THE ROLE OF TLR-4 AND GALECTIN-3 INTERACTION IN ACUTE PANCREATITIS

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ULOGA TLR-4 I GALEKTIN-3 IN RETAKCIJE U AKUTNOM PANKREATITISU

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ABSTRACT

Toll-like receptor-4 (TLR-4) is a member of evolutionarily conserved type I transmembrane proteins that can initiate sterile inflammatory cascade in the pancreas. Expression of TLR-4 is up-regulated in pancreatic tissue, as well as, on peripheral blood innate immune cells in human and experimental models of acute pancreatitis. TLR-4 plays important pro-inflammatory roles during development of acute pancreatitis: it recognizes alarmins released from injured acinar cells and promotes activation and infiltration of innate immune cells after the premature and intra-acinar activation of trypsinogen. Galectin-3 is β -galactoside-binding lectin that plays pro-inflammatory roles in a variety of autoimmune diseases, acute bacterial infections and during tumorigenesis. It is reported that Galectin-3 is an alarmin in experimental models of neuroinflammation and binds to TLR-4 promoting the pro-inflammatory phenotype of microglia. Also, in experimental model of acute pancreatitis Galectin-3 is colocalized with TLR-4 on innate inflammatory cells resulted in enhanced production of inflammatory cytokines, TNF- α and IL-1 β , increased infiltration of pro-inflammatory N1 neutrophils, macrophages and dendritic cells and increased damage of pancreatic tissue. This review paper discusses the role of TLR-4/Gal-3 axis in the pathogenesis of acute pancreatitis.

Keywords: TLR-4, Galectin-3, acute pancreatitis.

SAŽETAK

TLR-4 je receptor koji pripada konzervisanoj familiji transmembranskih proteina tipa I i ovaj receptor može pokrenuti sterilnu inflamacijsku kaskadu u pankreasu. U toku humanog i eksperimentalnog akutnog pankreatitisa, ekspresija TLR-4 je povećana u parenhimu pankreasa i na ćelijama urođene imunosti u perifernoj krvi. TLR-4 igra važnu pro-inflamacijsku ulogu u patogenezi akutnog pankreatitisa: detektuje alarmine koji su oslobođeni iz oštećenih acinusnih ćelija pankreasa i pospešuje aktivaciju i infiltraciju ćelija urođene imunosti u oštećeno tkivo pankreasa nakon prerane i intra-acinusne aktivacije tripsinogena. Galektin-3 je lektin koji vezuje β -galaktozid i igra pro-inflamacijsku ulogu u brojnim autoimunskim bolestima, akutnim bakterijskim infekcijama i tokom tumorigeneze. Galektin-3 je alarmin u eksperimentalnim modelima neuroinflamacije, vezuje se za TLR-4 i pospešuje razvoj pro-inflamacijskog fenotipa mikroglija. Takođe, u eksperimentalnom modelu akutnog pankreatitisa, Galektin-3 je kolokalizovan sa TLR-4 na površini ćelija urođene imunosti rezultujući povećanom produkcijom inflamacijskih citokina, TNF- α i IL-1 β , infiltraciji pro-inflamacijskih N1 neutrofila, makrofaga i dendritskih ćelija i povećanom oštećenju pankreasa. Ovaj pregledni rad govori o ulozi TLR-4/Gal-3 osovine u patogenezi akutnog pankreatitisa.

Cljučne reči: TLR-4, Galektin-3, akutni pankreatitis

ABBREVIATIONS

AP- Acute pancreatitis
DAMPs- Damage-associated molecular patterns
NF-K β - Nuclear factor kappa B
TLR-4- Toll-like receptor-4
IL- Interleukin
PAMPs- Pathogen-associated molecular patterns
LPS- Lipopolysaccharide
Gal-3- Galectin-3
SIRS- Systemic inflammatory response syndrome

TNF- α - Tumor necrosis factor alpha
TLR4 KO- Toll-like receptor-4 knockout
SAP- Severe form of AP
WT- Wild-type
HMGB1- High mobility group box 1 protein
MPO- Myeloperoxidase
PBMCs- Peripheral Blood Mononuclear cells
NO- Nitric oxide
DSc- Dendritic cells

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INTRODUCTION

Acute pancreatitis (AP) is a complex inflammatory disease of pancreas initiated by acinar cell injury and followed by increased production of pro-inflammatory mediators that play a pivotal role in the development of local inflammation and systemic inflammatory complications (1). AP initially develops as a chemical and nonspecific inflammatory process caused by several factors, including acinar hypersecretion, obstruction of pancreatic duct and ischemia of pancreata (2). The first event in the development of AP is the injury of the acinar cell caused by premature and intra-acinar activation of trypsinogen in the cathepsin-B-dependent manner (3). Injured acinar cells discharge intracellular content into the extracellular space that serves as damage-associated molecular patterns (DAMPs) that recruit more inflammatory cells in the process of “sterile inflammation” which then cause further injury of the pancreas (4). Previous reports indicate that events developed after acinar cell injury determine the severity and outcome of pancreatitis (5). Thus, these events are a degree of activation of transcriptional factors, such as nuclear factor kappa B (NF- κ B), then the production of pro-inflammatory mediators, and degree of activation of innate immune cells, especially macrophages (6).

