Review Article

Toll-Like Receptor 4 in Acute Respiratory Distress Syndrome: Good Time to Target?

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Abstract

Acute respiratory distress syndrome (ARDS) is considered a major cause of death in the world. ARDS is defined by life-threatening pulmonary edema and hypoxemia with a mortality rate of up to 40%. Recent advances in understating cellular aspects of the syndrome have shed light on possible new treatments and reduction of mortality. Toll-like receptors (TLRs) are pattern recognition receptors (PRRs) involved in adaptive and innate immunity. They are present in the alveoli and their activation can lead to inflammatory responses and finally acute lung injury. Among them, Toll-like receptor 4 (TLR4) is abundantly available on the epithelial cells of the alveoli and also on resident monocytes. TLR4 is one of the players that can promote alveolar damages during ARDS. Here, we focus on the TLR4 role in ARDS pathophysiology. We also present the potential therapies for the syndrome based on TLR4 inhibition.

Keywords: ARDS, Toll-like Receptor 4, Infection

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Introduction

The initial definition of acute respiratory distress syndrome (ARDS) was developed by the American-European Consensus Conference (AECC) in 1994. However, there were some issues bout this definition, and years later in 2011 a new definition was developed by the European Society of Intensive Care Medicine endorsed by the American Thoracic Society and the Society of Critical Care Medicine, which was considered as Berlin Definition; the latter is more feasible and well-known term. ARDS definition by Berlin criteria includes radiologic changes including pulmonary edema and bilateral patchy infiltration in chest X-ray or pulmonary CT scan with moderate to severe impairment of blood oxygenation. The amount of arterial oxygen tension to the fraction of inspired oxygen content (PaO2/FiO2) is also included (1, 2).

- mild (200mmHg<Pao2/Fio2≤300mmHg)
- moderate (100mmHg<Pao2/Fio2≤200mmHg)
- severe (Pao2/Fio2≤100mmHg)

Besides, there are 4 ancillary variables for severe ARDS:

- radiographic a severity
- respiratory system compliance ≤40ml/cmH2O
- positive end-expiratory pressure ≥10cm H2O
- the corrected expired volume per minute ≥10 l/min

Toll-Like Receptor 4 (TLR) Structure

All Toll-Like Receptors (TLR) have a glycoprotein structure weighed between 90 and 150 kDa (3). In terms of the structure, TLR4 is a member of the large interleukin receptor family (IL-IR). TLR4 consists of three components:

1. The outer part of the N-terminal cell is composed of two components of leucine-rich repeat (LRR) and 60 amino acids cysteine.
2. Membrane part
3. The C-terminal intracellular part that is IL-IRhomolog.

To bind to lipopolysaccharides (LPS), TLR4 needs a co-receptor called MD-2. After stimulation, the TLR4-MD-2 complex forms a dimer and makes a cellular response. TLR4 can bind to LPS in both hydrophobic and hydrophilic ways (4).

Ligands

Many studies have been carried out on TLR4-stimulating factors. There are two ligand classes for TLR4: exogenous and endogenous. Endogenous ligands, also called damage-associated molecular pattern (DAMP), are not produced under physiological conditions; but rather are primarily released from degrading and dying cells. The most important endogenous ligands are heat shock proteins (HSPs); and fibrinogen. Exogenous ligands, also called pathogen-associated molecular patterns (PAMP), are released from microorganisms. These most importantly include LPS, peptidoglycan, and lipoteichoic acid compounds (5,6).

Expression and Signal Transduction

Overall, TLR4 is expressed in many tissues but more importantly, and with varying degrees, it is present on macrophages, dendritic cells, and lymphocytes (7,8). The CD14 marker, which is mainly found on the surface of the monocytes, transfers ligands like LPS to TLR4. Following stimulation, TLR4 forms a dimer; then the cellular response is initiated. The intracellular signals initiated by TLR4 are divided into two Myd88 protein-dependent and Myd88 protein-independent categories. In the dependent form, which initiates earlier, after Myd88 activation, downstream kinase enzymes such as IRAK4 are activated (9). Subsequently, IRAK4 activates another enzyme called IRAF6. Activation of IRAF6 activates the Nuclear factor-kappa B transcription factor (NF-κB) and MAP kinase pathway. NF-κB entry into the cell nucleus and activation of the MAP kinase pathway produces inflammatory cytokines like TNF-α, IL1-β, and IL-6 (9-10).

The second pathway, Myd88-protein-independent, starts later and induces the downstream kinases such as TRAM, TRIF, and TRAF are activated. These activations will result in translocation of NF-κB and IRF3 to the nucleus (10,11).

