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The role of TLR4 in the pathogenesis of indirect acute lung injury

Rong Hu[1], Hui Xu[1], Hong Jiang[1], Ying Zhang[1], Yu Sun[1]

[1]Department of Anesthetics, Shanghai Ninth People's Hospital affiliated with Shanghai Jiao Tong University, School of Medicine, N.O. 639, Zhizaoju Road, Shanghai 200011, China

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1. ABSTRACT

Indirect acute lung injury (IALI) manifests as rapid-onset respiratory failure following secondary clinical events to the parenchyma or lung vasculature, such as hemorrhage shock, extra-pulmonary sepsis, trauma, ischemia-reperfusion, and burn injury. Accumulating evidence demonstrates the pivotal role of pattern recognition receptors (PRRs) in the innate immune system of lung diseases. Toll like receptor 4 (TLR4), one of the well characterized PRRs, recognizes not only the lipopolysaccharide (LPS) of Gram-negative bacteria, but also the endogenous ligands in IALI. In this review, we summarize a variety of reports concerning the role of TLR4 and IALI pathogenesis.

2. INTRODUCTION

Acute lung injury (ALI) is a progressive, devastating disease that exhibits typical physiological changes and radiological manifestations (1). It is characterized as continuous hypoxemia refractory to oxygen supplementation. As the American-European Consensus Committee recommends, indirect acute lung injury is a secondary or extra-pulmonary insult resulting from acute systemic inflammatory response (2). Following indirect insult, systemic circulating mediators released from extra-pulmonary foci target lung parenchyma or vasculature, leading to lung lesions (3-6). Several triggering conditions, including hemorrhage shock, extra-pulmonary sepsis, trauma, ischemia-reperfusion, and burn

injury, contribute to IALI and exaggerate the inflammatory process of ALI (7, 8).

Pattern recognition receptors (PRRs) are highly evolutionarily conserved receptors that trigger both pathogen-associated molecular patterns (PAMPs) and damage-associated molecular patterns (DAMPs). Accumulating evidence demonstrates that it plays a key role in innate and adaptive immune response to ALI (9-12). Toll like receptors (TLRs), principal members of PRRs, comprise a family of type I transmembrane proteins that belong to the superfamily of interleukin (IL) receptors. The TLRs recognize certain structural components (e.g., peptides, lipids and nucleic acids) unique to bacteria, fungi, and viruses; they then transduce and activate the host inflammatory response (13). Toll like receptor 4 is one of the most extensively investigated TLRs. In addition to reports that have demonstrated the importance of TLR4-dependent cascade of events in IALI pathogenesis in gram negative sepsis (14), an increasing number of reports have indicated that TLR4 contributes to non-septic acute organ dysfunction (15, 16).

In this review, we aim to discuss the potential role of TLR4 in the pathogenesis of IALI and the potential utility of TLR4-associated mechanisms in improving the clinical outcome of IALI.

3. TLR STRUCTURE

TLRs are composed of an ectodomain with tandem leucine-rich repeats (LRRs) and a highly conserved cytoplasmic domain, known as the Toll/interleukin-1 receptor domain(17). The extracellular regions differ markedly and bind to various ligands with or without accessory molecules, whereas the homologous TIR domain contains a ~200 amino acid conserved region in the cytoplasmic tail and interacts with TIR domain containing adaptors (18). Once PAMPs are recognized by the ectodomain, changes in TIR domain initiate a signal pathway that leads to relevant inflammatory responses.

4. TLR4 SIGNALING PATHWAY

As a major agonist of TLR4, LPS initially associates with the LPS-binding protein (LBP), a serum molecule that increases the sensitivity of monocytes in response to LPS. This complex then binds to CD14, a myeloid cell specific GPI-linked molecule, and in turn associates with TLR4. It has been previously demonstrated that TLR4 must associate with MD-2 because of its marked surface expression (19). In addition, LPS-CD14-TLR4-MD-2 complexes anchor in cholesterol-enriched-lipid rafts of cell membranes and allow for LPS signaling (20). The engagement of TLR4 homodimers by LPS or other protein cognate ligands initiates a signaling cascade and thus induces genes involved in immune response against pathogens. Five intracytoplasmic adaptor molecules have been identified: myeloid differentiation protein 88 (MyD88), TIR-associated protein (TIRAP)/MyD88 adaptor-like (Mal), TIR domain containing adaptor protein inducing IFNβ (TRIF)/TIR domain containing molecule 1 (TICAM1), TRIF related adaptor molecule (TRAM; also known as TCIAM2), and sterile alpha and HEAT-Armadillo motifs (SARM) (Figure 1).

