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Concise Review: Mesenchymal Stem Cells for Acute Lung Injury: Role of Paracrine Soluble Factors

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Abstract

Morbidity and mortality have declined only modestly in patients with clinical acute lung injury (ALI) and acute respiratory distress syndrome (ARDS), despite extensive research into the pathophysiology. Current treatment remains primarily supportive with lung-protective ventilation and a fluid conservative strategy. Pharmacologic therapies that reduce the severity of lung injury in preclinical models have not yet been translated to effective clinical treatment options. Consequently, further research in trans-lational therapies is needed. Cell-based therapy with mesenchymal stem cells (MSCs) is one attractive new therapeutic approach. MSCs have the capacity to secrete multiple paracrine factors that can regulate endothelial and epithelial permeability, decrease inflammation, enhance tissue repair, and inhibit bacterial growth. This review will focus on recent studies, which support the potential therapeutic use of MSCs in ALI/ARDS, with an emphasis on the role of paracrine soluble factors.

Keywords

Acute lung injury; Angiopoietin-1; Keratinocyte growth factor; LL-37; Mesenchymal stem cells; Pulmonary edema

Introduction

Adult stem cells are tissue specific cells that have retained the ability to differentiate into a distinct variety of cell lineages and have self-renewal capability. Although adult stem cells do not possess the full plasticity of embryonic stem cells (ESCs), they offer practical advantages including ease of isolation and propagation. More significantly, they have a limited risk of tumor formation and are not associated with the ethical controversy that surrounds ESC research. Mesenchymal stem cells (MSCs) are one class of adult stem cells that have generated a significant amount of interest as a potential therapy for lung disease. MSCs were first discovered in 1968 by Friedenstein et al. [1], who found bone marrow

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stromal cells that were adherent, clonogenic, and fibroblastic in appearance. MSCs, also called multipotent mesenchymal stromal cells, can now be isolated from a variety of human tissues including the bone marrow, adipose tissue, and placenta. Bone marrow-derived MSCs reside near the sinusoids and function as support cells for hematopoietic stem cells. Although MSCs comprise less than 0.1% of all bone marrow cells, they can be isolated from whole bone marrow aspirates by their ability to adhere to plastic and form colonies. Although no unique MSC surface markers have been identified, the International Society of Cellular Therapy defined MSCs in 2006 by three criteria: (1) MSCs must be adherent to plastic under standard tissue culture conditions; (2) MSCs must express certain cell surface markers such as CD73, CD90, and CD105, but must not express other markers including CD45, CD34, CD14, or CD11b; and (3) MSCs must have the capacity to differentiate into mesenchymal lineages including osteoblasts, adipocytes, and chrondoblasts under in vitro conditions [2].

The current enthusiasm surrounding the potential use of MSCs for therapeutic purposes is based on their low immunogenicity, their immunomodulatory properties, and their ability to secrete endothelial and epithelial growth factors and, more recently, antimicrobial peptides (Table 1). Allogeneic MSCs are able to evade clearance by the host immune system through a variety of mechanisms including low expression of the major histocompatibility complex (MHC) I and II proteins as well as lack of the T-cell costimulatory molecules, CD80 and CD86, a characteristic of MSCs often referred to as being “immunoprivileged” [13]. MHC II molecules are designed to enable T4-lymphocytes to recognize epitopes of exogenous antigens and discriminate self from nonself. This property makes MSCs attractive for cell-based therapy because they can be administered to patients without human leukocyte antigen matching. However, recent studies have shown that MSCs can express higher levels of the MHC class II proteins than originally thought [14–16]. In addition, Nauta et al. [17] demonstrated that infusion of allogeneic MSCs elicited a host response and led to graft rejection. It has now become apparent that the original belief that MSCs have low immunogenicity due to a lack of MHC II antigen is not entirely correct. However, the concern over the potential immunogenicity of MSCs must be interpreted in light of how MSCs affect both the innate and adaptive immune systems, often suppressing T and B lymphocyte activations (see below). Although these are different issues and seem to have opposing functions, they clearly affect each other’s behavior in vivo and must be studied further.

