Journal of Microbiology (2013) Vol. 51, No. 6, pp. 724–730
DOI 10.1007/s12275-013-3429-6

MINIREVIEW

Shiga Toxins Expressed by Human Pathogenic Bacteria Induce Immune Responses in Host Cells

Moo-Seung Lee[1*], Myung Hee Kim[1], and Vernon L. Tesh[2]

[1]*Infection and Immunity Research Center, Korea Research Institute of Bioscience and Biotechnology, Daejeon 305-806, Republic of Korea*
[2]*Department of Microbial and Molecular Pathogenesis, Room 3002, Medical Research and Education Building College of Medicine, Texas A&M Health Science Center, 8447 State Highway 47 Bryan, TX 77807-1359, USA*

(Received August 9, 2013 / Accepted October 22, 2013)

Shiga toxins are a family of genetically and structurally related toxins that are the primary virulence factors produced by the bacterial pathogens *Shigella dysenteriae* serotype 1 and certain *Escherichia coli* strains. The toxins are multifunctional proteins inducing protein biosynthesis inhibition, ribotoxic and ER stress responses, apoptosis, autophagy, and inflammatory cytokine and chemokine production. The regulated induction of inflammatory responses is key to minimizing damage upon injury or pathogen-mediated infections, requiring the concerted activation of multiple signaling pathways to control cytokine/chemokine expression. Activation of host cell signaling cascades is essential for Shiga toxin-mediated proinflammatory responses and the contribution of the toxins to virulence. Many studies have been reported defining the inflammatory response to Shiga toxins *in vivo* and *in vitro*, including production and secretion of tumor necrosis factor alpha (TNF-α), interleukin-1β (IL-1β), macrophage inflammatory protein-1α/β (MIP-1α/β), macrophage chemoattractant monocyte chemoattractant protein 1 (MCP-1), interleukin 8 (IL-8), interleukin 6 (IL-6), and Groβ. These cytokines and chemokines may contribute to damage in the colon and development of life threatening conditions such as acute renal failure (hemolytic uremic syndrome) and neurological abnormalities. In this review, we summarize recent findings in Shiga toxin-mediated inflammatory responses by different types of cells *in vitro* and in animal models. Signaling pathways involved in the inflammatory responses are briefly reviewed.

Keywords: inflammatory response, Shiga toxin, signaling, HUS, animal model

*For correspondence. E-mail: msl031000@kribb.re.kr; Tel.: +82-42-879-8224; Fax: +82-42-879-8595

Introduction

Shiga toxin (Stx) is an exotoxin secreted by *Shigella dysenteriae* serotype 1. Both the genus name *Shigella* and the toxin are named after the Japanese bacteriologist Kiyoshi Shiga who first identified the microorganisms as the cause of inflammatory dysentery or bloody diarrhea in 1897 (Trofa *et al*., 1999). All members of the *Shigella* species are Gram-negative, non-motile, rod-shaped, invasive, and facultative intracellular pathogens. Genomic analysis has revealed that the *Shigella* spp. are very closely related to *Escherichia coli*. Only *Shigella dysenteriae* serotype 1 has been reported to express Shiga toxin. Recently, select serotypes of *E. coli*, collectively referred to as Shiga toxin-producing *E. coli* (STEC), have been shown to express one or more toxins that are genetically and structurally related to Shiga toxin (Gyles, 2007). *Shigella dysenteriae* serotype 1 and STEC are major public health concerns in the developed and developing countries. Infections with *Shigella dysenteriae* serotype 1 (termed shigellosis) and STEC may result in bloody diarrhea and the subsequent development of life threatening sequelae, including acute renal failure and neurological abnormalities such as seizures, paralysis, blindness, and death (Proulx and Tesh, 2007). Infections with Shiga toxin-producing bacteria are still a significant cause of morbidity and mortality. Annually in the U.S., *E. coli* serotype O157:H7 is estimated to cause 63,153 cases of illness and STEC of serotypes other than O157:H7 cause 112,752 cases (Scallan *et al*., 2013).

Upon infection with STEC or *Shigella dysenteriae* serotype 1, multiple clinical outcomes may follow including hemorrhagic colitis and systemic extra-intestinal complications involving the kidneys and central nervous system (CNS). In many cases, the activation of innate immunity with the elicitation of an inflammatory response has been observed. In particular, pro-inflammatory cytokine production in response to Shiga toxins is a topic of interest and has been studied by many investigators using *in vitro* and animal models (Ramegowda and Tesh, 1996; Harrison *et al*., 2004; Keepers *et al*., 2006; Lentz *et al*., 2011; Mohawk and O'Brien, 2011; Stearns-Kurosawa *et al*., 2013). The activation of the innate immune response is critical for the elimination of infectious agents and toxins, leading to healing or regeneration of damaged tissues. Tissue healing may include the activation of cell survival and apoptotic signaling cascades. The inflammatory response which may ensue following the successful recognition of "danger signals" such as bacterial cell membrane components, toxins, flagella, or viral RNA

and DNA, is mediated in part by the increased synthesis and release of low molecular weight (glyco)proteins, collectively termed cytokines (Horvath *et al*., 2011; Kumar *et al*., 2011; Franchi *et al*., 2012). Cytokines are essential for cell-to-cell communication regulating the initiation, maintenance, and resolution of innate immunity, and generally act at short distance and at low concentrations in autocrine, paracrine or endocrine fashion. Usually, cytokines act by binding to specific membrane receptors, which in turn, signal via second messengers, often tyrosine kinases, to alter gene expression. Physiological responses to cytokines may include increasing or decreasing expression of membrane proteins (e.g., regulation of the cytokine receptors that initiated the response), cell proliferation, and the *de novo* synthesis and secretion of a large number of additional cytokines that may further enhance or diminish inflammation. The successful orchestration of inflammation leads to alterations in cellular physiology that mediate wound healing and pathogen elimination. However, excessive inflammation may lead to deleterious effects and is linked to several diseases such as rheumatoid arthritis, Crohn's disease, and septic shock (Moritz *et al*., 2005; Ezri *et al*., 2012).