4.1. MyD88 dependent pathway

MyD88 contains an N-terminal death domain and a C-terminal TIR domain. When stimulated, MyD88 is recruited and, in the early phase, interacts with the cytoplasmic TIR domain of TLR4. Then, its death domain associates with IL-1 receptor associated kinase4 (IRAK4), which mediates phosphorylation of IRAK1 (21, 22). The activated IRAK1 binds with TNF receptor associated factor 6 (TRAF6), which acts as a ubiquitin protein ligase, leading to two different signaling pathways (23). IRAK-M/IRAK-3, a negative regulator of LPS-induced inflammation, is essential for endotoxin tolerance (24), whereas TRAF6 is critical for polyubiquinating TGF-β activated kinase1 (TAK1). In this pathway, the inhibitor of nuclear factor-κB kinase (IKK) complex, consisting of IKKα, IKKβ, and IKKγ (also known as NF-κB essential modulator, NEMO), is activated via phosphorylation once TRAF6/TAK1/TAB1/TAB2 (TAK binding protein) complex associates with the ubiquitin ligases and induces ubiquitylation of TRAF6 (25). The subsequent phosphorylation and degradation of IkB causes the nuclear translocation and transcription of NF-κB (26). On the other hand, Mitogen-activated protein kinases (MAPKs) family members, including P38, c-Jun N-terminal kinases (JNKs), and extracellular-signal-regulated kinases (ERKs), are phosphorylated and lead to the activation of adaptor protein-1 (AP-1). These chain reactions induce gene transcription of inflammatory cytokines.

TIRAP/MAL has been previously reported to be an essential molecule for MyD88 dependent pathways (27, 28). TIRAP deficient mice have been shown to exhibit impaired TLR4 induced responses (29). In addition, TIRAP/Mal is essential to mediate MyD88 dependent TLR4-specific signaling to induce inflammatory cytokines (30).

4.2. MyD88-independent pathways

Kawai reported that in response to lipopolysaccharide (LPS), TLR4 induced delayed expression of NF-κB, whereas it failed to activate gene expression of inflammatory cytokines in MyD88 deficient mice (31). Further investigation revealed that TLR4 induced expression of IFN-inducible gene in MyD88 deficient bone marrow-derived dendritic cells. IFN-β production was shown to be a consequence of LPS induction in IFN-α/β-receptor-deficient mice (32). These studies demonstrated the existence of a MyD88 independent pathway in TLR4 signaling.

The function of TRIF/TICAM-1 was initially identified in TRIF deficient mice. TLR3 and TLR4 failed to induce expression of IFN-β and activate transcription factors interferon regulatory factor 3 (IRF-3) or NF-κB in these mice (33). Additionally, TRIF is essential for the maturation of dendritic cells via TLR4-MyD88 independent pathway induced by Escherichia coli (34). In the TLR4-TRIF pathway, TRAM (TRIF related adapter molecule) is