Toll-like receptor-4 (TLR-4) is a membrane protein that can initiate this inflammatory cascade in the pancreas (2). TLR-4 is one of ten known Toll-like receptors (TLRs), a large family of evolutionarily conserved type I transmembrane proteins and belongs to the superfamily of interleukin (IL) receptors (7). Apparently, TLRs have a basic function to react to triggers and induce the activation of powerful pro-inflammatory processes against the invasion (8). On the other hand, inappropriate activations of this receptor are one of the key characteristic of autoimmune diseases, protracted infections, and several types of cancers (9). TLRs are expressed on the immunocompetent hematopoietic cells involved in the first line of defense, such as neutrophils, dendritic cells, macrophages, but also, on T cells, and B cells; or other nonhematopoietic cells, including epithelium, fibrocyte, endothelial cells, cardiac myocytes, and cells of the central nervous system (10-12). Recent research has suggested that TLR-4 is extensively expressed on the epithelial cells and vascular endothelial cells of the pancreas (13, 14). TLR-4 can identify several structural molecular patterns which are highly conserved between diverse microorganism (also known as pathogen-associated molecular patterns, PAMPs), especially the molecules from gram-negative bacteria (e.g., Lipopolysaccharide, LPS) (Table 1). Also, TLR-4 can detect endogenous molecules released from injured tissue and necrotic cells, called damage-associated molecular pattern molecules or alarmins (15).

Galectin-3 (Gal-3) is glycan-binding lectin that selectively binds β -galactoside by conserved carbohydrate-recognition domains (45). Gal-3 has a unique chimeric structure in the galectin family, which consists of both carbohydrate-recognition domain and lectin-like domain (46). Gal-3 can be localized on the cell surface, in the cytoplasm and the

nucleus, and can also be excreted in extracellular spaces and systemic circulation (46). The expression of Gal-3 is highly increased during several inflammatory processes, but its role in regulating inflammation mainly depends on the localization of Gal-3 (47). Thus, Gal-3 plays pro-inflammatory roles in a variety autoimmune diseases, fulminant hepatitis, acute bacterial infections and during tumorigenesis (48-51). It is suggested that Gal-3 may function as an alarmin, an immunomodulatory molecule that belongs to the larger family of danger-associated molecular patterns (52).

TLR-4 AND ACUTE PANCREATITIS

Previous research has indicated that TLR4 is localized in the pancreatic duct and acinar cells, as well as cells and endothelial cells of normal human and rat pancreas (53). The expression of TLR4 on the epithelial cells of pancreatic ducts exhibit the potential role of pancreatic ducts as a place for initiation of inflammatory processes (53). Also, TLR-4-mediated endothelial response participates in the sequestration of leukocyte during the early stage of AP (54). Further, it has been demonstrated that acinar cells express TLR4 and react to LPS by triggering inflammasome and producing pro-inflammatory cytokines and mediators (55).

Convincing data have shown that the association between TLR4 and alarmins plays a significant role at the beginning of AP (2). Experimental studies showed that enzyme pancreatic elastase binds to the TLR4 on the surface of mononuclear inflammatory cells and activates NF- κ B which results in increased production of Tumor necrosis factor-alpha (TNF- α) (56, 57). Similar results are presented by Hietaranta *et al* (58) with increased expression of NF- κ B in human myeloid cells treated with porcine elastase. There is evidence that activation of a signaling pathway in the early stage of AP is also caused by degradation products of heparan sulfate produced by the action of pancreatic enzymes in absence of bacterial infection (2). The development of systemic inflammatory response syndrome (SIRS) is mainly induced by the significant activation of TLR4 by these degradation products (9). Moreover, prematurely activated of trypsin and elastase cleaves heparin sulfate expressed on the cell plasma membrane or in the extracellular matrix and discharge endogenous heparin sulfate (59). In turn, heparin sulfate binds and activates TLR4 and these associations contribute to the development both and local pancreatic inflammation and SIRS-like reaction in mice with early increased serum levels of TNF- α serum (60).

The other proposed factors that trigger TLR4 are PAMPs from translocated bacteria (2). Namely, bacterial translocation is an event that precedes bacterial infection of pancreatic necrosis (61). After activation TLR4 by PAMPs derived from invading bacteria, expression of several cytokines and costimulatory molecules is increased in the antigen-presenting cells and therefore promote activation of the specific immune response (2).

