**Review Article**

**Toll-Like Receptor 4 in Ventilator-Induced Lung Injuries: Mechanism of Disease**

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**Abstract**

Toll Like Receptors (TLRs) are pathogen recognition molecules with widespread expression in the cells and tissues. Among them, TLR4 is involved in several important diseases. TLR4 can be stimulated by endogenous ligands as well as bacteria and viruses. TLR4 stimulation on the surface of monocytes leads to production of pro-inflammatory cytokines. It is documented that mechanical ventilation with tidal volumes of 40 mL/kg is able to induce inflammation. The mechanism of action of mechanical ventilation may involve increasing in TLR2 and TLR4 expression. Here we review the current understanding about TLR4 and lung injuries in basic and clinical science.

*Key words: Toll-like receptor 4, ventilation, lung*

**Introduction**

Toll like receptors (TLRs) recognize pathogens and generate an immediate defense response by inducing the production of pro-inflammatory cytokines, which rapidly destroy or limit the pathogens (1). In their bridging role, TLR downstream signals link innate and adaptive immunity, particularly by mediating dendritic cells (DCs) maturation and activation of pathogen specific T lymphocytes. These pathways lead to the activation of professional antigen presenting cells (APCs), which is followed by enhanced expression of surface molecules, MHC and co-stimulatory molecules [CD40, CD80, CD86 and CD70] (2). TLRs are expressed in a variety of cell types, mostly within the immune system where they have been linked to different cellular activation states, immune defense, maintenance of homeostasis, and various diseases (3). TLRs and related immunological pathways are being extensively studied for research, diagnostic and therapeutic purposes. Most mammalian species have between ten and fifteen types of TLRs. Ten functional TLRs (TLR1-10) have been identified in human.

**Structure of TLRs**

TLR family is a member of interleukin-1 receptor (IL-1R)/TLR superfamily, as a family of type I transmembrane proteins that contain a TIR intracellular domain. All TLRs have a common basic structure (4, 5):

1. N-terminal extracellular or ectodomain (ECD) containing leucine rich repeat (LRR) modules and a 60 amino acid (AA) domain rich in cysteine
2. Transmembrane domain
3. C-terminal intracellular globular domain containing a conserved region which has high homology with mammalian IL-1R family members. This region is called the Toll/IL-1 receptor (TIR)
domain

Both TLR dimerization and the TIR domains are required for signal transduction. Both extracellular and intracellular membrane-bound (endosomal) TLR4 requires its co-receptor MD2 to recognize LPS. Dimerization of the TLR4-MD2 complex with another TLR4-MD2 complex occurs following binding of LPS. The structure showed that LPS bound to two symmetrically arranged copies of TLR4 and MD2. TLR4 forms hydrophobic and hydrophilic interactions with LPS, which is required for TLR4 dimerization to occur (5, 6).

Ligands of TLR4

A large body of interest has centered on ligands of TLR4. There are 2 types of ligands for TLR4: endogenous and exogenous. Endogenous ligands are not produced under physiologic conditions. Protozoa, LPS of bacteria, fungi, and viruses are considered as exogenous ligands for TLR4 (7, 8).

It is well recognized that ligand-induced TLR activation is a double-edge sword. On the one hand, exogenous ligands initiate a quick immune response through the TLR system and also excessive TLR activation can lead to massive inflammation, tissue damage and cell death. The injured and dying cells release endogenous ligands that in turn can activate TLR pathways, causing more and more tissue damage and ligand release, thereby causing more destructions (7-9).

Expression

In general, TLRs are vastly expressed in immune cells, including macrophages, dendritic cells, neutrophils, mucosal epithelial cells and dermal endothelial cells. However, TLRs have also now been identified in numerous other cell types and tissues. The most important sites of TLR4 expression are summarized in table 2. Monocytes and macrophages are important for this context.

TLR4 Signal transduction

Circulating LBP recognizes LPS in the plasma and brings it to CD14. This aids the loading of LPS onto the LPS receptor complex, which is composed of dimerized TLR4 receptors and two molecules of the extracellular adapter MD-2. Subsequent signals activated by TLR4 can be subdivided into those dependent on MyD88 and MAL, which occur early and those independent of MyD88, which occur later and use the adapters TRIF and TRAM. Signaling caused by LPS leads to the early activation of NF-κB, IRF3 and MAP kinase pathways which are mediated by the adapters MyD88 and Mal. After the subsequent activation and phosphorylation of IRAK, TRAF6 becomes activated which gives rise to the expression of numerous pro-inflammatory genes. As a later response to LPS, TLR4 gives rise to the activation of TRAF6 and TBK1, an event mediated by the adapters TRIF and TRAM (10).