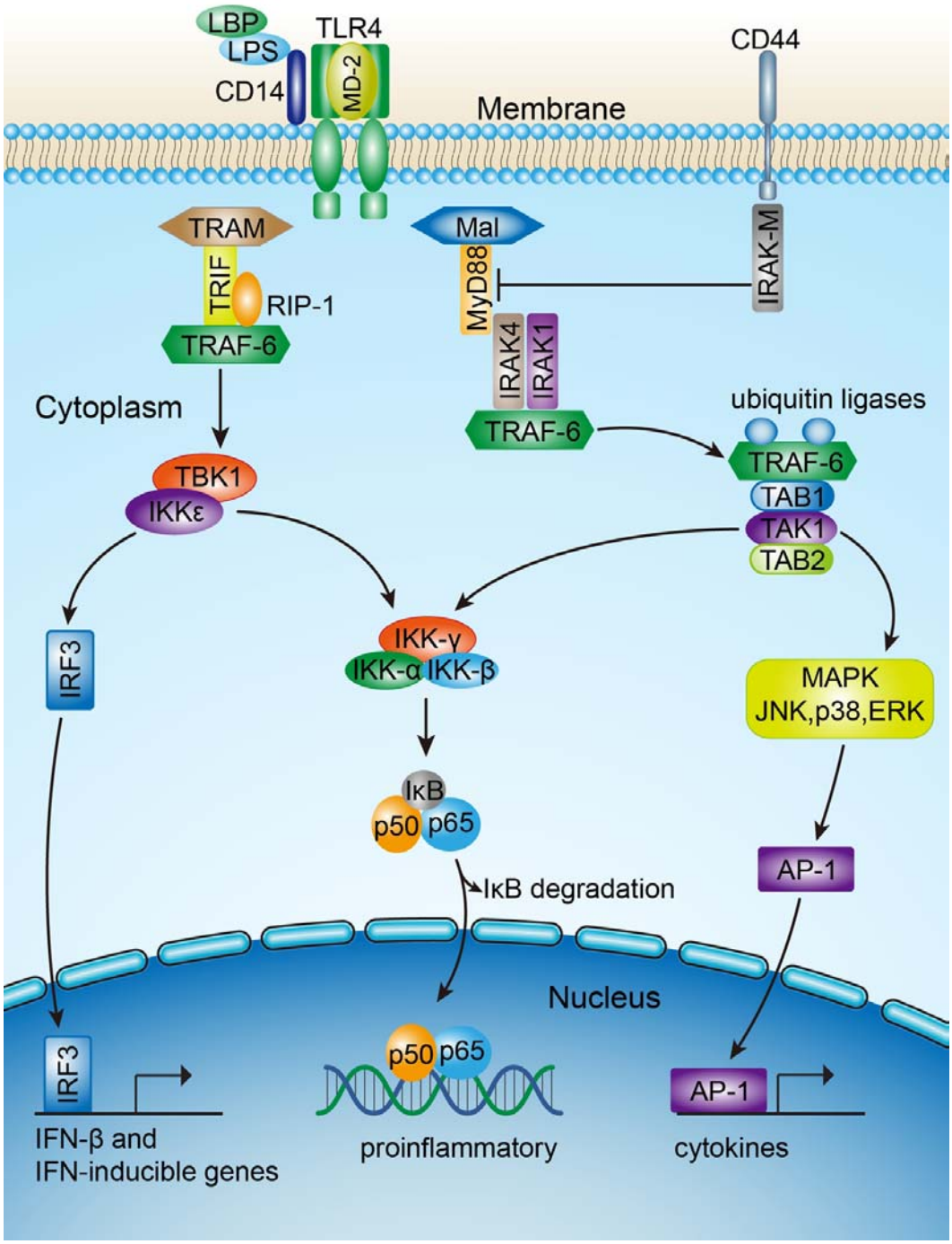

Figure 1. TLR4 signaling pathway. LPS binds to CD14 after association with LBP. TLR4 associates with MD2 to exert its surface expression. LPS-CD14-TLR4-MD2 complex anchors in cell membrane for LPS signaling. The TIR domain of TLR4 binds to MyD88/Mal, which in turn activates IRAK4 and IRAK1. IRAK dissociates from the complex and associates with TRAF6. IRAK-M prevents the dissociation of IRAK4/IRAK1 from TLR4/MyD88 complex and subsequent recruitment with TRAF6. TRAF6/TAK1/TAB1/TAB2 complex translocates to cytosol and the ubiquitylation of TRAF activates TAK1, which in turn phosphorylates the MAPK and IKK complex, respectively. The following phosphorylation of IκB causes NF-κB to translocate to the nucleus and subsequent induction of proinflammatory cytokine expression. The phosphrylated MAPK, such as JNK, p38, and ERK, activate AP-1 and subsequently induce the expression of cytokines in the nucleus. A later response refers to TRIF/TRAM, which activates IRF3 in the presence of IKK(and TBK1. IKF-3 translocates to the nucleus and expresses IFN-β and IFN-inducible genes. IKKε/TBK1 potentially also leads to the degradation of IκB and activates the translocation of NF-κB.

necessary for the association of TRIF. In TRAM-deficient mice, TLR4 ligand did not induce cytokine production via MyD88 independent pathway (35). SiRNA treatment of TRAM revealed its essential role in inducing IFN-β and IFN-inducible genes via TLR4 pathway (36).

Non-canonical IκB kinases (IKKε) and TANK (TRAF-associated nuclear factor κB activator) binding kinase (TBK1) are required for the activation of IRF-3 mediated by TRIF. After phosphorylation by these kinases, IRF-3 translocates to the nucleus and induces subsequent cytokine genes. The activation of IFNβ and the translocation of IRF3 are enhanced via expression of IKKε and TBK1, whereas knockdown of IKKε or TBK1 severely reduces the induction of IFN-β reporter genes (37). In addition, embryonic fibroblast cells exhibited unimpaired response to TLR3 and TLR4 ligands in IKKε deficient mice (38).

