mismatching. In addition, inhaled NO may also lessen the inflammatory component of ARDS by modulating PMNs and macrophage function. However, in practical patients with ALI/ARDS the effect of inhaled NO is still marginal and variable. Patients with higher pulmonary arterial pressure presumably appear to benefit the most. Inhaled NO is usually used in lower concentration (5×10^{-6}~10×10^{-6}) to avoid possible systemic effect and toxicity of its breakdown products, such as high reactive free radicals (peroxynitrate) and methemoglobin. Moreover, the rebounding phenomenon manifested as pulmonary hypertension after sudden discontinuation of inhaled NO use necessitates a slow weaning process. Corticosteroids are usually used in patients with late fibro-proliferative phase of ALI/ARDS. Patients treated with corticosteroids for ARDS-related pulmonary fibrosis demonstrated improved gas exchange and lower mortality rates (Meduri *et al*., 1994). Larger controlled clinical trials will be necessary before making recommendations for steroid therapy in patients with ALI/ARDS.

CELL THERAPIES FOR ALI/ARDS

The reparative stage (fibro-proliferative phase) in patients with ALI/ARDS is usually ineffective and inadequate, resulting in the consequences of pulmonary fibrosis. This is regarded as an irreversible process with the manifestations of worsening pulmonary function and gas exchange. Mechanical ventilation is administered to improve tissue oxygenation but causes further pulmonary injury. This vicious cycle makes patients with ALI/ARDS die of uncompensated respiratory failure. Therefore, if we can find ways to regenerate the alveolar epithelium or vascular epithelium, patients with ALI/ARDS will escape this vicious cycle and have more possibilities to recover (Slutsky and Tremblay, 1998; Ware and Matthay, 2002; Kuebler *et al*., 1999; 2000; Zimmerman *et al*., 1996).

Although the lung has been thought of as a complex organ with limited regeneration capacity, there is still substantial evidence that adequate lung tissue repair (regeneration of endothelial or epithelial cells, rather than fibroblasts) might occur under controlled circumstances (Brown *et al*., 2001). Previous

studies attributed this growth to in situ proliferation and conversion of resident cells, such as type II pneumocytes or Clara cells within the lung (Otto, 2002). Recent developments in the field of stem cell biology revealed that many of the body's tissues previously believed to be non-regenerative or only locally regenerative may be replaced by circulating stem cells (Poulsom *et al*., 2002). Animal models have demonstrated the derivation of endothelium, neural, hepatic tissue, skeletal or cardiac muscle from circulating stem cells (Asahara *et al*., 1997; Kocher *et al*., 2001; Ferrari *et al*., 1998; Brazelton *et al*., 2000; Eglitis and Mezey, 1997; Petersen *et al*., 1999; Lagasse *et al*., 2000). Human studies revealed that after allogenic hematopoietic stem cell (HSC) and solid organ transplantation, the appearance of endothelium, epithelium, hepatic and renal cells of donor (HSC transplantation) or recipient (solid organ transplantation) origin, occurred, suggesting derivation of these cells from circulating stem cells (Krause *et al*., 2001; Gao *et al*., 2001; Korbling *et al*., 2002; Theise *et al*., 2000; Grimm *et al*., 2001).

This "chimerism" phenomenon can also be shown in the lungs of mice with type I or II pneumocytes regeneration after HSC or cultured bone marrow progenitor cell (BMPC) infusion after cytoablative therapy (chemo- or radiotherapy) (Grove *et al*., 2002; Kotton *et al*., 2001; Suratt *et al*., 2003). Grove *et al*.(2002) used an approach of retrovirally transduced bone marrow stem cells (BMSCs) to deliver gene therapy to lung epithelium, and up to 20% of lung epithelial cells can be derived from BMSCs. Kotton *et al*.(2001) applied the mice bleomycin-induced lung injury model with lacZ-labelled cells being delivered into wild-type animals. It was found that marrow-derived cells engrafted in recipient lung parenchyma as cells with the morphological and molecular phenotype of type I, rather than type II, pneumocytes. These observations challenge the traditional belief that type I pneumocytes invariably arise from local precursor cells. Suratt *et al*.(2003) found significant chimerism of the human lung epithelial (2.5% to 8.0%) and endothelial (37.5% to 42.3%) cells following human allogenic HSC transplantation, and this could play a therapeutic role in treatment of the damaged lung.

There are many studies demonstrating the roles of HSC or BMPC in lung repair after various types of ALI/ARDS. Yamada *et al*.(2004) applied the mice lipopolysaccharide (LPS)-induced lung injury model to show the rapid mobilization of BMPCs into the circulation. BMPCs accumulate within the inflammatory site and differentiate into endothelial or epithelial cells. These data supported the important role of BMPCs in the lung repair after LPS-induced injury. Burnham *et al*.(2005) found that endothelial progenitor cells colony number was significantly higher in patients with ALI/ARDS compared with healthy control cases ($P<0.05$), and that improved survival after ALI/ARDS correlated with higher colony number.