Shigella dysenteriae serotype 1 expresses Stx, while STEC may express one or more related toxins. The toxins expressed by STEC are categorized into two toxin types depending on their antigenic and genetic relationship to Stx. Shiga toxin type 1 (Stx1) is essentially identical to Stx, while Shiga toxin type 2 (Stx2) displays approximately 56% sequence homology to Stx/Stx1 at the deduced amino acid sequence level (Strockbine *et al*., 1986; Jackson *et al*., 1987). All members of the Shiga toxin family are ribosome-inactivating proteins with an AB_5 molecular structure; that is, all the Shiga toxins contain one large monomeric A subunit in non-covalent association with a homo-pentameric B subunit. The toxin A subunit is responsible for an *N*-glycosidase activity that cleaves a single adenine residue from the 28S rRNA subunit of eukaryotic ribosomes, thereby inhibiting protein biosynthesis. The toxin pentameric B-subunit is essential for binding to target cells expressing the toxin receptor, the membrane glycolipid globotriaosylceramide (Gb_3) (reviewed in Tesh, 2012). Following membrane binding to Gb_3, Stxs are internalized and routed within the cell in a retrograde manner to reach the endoplasmic reticulum (ER) lumen. Furin or a furin-like protease cleaves the toxin A-subunit to produce the enzymatically active A1-fragment which is then delivered across the ER membrane into the cytoplasm. The A1-fragment possesses the *N*-glycosidase activity that cleaves a single adenine residue from the α-sarcin/ricin loop within the 28S rRNA component of eukaryotic ribosomes (Endo *et al*., 1988; Sandvig and Van Deurs, 2002; Lord *et al*., 2005; Johannes and Römer, 2010). While numerous studies have been carried out to characterize the mechanism of action and intracellular trafficking of Shiga toxins, only more recently studies have been initiated to define the inflammatory response to Stxs *in vitro* and *in vivo*. Furthermore, the contribution of the innate immune response in damage to the colon and the development of potentially fatal complications such as the hemolytic uremic syndrome (HUS) remains to be fully explored. This review presents recent findings on immune responses triggered by Stxs and provides the context to understand pro-inflammatory cell signaling mechanisms behind the toxin-mediated disease progression.

Inflammatory response to Shiga toxins

An inflammatory response consists, in part, of synthesis and release of cytokines and chemokines by immune cells, following recognition of danger signals such as bacterial membrane components, toxins, or flagella to eliminate pathogens and/or induce wound healing. However, excess inflammation may lead to deleterious effects, such as septic shock and multiple organ failure (Gustot, 2011; Girardis and Cossarizza, 2013). In 1996, Raqib *et al*. (1996) showed that levels

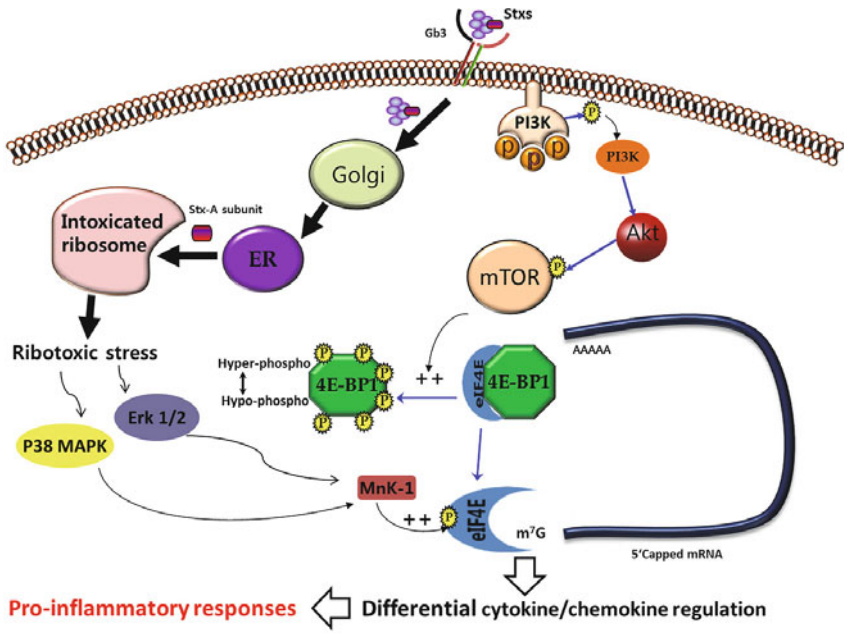

Fig. 1. Summary of Stxs-activated translational signaling pathways by which the ribotoxic stress induces proinflammatory cytokine regulation. Following retrotranslocation of Stx A-subunit across the ER membrane through Golgi apparatus, the depurination reaction involving a critical adenine residue within the α-sarcin/ricin loop of the 28S rRNA ribosomal subunit induces ribotoxic stress response to the toxin, rendering host ribosome inactive. Conformational changes of the intoxicated ribosome may be recognized by the stress-activated MAPKs. Stxs appear to be capable of activating host mitogen-activated kinases (p38 MAPK isoforms and ERKs) by phosphorylations. Both p38 and ERK are the upstream molecules for the activation of MAP-kinase signal-integrating kinase 1 (MnK1), which sequentially phosphorylates eIF4E. Transducing signal from PI3K/Akt/mTOR pathway activated by Stxs leads to phosphorylation of 4E-BP 1 to recruit 5′ capped mRNAs to host ribosomes. Ultimately, these two phosphorylating events may regulate cytokine/chemokine gene expression at transcriptional level and post-transcriptional level for the proinflammatory response to induce innate immunity.