Compared with wild-type mice, NF-κB can be activated in a delayed phase in MyD88 deficient mice when stimulated with LPS. TRIF associates with IKKε and TBK1 through its N-terminal region and activates IFNβ promoter (39). Additional reports indicated that TRIF interacted with TRAF6 through its response in the N-terminal portion. After complete mutation of TRAF6-binding motifs, the activation of NF-κB could only be partially decreased (40). In addition, a previous report suggested that the C-terminal region of TRIF participated in the activation of NF-κB by associating with receptor-interacting protein 1 (RIP1) (41). In RIP-deficient mice, embryonic fibroblast cells exhibited attenuated activation of NF-κB in response to the TLR3 ligand.

SARM is the fifth member of TIR domain family (42). It has been shown to be a negative regulator of TLR signaling; RNAi treatment of endogenous SARM enhanced the expression of TRIF-dependent cytokine production (43).

5. TLR4 IN INDIRECT ACUTE LUNG INJURY

It has been previously demonstrated that TLR4 is expressed in various types of lung cells, such as vascular endothelial and airway epithelial cells (44, 45). It plays an important role in the pathogenesis of IALI. TLR4 responds to not only PAMPs from extrapulmonary invading microbes, but also DAMPs released in response to trauma hemorrhage shock, ischemia-reperfusion, and burn injury.

5.1. TLR4 in trauma hemorrhage shock (TSH) induced IALI

Hemorrhagic shock (HS), which usually results from major trauma, promotes the development of the inflammatory response in lung tissue by initiating the innate immune system, which is often followed by an exaggerated inflammatory response and injury (46). Originally a LPS receptor, functional TLR4 has assumed a key PPR in the development of systemic inflammation induced by HS (47, 48).

5.1.1.. THS induced activated PMN priming via TLR4 signaling

A previous study indicated that hemorrhage primed lung inflammation is necessary for ALI pathogenesis. Polymorphonuclear leukocytes (PMN) are the primary cells involved in lung inflammation. The activated PMN priming plays a predominant role in the development of ALI (49). In animal studies in which animals are hemorrhaged followed by CLP challenge, neutrophil apoptosis is suppressed whereas the capacity of PMN to produce a respiratory burst is enhanced. Neutrophils mediate priming for ALI via the TLR4 pathway (46, 50). In TLR4-mutated (C3H/HeJ) mice, mesenteric lymph after HS fail to prime PMNs to induce ALI (51). In addition, as with mice stimulated by LPS, time-dependent accumulation of lung neutrophils has been shown to be associated with lung leakage in unresuscitated HS induced TLR4 wild type mice (52). Interestingly, HS differs from endotoxemia in inducing TLR-4-dependent intracellular activation. In lung neutrophils, distinct proinflammatory cytokines are expressed in THS and endotoxemia induced ALI. Xanthine oxidase derived reactive oxygen species merely appear to be involved in the expression of proinflammatory cytokines in neutrophils of ALI associated with hemorrhage (47, 53).

5.1.2. THS induced lung endothelial activation via TLR4 signaling

Endothelial alteration is an important early event in the progress of systemic inflammatory response. A previous report demonstrated that TLR4 plays a key role in hemorrhage induced endothelial dysfunction (54). The lung endothelium contributes to THS induced ALI by generating ROS and thereby affecting the release of various inflammatory mediators such as intercellular adhesion molecule-1 (ICAM-1), which regulates the sequestrating of PMN. Reduced nicotinamide adenine dinucleotide phosphate (NADPH), one of the ROS mediating enzymes, has been shown to prime for organ injury induced by HS. PMN NADPH oxidase is reported to prime the augmented activation of HS induced NADPH oxidase in lung endothelial tissue (55). Further studies revealed that in lung endothelial tissue, the activation of NADPH oxidase depends upon TLR4 signaling in the early phase, whereas the activation is dependent upon TLR2 pathway in the late phase. Additionally, activated PMN leads to the upregulation of TLR2 through TLR4 signaling. TLR2 expression, which is regulated by HS-stimulated PMN, is closely associated with pulmonary sequestration and subsequent infiltration of PMN (56).