Induction of AP by taurocholate in TLR4 knockout (TLR4 KO) mice was accompanied by lower activity of myeloperoxidase as a marker of neutrophil infiltration, as well as, decreased serum amylase levels compared with diseased wild-type (WT) (62). Also, it is noticed that histological scores of pancreatic edema, hemorrhage, and acinar cell necrosis were significantly decreased in TLR4 KO mice with a severe form of AP (SAP) (62). Similarly, the treatment of TLR4 KO mice with cerulein or L-arginine resulted in the lower histological score of pancreatic and lung damage (13). Namely, serum levels of amylase and edema of the pancreas are lower in TLR4KO mice in comparison to WT mice (13). Neutrophils infiltration and necrosis of acinar cells were less prominent in TLR4 KO mice (13). Contrarily to these findings, recent studies suggest that there was no significant difference in histological scores of AP and serum amylase levels between TLR4 KO mice and WT mice with SAP (63). Interestingly, Ding *et al* (63) showed that deletion of TLR4 significantly decreased levels of pro-apoptotic proteins in the pancreas 2 h after taurocholate administration and it is known that apoptosis is a beneficial event in the course of AP prevents occurrence severe necrotizing form of the disease (64).

The deletion of TLR4 contributed to repair of kidney and liver damage, but enhanced intestinal translocation of gram-negative bacteria to the pancreatic necrotic tissue in the experimental form of SAP (65). Additional experimental studies suggest that TLR4 plays a major role in high mobility group box 1 (HMGB1) induced acute lung injury (66). In the lung, myeloperoxidase (MPO) activity and neutrophil infiltration were markedly lesser in TLR4 KO mice (13). Significantly, translocation of gram-negative bacteria into the pancreata is increased in TLR4 mice with SAP (65). These reveal that TLR4 initiates the inflammatory reaction defensively against infection in SAP (65).

TAK-242 is the antagonist of TLR4 and the application of this molecule in mice with taurocholate-induced AP had a protective effect with diminished apoptosis and cytotoxicity associated with preserved mitochondria and less production of oxidative stress (67). Contrary, the application of anti-TLR4 monoclonal antibody can exacerbate pancreatic injury during SAP due to activation of NF- κ B by this antibody that imitates LPS and results in enhanced production of pro-inflammatory cytokines and neutrophil activation (2).

In humans, it has been reported that the expression of TLR4, IL6, and TNF- α is signed in on the innate immune cells of patients during the early phase of AP compared with control volunteers (68). At the beginning of a human mild form of AP, expression of TLR4 on the peripheral blood mononuclear cells (PBMCs) is initial up-regulated, but then progressively decreases during the first week of disease (68). Production of the pro-inflammatory cytokine, TNF- α , significantly correlated with the change in TLR4 expression (68). Furthermore, expression of TLR is down-regulated in the late stage of SAP which may result in increased bacterial translocation and subsequent infection of pancreatic necrotic tissue (65).

We would mention the Chinese study which enrolled 310 patients with AP (69). At half of the patients with severe form, AP with pancreatic necrosis was TLR4 mutation (896G *allele*) carriers (69). Actually, percent of TLR4 mutation was strikingly higher in patients with a severe form of AP and formation of pancreatic abscesses (69). It is demonstrated that TLR4 mutation increased morbidity and mortality in patients with severe AP and simultaneously Gram-negative infection (69).

Also, polymorphism of TLR4 Asp299Gly correlated with increased occurrence infection of pancreatic necrosis. Later, in the study Zhang *et al* (70) which included 238 patients with AP and 121 healthy controls, there was no statistically significant difference in the incidence of Asp299Gly polymorphism between patients with AP and healthy volunteers. Further, in a Hungarian study 92 patients were diagnosed with AP and there was observed occurrence Asp299Gly polymorphism and Thr399Ile polymorphism (71). It is showed that there no significant difference in the occurrence of any polymorphism analyzed between patients with severe form and a mild form of AP, as well as, between 92 patients with diagnosed AP and 200 healthy volunteers. Recent, in a case-control study, Guenther *et al* (72). reported that there were no significant differences in the occurrence of Thr399Ile and/or Asp299Gly polymorphism between 521 patients with AP and 393 healthy volunteers. Hence, clinical data show mentioned polymorphism of TLR4 is not linked with the development and outcome of AP, although the mutation of the TLR4 gene is associated with deterioration of immune response initiated with LPS-TLR4 interaction (73). Additional experimental studies suggest that human monocytes *in vitro* treated with alcohol reduced expression of TLR4 and secretion of pro-inflammatory cytokines after challenge with alcohol and then LPS (74).

