Critical Review

Advances in Pathogenesis and Therapy of Hemolytic Uremic Syndrome Caused by Shiga Toxin-2

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Abstract

Shiga toxin (Stx) producing *Escherichia coli* (STEC) is responsible to bloody diarrhea (hemorrhagic colitis) and the hemolytic uremic syndrome (HUS). STEC strains carry inducible lambda phages integrated into their genomes that encode Stx 1 and/or 2, with several allelic variants each one. O157:H7 is the serotype that was documented in the vast majority of HUS cases although non-O157 serotypes have been increasingly reported to account for HUS cases. However, the outbreak that occurred in central Europe during late spring of 2011 showed that the pathogen was *E. coli* O104:H4. More than 4,000 persons were infected mainly in Germany, and it produced more than 900 cases of HUS resulting in 54 deaths. *E. coli* O104:H4 is a hybrid organism that combines some of the virulence genes of STEC and enteroaggregative *E. coli* specially production of Stx2 and the adherence mechanisms to intestinal epithelium. The differences in the epidemiology and presentation of *E. coli* pathogen meant a challenge for public health and scientific research to increase the knowledge of HUS-pathophysiology and to improve available therapies to treat HUS. © 2013 IUBMB Life, 65(10):827–835, 2013

Keywords: *hemolytic uremic syndrome; O104:H4; Shiga toxin-2*

Introduction

Shiga toxin (Stx) producing *Escherichia coli* (STEC) were first described as Vero toxin producing *E. coli* (VTEC) leading to bloody diarrhea (hemorrhagic colitis) and the hemolytic uremic syndrome (HUS) about 30 years ago (1). STEC strains carry inducible lambda phages integrated into their genomes that encode Stx 1 and/or 2, with several allelic variants each one. The entire phage and specific regions within the phage can be gained or lost through horizontal gene transfer (2). Because Stx-bacteriophage are mobile, their evolutionary history may differ from that of their bacterial host. Enterohemorrhagic *E. coli* (EHEC), the "classically pathogenic" subset of STEC, possess the *eae* gene of enteropathogenic *E. coli* as an additional virulence factor conferring adherence to the intestinal mucosa (3). STEC not harboring *eae* were long believed to be less virulent but have also been shown to be the causative agent of STEC outbreaks. O157:H7 is the serotype that was documented in the vast majority of HUS cases (4). However, non-O157 serotypes have been increasingly reported to account for HUS cases (5).

The major pathogenic *E. coli* O104:H4 (STEC O194:H7) outbreak that occurred in central Europe during late spring of 2011 highlighted that pathogenesis of HUS is incompletely understood. More than 4,000 persons were infected mainly in Germany, and it produced more than 900 cases of HUS resulting in 54 deaths (6). A few weeks later, a smaller outbreak occurred in southwest France causing 15 cases of bloody diarrhea of which 9 progressed to HUS.

Several assays demonstrated that the 2011 outbreak strain is a hybrid organism that combines some of the virulence genes of EHEC (Stx2 encoding gene, but not the type III secretion and Tir/intimin system) and enteroaggregative *E. coli* (EAEC) specially the adherence mechanisms, and expresses

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the corresponding phenotypes including production of Stx2 and aggregative adherence to cultured intestinal epithelial cells (7). Moreover, this strain shows an extended-spectrum beta-lactamase phenotype. Despite the observation that the constitutive Stx production of STEC O104:H4 was lower, as compared with "classical" O157:H7 strains (2), the proportion of patients developing HUS was greater than in most outbreaks before. This led to speculation about a possible hypervirulence of STEC O104:H4, potentially mediated by a facilitated Stx uptake due to the very efficient adherence of EAEC to the intestinal mucosa (7).

The specific combination of enhanced adhesion, survival fitness, Stx2 production, and antibiotic resistance illustrates the high genome plasticity of this *E. coli* pathogen. On the other hand, the differences in the epidemiology and presentation meant a challenge for public health and scientific research to be prepared for new non-classical STEC-HUS, to increase the knowledge of HUS-pathophysiology and perhaps, improve treatments.

