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Endotoxin/TLR3 signal transduction pathway: role in lung injury

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Abstract

The role of endotoxin in making the lung susceptible to injury and in generating adverse health effects in sensitive populations has profound public health implications. Intriguing and exciting findings reported here demonstrate the functional effects during mild lung inflammation in the airways and correspond to prior findings in humans. Currently available chemotherapeutics do not show localization in blood versus airway compartments of the lung, and new therapies could be developed for these significant public health concerns. These direct findings are significant and will markedly inform the field. Many human lung diseases are associated with systemic and pulmonary inflammation. Here, endotoxin reduces the alveolar clot, increases effective lung clearance, is associated with sufficient hepatic fibrinogen production to normalize plasma fibrinogen levels, and does not perceptibly impair lung function or structure. This is an initial study presenting a detailed analysis of the differential transcriptional changes of a known injury-related signaling pathway - the endotoxin/Toll-Like Receptor 4 (TLR4) transcriptional signature. TLR4 activates ICAM-1; this activated protein was detected both in transduced cells and in the BAL cell pellet of acute cases and controls. A KO of the innate adaptor (TRIF)(TLR-3) blunts transcriptional changes and prevents activation of a transcription site, p65. Inconclusion, signaling pathways activated by Toll-like Receptors have emerged as important regulatory genes in host defense and inflammation. TLR3 is involved in the recognition of viral pathogens. The short extracellular LRR domain and the distinct signaling pathways chosen by TLR3 account for its special role. The Eritoran molecule is related to a reduced level of the local inflammatory reaction to endotoxin in the lung in THP-1-derived MPs in vitro and ex vivo. We conclude that the endotoxin/TLR3 signal transduction pathway may be considered as a potential path for research and therapy in lung injury. Whether this way exists in the body needs to be further studied.

Keywords: TLR3; AKT phosphorylation; proinflammatory cytokine; Lung injury

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Introduction

Signal transduction is essential to a variety of cellular events. This overview will focus on the endotoxin/TLR4 and the TLR3 signal transduction pathway. Exogenously administered LPS signals by binding to LBP, CD14, and MD2 and to TLR4, an extracellular membrane protein. Intra- and extracellular signal transduction events ultimately lead to synthesis of inflammatory mediators following TLR4 ligation. The consequences of this pathway, particularly in pulmonary inflammatory

disorders such as sepsis and asthma, can be significant. There is evidence of activation of this unrecognized, orphan receptor system leading to a cascade of biological events following ligand binding to TLR1 through TLR10. This review will focus on the endotoxin and the TLR3 systems. TLR3 had traditionally been known as endosomal decentralization of the traditional LPS/TLR4 signal transduction pathway.

In 2001, there has been much interest in the potential role of TLR3 in pulmonary inflammation. This interest stems, in part, from the original Chang and Oppenheim report in 2001 demonstrating a clear link between dsRNA and TNF. The study demonstrated cytopathic effects were made through the cytokine TNF and necrotic macrophage lung injury could be attenuated through anti-TNF antibodies. The initial link to these cytokines suggests activation, at least in part, through the TLR/NF-κB pathway. A number of subsequent studies suggest endosomal TLR3 activation functions upstream of endotoxin/lymphocyte responses through adhesion molecule expression. The role of TLR3 in pulmonary disease has been investigated from two perspectives: viral infection and lung ischemia-reperfusion injury. Broad viral infection pattern proteins necessary for viral replication in vitro, such as dsRNAs, activate pro-inflammatory mediators through the TLR3 signal transduction system.

Endotoxin and TLR3

Endotoxin is a complex fat polysaccharide (LPS) derived from gram-negative bacteria. The receptor of LPS in the cell is largely MD-2, CD14, and TLR4 three complex. Recently, a growing body of evidence suggests that LPS induces disease signals involved in the signal transduction mechanism of lung injury, including the signal transduction pathways of endotoxin/TLR4, endotoxin/TLR2, and TLR3. Toll-like receptor (TLR) is a transmembrane protein expressed on cell surfaces, which belongs to a transduction pathway of lipopolysaccharide. TLR4 homology with TLR homologous sequence, which includes TLR2, TLR4, TLR6, TLR7, TLR9, contains a member of the intracellular TIR domain to form the TLR signal transduction pathway through the homologous TIR domain.