A novel therapeutic approach, cell-based gene therapy with the combination of concepts of cell [eg. the endothelial progenitor cells (EPCs)] and gene [eg. DNA of aderenomedullin (AM), a potent vasodilator peptide] therapy, has been used in experimental animal models or clinical cases with pulmonary vascular disease from ALI/ARDS or primary pulmonary hypertension (Campbell *et al*., 1999; 2001; Zhao *et al*., 2003; Kugathasan *et al*., 2005; Nagaya *et al*., 2003a). Campbell *et al*.(1999; 2001) used rat smooth muscle cells (SMC) in ex vivo transfection of DNA of nitric oxide synthase (NOS) or endothelial growth factor to the syngeneic recipient. Their results showed overexpression of NOS in the recipients' pulmonary vascular endothelium and these can effectively attenuate monocrotaline-induced pulmonary hypertension. Zhao *et al*.(2003) applied the rat model with SMC transfection of angiopoietin-1 (Ang-1), which can protect animals from pulmonary arterial hypertension. Cell-based gene therapy with the Ang-1 (Kugathasan *et al*., 2005) or AM (Nagaya *et al*., 2003a) cDNA vector has been successfully applied in human pulmonary arterial hypertension.

Embryonic stem (ES) cells, which are self-renewable and pluripotent cells derived from the inner cell mass of blastocyst-stage embryos (Evans and Kaufman, 1981; Martin, 1981), can open new perspectives for cell therapy for diseases of various organ systems (Jones *et al*., 2002; Lumelsky *et al*., 2001; Ali *et al*., 2002). They can replicate indefinitely if left undifferentiated, and can be differentiated into a broad spectrum of derivatives of all three germ layers under certain conditions, such as the addition of matrix components and/or growth factors (Odorico *et al*., 2001; Keller, 1995). In contrast to convincing results

of skin reconstruction (Coraux *et al*., 2003), the derivation from ES cells of cell types from endoderm origin has still been rarely documented. The capacity of ES cells to generate hepatocytes, pancreatic islets, or type II pneumocytes had been reported before (Jones *et al*., 2002; Lumelsky *et al*., 2001; Ali *et al*., 2002). Recently, its capacity to generate cells of differentiated airway epithelial tissue, including basal cells, ciliated cells, intermediate cells, or Clara cells, have been uncovered (Coraux *et al*., 2004). In this experiment, RT-PCR and immunocytochemistry demonstrated that murine ES cells can differentiate into non-ciliated secretory Clara cells, with the expression of Clara cell 10-kD protein (CC10) as well as surfactant proteins (SP). In the next step, Clara cells can give rise to a fully differentiated airway epithelium when cultured at the air-liquid interface. Quantitative histological examination, immunohistochemistry and scanning electron microscopy showed that this bioengineered epithelium is composed of all components of native tracheobronchial airway epithelium. These promising results can provide future perspectives for cell therapy of injured epithelium in respiratory diseases, such as cystic fibrosis (Spencer and Jaffe, 2004), bronchiolitis obliterans (Klepetko *et al*., 2004), or ALI/ARDS (Kubo, 2005).

Some pulmonary vascular diseases resulting in pulmonary hypertension are associated with ALI/ARDS. Dysfunction of pulmonary vascular endothelium may play a role in the pathogenesis of pulmonary hypertension (Archer and Rich, 2000). EPCs which are primarily located in the bone marrow could also be discovered in the peripheral blood, can migrate to the capillary wells and differentiate into mature endothelial cells in response to tissue injury (Gill *et al*., 2001; Takahashi *et al*., 1999). These findings raised the possibility of cell based therapy with the use of EPCs, which could play the role not only as a tissue-engineer to reconstruct pulmonary vasculature but also serve as a vehicle for gene delivery to injured endothelium. EPCs manipulated with gene transfer (such as nitric oxide, prostacyclin, and adrenomedullin) (Kitamura *et al*., 1993), which can be added to or replace the target genes, could further protect the tissue from ALI.

Cell therapy could also be applied in various types of subacute or chronic lung injuries. For example, patients undergoing lung transplantation would encounter chronic allograft deterioration while being followed up for a long period. This deterioration, mainly manifests as small airway obstruction (bronchiolitis obliterans on histological examination), is related to various types of previous injuries, including graft ischemia, reimplantation injury, allograft rejection or viral infection (Frost, 2002; Chan and Allen, 2004; Ezri *et al*., 1994). Cytomegalovirus (CMV) is the most common allograft infection after lung transplantation, and related strategies on its control are very important to prevent allograft dysfunction years later. However, there is still no satisfactory method for CMV control (Avery, 1999; Chaparro and Kesten, 1997; Paradis *et al*., 1993). CMV disease usually developed if T lymphocyte response is compromised (Harari *et al*., 2004; Moss and Khan, 2004). Nagaya *et al*.(2003b) demonstrated the correlation between specific T lymphocyte and CMV disease. It was found that CMV disease developed exclusively in patients with dominant p65-specific CD8 T cell response. These results have important implications for adoptive cell therapy or vaccine design.

In conclusion, extensive investigations in experimental models and humans with ALI/ARDS have revealed many molecular mechanisms that offer therapeutic opportunities for cell or gene therapy. Several lines of evidence suggest that cell therapy can be used to improve the reparative process of pulmonary tissues, as well as augment tissue defense mechanisms. The lack of effective therapies for ALI/ARDS and the feasibility and accessibility of the lung for cell or gene transfer made it suitable for the development of cell-based therapy.

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