Pathophysiology of STEC-HUS

Shiga-toxin Production

Nowadays there is no doubt about the importance of Stx2 and Stx2 phage biology to understand HUS. Stx is a member of the AB5 family of bacterial toxins. The A subunit (StxA) possesses *N*-glycosidase activity against 28S rRNA of 60S ribosomes in the cytosol, resulting in inhibition of protein synthesis in eukaryotic cells and activation of proinflammatory signaling cascade referred to as the ribotoxic stress response (RSR) (8). It fact, it has been demonstrated that Stx induces both primary response genes *c-jun* and *c-fos* and activates the stress-activated protein kinases, JNK/SAPK and p38, in intestinal epithelial cells. Stx enzymatic activity is also required for the referred kinase activation. The five B subunits (StxB) form a pentamer that binds to globotriaosyl ceramide receptors (Gb3) on the cell membrane (9). STEC express two types of Stx proteins (Stx1 and Stx2) and their variants, being Stx2 more virulent and epidemiologically more relevant than Stx1 (10).

In most of the STEC strains identified, the toxin genes, *stxAB,* are located in the genome of prophages that resemble the coliphage lambda (11). Phage induction is critical to toxin gene expression and to the ability of STEC to cause disease (12). The toxin genes are late-stage genes that are transcribed only during the lytic stage of the phage. The production of Stx is linked to the replication cycle of Stx phages, and the release of Stx is dependent on the lytic phase (13), which is induced under stress conditions (14,15). In this stage, the viral progeny is also able to infect and transduce *in vivo* and *in vitro* other bacteria present in the gut (16). In fact, Stx-converting bacteriophages are able to infect and lysogenize laboratory strains of *E. coli* as well as *E. coli* strains derived from the human and ruminant intestines (17,18). The resulting lysogenic strains are able to produce toxins and infectious phage particles, facilitating the spread of toxin genes among *E. coli* strains and other *Enterobacteriaceae*. On the other hand, different lines of evidence have shown that bacteriophage lambda is able to transduce mammalian cells, and that bacteriophage lambda vectors containing a mammalian gene expression cassette are able to express encoded genes in mammalian target cells *in vitro* and *in vivo* (19). The ability of lambda phage particles to transduce mammalian cells *in vivo* depends on the phagocytic and non-phagocytic uptake of the phage, possibly including macropinocytosis, and is increased through a Fc receptor-mediated antibody-dependent mechanism (20). Adding more complexity to this scenario, it was recently shown the existence of putative eukaryotic promoter-like sequences located upstream of the genes encoding for the Stx2A and B subunits, and that the eukaryotic machinery is able to recognize these sequences and to initiate the transcription and translation. The capacity of mammalian cells to transcribe and translate *stx2* genes could have important pathological implications, by adding a putative alternative source of toxin (21). It has been proposed that differential Stx2 expression may account for differences in virulence. In this regards, STEC have been classified in pathotypes and clades according to Stx-phage type that determines Stx-variant and level of Stx-production (22). In particular, Stx2 overexpression is common to STEC strains from clade 8, which are highly associated with HUS (23). In this context, the importance of the specific Stx2-phage features in the virulence of the *E. coli* O104:H4 outbreak strain started to be studied (24). Recently, Laing et al. (2) found that an *E. coli* O104:H4 outbreak-related isolate (ON-2011) produced significantly more Stx2 and Stx2-mRNA than outbreak-related lineage I *E. coli* O157:H7 strains after mitomycin C induction. However, without mitomycin C added to the culture, O104:H4 strain ON-2011 produced very little toxin. While several groups have analyzed factors and conditions in the human gastrointestinal tract able to induce Stx2-phage and the corresponding increase in Stx2 production, further study is required to determine whether Stx2 production by *E. coli* O104:H4 is greater or lesser than for *E. coli* O157 strains *in vivo*, particularly since other work has shown downregulation of toxin-production by *E. coli* O157:H7 strains due to microbiota secreted factors present in the human gut (25).