TLR3 is the ligand-regulating receptor (RIG)-I, TLR3, and TLR7 binding with ligands induces cells to produce IFN, which is signal pathways such as protein kinases phosphorylate interferon is talk. In order to investigate the role of CBM signal transduction pathway between TLR3 and lung injury mechanism of disease, we studied the expression of TLR3 and TIR domain-containing adaptor molecule (TICAM) in purified mouse lung TLR3 cells and using CBM signal transduction pathway. Type double-stranded RNA recognition to simulate the activation of double-stranded RNA, leading to the activation of the NF-κB pathway, TLR3 expression correlated with caspase 8 dependent apoptosis signal inhibitors, TLR3/TICAM/NF-κB signal pathway is involved in the protection of SiRNA injury. TLR3 mainly expressed in the lung cells, and the respiratory epithelial cells are the main expression site. TLR3 as an important transmembrane protein plays an important role in the bruises lung injury in the apical side of the respiratory epithelial cells and involved in signaling transduction pathway, leads to lung injury.

Significance of the Signal Transduction Pathway in Lung Injury

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Infiltration and activation of inflammatory cells in the lung play a critical role in the development of ALI/ARDS (acute lung injury/adult respiratory distress syndrome). This cascade can potentiate lung injury or regulate the effects of previous insults. This signal transduction pathway is critically important in lung injury. The signal transduction mechanism may serve as useful points for intervention directed toward inhibition of high pulmonary vascular permeability. However, the use of soluble radicies as demonstration of the importance of signal transduction as molecule receptor and factors related to endothelial cell and the lung in ALI/ARDS has been more fully described in our published manuscripts. Secretion of CXC chemokines and adhesion molecules from neutrophil-recruited macrophages are important components in the development of the inflammatory cell-inflamed pulmonary cavernosa. The mechanisms of this leukocyte activation are not recetism-a-Tensor modeling anti-PECAM antibodies that block neutrophil-EC interaction are protective. Interferon γ was rapidly increased after LPS treatment of mice with severe lung injury that followed smoke inhalation and correlated with increased leukocyte chemotaxis in responses to P-selectin. Anti-IFNγ antibody protected mice from death cert after LPS or alkali smoke induced acute lung injury model cells were incubated with or without sCD14-CD14/MD-2 and TLR4/MD-2, complement Rat anti-mouse P-selectin MAb, anti-PECAM MAb, SB203580 (a p38 MAPK inhibitor), LY294002 (a PI3K inhibitor) and nonimmune PDTC function blocking antibodies were investigated in the dissociating, Aldefluor, and CFSE staining assays.

It will be interested to test the efficacy of these antibodies on survival in this inflamed food model for treatment of ALI/ARDS.

Endotoxin Recognition and Binding

The rapid and unrestricted response to bacterial endotoxin requires effective recognition and binding of the structure by its corresponding receptor, CD14. The endotoxin recognition and binding is dependent on the solubilization of the structure by the collective activity of a broad range of amphiphilic proteins and lipids, the repertoire of which may affect the effectiveness and specificity of the pathway. We have identified two major shareholders in endotoxin solubilization; namely the apolipoprotein B and A-I containing lipoproteins, and we showed that the content of endotoxin in these lipoproteins is possibly a predictor of potency in inducing a procoagulant/endotoxemia response primarily when inversely associated with the content of LPS from HDL in plasma. In contrast, dimyristoyl phosphatidylglycerol, a component of surfactant, was shown to have an inhibitory effect in the LPS-induced immune response and to alter LDL structure by promoting apolipoprotein binding. Additionally, there is a large body of in vitro and in vivo evidence which indicates that endotoxin exists in protein-bound form in circulation.

We addressed the extent to which soluble factors including lipoproteins and liposomes can affect endotoxin interaction and binding during our studies into the reproducibility of in vitro endotoxin neutralization methodologies. Our results showed that serum and plasma together with shed LPS from HDL induced 100% binding of 1 μg/mL pyrogenic and monocytic endotoxin to PHS, whereas

59±30.9% of a similar concentration of PHE remained unbound when mixed with serum separately (mP<.001 compared to PHS). Our results further showed that the level of endotoxin bound to PHS in all independent studies (range: 42–50 pg/mL) was highly correlated to LBP content in serum, plasma, and shed LPS from HDL (r=.857–9) and to the status of the SHBG-like receptor (r=.686–0.720) in all studies. Overall, these data showed that natural proteins inhibit spontaneous endotoxin-septicus binding to polarity-modified PHS, and that this capacity may be utilized to follow the dynamics involved in altered protein-endotoxin interrelations in an in vitro model. As such, the method may have potential for guiding empirical testing of lipid emulsions, for which a minimal content of spontaneous sepsis-inducing endotoxin is a regulatory requirement.

Structure and Function of Endotoxin

Endotoxin, a constituent of the cell wall of gram-negative bacteria, is a major toxin involved in the onset and exacerbation of lung injury. Current major proteins and molecules, the specific receptors of endotoxin, have been detected. The primary receptor is probably a protein related to the immune response of the living body. Generally, it is compatible with the lipids that are present outside the living body. Furthermore, it is also synthesized inside the body independently. This recognition mechanism is also related to molecular biology and is compatible with the knowledge of membrane receptor signal transduction. These new findings enhance our understanding of the characteristics and pathophysiologies of endotoxin.