5.1.3. HMGB1-TLR4 signaling mediates TSH-induced ALI

Certain mediators of protein binding, such as TNFα and IL-1β, or high mobility group box 1 (HMGB1), which was initially recognized as a DNA-binding protein, have been reported as potent proinflammatory cytokines (57, 58). When exposed to neutrophils or macrophages, HMGB1 induces the translocation of NF-κB and amplifies the proinflammatory cytokine production, in part, via TLR4/TLR2 signaling (47, 59). Although it has been reported as a late acting mediator in endotoxemia, HMGB1

is also released by injured cells to associate with early proinflammatory mediators and serves as an emerging DAMP. A recent study found that the serum level of HMGB1 increased within 6 h in humans who underwent accidental trauma (60). HMGB1 expression is found to be elevated within 4 h and increases over the next 72 h in the lung following hemorrhage. The mice treated with delayed anti-HMGB1 antibodies exhibited ameliorated lung leakage and decreased lung MPO levels in hemorrhage-induced ALI (61). In addition, HMGB1 contributed to the progression of hemorrhage-induced ALI in an early phase. It activates PMN NADPH oxidase via TLR4-MyD88-IRAK4-Akt/p38 signaling pathway and subsequently leads to lung dysfunction by generating ROS following HS. Moreover, the oxidants derived from PMN NADPH oxidase augment PMN infiltration by mediating TLR4-TLR2 cross talk in alveolar macrophages (62).

5.1.4. HLA-TLR4 signaling mediates Trauma-Induced ALI

Hyaluronic acid (HA), a major endogenous non-sulfated glycosaminoglycan, has been reported to be distributed widely in mammal organs, such as heart valves, skin, and synovial fluid. During tissue injury, HA is released from extracellular matrix and accumulates at the sites of inflammation, causes damage due to low molecular weight fragments, and induces gene expression of inflammation (63). Soluble HA fragments initiate innate immunity by stimulating macrophages to produce inflammatory mediators in trauma induced lung injury. Moreover, it also acts as a danger signal and triggers recognition of injury and induction of repair response (64). In a report of bleomycin treated mice, it was suggested that TLR2/4 or MyD88 deficient mice were more susceptible to ALI. Hyaluronan interacts with TLR2 and TLR4 to maintain epithelial cell integrity and protects against epithelial apoptosis (65). In contrast with LPS, small HA fragments are reported to require MD2 rather than CD14 to activate TLR4 signal pathway. As an accessory molecule, CD44 plays a role in stabilizing and augmenting the interactions between TLR4 and HA fragments following sterile injury (66). Additionally, *in vivo* studies have demonstrated that CD44 plays a key role in removing small fragments of HA. CD44 deficient mice exhibit more severe pulmonary injuries due to the failure in remitting the accumulation of low MW HA (67). Although both HA and LPS induce subsequent inflammatory expression dependent upon the TLR4 pathway, stimulated monocytes exhibit different patterns of gene production; this indicates that sterile lung injury involves a different pattern of cellular mechanisms of action in TLR4 pathway compared to sepsis induced ALI (66).

5.2 TLR4 in extra-pulmonary sepsis induced ALI

5.2.1. Role of TLR4 in LPS induced ALI

LPS, a constituent of the cell wall in gram-negative bacteria, is a major cause of endotoxin shock and leads to increased mortality in ALI patients. TLR4 plays a pivotal role in recognizing LPS and binds with some accessory molecules to prime the signal pathway. After associating with LPS binding protein (LBP) and CD14 {a glycosylphosphatidylinositol (GPI) anchored molecule}, the complex binds to MD2 and leads to subsequent TLR4 aggregation and response. In addition, high dose LPS induces CD11b instead of CD14 via TLR4 pathway (68, 69). MyD88 has been shown to be important in endotoxin induced lung inflammation. In MyD88 deficient mice, acute bronchoconstriction, cytokine production, protein leak, and neutrophil recruitment are abolished. TIRAP, rather than TRIF, is indispensable for LPS induced inflammatory response in lung (70). Following induction by endotoxin, the inhibition of p38 MAPK results in a blockade of lung inflammation (71). Additionally, the expression level of TLR4 has been reported to be associated with the severity of inflammatory response. Damage of microarchitecture, injury of alveolar epithelial and vascular endothelial tissue, and PMN recruitment seem to be dependent on Tlr4 gene dosage (72).