of TNF-α, IL-1β, IL-6, IL-8, and GM-CSF directed into the stool are significantly higher in *Shigella dysenteriae* serotype 1-infected patients compared to patients infected with *S. flexneri*. In contrast to studies showing that Stx-producing *Shigella dysenteriae* serotype 1 induces gut inflammation, STEC colonization may initially suppress the proinflammatory cytokine response in the gut. Following colonization by STEC, the expression of virulence genes encoded within the LEE pathogenicity island (McDaniel *et al*., 1995) mediates profound changes in cytoskeletal elements in the colonic epithelium resulting in microvilli retraction, the assembly of filamentous actin in the form of raised pedestals subjacent to the attached bacteria, and an increase in intestinal barrier permeability. The unique structures formed at the STEC-epithelial cell interface are termed attaching and effacing lesions (McDaniel *et al*., 1995; Dean-Nystrom *et al*., 1997). Upon attaching and effacing lesion formation by STEC capable of producing the translocated bacterial effector protein EspB, NF-κB activity was shown to be suppressed and the bacteria induced significantly lower mRNA levels of IL-8, IL-6, and IL-1α, in effect, counteracting the host defensive response during colonization (Hauf and Chakraborty, 2003). During the course of HUS following STEC or *Shigella dysenteriae* serotype 1 infection, there is evidence of an acute inflammatory response with increased numbers of neutrophils, and raised concentrations of C-reactive proteins and cytokines, including TNF-α, IL-1, and IL-6 in the serum (Tesh, 1998). Heyderman *et al.* (2001) noted in an opinion article that, in the gut, Stxs produced by STEC mediate immune cell activation, particularly T-cell activation, to determine disease progression from the diarrheal phase to acute renal failure. *Shigella* species are human adapted pathogens, and studies on the role of cytokines in the pathogenesis of shigellosis have been limited by the lack of an animal model. In a murine model of Stx-mediated renal damage, macrophages were recruited to the kidneys of mice injected with Stx2 with or without lipopolysaccharides (LPS), in association with elevated renal production of chemoattractants including MIP-1α (Keepers *et al*., 2007). Jeong *et al.* (2010) induced acute gastroenteritis in gnotobiotic piglets by oral administration of *Shigella dysenteriae* serotype 1 and many of the histopathological changes observed in the colon of humans with shigellosis were reproduced in this model. Elevated levels of IL-8 and IL-12 were detected in the stool, and the amounts of pro-inflammatory cytokines IL-8, TNF-α, IL-1β, and IL-6 were increased up to three days after challenge, while levels of Th1 cytokines IL-12 and IFN-γ, and Th2 cytokine IL-10 were elevated at later time points in gut contents (Jeong *et al*., 2010). Clinical observations and animal studies suggest that Stxs play a direct role in activating gut pro-inflammatory responses during infection with STEC (reviewed in Thorpe *et al*., 2002). Recently, extremely high mRNA levels for IL-8, MCP-1, and MIP-1α in tissue samples obtained from multiple organs, including small intestine, colon, lung, and spleen, and elevated urine chemokines were detected from Stx1 (100 ng/kg) or Stx2 (50 ng/kg)-challenged baboons (Stearns-Kurosawa *et al*., 2013).

In vitro/*In vivo* evidence of inflammatory responses to Stxs

Shiga toxins may elicit the expression of host proinflammatory cytokines which contribute to the enhancement of cytotoxicity in many cell types. In human intestinal microvascular endothelial cells (HIMEC), Stx1 has higher binding affinity than that of Stx2, although Stx2 is more toxic to the cells than equivalent amounts of Stx1 and induces IL-8 with no difference between primary and transformed cells (Jacewicz *et al*., 1999). Studies using polarized T84 human intestinal epithelial cells showed that IL-8 secretion was induced upon Stx translocation across the cell membrane (Thorpe *et al*., 2001). Similarly, in Caco2 cells, both Stx1 and Stx2 induce proinflammatory cytokines, in particular, IL-8, via a mechanism requiring *N*-glycosidase activity, whereas levels of IL-8 were not enhanced in the non-toxic mutant Stx1-treated cells (Yamasaki *et al*., 1999). Thorpe *et al.* (1999) have published data showing that stimulation of human epithelial cells with Stxs results in stabilization of IL-8 transcripts, which may lead to prolonged IL-8 production, and increased neutrophil infiltration into the intestinal lumen (Hurley *et al*., 2001).