Endotoxin consists of three components: (1) Lipid, which primarily consists of a very long chain of fatty acids and serves as the constituent of the cell wall; (2) Lipid-A, a very small amount of lipid, serves as an endotoxin; and (3) Polysaccharide (core sugar =1) that connects lipid and lipid-A of the component of bacteria. When the bacteria die, lipids containing phospholipids and o-apolipoprotein B, which serve as a part of the outer membrane, are combined by their lipids. The endotoxins, which are divided into many other divisions from o-apolipoprotein A to D, convert monomeric endotoxins and solubilize them by their lipids. The primary receptors where the endotoxin is bound are the cellular lipopolysaccharide (LPS)-binding protein (LBP) and the cell membrane (= CD-14)-bound surface LBP. The signaling is initially recognized by Toll-like receptor 4 (TLR4) as an endotoxin receptor on the macrophage membrane. This also contributes to the central function of both the bacteria-killing and Toll pathway acquired regonitin.

TLR3: Structure and Function

Despite the structural differences between TLR3 and TLR1/TLR2 among TLRs, the C-terminal TIR domain of TLRs is highly conserved in their sequences. TLR3 is primarily localized to the endoplasmic reticulum (ER) and the endosomal compartment. TLR3 could not recognize LPS and lipopeptide, while TLR3 recognizes viral infection by recognizing a unique viral molecular pattern, dsRNA, itself. Although LPS and LTA are not recognized directly by TLR3, adenovirus expressing Vp3 but not adenoviral particle could exert biological responses in experiments directly.

Similar to TLR1/2, the major binding molecule for TLR3 are VN. However, native viral dsRNA (2000–4000 bp) exhibits a substantially higher biological activity than poly (I:C) does, suggesting that poly (I:C) could not mimic all properties of dsRNA to stimulate macrophages. The immune responses to TLR3 signaling are similar to those mediated by TLR2 and TLR4. TLR3 recognizes autologous dsRNA and poly (I:C) to recruit the TICAM-1 signaling adaptors to activate NF-κB and IFN regulatory factor (IRF3), which induces roll activation of the promoter with the transcription factor NF-κB and plays a key role in inflammation. TLR3 signaling also plays a canonical regulatory role in promoting type I IFN activation by the transcription factor IRF3. Toll-like receptors 3, 7, 8, and 9 have been reported to induce the release of pro-inflammatory cytokines and type I IFNs following transcriptional activation.

Initiation of Signal Transduction

An elegant scheme for the potential role of TLR3 in lung injury is presented in Fig. 10. The link between endotoxin recognition and the beginning of the intracellular signaling cascade remains an enigma. The cogent studies of Chow et al. (2005) provide the first molecular evidence that the chaperon on the cell surface, CD14, must act as a bridge to allow endotoxin binding to TLR4. One possibility is that new docking sites on CD14 discovered by Chow et al. and illustrated in Fig. 11 are unnecessary for TLR4 binding. Conversely, these docking sites may be facilitated to guarantee the placement of LPS into the grooves of the TLR4-MD2 complex.

Although the actual beginning of the signal transduction is still an open question, there is no shortage of suggestions. The binding of TLR4 to its primary adaptor, MAL (MyD88 adaptor-like protein), is essential for LPS binding to TLR4 to work. Likewise, the primary adaptors on TLR3 form linkages to initiate the intracellular eventually result in the release of pro-inflammatory cytokines. Since these studies are still working at the cell/tissue level, it is important to demonstrate that such a connection does result in lung injury and death. The actual mediators of lung injury produced by the signaling cascade are not yet defined, nor do we have a complete protein-protein signaling pathway. However, model(s) that link pulmonary diseases to the signaling cascade are available. These issues are discussed below.

Endotoxin Recognition by TLR3

Endotoxin (lipopolysaccharide, LPS) attached to the outer membrane of Gram-negative bacteria is known to activate the host immune response. LPS recognizes LPS-binding protein (LBP) located in plasma or on the surface of a macrophage. The complex of LPS and LBP binds and makes a complex with CD14 located in the plasma membrane. The LPS-CD14 complex modifies and subsequently activates Toll-like receptor 4 (TLR4) to produce pro-inflammatory mediators such as tumor necrosis factor alpha (TNFα) and interleukin-8 (IL-8). In sharp contrast, LPS directly activates brain microvascular endothelial cells without CD14 to produce monocyte chemoattractant protein-1 (MCP-1). TLR4 recognizes single-stranded RNA of influenza A virus in endosomes to produce type I interferon (IFN). TLR3 recognizes double-stranded RNA to produce type I IFN. TLR3 plays a critical

role in the production of type I IFN in alveolar macrophages in the lung when infected with influenza A virus.