Table 1: TLR4 expression in cells and tissues (7, 8).

<table>
<thead>
<tr>
<th>Endogenous ligands</th>
<th>Exogenous ligands</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fibrinogen/fibrin</td>
<td>Fusion protein (RSV)</td>
</tr>
<tr>
<td>Heat shock proteins</td>
<td>LPS</td>
</tr>
<tr>
<td>Minimally modified LDL</td>
<td>Lipoteichoic acids</td>
</tr>
<tr>
<td>OxLDL</td>
<td>Taxol</td>
</tr>
<tr>
<td>Heparan sulfate</td>
<td>Mannuronic acid polymers</td>
</tr>
<tr>
<td>Soluble hyaluronan protein</td>
<td>Soluble murine retroviral envelope</td>
</tr>
</tbody>
</table>

CD14

CD14 is widely used as a monocyte/macrophage marker in flowcytometry as well as immunohistochemistry. CD14 is a 55 kDa glycoprotein with multiple leucine-rich repeats. It is encoded on chromosome 5q23–31, together with IL-3, GM-CSF, epidermal growth factor (EGF) receptor,
β adrenergic receptor and platelet derived growth factor (PDGF). CD14 is attached to the cell membrane via a glycosylphosphatidylinositol (GPI) anchor. Monocytes or macrophages shed CD14 to facilitate LPS signaling for all other cells in conjunction with lipopolysaccharide binding protein (LBP) and, TLR4 (11).

### Table 2: TLR4 expression in cells and tissues (7-9)

<table>
<thead>
<tr>
<th>Cell type</th>
<th>Tissue</th>
</tr>
</thead>
<tbody>
<tr>
<td>B cells</td>
<td>Active pouchitis</td>
</tr>
<tr>
<td>Basophiles</td>
<td>Aortic valve cells</td>
</tr>
<tr>
<td>CD4+ T cells</td>
<td>Bone marrow</td>
</tr>
<tr>
<td>CD8+ T cells</td>
<td>Brain (microglia cells)</td>
</tr>
<tr>
<td>Endothelial cells</td>
<td>Colonic epithelium</td>
</tr>
<tr>
<td>Immature DCs</td>
<td>Female reproductive system</td>
</tr>
<tr>
<td>Macrophages</td>
<td>Ileum</td>
</tr>
<tr>
<td>Mast cells</td>
<td>Lymph node</td>
</tr>
<tr>
<td>Monocytes</td>
<td>Ovary</td>
</tr>
<tr>
<td>Myeloid DCs</td>
<td>Pulmonary epithelium</td>
</tr>
<tr>
<td>Neutrophils</td>
<td>Rectum</td>
</tr>
<tr>
<td>Platelets</td>
<td>Renal epithelium</td>
</tr>
<tr>
<td>Splenocytes</td>
<td>Small intestinal epithelium</td>
</tr>
<tr>
<td>T cells</td>
<td>Ureter epithelium</td>
</tr>
<tr>
<td>TH-1 cells</td>
<td>Bladder epithelium</td>
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</table>

Products of mononuclear cells

Mononuclear are capable of producing many substances that can influence the host. Presence of a stimulus is required for activation of these cells. Following activation, signaling cascades produce various substances. Enzymes, reactive oxygen species, reactive nitrogen species, bioactive lipids and cytokines are produced by macrophages. Macrophages are particularly important sources of TNF-α, IL-1, IL-6, IL-8, and IL-12 (12, 13). IL-1 is a major mediator of local and systemic inflammation. It is secreted in response to diverse stimuli including bacterial infection, viral infection, tissue trauma, and tumors. It can stimulate proliferation of T and B lymphocytes; cause hyperthermia by an action through hypothalamic cells; alter synovial cell synthesis of prostaglandins, collagenase, and plasminogen activator; enhance fibroblast proliferation; enhance catabolic activities in muscle; cause specific granule release from neutrophils; and cause hepatocyte synthesis of acute-phase reactants (14). TNF-α is the prototype for a large family of cytokines, which have important roles in immunity. TNF-α is secreted by macrophages after exposure to endotoxin. It binds either a 55-kD or 75-kD receptor. The members of TNF-α family have multiple activities that include beneficial functions for organogenesis, inflammation, and host defense. However, overproduction of TNF-α can lead to cachexia, sepsis, and autoimmune disease (15).