In a LPS induced IALI model, NF-κB has been shown to be activated, and the gene expression level of pulmonary cytokines, such as TNF, IL-6, and IL-1β, is significantly increased in wild type mice compared with TLR4 deficient mice (73). A report concerning pancreatitis-associated lung injury demonstrates that TLR4 plays a key role in endotoxemia induced lung injury, whereas TLR4 seems to exhibit no impact on the pathogenesis of acute pancreatitis and secondary lung injury induced by cerulean and follow-up LPS (74). In another two hit model (hemorrhage followed by CLP challenge), it was reported that TLR deficient mice exhibit attenuated neutrophil priming influx into the lung and no evident change in chemokine/cytokine levels (50).

5.2.2. PMN activation via TLR4 signaling in sepsis

Neutrophils are the pivotal and primary cells that provide host defense against LPS induced ALI. In endotoxemia-induced ALI, neutrophils, which infiltrate and migrate to the lung parenchyma and express proinflammatory cytokines, result in loss of epithelial integrity and cause oxidant induced injury (53). A previous report indicated that PMN is recruited to the lung via TLR4-NF-κB signaling pathway in endotoxemia. Phosphatidylinositol 3-kinase (PI3-K) phosphorylates and activates Akt through phosphatidylinositol-dependent kinases (PDK1 and PDK2), and then modulates neutrophil chemotaxis. Furthermore, it has been suggested that p38 MAPK contributes to the modulation of NF-κB pathway and neutrophil adhesion (49). Fan *et al.* reported that LPS mediated TLR4 transcriptionally reduces the expression of G-protein-coupled receptor kinases (GRK2 and GRK5) induced by macrophage inflammatory protein 2 (MIP 2) and amplifies PMN migration (75). In a model of LPS dependent sepsis, E3 ubiquitin ligase Cblb, which controls the association of TLR4 and MyD88, was shown to modulate the microvascular endothelial integrity of the lung and to prevent PMN sequestration. The loss of Cblb expression increased expression of inflammatory chemokines and cytokines and exacerbated ALI inflammation (17). In a recent report, mTOR complex 1 (mTOR1) was described as a regulator of PMN activation via TLR4 pathway; pretreatment of rapamycin, an inhibitor of mTOR1, attenuated the severity of lung injury and reduced neutrophil recruitment in LPS induced ALI (76).

5.2.3. Endothelial cell activation via TLR4 signaling in sepsis

The pulmonary vascular endothelium is a crucial target that plays a critical role in the development of sepsis-induced ALI. It has been reported to maintain vascular hemostasis, mediate PMN infiltration and sequestration in the lung and to secret cytokines and chemokines and thus exacerbate lung inflammation. Following exposure to LPS induced endotoxemia these functions are mediated by the TLR4 signaling cascade (77).

A recent study revealed that endothelial cells are more critical as sentinel cells than previously anticipated for PMN recruitment in ALI; in LPS sepsis, $TLR4^{+/+}$ endothelium recruits neutrophils to the lungs without the expression of selectin molecules and CD18 integrin when lacking $TLR4^{+/+}$ neutrophils (45). TLR4 and CD14 dependent endothelial responses induced by LPS are crucial for neutrophil sequestration into the lung (78). Interaction between neutrophils and endothelial cells seems to be required for PMN migration into the lung. Additionally, it may contribute to the mediation of endothelial cell responses in innate immunity. PMN NADPH oxidase and neutrophil adhesion to endothelial cells are critical for amplification of the expression of TLR2 challenged by LPS and peptidoglycan. TLR4-TLR2 cross talk results in augmented endothelial activation challenged by invading pathogens (79).

5.2.4. Epithelial cell activation via TLR4 signaling in sepsis

TLR4 has been reported to be expressed in bronchial and alveolar epithelial cells (ECs); furthermore, it has been reported as an important PPR in recognizing airway epithelial cells. IRAK, MAPKs, TRAF6, and activation of NF-κB appear to be involved in the TLR4-MyD88 dependent signal pathway in LPS induced ECs. TLR4 is expressed in airway cells and mediates the secretion of inflammatory cytokines upon exposure to LPS (80). Type II alveolar epithelial cells are reported to be activated by LPS via TLR4 signaling and subsequently amplify the pulmonary inflammatory process (81). In a transgenic mouse model, NF-κB is selectively inhibited by a mutant IκB-α construct. Nuclear translocation of RelA was demonstrated in the airway epithelium of challenged, transgenic negative control mice but not in transgenic mice following LPS inhalation; moreover, expression of TNF-α within bronchial ECs was blunted in transgenic mice. NF-κB activation involved the lung inflammatory response in distal airway epithelium following LPS challenge (82).