Mscs in Preclinical Ali Models

Previous studies suggested that bone marrow-derived MSCs may have therapeutic applications in several clinical disorders including myocardial infarction [18–21], diabetes [22], sepsis [9, 11], hepatic failure [23], and acute renal failure [24]. Bone marrow-derived MSCs have been investigated in several in vivo models of lung disease [5, 8–10, 25–30]. In a mouse model of bleomycin-induced lung injury and fibrosis, syngeneic MSCs improved survival and lung inflammation when given intravenously [25, 27]. In a follow-up study, Ortiz et al. [8] found that a subpopulation of mouse MSC produced interleu-kin-1 receptor antagonist (IL-1ra) that was capable of attenuating the severity of bleomycin-induced lung injury. To determine the effect of MSCs on lung injury from endotoxin, several groups studied the therapeutic effect of MSCs following intraperitoneal [28] or intratracheal *Escherichia coli* endotoxin [5, 30] in mice. Xu et al. [28] found that intravenous administration of syngeneic MSCs following intraperitoneal lipopolysac-charide (LPS) prevented endotoxin-induced pulmonary inflammation, injury, and edema as well as the influx of neutrophils into the injured alveoli. In addition, Xu et al. [30] and Mei et al. [5] also discovered that transfection of mouse MSCs with and without human angiopoietin-1 (Ang-1) both reduced the severity of *E. coli* endotoxin-induced lung injury. In all these

studies, the therapeutic effect could not be accounted for by the level of lung MSC engraftment, suggesting the importance of paracrine soluble factors or direct interaction with host cells.

One major limitation to these studies was that MSCs were not used as a treatment modality; the cells were given concurrent with the injury or before the injury. Recently, we reported that intrabronchial instillation of syngeneic MSCs 4 hours after endotoxin delivery to the lung improved survival and reduced the extent of pulmonary edema formation in *E. coli* endotoxin-induced acute lung injury (ALI) in mice [10]. To further define the therapeutic potential of MSCs, we developed two human models of ALI: an ex vivo human lung preparation perfused with human blood and injured by *E. coli* endotoxin and primary cultures of human alveolar epithelial type II cells grown in a Transwell plate with an air-liquid interface injured by an inflammatory insult. In the ex vivo perfused human lung, the intrabronchial instillation of human MSCs 1 hour after endotoxin-induced lung injury restored alveolar fluid clearance (AFC) or the ability to resolve pulmonary edema fluid in part by the secretion of keratinocyte growth factor (KGF) [3]. In primary cultures of human alveolar type II cells, human MSCs grown without cell contact in a Transwell plate restored the increase in epithelial permeability to protein caused by exposure to inflammatory cytokines in part by the secretion of Ang-1 [6]. In addition, new preliminary data from a meeting suggest that MSCs may enhance repair to the injured alveolar epithelium in a LPS-induced lung injury model in rats by the mitochondrial transfer of material from one cell to the other [31]. In summary, these studies supported the theme that MSC paracrine factors with or without direct interaction with injured lung cells were a key mechanism by which MSCs promoted repair in the injured tissue beds.

Mechanism: Engraftment

Much of the initial interest in adult stem cell therapy originated from the multipotent nature of the bone marrow-derived cells. Krause et al. [32] found that a single bone marrow-derived hematopoietic stem cell could give rise to cells of multiple different organs including the lung. She reported up to 20% engraftment of bone marrow-derived cells in the lung, including epithelial cells, from a single hematopoietic precursor. This report stimulated additional investigations into the possibility that bone marrow-derived MSCs might be able to regenerate the lung epithelium and/or endothelium as well. However, these results were questioned by multiple groups, who observed only engraftment of leukocyte lineages [33] or low engraftment rates in lung injury models with observed rates of <1% [25, 27, 34, 35]. Despite initial interest in their multipotent properties, engraftment in the lung now does not appear to play the major beneficial role. The beneficial effect of MSCs appears to derive more from their capacity to home to injured tissue beds, interact with injured host cells, and secrete paracrine soluble factors that modulate immune responses as well as alter the responses of endothelium or epithelium to injury through the release of growth factors and antimicrobial peptides [3, 6, 8, 9, 12].