Primary human blood monocytes have been shown to be relatively resistant to the cytotoxic action of Stxs (Ramegowda and Tesh, 1996; Falguières *et al*., 2001; Lee *et al*., 2011). After crossing the epithelial barrier to reach the submucosa and gaining access to the bloodstream, Stxs stimulate circulating human monocytes to secrete cytokines (Hurley *et al*., 1999). For example, in human primary monocytes/macrophages, Stxs have been shown to induce the secretion of TNF-α, IL-1, IL-6, and IL-8 (Ramegowda and Tesh, 1996; Harrison *et al*., 2004; Yamasaki *et al*., 2004). However, monocytic cell line THP-1 cells are more sensitive to Stx1 (CD50 < 18 pg/ml) with no inflammatory cytokine induction, in part because the cells undergo programmed cell death (apoptosis). Apoptosis is triggered through ER stress response sensors (Harrison *et al*., 2005; Lee *et al*., 2008). Vero and HeLa cells are also highly toxin-sensitive cells which undergo rapid cytotoxicity (Fujii *et al*., 2003). In contrast to studies examining apoptosis in the toxin-sensitive Vero, HeLa and undifferentiated monocytic cell line THP-1 cells, differentiated macrophage-like THP-1 cells are relatively refractory to the cytotoxic action of the toxins with only approximately 30% of the cells undergoing apoptosis. However, macrophage-like THP-1 cells are competent to secrete the proinflammatory cytokines TNF-α, IL-1β and other chemokines when treated with Stxs (Ramegowda and Tesh, 1996; Sakiri *et al*., 1998; Harrison *et al*., 2004, 2005). Macrophages and macrophage cell lines are known to produce tissue factors and cytokines in response to Stxs, suggesting that infiltrating macrophages may further exacerbate inflammation, thrombogenesis, and tissue damage (Murata *et al*., 2006). These host factors, made in response to Stxs, may contribute to the progression of disease by upregulating Gb3 synthesis in endothelial cells.

The primary target organs of Stxs following entry into the bloodstream are the kidneys. The toxins have been shown to induce secretion of IL-1β, IL-6, and IL-8 from human proximal tubular cells (Hughes *et al*., 1998) and human glomerular epithelial cells (HGEC) (Hughes *et al*., 2001). In an immortalized adult human proximal tubule epithelial cell line termed Human Kidney 2 (HK2), Stx1 did not induce cytokine and chemokine expression, but Stx2 selectively in-

duced two chemokines: MIP-1α (CCL3) and MIP-1β (CCL4) (Lentz *et al*., 2011). In this regard, localized upregulation of chemokine secretion may facilitate the recruitment of activated immune cells, such as macrophages, into sites of initial tissue damage.

In baboon model of Stx-mediated HUS, systemic inflammatory response was minimal after intravenous challenge of Stx1, but cytokine production was elevated significantly and glomerular thrombotic microangiopathy was found at necropsy in the primate model Stx2 given (Siegler *et al*., 2003). Clinical manifestations elicited by Stx1 and Stx2 were confirmed in a non-human primate toxemia model to identify similarities and differences of pathologic consequences after intravenous challenge of each toxin (Stearns-Kurosawa *et al*., 2010). Glomerular platelet-rich thrombi, interstitial hemorrhage, and tubular injury were observed in detailed pathological examinations of kidney tissue isolated from baboons challenged with Stx1 or Stx2 (Stearns-Kurosawa *et al*., 2013). Stearns-Kurosawa *et al.* (2013) also looked at cytokine/chemokine expression in baboons treated with Stx1 or Stx2. After intravenous challenge of Stx1 (100 ng/kg) or Stx2 (50 ng/kg), analysis of the baboon kidney and other organs including small intestine, colon, lung, and spleen revealed a striking chemotactic profile with extremely high mRNA level for IL-8, MCP-1, and MIP-1α and elevated urine chemokines at 48 h after challenge. These pathophysiologic manifestations support earlier studies showing that proinflammatory cytokine responses to Stxs may contribute to toxin-mediated disease pathogenesis (Siegler *et al*., 2001).

Regulation of Stx-mediated cytokine synthesis and release

It is well known that human macrophages secrete soluble cytokine and chemokines in response to microbes and microbial products. It has been well established that Stxs are potent inhibitors of host protein synthesis by acting as an *N*-glycosidase and cleaving off a single adenine residue located on a stem-loop structure near the 3′ end of 28S rRNA of the 60S ribosomal subunit. The depurination reaction mediated by ribosome-inactivating proteins such as Shiga toxins and ricin, may result in the activation of the ribotoxic stress response (Endo *et al*., 1988; Saxena *et al*., 1989; Iordanov *et al*., 1997). The ribotoxic stress response leads to the activation of multiple mitogen-activated protein kinase (MAPKs) signaling pathways. Yamasaki *et al*. (2004) showed that the *N*-glycosidase activity of Stx, ricin and modeccin was required for the enhanced production of IL-8 by human colonic epithelial cells. Thus, Stxs, in addition to their role as protein synthesis inhibitors, may also induce cytokine and chemokine expression through the activation of multiple cell signaling pathways (Thorpe *et al*., 1999; Foster *et al*., 2000; Cameron *et al*., 2003; Harrison *et al*., 2004). While many details of the mechanisms of proinflammatory cytokine response induced by Stxs remain to be elucidated, some pathways appear to be recapitulated in multiple cell types.