Shiga-toxin Action in the Gut

After passage through the acidic barrier, STEC colonizes the mucosal epithelium at the human colon promoting production and absorption of Stx. It has been suggested that the increased virulence of O104:H4 strain could in part be related to the EAEC bacterial host genome, which has evolved adaptations for attachment and survival in the human intestine and that these adaptations have facilitated the systemic absorption of Stx2, which in turn increased the risk of developing HUS (3,6). Supporting the importance of colonization adaptations to improve pathogenicity, we recently demonstrated in a mouse model of EHEC infections, that stool-recovered strains are able to generate a more generalized and persistent colonization

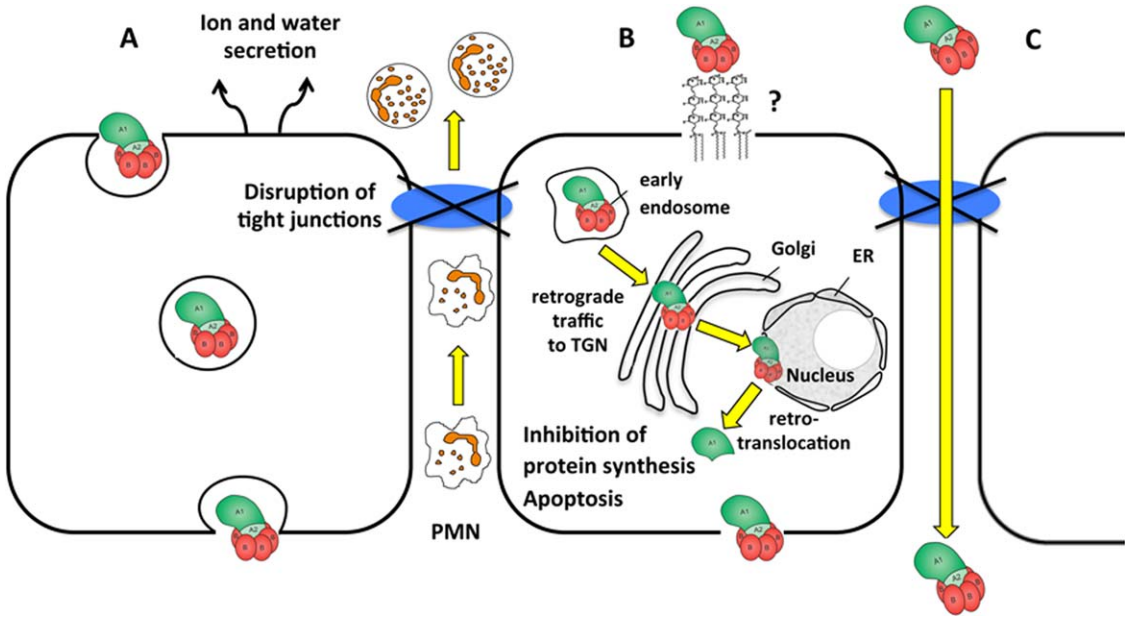

Fig 1 *Transcellular and paracellular pathways of Stx across human colonic epithelium. A: Gb3-independent macropinocytosis, (B) Gb3-dependent translocation by retrograde transport and Stx release after cell death. C: Paracellular transport during neutrophil (PMN) transmigration. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]*

than the parent strain. Furthermore, we showed that the use of significantly lower inoculums of the stool-recovered strains (10^4-fold-reduced) still results in gut colonization and Stx2-dependent mouse mortality. These results suggest that stool-recovered strains have a higher pathogenic capacity even though Stx2 production was similar or even lower, by increasing the colonization and persistence of bacterial strain. These results highlighted the importance of bacterial adaptation to the host gut environment for the colonization process and HUS development upon the establishment of STEC infections (26).

Although accumulating evidence have greatly advanced in the knowledge of the complex and dynamic colonization of human colon caused by STEC/EHEC, the precise mechanism by which Stx contributes to the intestinal pathology are not well understood. As STEC strains are generally non-invasive, the HUS systemic development upon establishment of STEC infections results from the systemic uptake of Stx and possibly other virulence factors from the intestinal lumen. It was demonstrated that Stx could cross the intestinal epithelium by both transcellular and paracellular route (Fig. 1). The transcellular trancytosis via receptor independent macropinocytosis may represent the major pathway, at least during the early stages of infection (27), while the paracellular pathway may be the entry of Stx during the acute inflammation of the colonic mucosa (28). However, the exact mechanism(s) by which Stx itself contribute to the compromise of gut barrier function remains unclear.