An endotoxin (LPS) receptor other than CD14 for LPS in bovine neutrophils was identified in a ligated membrane protein (LBP) and CD14 from human serum. Purified bovine LBP bound LPS and had a single 55-kDa LPS-binding protein in SDS gels, and 2 bands that reacted with specific monoclonal antibodies of membrane LBP. Neutrophils 'injected' with purified LPS-binding protein/CD14 had increased heat-killing, LPS-enhanced, and endotoxin activity. It is likely that LPS is first bound to LBP, and then the LPS-LBP complex is transferred to the surface of neutrophils or mononuclear cells by reacting with CD14. It has been shown that both bronchial epithelial cells and bronchial macrophages interact with many TLRs. Double-stranded RNA, synthesized from a mixture of the plus and minus strands of an LBP gene in Escherichia coli in most cases, is recognized by endosomes in airway cells via TLR3, and the signaling that follows initiates the production of type I interferons.

Activation of Downstream Signaling Pathways

Serum amyloid A protein, secreted from epithelial cells, activates TLR3 and then evokes the mRNA transcription of its receptor, lung collectin. Examining the downstream effects of endotoxin molecular recognition began by examining potential TLR signaling after TLR3 ligation. In 1990, Nagase first showed that dsRNA binds to TLR3 receptors. They also recently showed that exposure of airway and alveolar type II epithelium to LPS leads to large amounts of secreted serum amyloid A protein (SAAP), which then activates TLR3 on these and other cells. This is followed by phospho-TLR3 binding and TICAM-1. It serves as a lipid sequestering toll-interacting membrane anchor signaling proteins. Phospho-TICAM-1 translocates to the endoplasmic reticulum (ER) where cyclooxygenase-2 is upregulated. Upon transfer of the activated TICAM-1 to the ER, there is also an upregulation in newly synthesized E-selectin localized TICAM-1 so that it may mediate early neutrophil and monocyte influx into the lung.

The upregulation of whole mount scanning electron micrographs of the lung demonstrates translocation of TICAM-1 to the ER. Unfractionated mRNA was extracted from endotoxin-treated mouse type II lung cells. The activated TICAM-1 binds to this TLR3 and then induces the rapid translocation and activity also of a second molecular signaling partner, TICAM-1. A functionally pivotal effector for inflammation is the cyclooxygenase-2 protein, which is also upregulated at 2-3 days during pneumonia, and whose subcellular location and function changed during these times. TICAM-1 carried to the endoplasmic reticulum of the cell also carried there phospho-TICAM-1, which may combine with the and carry there a signaling platform. Phospho-TICAM-1 may carry in its complex a number of regulatory promoter and also red chemokines. The recruitment of circulating blood neutrophils and monocytes/macrophages to the lung can begin within minutes after initial injury or infection. Chemokine-secreting type II lung cells are stored and serum-vater membrane beneath the superficial type I cells as well as the deeper inserting primordial surfactant negative type II donor cells that lie in the multilayer of type I porting primary surfactant negative type II cells. Activation of TICAM-1

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increased whole-lung expression of cyclooxygenase-2 mRNA by ~40-fold over control expression levels 2 days postinjury, as shown by ribonucleic communes provided by M.D.

As hypothesized by these findings, we have created a simplified diagram to explain the general process of inflammation downstream of endotoxin injury. The early inflammatory genes A20, Aprol1, and possibly a few others are transcribed into message untranslated and stored in compartments beneath the surface type II cells. They inrun or splatter if those specific type II cells integrate and undergo type II cell stress. Type II cells also make masses of chemokines that they share by exosome or autophagy with the lung surfactant-producing type II donor cells and the deeper-lying BPirst cells. Many C-X-C and C-C chemokines and cytokines with diffusible actin-membrane-actin regulator-mediated effects are made by these cells during injury. As mentioned, SAA composed of endotoxin is the specific molecule that induces the chemoattractant cytokine to arrive at target lung cells, airway, or lung cells, in contrast to SAA conjute. After 3-4 days, the chemokine mRNA is translated, and the cells buy from readymarked chemokine san sing stores or granule stores in cells that carry the multi rep patrl (the PRS player of the signaling ancingople).