In human macrophage-like THP-1 cells, Stx1 induced the production of the pro-inflammatory cytokine TNF-α which was associated with the phosphorylation (activation) of p38 MAPK and c-Jun NH_2 terminal kinase. Treatment of cells with a p38 MAPK inhibitor abrogated TNF-α production induced by Stx1 (Foster and Tesh, 2002). Similar observations were reported in human brain microvascular endothelial cells by utilizing ^{125}I-labeled Stx, showing that Stx1/Stx2 mediated-inflammatory cytokine production was abrogated by a p38 MAPK inhibitor but not by a JNK inhibitor (Stricklett *et al*., 2005). Murata *et al*. (2006) found that Stx1 stimulation of human macrophage THP-1 cells rapidly activated the tyrosine kinase c-YES, which subsequently increased the expression of tissue factor, a key player in the coagulation-inflammation-thrombosis circuit. Tissue factor expression was induced through signaling cascades involving phosphotidylinositol-3-kinase (PI3K)-mediated activation of the inhibitor of NF-κB kinase subunit-β (IKK-beta)-proteasome-nuclear factor-κB-REL and MAPK-ERK kinase (MEK)-extracellular signal-regulated kinase 2 (ERK2)-early growth response protein 1 (Egr-1) pathways. Moreover, specific inhibition studies of dual-specificity phosphatase 1 (DUSP1), also known as MAP kinase phosphatase, utilizing pharmacological reagents and small interfering RNA (siRNA), confirmed that DUSP1 was involved in regulation of ERK, p38, and JNK MAPKs to result in differential production of cytokines and chemokines induced by Stxs (Leyva-Illades *et al*., 2012). These findings suggest that targeting DUSP1 activity in susceptible cells responding to Stxs could be exploited to develop interventional strategies to regulate pro- and anti-inflammatory effects of Stxs. In the human ileocecal adenocarcinoma cell line, HCT-8, a MAP3K termed zipper sterile-alpha-motif kinase (ZAK) was suggested to be a transducer of signals from the ribotoxic stress response to activate MAPKs and play a role in IL-8 up-regulation in response to toxin treatment (Jandhyala *et al*., 2008). It is also interesting that Stx1 can affect a translational regulation pathway by enhancing the phosphorylation of eukaryotic translation initiation factor-4E (eIF4E) and eIF4E-binding proteins (4E-BP1) through a p38 MAPK/Mnk1/eIF4E signaling pathway that is involved in maintaining host cell translation despite profound inhibition of protein synthesis due to the toxin-induced ribotoxic stress (Colpoys *et al*., 2005; Cherla *et al*., 2006). In 2001, Thorpe and colleagues recognized that Stx-mediated translational control of cytokine expression involves, in part, stabilization of specific cytokine and chemokine mRNA transcripts (Thorpe *et al*., 2001). Recent studies also demonstrated that Stx1 activates the (PI3K)/Akt/mammalian target of Rapamycin (mTOR) signaling pathway leading to 4E-BP hyperphosphorylation which may ultimately have a negative influence on cytokine expression due to the increased phosphorylation and inactivation of glycogen synthase kinase 3 α/β (GSK-3α/β) (Cherla *et al*., 2009). These data suggest that signaling pathways activated to induce cytokine expression by Stxs include signals which restore cytokine expression to normal, baseline levels. A proposed model of the regulation of proinflammatory cytokine production by Stxs is shown in Fig. 1.

Conclusion and future studies

Experiments described above utilizing *in vitro* and *in vivo* studies suggest that Stxs induce inflammatory cytokines and chemokines from various types of primary and transformed cells. Cell types that have been used in these studies include human monocytes/macrophages, endothelial cells, renal epi-

thelial cells, and neuronal cells. *In vivo* evidences using animal models including mice, piglets, rabbits, and baboons have provided unique insights into pathologic consequences of Stxs-activated inflammatory response in a near human setting and offered potential target pathways for effective therapeutic interventions to treat HUS. Unfortunately, there are no satisfactory therapeutic vaccines available for amelioration of diarrhea-associated hemolytic uremic syndrome (D+HUS) which is a leading cause of pediatric acute renal failure. Although many reports have shown that various kinase pathways, including p38 MAPKs, JNKs, ERKs, MK2, and ZAK were activated by both Stx1 and Stx2, characterization of unidentified or unknown downstream substrates for each kinase pathway has proven to be a hurdle in the development of therapeutic drugs because of the difficulty in achieving target specificity. Moreover, the characterization of intermediate signaling molecules involved in the inflammatory response may represent targets to intervene in Stx-mediated renal damage. Further studies are needed to evaluate inhibitors targeting the kinases activated by the toxins using both *in vitro* and *in vivo* models. A more refined and improved understanding of the pro-inflammatory cytokine-mediated signaling mechanisms activated by Stxs will be critical for the development of adequate therapeutic approaches for treating the emerging infectious diseases caused by *Shigella dysenteriae* serotype 1 and STEC.

Acknowledgements

This work was supported by the Korea Research Institute of Bioscience and Biotechnology (KRIBB) Research Initiative Program.

References

Cameron, P., Smith, S.J., Giembycz, M.A., Rotondo, D., and Plevin, R. 2003. Verotoxin activates mitogen-activated protein kinase in human peripheral blood monocytes: role in apoptosis and proinflammatory cytokine release. *Br. J. Pharmacol.* **140**, 1320–1330.

Cherla, R.P., Lee, S.Y., Mees, P.L., and Tesh, V.L. 2006. Shiga toxin 1-induced cytokine production is mediated by MAP kinase pathways and translation initiation factor eIF4E in the macrophage-like THP-1 cell line. *J. Leukoc. Biol.* **79**, 397–407.

Cherla, R.P., Lee, S.Y., Mulder, R.A., Lee, M.S., and Tesh, V.L. 2009. Shiga toxin 1-induced proinflammatory cytokine production is regulated by the phosphatidylinositol 3-kinase/Akt/mammalian target of rapamycin signaling pathway. *Infect. Immun.* **77**, 3919–3931.

Colpoys, W.E., Cochran, B.H., Tessa, M., Carducci, M., and Thorpe, C.M. 2005. Shiga toxins activate translational regulation pathways in intestinal epithelial cells. *Cell. Signal.* **17**, 891–899.