While most of the data regarding the trafficking of Stx and cytotoxicity are based on studies in cells carrying the specific Stx-receptor Gb3, the finding of retrograde transport of Stx in the T84 human intestinal cell line that lacks receptor Gb3 is of particular interest. In these cells, Stx1 was detected in endosomes, trans-Golgi network (TGN), endoplasmic reticulum (ER), and nuclear membrane (29). Although the cleavage of the StxA subunit occurs after 6 h of incubation, no cytotoxicity was observed even in periods of 24 h. On the contrary, in Caco-2 cells, a human intestinal cell line expressing Gb3 receptors, Stx1 and Stx2, are transported to the ER, and both StxA subunits are activated by furin-dependent cleavage and produce RSR with the consequent protein synthesis inhibition and induction of cellular apoptosis (30) (Fig. 1). It has been also described a movement of Stx through the intestinal barrier without apparent cellular damage probably via an active transcellular route, that may be increased by transmigration of neutrophils (PMN) across the endothelium (31) (Fig. 1). Taken together, these evidences suggest that Stx cytotoxicity is associated with the presence of Gb3 receptor. However, its expression in the apical membrane of epithelial cells of the human colonic mucosa is discussed. The existence (32,33) and the absence of Gb3 (30) have been shown by different groups using different experimental conditions.

Independently of Gb3 presence in the gut, structural and functional alterations in human intestinal epithelium by action of Stx have been broadly described (34). Studies in intestinal models suggest that Stx can modulate disease severity, including the production of diarrhea and hemorrhagic colitis. Intraintestinal inoculation of rabbits with Stx or STEC has been used as models of both hemorrhagic colitis and HUS (35). Inoculation of purified Stx1 in adult rabbit's ileum loops induced fluid accumulation in association with the presence of apoptotic intestinal villous epithelial cells (36). Crude and purified Stx2 holotoxin also induced a significant inhibition of water