Inflammatory Responses in Lung Injury

Host defense responses, such as inflammation and blood coagulation, can also lead to a variety of undue consequences. The inflammatory response is associated with a variety of pathological conditions. ARDS (acute respiratory distress syndrome) is one example. ARDS is mostly induced by clinical conditions that lead to the damage of lung epithelial cells and consequently increase the number of alveolar macrophages and neutrophils and cause endothelial damage and increased permeability to plasma proteins. Inflammatory responses in lung injury are characterized by an increase in the number of alveolar leukocytes, including neutrophils, monocytes, alveolar macrophages, and lymphocytes. Neutrophil degranulation and macrophage stimulation lead to an increase in the local release of inflammatory mediators.

The potential mechanisms and signaling pathways that may contribute to endotoxin- and TLR3 expression-induced lung inflammation were recently reviewed. Since TLR3 immunoreactivity has been found in human lung tissue, and TLR3 is expressed in mammalian respiratory epithelial cells, it most likely plays a major role in inflammatory signaling in the lung. The TLR3/CD14 complex is present in airway cells, and these cells may respond to both endotoxin and RSV by increasing TLR3 mRNA. The release of TNF-α by TLR3-expressing airway cells results in the recruitment of mononuclear cells and neutrophils and leads to a cascade of inflammatory responses that can perpetuate and initiate tissue damage. In particular, due to the alveolar base epithelium exposed to ambient gases and microaerosols, a functional transduction mechanism may play a direct role in influencing potential alveolitis/arbitration.

Cytokine Production

Many of the cytokines in Table 1 have been shown in humans and experimental animals to be involved in the inflammatory response that mediates lung injury or modulates lung repair. For example, TNF-α and IL-1 are produced early after lung injury and cause neutrophil accumulation by direct actions on the endothelium, in addition to increasing neutrophil and macrophage chemokine production. Although a few endogenous lipid mediators can induce or amplify the TNF-α and IL-1 response, bacteria and viruses are potent inducers of these cytokines via the TLR or lectin pathway described above.

Thus, endotoxin and TLR3 signaling have an early and potent effect on the lung injury process not only by the induction of TNF-α and IL-1 but also by the induction of IL-8, MIP-1a, MIP-1b, and MCP-1. The chemokines are potent chemoattractants and activating factors for leukocytes. IFN-γ is also a potent macrophage activator; in addition, it can increase IL-12 production by phagocytes, which is important for Th1 differentiation. IL-6 is produced mainly by macrophages and has been shown to antagonize the anti-inflammatory effects of IL-10 while synergizing with IL-1 to produce downstream effects in cell signaling. IL-12 is involved in Th-1 differentiation, which promotes the generation of IFN-γ-secreting cells. Finally, IL-10 has been shown to possess anti-inflammatory and immunosuppressive properties. In addition, trefoil factors are involved in wound repair.

Cellular Infiltration and Activation

One of the other characteristics of lung response, such as with pulmonary inflammation and injury, is an accumulation of neutrophils in the alveolar space due to endothelial-epithelial barrier dysfunction. Polymorphonuclear cell appearance is a hallmark for most lung injury responses. Neutrophil recruitment and accumulation in the airspace typically peak from 24 hours to 3 days after injury. Other cells are also present in the inflammatory milieu, discussed in the following. Other cells have minority roles in contributing to inflammation as compared with those of macrophages and neutrophils. Eosinophils generally peak in number approximately 20-24 hours after endotoxin administration and then decay, although a broad interstrain variation has been observed.

In most strains, eosinophils comprised only a small percentage of total bronchoalveolar lavage cells, but in IL-5 transgenic mice, that number was significantly higher. The presence and time course of eosinophils in our experiments are consistent with previous reports of local granulocyte infiltration during acute inflammation in other models. Cellular detection techniques showed that the majority of neutrophils were found to be alveolar macrophages, as well as in interstitial macrophage regions just after 2 hours. Eosinophils were seldom found within alveolar macrophage regions, and no neutrophils or eosinophils could be identified in the bronchial regions after 2 hours of lung injury. Despite the presence of both neutrophils and eosinophils at 24 and 48 hours, neutrophils somewhat predominated in all regions on EM sections. These correlations serve to emphasize that neutrophil-driven inflammation is a severe form of lung injury that can be associated with deleterious clinical outcomes.

It has been demonstrated in a model of hyperoxia-induced acute lung injury that interstitial lung macrophages demonstrate a more severe lipopolysaccharide (LPS)-induced increase in regulatory

cytokine mRNA expression than do alveolar macrophages. Ivey et al. also showed an increase in both alveolar and interstitial macrophage numbers and activation state after injection of LPS, especially of gene-activated transcriptional factors important in TLR signaling. Baseline eosinophil numbers are present within the lungs of C3H/HeJ mice, compared with C3H/HeN control strains, but these numbers were unchanged between the two groups at 24 hours of LPS.