However, the role of stem cell engraftment in repair following lung injury requires further research. Sueblinvong et al. [36] found that human umbilical cord MSCs when cultured in vitro with specialized growth medium expressed Clara cell secretory protein (CCSP), surfactant protein-C (SP-C), and cystic fibrosis transmembrane conductance regulator (CFTR), important functional markers of lung alveolar epithelial cells. After systemic administration to immunotolerant, nonobese diabetic/severe combined immunodeficiency mice, rare cells were localized in the lung airway epithelium that expressed cytokeratin and human CFTR. Wong et al. [37, 38] found a subpopulation of adherent human and murine bone marrow cells that expressed CCSP as well, and when cultured ex vivo with an air-liquid interface, these CCSP+ cells expressed alveolar type I and II markers such as pro-SP-

C, CFTR, and epithelial sodium channel (ENaC). CCSP+ cells preferentially homed to naphthalene-damaged airways when delivered transtracheally or intravenously. Although further research is needed, these publications highlight the potential of in vitro modification of MSCs, which may increase lung engraftment and/or regeneration in vivo.

Several groups have also identified adult stem cells within the lung, some with the features of MSCs. Kim et al. [39] identified CCSP+ SP-C+ Sca-1 CD34 cells at the bronchoalveolar ductal junction in mice,+which were + capable of differentiating into Clara cells or alveolar type I or II in response to lung injury. In addition, fibroblast-like cells have been found in the lungs of premature human infants as well as the lungs of human allograft following lung transplantation, which have characteristics of MSCs [40, 41]. Jarvinen et al. [41] demonstrated that these lung resident MSCs from human lung allografts, similar to bone marrow-derived MSCs, suppressed T-cell activation in vitro, primarily through the secretion of prostaglandin E_2 (PGE_2). Although promising, the roles of these lung resident stem cells during ALI will need to be investigated further. One potential mechanism of benefit by MSC therapy in ALI may be through the support of these endogenous stem cells.

Mechanism: Immunomodulation

A major characteristic of MSCs has been the immunomodulatory properties of the cells. Multiple studies have demonstrated that MSCs possess potent immunosuppressive effects by inhibiting the activity of both innate and adaptive immune cells [42–45]. This immunosuppression has been shown to be mediated by cell contact-dependent and cell contact-independent mechanisms through the release of soluble factors. The list of candidate mediators released or induced by MSCs include transforming growth factor-b, tumor necrosis factor a (TNFα)–stimulated gene/protein 6 (TSG-6) [21], PGE_2, indoleamine 2,3-dioxygenase, interleukin-10 (IL-10), and IL-1ra among others. In a model of sepsis following cecal ligation and puncture (CLP) in mice, Nemeth et al. [9] found that bone marrow-derived mouse MSCs, activated by LPS or TNFα, secreted PGE_2, which reprogrammed alveolar macrophages to secrete IL-10. The beneficial effect of MSCs on mortality and improved organ function following sepsis was eliminated by macrophage depletion or pretreatment with antibodies to IL-10 or the IL-10 receptor, suggesting an essential role for IL-10 in these experiments; IL-10 is a cytokine secreted predominantly by monocytes that downregulates the expression of T helper 1 cytokines, MHC class II antigens, and costimulatory molecules on macrophages. IL-10 has also been reported to inhibit the rolling, adhesion, and transepithelial migration of neutrophils [46] (Fig. 1).

In a model of ALI following intrabronchial *E. coli* endotoxin in mice, we [11] found that syngeneic MSCs improved survival and lung injury in association with a decrease in macrophage inflammatory protein-2 and TNFα levels in the bronchoalveolar lavage (BAL) fluid and elevated levels of IL-10 in both the plasma and BAL fluids. In bleomycin-induced lung injury and fibrosis in mice, Ortiz et al. [8] found that mouse MSCs decreased subsequent lung collagen accumulation, fibrosis, and levels of matrix metalloproteinases in part by IL-1ra secretion; IL-1ra is a cytokine that competitively competes with IL-1b for IL-1 receptor binding. IL-1b is one of the major inflammatory cytokines in pulmonary edema fluid in patients with ALI/acute respiratory distress syndrome (ARDS) [47]. These studies confirmed the anti-inflammatory effect of MSCs in multiple lung injury experiments in mice [5, 25, 27, 28, 30].