GALECTIN-3 IS A LIGAND FOR TLR-4

Emerging evidence indicates that activation of TLR-4 plays a crucial role during the development of sterile inflammation (75). Recently, it is identified that Gal-3 is an endogenous ligand for TLR-4 and interaction between Gal-3 and TLR-4 might be important in the setting of sterile inflammation, such as acute pancreatitis or stroke. Burguillos *et al.* (21) detected that Gal-3 is highly expressed and discharged by microglial cells during experimental neuroinflammatory models that mimic brain ischemia. Thus, Gal3 released from microglia serve as an endogenous paracrine ligand for TLR4. In these models, it is noticed that Gal-3 binds to TLR-4 via the carbohydrate recognition domain and thus induce a pro-inflammatory response in microglial cells during neuroinflammatory diseases. In humans, there is evidence of the interaction of Gal-3 and TLR4 in patients with diagnosed ischemic stroke. The deletion of Gal-3 led to anti-inflammatory effects with a neuroprotective response (21).

TLR-4 is composed of domain with β -galactosides and Gal-3 can be associated with this domains of TLR4. Gal-3 can activate TLR-4 by dimerization of these receptors

through a capacity of Gal-3 to form oligomers, or by promoting internalization of TLR-4 which is required for full activation of TLR4 (76). In agreement with the previous studies, it is noticed that Gal3 induce activation of TLR-4 signaling pathways in lung cancer cells (77). It is described that Galactin-3 improves TLR4 protein expression (77). Also, Gal-3 mediates in chemoresistance of ovarian cancer cells by TLR4 signal activation (78). Treatment with neutralizing antibodies against galectin-3 decreased the expression of $\text{TNF-}\alpha$, IL-1 β , IL-6, and nitric oxide (NO) synthase 2 and thus exerted neuroprotective effects on the cortical and hippocampal cell populations after injury of head (79). These indicate that released galectin-3 may act as an alarmin, binding, among other receptors, to TLR-4 and promoting inflammation after head injury (79). Gal-3-dependent-TLR4 activation contributes to sustained microglial activation, prolonging the pro-inflammatory response in the brain.

GALECTIN-3/TLR4 BINDING AND ACUTE PANCREATITIS

In our study (80) we have discovered that severe form of experimental acute pancreatitis induced by ligation of bile-pancreatic duct increased expression of Gal-3 in the infiltrating leukocytes indicating the role of Gal-3 in the development of acute pancreatitis. Also, we revealed that Galectin-3 and TLR-4 are co-localized on the surface of the same infiltrating leukocytes.

Target deletion of Gal-3 attenuates acute pancreatitis due to a lack of Gal-3 interaction with TLR-4 after initial BPD-induced destruction of acinar cells. Thus, Gal-3 knockout mice survival longer after induction of severe form of AP due to milder inflammatory response in pancreata. We found that lack of Gal-3 decreased the expression of TLR-4 on infiltrating leukocytes, and the diminished influx of macrophages and neutrophils that express TLR-4, in particular, pro-inflammatory N1 neutrophils. Similarly, the deletion of Gal-3 reduced the production of pro-inflammatory $\text{TNF-}\alpha$ and IL-1 β from neutrophils, macrophages, and dendritic cells (DCs). Hence, lack of galectin-3 binding to TLR-4 results in diminishing severity of AP by attenuating the early influx of neutrophils and inflammatory mononuclear cells of innate immunity (80). The interaction between Gal-3 and TLR-4 in acute pancreatitis is depicted in Figure 1.

Our results were also in accordance with those of Pan LL et al. (81). It has been reported that the application of lactose, an inhibitor of galectins, diminished severity of experimental AP which was accompanied by significantly lower pancreatic edema score and pancreatic damage score; as well as reduced serum amylase levels and pancreatic myeloperoxidase activities (81). Administration of lactose led to regulatory cytokine milieu in the pancreas with enhanced production of IL-10 from macrophages and reduced infiltration of inflammatory neutrophils and macrophages. Additionally, treatment with lactose after induction of AP diminished infiltration and activation of neutrophils and reduced tumor necrosis factor-alpha production (81).

Figure 1: The Galectin-3 /TLR-4 axis promotes inflammation and cell destruction during induction of acute pancreatitis.

The induction of acute pancreatitis by BPD ligation leads to the destruction of some acinar cells and subsequent release of DAMPs, cytokines, and Galectin-3. Released Galectin-3 binds to nearby neutrophils. Macrophages and dendritic cells via TLR-4 receptor, inducing development and predomination of the pro-inflammatory phenotype of innate inflammatory cells. This is followed by intensive inflammation and severe tissue damage.

CONCLUSION

In summary, this study shows for the first time the current understanding of the role of TLR-/Galectin-3 interaction in the pathogenesis of acute pancreatitis. Obviously, further investigation and elucidation of the precise mechanism of the TLR-4/Gal-3 axis in course of acute pancreatitis and other pancreatic diseases is warranted and its potential future clinical implications, such as in chronic pancreatitis and pancreatic cancer.

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