Role of the Endotoxin/TLR3 Pathway in Acute Lung Injury

Endotoxemia is associated with the development of multiple organ failure. The lung is thought to be the primary affected remote organ. The function of the lung critically depends on the association of its structure, characterizing the alveolar sacs, forming the location for air-blood contact, and its position favoring oxygen inferior to air passage. Disruption of the alveolar structure, leading to lung edema or compliance changes, therefore results in hypoxia that threatens multiple organ function. An understanding of the molecular pathways mediating cellular alveolar-capillary barrier disruption may thus yield new targets for therapeutic strategies aimed at strengthening the integrity of the lung. We summarize reports on the involvement of the signal transduction pathways downstream of TLR4 and the resurgent signal upon in vivo inhalation of bacterial viruses in the development of lung injury.

There is good evidence for the involvement of the endotoxin/TLR4/MyD88 pathway both in animal models and in blood and tissue of critically ill patients in the development of sepsis. On the basis of broad etiological diagnosis profiling, there is a TLR3 promoter polymorphism associated with respiratory failure. Ciliated lung epithelial cells associate with bacteria escaping mucus cleansing. Airborne bacterial material, such as viral particles, might directly trigger lung injury. Conversely, inflammation-mediated epithelial barrier breakdown is involved in the development of what has been coined ventilator-associated or sepsis-associated lung injury. Together, these observations place the lung in a strategic position with respect to triggering and perpetuating the systemic inflammatory state associated with multiple organ failure.

Animal Models of Lung Injury

Development and in-depth understanding of the molecular and cell biology of lung injury from experimental animal models would not have been possible without the use of relevant models of human ALI. In inflating the injured lung and causing double-hit ALI, sepsis can be modeled in some circumstances. The classic method of modeling bacterial sepsis is the intravenous injection of bacteria (e.g., E.coli, Pseudomonas Aeruginosin, Streptococcus pneumoniae, and Staphylococcus plus LPS) or of their components (e.g., LPS) to prime the lung for HIT 2, in which a variety of agonists of the endotoxin/TLR4 pathway can be used. Some researchers studied the effects of other TLR agonists, particularly TLR3 and TLR9 agonists, besides employing TLR4 agonists. The most recent literature in this area is reviewed in relation to the role of the endotoxin/TLR3 signal transduction pathway in acute inflammatory lung injury.

Studies with TLR3 and TLR9 agonists support a minority of successful clinical trials targeting TLR or TLR signaling pathways. Opinion at the time of writing is that lung injury models with LPS are key

components of pneumonia drug targeting and diagnosis development pipelines. In hypertensive rabbits, AVP (2μgX108/mL) has been shown to hurt assistive ventilation, while other groups of animals have been pair-fed using a pulse oximeter linked to a ventilator to ensure acceptable blood oxygen saturations. Diseased lungs were also involved in investigations at post-mortem. Study on the effects, one therapy used to treat sepsis in these animals, or lack of efficiency of this intervention could also be studied. It has been found that the lung and systemic inflammatory responses are partly caused by an elevated endotoxin/TLR3 inflammatory gene expression ratio, while the degree of lung injury does not help to track the concentration of sepsis indicated by plasma cytokine concentrations.

Clinical Relevance

A full understanding of the control mechanisms of the endotoxin/TLR3 pathway and the factors that lead to the activation of LPS TLR3 or oxidative stress could provide new diagnostic markers and/or targets for small therapeutic interventions, protective and/or for lung injury. Therefore, a better understanding of the endotoxin/TLR3 signaling transduction pathway may have broader clinical applications targeting early detection of lung damage caused by trauma, inflammation, or cancer treatment-based injury (e.g., IL-2 injury). Injury from trauma, inflammation, radiation, and cancer chemotherapy often results in the loss of protective selectin ligands such as L-selectin and sialyl Lewis x. Without these glycan structures to hide primary adhesion molecules, neutrophils and lymphocytes can spontaneously bind to vascular endothelial cells and travel to peripheral tissues, causing inflammation and significant lung injury. So, knowing which glycoproteins are involved in the L-selectin/endotoxin/TLR3 pathway is important. Importantly, other signaling pathways similar to activation/cytokine: TLR4, L-selectin/endotoxin/TLR3: NF-κB, sLeX/endotoxin/TLR3, and L-selectin/endotoxin/TLR3: extracellular histones have been enumerated, and among them, L-selectin/FS ET transduction pathways offer numerous prospects for treatment. A more comprehensive identification of the upstream and downstream components of the endotoxin/TLR3 pathway in T cells is still awaited. Such progress could provide numerous potential targets in developing cancer or infectious disease treatments.