Dean-Nystrom, E.A., Bosworth, B.T., and Moon, H.W. 1997. Pathogenesis of O157:H7 *Escherichia coli* infection in neonatal calves. *Adv. Exp. Med. Biol.* **412**, 47–51.

Leyva-Illades, D., Cherla, R.P., Lee, M.S., and Tesh, V.L. 2012. Regulation of cytokine and chemokine expression by the ribotoxic stress response elicited by Shiga toxin type 1 in human macrophage-like THP-1 cells. *Infect. Immun.* **80**, 2109–2020.

Endo, Y.K., Tsurugi, T.Y., Takeda, Y.O., and Igarashi, K. 1988. Site of action of a Vero toxin (VT2) from *Escherichia coli* O157:H7 and of Shiga toxin on eukaryotic ribosome. RNA N-glycosidase activity of the toxins. *Eur. J. Biochem.* **171**, 45–50.

Ezri, J., Marques-Vidal, P., and Nydegger, A. 2012. Impact of disease and treatments on growth and puberty of pediatric patients with inflammatory bowel disease. *Digestion* **85**, 308–319.

Falguières, T., Mallard, F., Baron, C., Hanau, D., Lingwood, C., Goud, B., Salamero, J., and Johannes, L. 2001. Targeting of Shiga toxin B-subunit to retrograde transport route in association with detergent-resistant membranes. *Mol. Biol. Cell.* **12**, 2453–2468.

Foster, G.H., Armstrong, C.S., Sakiri, R., and Tesh, V.L. 2000. Shiga toxin-induced tumor necrosis factor alpha expression: requirement for toxin enzymatic activity and monocyte protein kinase C and protein tyrosine kinases. *Infect. Immun.* **68**, 5183–5189.

Foster, G.H. and Tesh, V.L. 2002. Shiga toxin 1-induced activation of c-Jun NH(2)-terminal kinase and p38 in the human monocytic cell line THP-1: possible involvement in the production of TNF-alpha. *Infect. Immun.* **71**, 107–114.

Franchi, L., Munoz-Planillo, R., and Nunez, G. 2012. Sensing and reacting to microbes through the inflammasomes. *Nat. Immunol.* **13**, 325–332.

Fujii, J., Matsui, T., Heatherley, D.P., Schlegel, K.H., Lobo, P.I., Yutsudo, T., Ciraolo, G.M., Morris, R.E., and Obrig, T.G. 2003. Rapid apoptosis induced by Shiga toxin in Hela cells. *Infect. Immun.* **71**, 2724–2735.

Girardis, M. and Cossarizza, A. 2013. Early alterations of B cells in patients with septic shock: another piece in the complex puzzle of the immune response in sepsis. *Crit. Care.* **17**, 162.

Gustot, T. 2011. Multiple organ failure in sepsis: prognosis and role of systemic inflammatory response. *Curr. Opin. Crit. Care.* **17**, 153–159.

Gyles, C.L. 2007. Shiga toxin-producing *Escherichia coli* : an overview. *J. Anim. Sci.* **85**(13 Suppl), E45–62.

Harrison, L.M., Cherla, R.P., van den Hoogen, C., van Haaften, W.C., Lee, S.Y., and Tesh, V.L. 2005. Comparative evaluation of apoptosis induced by Shiga toxin 1 and/or lipopolysaccharides in human monocytic and macrophage-like cells. *Microb. Pathog.* **38**, 63–76.

Harrison, L.M., van Haaften, W.C., and Tesh, V.L. 2004. Regulation of proinflammatory cytokine expression by Shiga toxin 1 and/or lipopolysaccharides in the human monocytic cell line THP-1. *Infect. Immun.* **72**, 2618–2627.

Harrison, L.M., van den Hoogen, C., van Haaften, W.C., and Tesh, V.L. 2005. Chemokine expression in the monocytic cell line THP-1 in response to purified shiga toxin 1 and/or lipopolysaccharides. *Infect. Immun.* **73**, 403–412.

Hauf, N. and Chakraborty, T. 2003. Suppression of NF-kappa B activation and proinflammatory cytokine expression by Shiga toxin-producing *Escherichia coli*. *J. Immunol.* **170**, 2074–2082.

Heyderman, R.S., Soriani, M., and Hirst, T.R. 2001. Is immune cell activation the missing link in the pathogenesis of post diarrhoeal HUS?. *Trends Microbiol.* **9**, 262–266.

Horvath, G.L., Schrum, J.E., De Nardo, C.M., and Latz, E. 2011. Intracellular sensing of microbes and danger signals by the inflammasomes. *Immunol. Rev.* **243**, 119–135.

Hughes, A.K., Stricklett, P.K., and Kohan, D.E. 1998. Shiga toxin-1 regulation of cytokine production by human proximal tubule cells. *Kidney Int.* **54**, 1093–1106.

Hughes, A.K., Stricklett, P.K., and Kohan, D.E. 2001. Shiga toxin-1 regulation of cytokine production by human glomerular epithelial cells. *Nephron.* **88**, 14–23.

Hurley, B.P., Jacewicz, M., Thorpe, C.M., Lincicome, L.L., King, A.J., Keusch, G.T., and Acheson, D.W. 1999. Shiga toxins 1 and 2 translocate differently across polarized intestinal epithelial cells. *Infect. Immun.* **67**, 6670–6677.

Hurley, B.P., Thorpe, C.M., and Acheson, D.W. 2001. Shiga toxin translocation across intestinal epithelial cells is enhanced by neutrophil transmigration. *Infect. Immun.* **69**, 6148–6155.