Despite the well documented anti-inflammatory effects of MSCs, recent literature described a dual role for MSCs as an immunostimulatory cell as well [16]. Several investigators have reported that MSCs can upregulate expression of MHC II when exposed to low levels of inflammation and function as antigen-presenting cells stimulating the adaptive immune

system [14, 15]. Recent evidence has also shown that MSCs can secrete IL-6 and induce production of IgG by B lymphocytes in an in vitro setting [48]. In addition, MSCs can prevent neutrophil apoptosis and degranulation in culture without inhibiting their phagocytic or chemotactic capabilities [49]. Thus, MSCs have more complex effects on the immune system than their classical role as immune suppressor cells. In the future, we will have to study the complex and often opposing relationship between the potential immunogenicity of MSCs and their ability to suppress the innate and adaptive immunity to understand the significance of immunomodulation during therapy for lung injury.

Mechanism: Afc

Impaired AFC (i.e., the resolution of pulmonary edema) is common in patients with ALI/ARDS. The level of AFC impairment has significant prognostic value in determining morbidity and mortality [50, 51]. Several experimental studies have studied the mechanisms that reduce AFC in ALI, and several pathways have been implicated [52, 53]. In ALI, we and other investigators have reported that pulmonary edema fluid contained high levels of several proinflammatory cytokines, including IL-1b, IL-8, TNFα, and TGFb1 [54–56]. Several of these proinflammatory cytokines have been studied in experimental epithelial fluid transport experiments, particularly the effect of the inflammatory cytokines on the major sodium channel (ENaC a, b, and c) and sodium-potassium ATPase (Na-K-ATPase), and CFTR, alveolar epithelial proteins involved in fluid transport or pulmonary edema resolution.

Bone marrow-derived MSCs are known to produce several epithelial specific growth factors, specifically KGF, the seventh member of the fibroblast growth factor (FGF) family. We have been particularly interested in KGF because multiple investigators have reported that KGF can reduce lung injury in small animal models of pulmonary edema. Recombinant human KGF pretreatment reduced mortality following intratracheal instillation of hydrochloric acid [57, 58], bleomycin [59, 60], hyperoxia [61, 62], and *Pseudomonas aeruginosa* [63]. In rat lung, KGF improved alveolar fluid transport in part by upregulating aENaC gene expression [64] and Na-K-ATPase activity [65]. In the ex vivo perfused human lung, the intrabronchial instillation of human MSCs 1 hour after endotoxin-induced lung injury restored AFC in part by the secretion of KGF [3], which increased vectorial fluid transport across the alveolar epithelium in part through increased trafficking of sodium transport proteins to the cell surface [66, 67]. These studies demonstrated how MSCs may reduce pulmonary edema, a key pathological feature in ALI with prognostic significance (Fig. 1).

Mechanism: Lung Protein Permeability

The integrity of the lung microvascular endothelium is essential to prevent the influx of protein-rich fluid from the plasma as well as inflammatory cells, which may further aggravate the ability of the lung epithelium to reduce alveolar edema. Several MSC paracrine soluble factors, such as Ang-1 and KGF, are potentially important in these effects. Ang-1, a ligand for the endothelial Tie2 receptor, is a known endothelial survival [68] and vascular stabilization factor that reduces endothelial permeability and inhibits leukocyte-endothelium interactions by modifying endothelial cell adhesion molecules and cell junctions [69–72]. We recently found that allogeneic human MSCs secreted a significant amount of Ang-1. Using small interfering RNA knockdown, the secretion of Ang-1 was essential to prevent the increase in epithelial protein permeability in primary cultures of human alveolar epithelial type II cells injured by an inflammatory insult [6]. The effect of MSCs secreted Ang-1 in lung permeability supported several recent studies, which

demonstrated the therapeutic use of MSCs (with and without transfection with human Ang-1) in mice injured by LPS [5, 7, 30] (Fig. 1).

The potential therapeutic role of KGF is intriguing given the recent study by Murakami et al. [4] that reported that FGFs FGF2, FGF4, and FGF8, which are specific for both FGF receptors 1IIIc and 3IIIc, are responsible for the maintenance of endothelial barrier homeostasis. In models of acute permeability edema such as α-naphthylthiourea [65, 73], *P. aeruginosa* [63], or ventilator-induced lung injury [74], KGF reduced lung edema and BAL protein levels. Another epithelial specific growth factor secreted by MSCs is hepatocyte growth factor (HGF). Previously, HGF was found to stabilize integrity of pulmonary endothelial cells by the inhibition of Rho GTPase and the prevention of actin stress fiber formation and paracellular gaps among pulmonary endothelial cells injured by thrombin [75, 76]. The effect of MSC-derived growth factors on restoring or maintaining lung permeability following injury is promising and will need to be studied further.