The integrity of the epithelial barrier is crucial to enable maintenance of the pulmonary physiologic condition. The impairment of epithelial integrity leads to exacerbated fluid influx into the alveoli and less tissue fluid reabsorption. In a FAS dependent ALI model, ECs tended to exhibit apoptosis and the alveolo capillary barrier was impaired upon exposure to a Fas activating antibody (83). In a TLR gene study, alveolar epithelial injury with airway protein leakage and destruction of lung microarchitecture were TLR4 gene dose dependent in endotoxin induced ALI (72).

5.3 TLR4 in ischemia-reperfusion induced ALI

Ischemia-reperfusion injury (I-R) is a complex pathogenetic condition that involves diverse molecular and cellular mechanisms. It potentially activates innate immunity via TLR4 signaling pathway by recognizing multiple endogenous ligands. Previous studies have demonstrated that TLR4 activation plays a pivotal role in mediating ischemia reperfusion in various organs, including liver, renal, heart, and lung (84-86). Given the continuous requirement for vascular supply and oxygen uptake, the lung is particularly susceptible to I-R injury regardless of whether it occurs in the lung or a remote organ. In a direct lung injury model, TLR4 null mice exhibited marked reduction of vascular permeability and myeloperoxidase activity following lung ischemia-reperfusion injury (LIRI) (87). TLR4 mutant mice displayed a lower level of neutrophil priming and infiltration in the left lung following the occlusion of the pulmonary artery. Lung inflammation appeared to require TLR4, not TLR2, to generate lung I-R injury (88). However, it has been reported that in intestinal I-R induced lung injury, both TLR2 and TLR4 mediate local and remote lung inflammatory responses without the involvement of TNFα (89). TLR/MyD88 pathway contributes to the epithelial damage and the lung inflammatory response. P38 kinase, NF-κB, and AP-1 appear to be involved in the TLR4 signal pathway and mediation of I-R lung injury (90, 91).

Reactive oxygen species (ROS) has been demonstrated to participate in activation and exacerbation of acute lung injury (74). During the progression of LIRI, ROS can be generated by mitochondrial, NOS, activated xanthine oxidase, and NADPH oxidase system (92). In a HS/Resuscitation lung injury model, xanthine oxidase has been shown to regulate the activation of cAMP response element binding protein and cytokine expression, such as IL-1b, TNF-a, and MIP-2 in neutrophils (53). In another global I-R model, HS/R induced a much lower level of ROS release in TLR4 mutant neutrophils. PMN NAD(P)H oxidase appears to be activated by HS/R via HMGB1/TLR4 signaling, which leads to inflammatory response and organ injury (62). Extracellular superoxide, a type of ROS, is primarily induced by Xanthine oxidase and NADPH oxidase in I-R injury. It acts as a key mediator of the proinflammatory response. Blockade of superoxide production derived from NADPH oxidase leads to inhibition of the proinflammatory processes initiated by ischemia-reperfusion injury (93). Additionally, xanethine oxidase generates extracellular superoxide to activate neutrophils and induce subsequent proinflammatory responses via TLR4-dependent signaling (94).

Heat shock protein (HSP), a highly conserved protein, exists in all prokaryotes and eukaryotes. Originally recognized as molecular chaperones, they are involved in folding naive polypeptides during protein synthesis (95). As a member of the HSP family, HSP70 has been investigated widely and demonstrated to play a critical role in I-R injury. A previous study found that HSP70 utilized both CD14/TLR2 and CD14/TLR4 in inducing proinflammatory cytokine production via MyD88/NF-κB

signal pathway (96). Extracellular heart shock cognate protein 70 (HSC70) has been shown to depress cardiac function by activating p38MAPK and NF-κB and expressing proinflammatory cytokines via TLR4-dependent pathway following global I-R injury (97). In a HS/Resuscitation model, HSP70 expression has been observed to increase quite early in lungs of rats subjected to I-R to enhance lung inflammation (98).

5.4 TLR4 in burn injury induces ALI

Apart from local inflammation and tissue damage, major burn injury tends to induce systemic innate immunity and subsequent inflammatory responses (99). As a result, the excessive synthesis of proinflammatory cytokines and chemokines contributes to the dysfunction of multiple organs and leads to ALI. It has been reported that p38 MAPK is involved in thermal induced ALI. Topical wound application of SB202190, a specific p38 MAPK inhibitor, has been shown to significantly diminish lung edema and pulmonary microvascular injury, accompanied with attenuated neutrophil sequestration and lower cytokine expression, including IL-6, MIP-2, and iNOS (100).