Iordanov, M.S., Pribnow, D., Magun, J.L., Dinh, T.H., Pearson, J.A., Chen, S.L., and Magun, B.E. 1997. Ribotoxic stress response: activation of the stress-activated protein kinase JNK1 by inhibitors of the peptidyl transferase reaction and by sequence-specific RNA damage to the alpha-sarcin/ricin loop in the 28S rRNA. *Mol. Biol. Cell.* **17**, 3373–3381.

Jacewicz, M.S., Acheson, D.W., Binion, D.G., West, G.A., Lincicome, L.L., Fiocchi, C., and Keusch, G.T. 1999. Responses of human intestinal microvascular endothelial cells to shiga yoxins 1 and 2 and pathogenesis of hemorrhagic colitis. *Infect. Immun.* **67**, 1439–1444.

Jackson, M.P., O'Brien, A.D., Holmes, R.K., and Newland, J.W. 1987. Nucleotide sequence analysis and comparison of the structural genes for Shiga-like toxin I and Shiga-like toxin II encoded by bacteriophages from *Escherichia coli* 933. *FEMS Microbiol. Lett.* **44**, 109–114.

Jandhyala, D.M., Ahluwalia, A., Obrig, T., and Thorpe, C.M. 2008. ZAK: a MAP3Kinase that transduces Shiga toxin- and ricin-induced proinflammatory cytokine expression. *Cell. Microbiol.* **10**, 1468–1477.

Jeong, K.I., Zhang, Q., Nunnari, J., and Tzipori, S. 2010. A piglet model of acute gastroenteritis induced by *Shigella dysenteriae* type 1. *J. Infect. Dis.* **201**, 903–911.

Johannes, L. and Römer, W. 2010. Shiga toxins-from cell biology to biomedical applications. *Nat. Rev. Microbiol.* **8**, 105–116.

Keepers, T.R., Gross, L.K., and Obrig, T.G. 2007. Monocyte chemoattractant protein 1, macrophage inflammatory protein 1 alpha, and RANTES recruit macrophages to the kidney in a mouse model of hemolytic-uremic syndrome. *Infect. Immun.* **75**, 1229–1236.

Keepers, T.R., Psotka, M.A., Gross, L.K., and Obrig, T.G. 2006. A murine model of HUS: shiga toxin with lipopolysaccharide mimics the renal damage and physiologic response of human disease. *J. Am. Soc. Nephrol.* **17**, 3404–3414.

Kumar, H., Kawai, T., and Akira, S. 2011. Pathogen recognition by the innate immune system. *Int. Rev. Immunol.* **30**, 16–34.

Lee, M.S., Cherla, R.P., Jenson, M.H., Leyva-Illades, D., Marinez-Moczygemba, M., and Tesh, V.L. 2011. Shiga toxins induce autophagy leading to differential signalling pathways in toxin-sensitive and toxin-resistant human cells. *Cell. Microbiol.* **13**, 1479–1496.

Lee, S.Y., Lee, S.Y., Cherla, R.P., and Tesh, V.L. 2008. Shiga toxin 1 induces apoptosis through the endoplasmic reticulum stress response in human monocytic cells. *Cell. Microbiol.* **10**, 770–780.

Lentz, E.K., Cherla, R.P., Jaspers, V., Weeks, B.R., and Tesh, V.L. 2010. Role of tumor necrosis factor alpha in disease using a mouse model of Shiga toxin-mediated renal damage. *Infect. Immun.* **78**, 3689–3699.

Lentz, E.K., Leyva-Illades, D., Lee, M.S., Cherla, R.P., and Tesh, V.L. 2011. Differential response of the human renal proximal tubular epithelial cell line HK-2 to Shiga toxin types 1 and 2. *Infect. Immun.* **79**, 3527–3540.

Lord, J.M., Roberts, L.M., and Lencer, W.I. 2005. Entry of protein toxins into mammalian cells crossing the endoplasmic reticulum membrane:co-opting basic mechanisms of endoplasmic reticulum–associated degradation. *Curr. Top. Microbiol. Immunol.* **300**, 149–168.

McDaniel, T.K., Javis, K.G., Donnenberg, M.G., and Kaper, J.B. 1995. A genetic locus of enterocyte effacement conserved among diverse enterobacterial pathogens. *Proc. Natl. Acad. Sci. USA* **28**, 1664–1668.

Mohawk, K.L. and O'Brien, A.D. 2011. Mouse models of *Escherichia coli* O157:H7 infection and shiga toxin injection. *J. Biomed. Biotechnol.* **2011**, 258185.

Moritz, F., Wagner, U., Distler, O., Seidel, W., Gay, S., and Hantzschel, H. 2005. Cardiovascular manifestations in rheumatoid arthritis. *Z. Rheumatol.* **64**, 222–228.

Murata, K., Higuchi, T., Takada, K., Oida, K., Horie, S., and Ishii, H. 2006. Verotoxin-1 stimulation of macrophage-like THP-1 cells up-regulates tissue factor expression through activation of c-Yes tyrosine kinase: Possible signal transduction in tissue factor up-regulation. *Biochim. Biophys. Acta.* **1762**, 835–843.

Proulx, F. and Tesh, V.L. 2007. Renal diseases in the Pediatric Intensive Care Unit: thrombotic microangiopathy, hemolytic uremic syndrome, and thrombotic thrombocytopenia purpura. pp. 1189–1203. *In* Wheeler, D.S., Wong, H.R., and Shanley, T.P. (eds.), Pediatric Care Medicine: Basic Science and Clinical Evidence, Springer-Verlag, London, UK.

Raqib, R., Ljungdahl, A., Lindberg, A.A., Anderson, U., and Anderson, J. 1996. Local entrapment of interferon γ in the recovery from *Shigella dysenteriae* type 1 infection. *Gut* **38**, 328–336.