Mechanism: Antibacterial Properties

One safety concern with MSC-based therapy, particularly in treating ALI/ARDS, is their effect on host defense against bacterial infection. Bacterial pneumonia and sepsis from a nonpulmonary cause are two of the most common etiologies of ARDS [77]. Given the preponderance of literature that describes the immunosuppressive effect of MSCs, there was a concern that this effect might diminish host defense against infections. However, recent in vivo studies have provided evidence for the beneficial effects of MSCs in the treatment of bacteria-induced sepsis. In the mouse model of sepsis (CLP), intravenous syngeneic MSCs reduced mortality, improved organ function, and decreased total bacterial counts in the blood and peritoneal fluid [9]. Survival benefits in this study were explained in part by the immunomodulatory properties of MSCs mediated by soluble paracrine factors such as IL-10 and PGE_2. In another study, Gonzalez-Rey et al. [78] reported the protective effect of subcutaneous adipose tissue-derived human and mouse MSCs in mouse experimental colitis and sepsis, which also was associated with improved bacterial clearance. Intraperitoneal MSC treatment suppressed acute inflammatory autoimmune responses in mice by inhibiting the inflammatory and Th1 responses. However, the actual mechanism of enhanced bacterial clearance was not clearly identified in these studies.

Macrophages and monocytes play a central role in the production of inflammatory mediators during sepsis, and they appear to be a major cell target in the protective effect of MSCs. Recently, Mei et al. [11] reported that the improvement in bacterial clearance in syngeneic MSC-treated septic mice following CLP could be in part explained by enhanced phagocytic activity of splenic monocytes and macrophages. Also, Kim and Hematti [79] reported that human MSCs improved phagocytic activity of monocyte-derived macrophages, when cocultured in vitro. They demonstrated that coculture of human MSCs and macrophages caused an alternative state (M2) of macrophage activation, which is characterized by anti-inflammatory properties and more potent phagocytic activity. The molecular mechanism of such macrophage “reprogramming” effect of MSCs in both of these studies is unclear. Interestingly, Mei et al. did not find any effect of MSCs on phagocytic activity of macrophages in vitro coculture experiments, but clearly demonstrated enhancement of phagocytosis in CD11+ cell population isolated from mouse spleen after MSCs treatment.

It is now well-established that MSCs have Toll-like and formyl peptide-like receptors and become activated in response to different bacterial products, suggesting the possibility that MSCs may be directly involved in innate immune response [10, 80]. Recently, we found that human bone marrow-derived MSCs can inhibit bacterial growth directly, and their antimicrobial effect is mediated in part through the secretion of an antimicrobial peptide

LL-37, which was upregulated upon bacterial stimulation [12]. We also demonstrated that LL-37 secretion by MSCs improved bacterial clearance in vivo in the mouse model of *E. coli* pneumonia, when MSCs were administered intrabronchially. These antimicrobial activities of MSCs suggest these cells may participants in host defense (Fig. 1).

Conclusion

ALI/ARDS is the most common cause of acute hypoxemic respiratory failure in critically ill patients [77, 81]. Current treatment for ALI/ARDS is only supportive [82, 83], and therefore, new treatments are needed. Recently, multiple investigators have demonstrated the beneficial effects of MSCs in preclinical models of ALI in both rodents and human tissue. Given the promising initial results, there has been enthusiasm to advance MSCs and/or cell-based therapy to patients with ALI/ARDS [84]. Currently, there are over 120 clinical trials registered with clinicaltrial.gov involving the use of MSCs as therapy in patients with cardiac, renal, and autoimmune diseases. Much of current interest of MSCs have focused on soluble factors due to their ability to secrete multiple paracrine factors such as growth factors, factors regulating endothelial and epithelial permeability, anti-inflammatory cytokines, and, more recently, antimicrobial peptides that can potentially treat the major abnormalities that underlie ALI, including impaired AFC, altered lung endothelial permeability, dysregulated inflammation, and infection. However, given the recent findings of in vitro modification of these MSCs to a lung epithelial phenotype and the potential to increase lung engraftment in vivo [36, 37] and the discovery of endogenous adult lung stem cells with features of MSCs [39, 41], further mechanistic studies are needed to maximize MSCs therapeutic effect. At a minimum, issues of potency (“what defines a MSCs”) and immunogenicity versus immunomodulatory behavior of MSCs needs further work. For instance, the effect of culture conditions on both MSCs growth and phenotype is significant but still not fully appreciated [85]; not all MSCs retain their “stem” cell-like properties in culture. It may be time to revise the definition of MSCs set forth by the International Society of Cellular Therapy from 2006 [2] to compare preclinical animal studies and clinical trials for efficacy better. Regardless, future research in this field should continue and focus on elucidating the basic mechanisms responsible for the beneficial effects of MSCs. In the process, a novel and safe therapy for ALI/ARDS might eventually emerge.