As a biosensor of tissue damage or noninfectious inflammatory stimulation, TLR4 has been reported to be indispensable in the pathogenesis of burn induced remote organ dysfunction. TLR4 knockout mice exhibit concentrated areas of occludin, a tight junction protein of the intestinal barrier, and less intestinal permeability compared with TLR4 wild type animals following thermal injury (101). Transendothelial electrical resistance, which is utilized to judge endothelial cell adhesive barrier function, rapidly decreases in TLR4 WT mice induced by burn injury, whereas the response is markedly reduced in TLR4 knockdown animals. Additionally, neutrophil adhesion in mesenteric venules also appears blunted in $TLR4^{-/-}$ mice (102). A recent study reported that lung injury appears quite early upon observation of histological changes, and PMN infiltration increases sharply in TLR WT mice within 24 h following induction by burn injury. TLR4 knockout mice seem to neither produce necessary inflammatory signals nor prime neutrophils in lung tissue (103). However, Oppeltz *et al.* reported that TLR4 responses are not augmented until 7 days following burn injury in accordance with the increased levels of IL-6, TNF-α, IL-17, MIP-1β, MCP-1, and RANTES in bronchoalveolar lavage cells (104). These differing results are potentially due to the distinct protocol and specific treatment of the particular study. Further studies are warranted to elucidate the intrinsic underlying mechanisms.

6. CONCLUSION

The diversity of phenotypes and specific conditions in IALI has been recognized as the crucial impediment to further research. To date, pharmacotherapy has not significantly improved the outcome of IALI. In this review, TLR4 is shown to be not only a receptor for microbial products, but also exhibits recognition of endogenous ligands leading to sterile inflammation in IALI. TLR4 signaling in pulmonary parenchyma and vasculature involves the priming of neutrophils, activation of lung stromal cells, and release of proinflammatory cytokines and chemokines. Direct blockade of TLR4 receptors and modulation of its signal pathway could potentially serve as effective therapeutic strategies for IALI. Further investigation is warranted to elucidate the cross talk between TLR4 and other PPRs relative to the complex mechanisms of IALI.

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Abbrevations: IALI, indirect acute lung injury; PRR, pathogen recognition receptor; LPS, lipopolysaccharide; DAMP, damage-associated molecular pattern; TLR, toll like receptor; LRR, leucine-rich repeat; TIR, toll/interleukin-1 receptor; LBP, LPS binding protein; MyD88, myeloid differentiation protein88; TIRAP, TIR-associated protein; Mal, MyD88-adaptor like; TRIF, TIR domain containing adaptor protein inducing IFNβ; TRAM, TRIF related adaptor molecule; SARM, sterile alpha and HEAT-Armadillo motifs; IRAK, IL-1 receptor associated kinase; TRAF, TNF receptor associated factor; TAK, TGF-β activated kinase; NF-κB, nuclear factor kappa B; IKK, inhibitor of NF-κB kinase; NEMO, NF-κB essential modulator; TAB, TAK binding protein; MAPK, mitogen-activated protein kinase; JNKs, c-Jun N-terminal kinases; ERKs, extracellular-signal-regulated kinases; AP-1, adaptor protein-1; IRF3, interferon regulatory factor3; IKKε, non-canonical IκB kinases; TANK, TRAF-associated nuclear factor kappa B activator; TBK, TANK binding kinase; RIP1, receptor-interacting protein 1; TSH, trauma hemorrhage shock; PMN, polymorphonuclear leukocytes; ICAM-1, intercellular adhesion molecule-1; NADPH, nicotinamide adenine dinucleotide phosphate; HMGB1, high mobility group box1; HA, hyaluronic acid; GPI, glycosylphosphatidylinositol; PI3-K, phosphatidylinositol 3 kinases; PDK, phosphatidylinositol-dependent kinases; MIP, macrophage inflammatory protein; GRK, G-protein-coupled receptor kinases; I-R, ischemia-reperfusion

Key Words: TLR4, IALI, LPS, Sepsis, Trauma Hemorrhage Shock, Ischemia-Reperfusion, Burn Injury, Review

Send correspondence to: Hong Jiang, Department of Anaesthetics, Shanghai Ninth People's Hospital affiliated to Shanghai Jiao Tong University, School of Medicine, N.O. 639, Zhizaoju Road, Shanghai, China, 200011; Tel: 86-21-23271699, Fax: 86-21-63136856, E-mail: dr_jianghong@yeah.net