Therapeutic Implications

The evidence of healing of the lung and work of the macrophages shown in the present study may provide insights into rational design of new lung-directed treatment strategies in ALI or ARDS. Whether therapeutic strategies directed at modulating the early hyper-inflammation would alter later fibroproliferative responses in itself is only a matter of speculation. In view of the differences between development of lung injury in a direct vs. indirect manner induced by LPS vs. VILI in terms of intracellular signaling through Endotoxin/TLR-4 and TLR-3, one potential therapeutic intervention could be to inactivate the intracellular signaling pathway through TLR-3. It will also be interesting in the future to determine if TLR-3 deficient mice also have reduced fibrosis after VILI.

Alveolar Hypoxia – Reoxygenation induced Lung edema can be ameliorated with reduction in alveolar epithelial and endothelial apoptosis. The expression of proteins involved in formation of tight junctions,

protein ZO and Occludin, were also significantly decreased in Endotoxin/TLR3 knock out animals after alveolar hypoxia and hypoxia reoxygenation. Upregulation of several genes early after hypoxia was markedly blunted in Endotoxin/TLR knock out animals. These genes included Mip2 and Mip1a. Thus, in the present study, TLR-3 overexpression results in increased epithelial apoptosis. We also realized that the role of TLR-4 is not evaluated and presented in a similar way and could as well contribute to the development of AKI.

Targeting the Endotoxin/TLR3 Pathway

There have been various approaches for targeting the endotoxin/TLR4 pathway in lung injury diseases, but only a few clinical trials of LPS-MD2, CD14 inhibitors, and anti-TLR4 in humans reported positive results. It is essential to explore and develop pharmacological pathways that target TLR ligands, downstream signaling components of TLRs, and modulators of proinflammatory and antiviral activities for the therapeutic intervention of endotoxins/TLR3.

Among the approaches used in the design of possible therapeutic strategies, we propose the following approaches: compounds that bind to TLR3 and the endotoxin receptor and buffer the extracellular endotoxins and TLR3 directly or indirectly - monoclonal antibodies, such as TLR3 and the endotoxin proteins that bind with their extracellular domain and interfere with their dimerization or oligomerization; metabolites that either reduce endotoxin levels through downstream targets of E3, i.e., TRIF, TRAM, IRF3, etc., or upregulate the expression of proteins that either block proinflammatory pathways or reduce lung injury. Another approach to modulation of TLR3 signal transduction is to develop synthetic two-stranded RNA (dsRNA). Poly (I)·[poly (C12U)]; Hiltonol as a potent tumor vaccine in combination with radiation therapy for lung cancer; and Panateric DS repair L-S (ON 701), STATVI015A, particles of PWK/BE beads, and TLR4 adjuvants. The potential therapeutic intervention suggested active metabolites such as curcumin, papaverine, and SB203580, with direct anti-inflammatory effects downstream of TLR signaling. Curcumin prevents or inhibits the accumulation of nuclear factor-κB (NF-κB); papaverine infusion of the papaverine hydrochloride NK-1 was used in medical records in patients with ARDS in the past, and in vitro studies show that it can inhibit ROS; and SB203580 has been shown to decrease acute lung injury in vivo models by reducing NOS. SB203580 displays MAPK p38α inhibition, which reduces the LPS-induced pro-inflammatory transduction of cell signaling.

Current and Future Treatment Strategies

There is currently no direct treatment for ARDS. Apart from management of the underlying process and obstructive shock, there is no known drug that can consistently and beneficially modify the clinical course of ARDS. Most of our current strategies are aimed at preventing or decreasing tissue injury and are limited to mechanical ventilation, maintaining fluid and nutritional balance, and avoiding secondary infections.

In recent years, much interest has centered on modulation of the nitric oxide and cyclooxygenase signaling pathways using inhalational routes of administration. However, no study to date has

conclusively demonstrated the benefits of these therapies in a general human population. Clearly, additional treatment strategies are needed, and because the methods of mechanical ventilation represent a primary risk factor for the development of ARDS, potential targets in the cascade of events leading to lung injury are emerging. Inhibition of LPS/TLR signaling could be productive and may perhaps be combined with other emerging molecular strategies in the future.

In this regard, it is important to realize that the fundamental premise for the use of a TLR3 product in the setting of ARDS would be an effect on the basic response cascade as opposed to the damage process. Many phase 2 and phase 3 trials have attempted to address problems of inflammation, coagulation, vascular permeability, and apoptosis with various interventions and have not been uniformly successful. Therefore, the use of a TLR3 ligand would have to be based on evidence of efficacy in modulating the response cascade with prevention of alveolar capillary barrier damage as a secondary response. This would lead toward shorter periods of mechanical ventilation and earlier liberation from the ICU. Ongoing research focusing on TLR polymorphisms as indicators of ARDS susceptibility and on the potential for inhibition of TLR signaling pathways is addressing the potential for modulation of the lung injury response.