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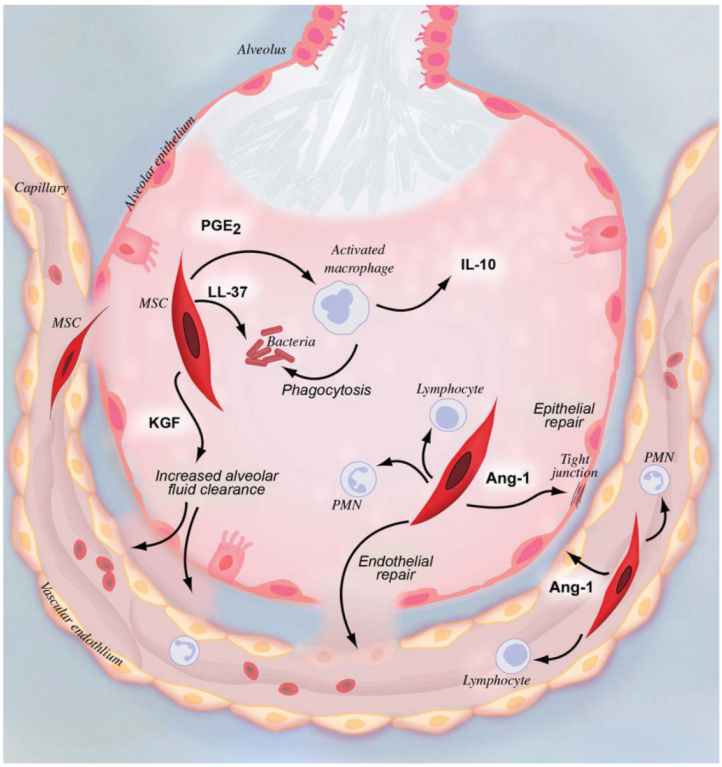

Figure 1.

In acute lung injury (ALI), the therapeutic properties of mesenchymal stem cells (MSCs) rely on both paracrine mechanism and through interaction with other cells. Multiple mechanisms have been identified through which MSC therapy may repair the alveolar epithelium and endothelium during ALI, such as (a) secretion of paracrine soluble factor, which restore alveolar fluid clearance, lung permeability, and inhibit bacterial growth, and (b) immunomodulation of innate and adaptive immune cells, which reduce alveolar inflammation. Although not fully characterized, the potential of engraftment by in vivo modified MSCs and the presence of endogenous adult stem cells with characteristics similar to MSCs may also contribute to this therapeutic effect. Abbreviations: Ang-1, angiopoietin-1; IL-10, interleukin-10; KGF, keratinocyte growth factor; MSC, mesenchymal stem cell; PGE_2, prostaglandin E_2; PMN, polymorphonuclear neutrophils.

Table 1

Mesenchymal stem cell paracrine soluble factors: Potential role in acute lung injury

Soluble factors[a]	Functional effects
Keratinocyte growth factor	Alveolar fluid transport [3]
	Lung protein permeability [4]
Angiopoietin-1	Lung epithelial and endothelial permeability [5–7]
Interleukin-1 receptor antagonist	Anti-inflammatory [8]
Interleukin-10	Anti-inflammatory [9, 10]
Prostaglandin E_2	Anti-inflammatory [9]
LL-37	Antimicrobial [9, 11, 12]

[a] Secretion of some soluble factors may depend on cell-cell contact or the alveolar milieu itself, such as interleukin-10 or prostaglandin E_2.