Ramegowda, B. and Tesh, V.L. 1996. Differentiation-associated toxin receptor modulation, cytokine production, and sensitivity to Shiga-like toxins in human monocytes and monocytic cell lines. *Infect. Immun.* **64**, 1173–1180.

Sakiri, R., Ramegowda, B., and Tesh, V.L. 1998. Shiga toxin type 1 activates tumor necrosis factor-alpha gene transcription and nuclear translocation of the transcriptional activators nuclear factor-kappaB and activator protein-1. *Blood* **92**, 558–566.

Sandvig, K. and van Deurs, B. 2002. Transport of protein toxins into cells: pathways used ricin, cholera toxin and Shiga toxin. *FEBS Lett.* **529**, 49–53.

Saxena, S.K., O'Brien, A.D., and Ackerman, E.J. 1989. Shiga toxin, Shiga-like toxinII variant, and ricin are all single-site RNA N-lycosidases of 28S RNA when microinjected into xenopus oocytes. *J. Biol. Chem.* **264**, 596–601.

Scallan, E., Mahon, B.E., Hoekstra, R.M., and Griffin, P.M. 2013. Estimates of illnesses, hospitalizations and deaths caused by major bacterial enteric pathogens in young children in the United States. *Pediatr. Infect. Dis. J.* **32**, 217–221.

Siegler, R.L., Obrig, T.G., Pysher, T.J., Tesh, V.L., Denkers, N.D., and Taylor, F.B. 2003. Response to Shiga toxin 1 and 2 in a baboon model of hemolytic uremic syndrome. *Pediatr. Nephrol.* **18**, 92–96.

Siegler, R.L., Pysher, T.J., Lou, R., Tesh, V.L., and Taylor, F.B.Jr. 2001. Response to Shiga toxin-1, with and without lipopolysaccharide, in a primate model of hemolytic uremic syndrome. *Am. J. Nephrol.* **21**, 420–425.

Stearns-Kurosawa, D.J., Collins, V., Freeman, S., Tesh, V.L., and Kurosawa, S. 2010. Distinct physiologic and inflammatory responses elicited in baboons after challenge with Shiga toxin type 1 or 2 from enterohemorrhagic *Escherichia coli*. *Infect. Immun.* **78**, 2497–2504.

Stearns-Kurosawa, D.J., Oh, S.Y., Cherla, R.P., Lee, M.S., Tesh, V.L., Papin, J., Henderson, J., and Kurosawa, S. 2013. Distinct renal pathology and a chemotactic phenotype after enterohemorrhagic *Escherichia coli* Shiga toxins in non-human primates models of hemolytic uremic syndrome. *Am. J. Pathol.* **182**, 1227–1238.

Strockbine, N.A., Marques, L.R., Newland, J.W., Smith, H.W., Holmes, R.K., and O'Brien, A.D. 1986. Two toxin-converting phages from *Escherichia coli* O157:H7 strain 933 encode antigenically distinct toxins with similar biologic activities. *Infect. Immun.* **53**, 135–140.

Stricklett, P.K., Hughes, A.K., and Kohan, D.E. 2005. Inhibition of p38 mitogen-activated protein kinase ameliorates cytokine upregulated shigatoxin-1 toxicity in human brain microvascular endothelial cells. *J. Infect. Dis.* **191**, 461–471.

Tesh, V.L. 1998. Virulence of enterohemorrhagic *Escherichia coli*: role of molecular crosstalk. *Trends Microbiol.* **6**, 228–233.

Tesh, V.L. 2012. Activation of cell stress response pathways by Shiga toxins. *Cell. Microbiol.* **14**, 1–9.

Thorpe, C.M., Hurley, B.P., Lincicome, L.L., Jacewicz, M.S., Keusch,

G.T., and Acheson, D.W. 1999. Shiga toxins stimulate secretion of interleukin-8 from intestinal epithelial cells. *Infect. Immun.* **67**, 5985–5993.

Thorpe, C.M., Ritchie, J.M., and Acheson, D.W.K. 2002. *E. coli*: Virulence mechanisms of a versatile pathogen. pp. 119. *In* Donnenberg, M. (ed.), Academic Press, New York, USA.

Thorpe, C.M., Smith, W.E., Hurley, B.P., and Acheson, D.W. 2001. Shiga toxins induce, superinduce, and stabilize a variety of C-X-C chemokine mRNAs in intestinal epithelial cells, resulting in increased chemokine expression. *Infect. Immun.* **69**, 6140–6147.

Trofa, A.F., Ueno-Olsen, H., Oiwa, R., and Yoshikawa, M. 1999. Dr. Kiyoshi Shiga: discoverer of the dysentery bacillus. *Clin. Infect. Dis.* **29**, 1303–1306.

Yamasaki, C., Natori, Y., Zeng, X.T., Ohmura, M., Yamasaki, S., Takeda, Y., and Natori, Y. 1999. Induction of cytokines in a human colon epithelial cell line by Shiga toxin 1 (Stx1) and Stx2 but not by non-toxic mutant Stx1 which lacks N-glycosidase activity. *FEBS Lett.* **442**, 231–234.

Yamasaki, C., Nishikawa, K., Zeng, X.T., Katayama, Y., Natori, Y., Komatsu, N., Oda, T., and Natori, Y. 2004. Induction of cytokines by toxins that have an identical RNA N-glycosidase activity: Shiga toxin, ricin, and modeccin. *Biochim. Biophys. Acta* **1671**, 44–50.