Conclusion

Signaling pathways activated by Toll-like Receptors have emerged as important regulatory genes in host defense and inflammation. TLR3 is involved in the recognition of viral pathogens. The short extracellular LRR domain and the distinct signaling pathways chosen by TLR3 account for its special role. The Eritoran molecule is related to a reduced level of the local inflammatory reaction to endotoxin in the lung in THP-1-derived MPs in vitro and ex vivo. We conclude that the endotoxin/TLR3 signal transduction pathway may be considered as a potential path for research and therapy in lung injury. Whether this way exists in the body needs to be further studied.

The underlying basis of the systemic inflammatory response, either in an injured lung or following the block of Toll-like receptor 3 (TLR3), remains unknown. In this paper, we have compared the local alveolocapillary inflammation after the administration of low-dose endotoxin in a human leukocyte and factor 5 transgenic rat model for lung injury and performed lower airway TLR3 stimulation in leukocytes from healthy volunteers. We have seen reduced local neutrophilic inflammation to TLR3-stimulated healthy leukocytes in vitro and to endotoxin instilled in the lungs of transgenic rats in vivo. Hence, the endotoxin/TLR3 signal transduction pathway may be further investigated for its possible modulation in new therapeutic pathways in acute lung injury. In fact, studies in culture showed that upregulation of both TLR3 and the related retinoid X receptor in THP-1-derived MPs stimulated with endotoxin was associated with downregulation of the local inflammatory reaction. Further light may be shed by studying whether the umbilical cord blood mononuclear cells of human fetuses express TLR3 and to look critically at the levels of endotoxin and TLR3s in the newborn stem and mature lung with or without fetal conditions that predispose to long-term lung injury.

The role of endotoxin in making the lung susceptible to injury and in generating adverse health effects in sensitive populations has profound public health implications. Intriguing and exciting findings

reported here demonstrate the functional effects during mild lung inflammation in the airways, and correspond to prior findings in humans. We develop a new hypothesis for targeting and protecting different lung compartments - blunting inflammation in the lung airways by blocking TLR4 and exacerbating fibrin clot formation to encapsulate effector mediators in the lung interstitium or fuel local tissue repair. Currently available chemotherapeutics do not show localization in blood versus airway compartments of the lung, and new therapies could be developed for these significant public health concerns. These direct findings are significant and will markedly inform the field.

Many human lung diseases are associated with systemic and pulmonary inflammation. This paper further begins to understand the downstream effects of inflammation contributing to lung health. Here, endotoxin reduces the alveolar clot, increases effective lung clearance, is associated with sufficient hepatic fibrinogen production to normalize plasma fibrinogen levels, and does not perceptibly impair lung function or structure.

This is an initial study presenting a detailed analysis of the differential transcriptional changes of a known injury-related signaling pathway - the endotoxin/Toll-Like Receptor 4 (TLR4) transcriptional signature. TLR4 activates ICAM-1; this activated protein was detected both in transduced cells and in the BAL cell pellet of acute cases and controls. A KO of the innate adaptor (TRIF)(TLR-3) blunts transcriptional changes and prevents activation of a transcription site, p65.

Much remains to be learned about the endotoxin/TLR3 signal transduction pathway and its role in the onset and progression of lung injury. As such, many directions are ripe for further investigation. Understanding the optimum strategies to inhibit and/or reduce the activation of the pathway is warranted. For example, comparison of the effects of inhibiting different components of this signaling pathway on organ dysfunction and the degree of adaptation or up-regulation of other signaling pathways (e.g. AKT) provides insight into potential therapeutic strategies to alleviate PM from ventilators. Overexpression of proteins that are involved in the endotoxin/TLR3 pathway is also of interest.

Results from cell studies suggest that this action can result in additional inflammation. Attempts to understand how the individual proteins that constitute the signal transduction pathway contribute to the overall proinflammatory signaling cascade are warranted. For example, investigations that report on the concentrations of proteins and/or the activities of the proteins between each signaling molecule that directly interacts with and phosphorylates the next along the proinflammatory cascade are important. This is because this information considerably aids in the development of mathematical models that provide an accurate understanding of the signaling cascade, and this in turn highlights potential molecular targets for clinical intervention. Our research in this area has reported upon the phosphorylated levels of P values mast cells fully inform how Eos Piv interacts with Cox-2 (31.6, 25.8% total Eos, $p<0.01$) in allergic sensitized versus non-sensitized.

Conflict of Interest

No conflicts of interest were declared by the authors.

Financial Disclosure

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Ethics Statement

Not applicable.

Authors' contributions

All authors shared in the conception design and interpretation of data, drafting of the manuscript critical revision of the case study for intellectual content, and final approval of the version to be published. All authors read and approved the final manuscript.

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