### DISCUSSION

#### Concepts of TLR4 in Disease

**TLR4 and stenosis of coronary vessels**

As plaque encroaches into the lumen, the coronary artery diameter decreases. Luminal narrowing of more than 60 percent may result in transient ischemia and angina. More importantly, there is a poor correlation between the severity of stenosis and its propensity to cause myocardial infarction or sudden cardiac death (18). TLR4 involvement in coronary stenosis is not mechanistically understood. It is proposed that gradual infiltration of TLR4+ monocytes in developing plaques and production of cytokines in concert with other important players can deteriorate atherosclerosis. Up regulation in TLR4 and pro-inflammatory cytokines and increased arterial remodeling may impair vasodilatation, reduce coronary flow and thus contribute to facilitate...
ischemic damages (19).

**TLR4 and lung injuries**

The stimulation of inflammatory cytokines can up-regulate the secretion of TLR4, MyD88 and NF-κB. TLR4-MyD88 signaling plays an important role in the development of ventilator-induced lung injury in rats (20). Current findings suggest that injurious mechanical ventilation (MV) may elicit an immune response that is similar to that observed during severe infections. Further studies are needed to fully address these questions. It is observed that mechanical ventilation with tidal volumes of 40 mL/kg increased lung permeability and induced inflammation. The mechanism of action of mechanical ventilation may involve increasing in TLR2, TLR4, and TLR9 and MyD88 expressions (21). Studies supports an interaction between TLR2, TLR4, and TLR9 and MyD88 signaling pathway for the expression and release of pro-inflammatory cytokines during VILI. Further investigation to identify the exact functions of TLR families will provide crucial insight into designing new interventions to limit lung injury induced by mechanical ventilation (21).

**TLR4 and drugs**

Glucocorticoids can up-regulate the cytoplasmic inhibitor of NF-κB, I-κB and thus inhibit translocation. In addition, glucocorticoids have inhibitory effects on genes for inducible nitric oxide synthase (NOS), cyclooxygenase-2 (COX-2) and inflammatory cytokines (22). These results highlight the distinguished role of glucocorticoids as anti-inflammatory and immunosuppressive agents. Significant data support the expression of TLR4 on the surface of monocytes not only in the blood but also in arterial intima during atherogenesis. Recent data suggest that angiotensin-converting enzyme 2 (ACE2) possess anti-inflammatory effects by suppression of TNF-α and IL-6 release (23). It might be possible that part of ACE2 mechanism of action is mediated through hTLR4 inhibition in atherosclerotic lesions. Our previous study showed that hydrocortisone was able to reduce monocyte expression of TLR4 in patients with stable angina (24). Multiple experimental and clinical studies support additional activity of statins beyond their serum cholesterol-lowering effects. However, little is known about the mechanisms underlying these anti-inflammatory effects of statins. Statins reduce TLR4 surface expression on CD14+ monocytes in vivo and ex vivo in a dose dependent fashion and reduced expression of pro-inflammatory cytokines. Previous observations showing that statins are able to suppress oxidized low-density lipoprotein induced NF-κB expression and that oxidized low-density lipoprotein up-regulates TLR expression in human macrophages. Among the potential pathways, inhibition of protein prenylation or of lymphocyte function-associated antigen-1 might be key components mediating the rapid effect of statins (25, 26). Quite recent data showed that simvastatin or the combination of simvastatin with ezetimibe reduced TLR4 expression and IL-6 and IL-1β production in monocytes of hypercholesterolemic patients (27). Additionally, Eritoran is a second-generation structural analog of the lipid A portion of LPS. In in vivo and in vitro models, eritoran has been shown to be a potent antagonist of the biochemical and physiological effects of LPS by blocking translocation of NF-κB, which results in decreased expression of inflammatory cytokines. It is shown in a mouse model that the inhibition of TLR4 by its antagonist eritoran attenuates the inflammatory response to MI/R, as evidenced by a significant reduction in infarct size decreased NF-κB nuclear translocation, and decreased expression of inflammatory mediators, such as TNF-α, IL-1β and IL-6 (28). Eritoran has been shown to be safe in humans and it is currently undergoing clinical development as a possible therapeutic agent for the treatment of sepsis and for myocardial protection during coronary artery bypass grafting. However, the primary results are not promising. Beneficial effects of glucocorticoids in reduction of TLR4 expression and incidence of ventilator-induced lung injuries should be adequately assessed. Furthermore, an effective and standard therapy to prevent such injuries should be ascertained.

**Conclusion**

TLRs are link between the development of ventilator-induced lung injuries and the immune system. Current evidence supports the theory that
TLR activation contributes to the development and progression of lung injuries during ventilation. The therapeutic potential of TLRs should be further studied in basic and clinical settings.

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Conflicts of Interest

The authors declare that there are no conflicts of interest.

References