TLR4 in ARDS

TLR4 is involved in several inflammatory conditions; from ischemic heart disease to ventilator-associated pneumonia (VAP) (11-14). Normally, there are two types of alveolar epithelial cells; alveolar type I (ATI) and alveolar type II (ATII). AT1 cells are frequently present and can be easily injured. Damage to type I cells causes fluid entry into the alveoli that can disrupt the normal alveolar clearance. The ATII cells are responsible for surfactant secretion, a critical factor that decreases the alveolar tension. Besides, ATII cells are involved in ion transportation. ATII cells are low in number yet are more resistant to the damages (13-15). Both alveolar epithelial cells and capillary vascular cells can be involved in the syndrome. However, endothelial damages are more common. In ARDS, due to increased permeability of the capillaries, there is a leakage of fluids and proteins to the interstitium. After that, there is an entry of fluids, red cells, and neutrophils into the alveolar space across the damaged epithelial cells. Interstitial and alveolar edema is usually seen in the exudative phase of ARDS (16,17). TLR4 is present on the alveolar macrophages and the alveolar epithelial cells. During ARDS propagation, TLR4 recognizes important ligands like hyaluronan, LPS, heat shock proteins, and also high mobility group box-1(HMGB) protein (17-19). As mentioned early, activation of TLR4 results in the production of pro-inflammatory cytokines which can promote the degree of injuries. During recent years many works have put their efforts to elucidate TLR4's exact role in ARDS. Of note, present data are mainly from experimental studies in mice and rats. A work by Deng showed that there is a considerable amount of TLR4 expression on alveolar macrophages; which can

be overexpressed by recombinant human HMGB-1. Sprague-Dawley rats were used for the induction of acute lung injury by rhHMGB-1 (19, 20). Another study provided evidence for TLR4 and TLR3 cross-talk in murine acute lung injury (20, 21). A work by Wu indicated that there was a marked reduction in IL-1β, and TNF-α production in TLR4 knocked down mice (21-23). This study indicated that LPS could not initiate vast inflammatory responses when TLR4 was silenced. Interestingly a fresh study in mice showed that angiotensin-converting enzyme 2 (ACE2) was able to attenuate the inflammatory responses following LPS induced lung injury (22-23). Taken together, many experimental investigations have provided a wealth of body of evidence about the TLR4 role in ARDS. Besides, limited works have been performed to explore TLR4 involvement and TLR4 inhibition in human ARDS. Go's study was one of the first studies which proved TLR4 overexpression on epithelial cells of patients with idiopathic interstitial pneumonia (23-25). Of note, some investigations have reported the role of TLR4 in the resolution of inflammation and also fibrosis (24-25).

TLR4 Inhibition

Evidence from different experimental and clinical investigations has shown that inhibition of TLR4 or its downstream pathways is useful in reducing inflammation. The study by Li et al. revealed that Celastrol, known as an anti-tumor compound in Chinese traditional medicine, may decrease TLR4 expression and reduce matrix metalloproteinase production in an experimental rheumatoid arthritis model in rats; moreover, coumarin derived from the plant Urtica Dentate Hand has been shown effective in reducing TLR4 expression and downregulating its downstream pathways in rats with type 1 autoimmune diabetes (25-27). In addition to the above herbal compounds, many antibodies have been made against TLR4. Eritoran is one of the initial antibodies developed against TLR4. The efficacy of this compound in the prevention of bacterial and viral sepsis in experimental models of inflammation has been successfully demonstrated; in conducted clinical trials, eritoran has not declined mortality from severe infection and septic shock (27-29). The last class of TLR4 inhibitors is recently studied small molecule inhibitors (SMIs). These molecules may enter the cells and affect the signaling pathways. Through inhibiting intracellular kinases, CPG-52364 inhibits the rapid production of cytokines. RDP58 inhibits the intracellular interaction of Myd88, IRAK4, and TRAF6. RDP58 has been associated with clinical improvement in experimental models of autoimmune encephalomyelitis; also, the results of some clinical trials confirm the efficacy of this compound in treating ulcerative colitis and in kidney transplantation (29-31). CLI-095 (also known as TAK-242), is another small molecule that has been shown as an efficacious agent for blocking human and murine TLR4 signaling and hence reducing cytokine production (31-33). Recent studies have shown that inhibiting TLR4 using TAK-242 can reduce the onset and development of sepsis in individuals who are at risk and there has already been a successful phase 1 clinical trial about TAK-242 effects in healthy volunteers; while TAK-242 prodrugs are currently under design for inhibition of TLR-4 and can be clinically tested (32-34). Interestingly, some Chinese traditional compounds have been found to reduce the alveolar and macrophage expression of TLR4 in mice or rat model of ARDS. Such compounds as Glycyrrhizin, Ruscogenin, Polygonatum sibiricum, Madecassoside, and Nuciferine (34-39). Bedsides, Nano-particles that can antagonize TLR4 are emerging. However, there are limited clinical investigations and their long term effects remain to be defined (39-41). Interestingly, a recent study provided evidence for the beneficial effects of dexmedetomidine in lung injury. Xue et al showed that preconditioning with dexmedetomidine (DEX) was able to reduce the TLR4 pathway in mice with lung injury. DEX could remarkably decrease levels of TNF- α, IL-1, and IL-6. It could decrease neutrophil infiltration, as well. The anti-inflammatory effects of DEX can be partially explained by α -2 adrenoceptor activation (40,41).

Conclusion

Many studies have demonstrated the role of TLR4 in ARDS. Like a double-edged sword, TLR4 acts both as a barrier against infectious diseases and as a predisposing factor to inflammatory damages. The main challenge is how to inhibit TLR4 with minimal effects on the immune system. Future studies and the

introduction of new drugs may overcome this challenge and provide deeper understandings of the effects of TLR4 inhibition.

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Conflicts of Interest

The authors declare that there are no conflicts of interest.

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