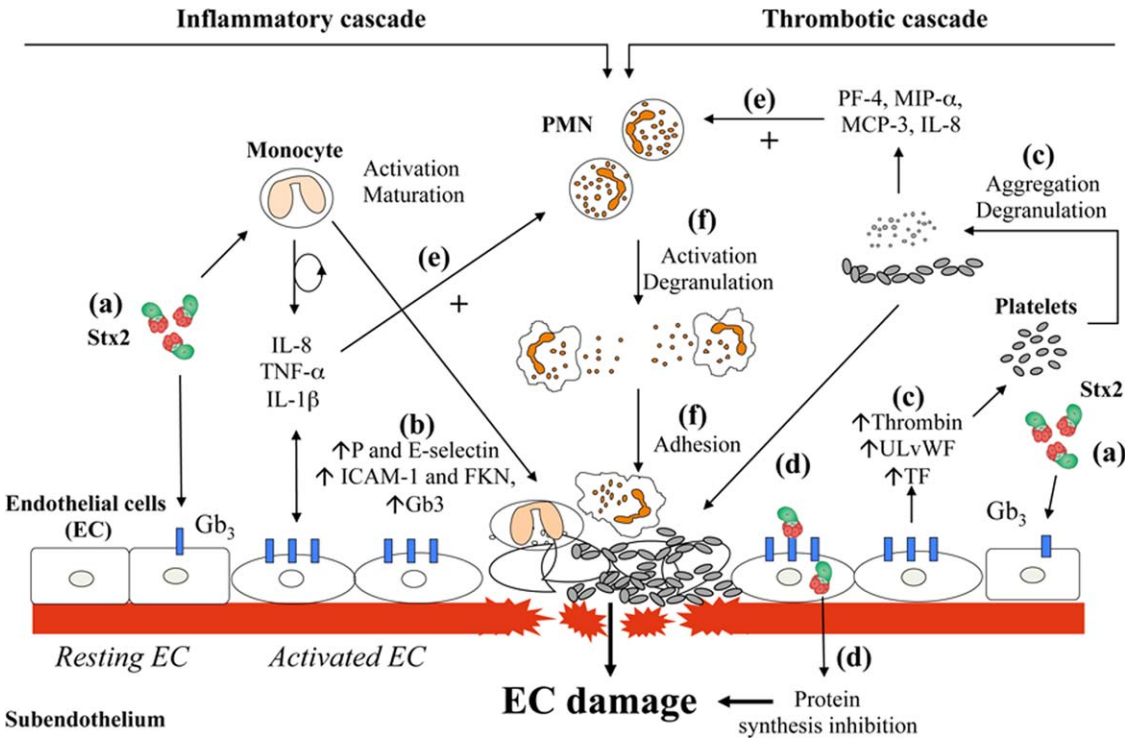

Fig 2

Schematic representation of the interaction between Shiga toxin and host responses leading to endothelial damage in HUS. (a) Stx binds to monocytes and endothelial cells (EC) promoting their activation, maturation, and secretion of cytokines and chemokines. (b) Cytokines released cause upregulation of adhesion molecules and of the Gb3 receptor in EC. (c) Activation of EC leads to secretion of thrombotic factors that induce platelet aggregation and degranulation. (d) Stx internalization in activated EC induces inhibition of protein synthesis. (e) Several factors released by activated endothelium, monocytes, and platelets collaborate in PMN activation. (f) Activated PMN release their granule content, produce reactive oxygen species, adhere to EC and, together with platelets and monocytes, potentiate Stx-induced EC damage. ULvWF: ultra-large von Willebrand factor, TF: tissue factor, TNF-α: tumor necrosis factor- α, IL-1β: interleukin-1 β, PF-4: platelet factor 4, MIP-α: macrophage inflammatory protein- α, MCP-3: monocyte chemoattractant protein-3, IL-8: interleukin-8, ICAM-1: intercellular adhesion molecule-1. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

transport across human colon *in vitro* (37) and evoked a hemorrhagic fluid accumulation in rat colon loop *ex vivo* in association with damage in the colonic epithelial cells (38). These studies have shown that Stx alters the usual balance of intestinal absorption and secretion toward net secretion because it acts *in vivo* on a specific cell population, the mature, differentiated absorptive epithelial cells.

Shiga-toxin Systemic Effects

Controversy exists regarding how Stx reaches target organs. Free Stx has not been detected in the serum and it is rapidly cleared from the circulation following intravenous administration (39). Instead, it has been suggested that Stx is transported on leukocytes that possess a surface receptor with an affinity for the toxin that is lower than the affinity for Gb3 receptor on the endothelial cell surface (40,41). However, a follow-up study failed to confirm this mechanism and it remains unknown how Stx transits by the systemic circulation to reach the microcirculation of the kidney and brain (40,42). Either way, the initial step in the pathogenesis of STEC-HUS is binding of the B subunit to Gb3 on the cell membrane (43). This step is followed by retrograde transport of the A subunit to the Golgi apparatus and inhibition of protein synthesis in the ribosome (44).

Besides intestinal symptoms, the most frequent serious consequence of STEC/EHEC infection is the progression to HUS linked to the renal and brain lesions caused by Stx. While STEC-HUS is a systemic process that affects the vasculature of every organ, Stx is particularly aggressive against small blood vessels, such as those found in the digestive tract, kidneys, lungs, and central nervous system (40). The vascular endothelium of the glomeruli in the kidney is a specific target for the toxin. In addition to its direct effects on the endothelium that promote cell injury, Stx induces broad inflammatory response that is triggered by much lower levels of Stx than the amount needed to inhibit protein synthesis (40). This process involves the RSR, upregulation of adhesion molecules for leukocytes, and promotion of a prothrombotic state in blood vessels. Besides Stx, putative virulence factors of STEC including adhesins, other toxins, proteases, and lipopolysaccharide (LPS) (10,40) would be required to the complete development of disease. Although the precise role of some of these factors in STEC disease remains to be fully established, it has been

largely demonstrated that LPS, directly or indirectly through triggering inflammatory response, upregulates the expression of Stx receptors and sensitize microvascular endothelial cells to Stx-induced injury (45,46).

Factors from the Host

Several authors have reported the importance of the inflammatory and thrombotic responses in the development of HUS. Initially, STEC colonization induces acute colonic inflammation (47). In this regards, the infiltration of the gut and the presence of leukocytes in feces are seen in many STEC-infected patients. Several pathogenic factors of STEC have been demonstrated to induce the expression of proinflammatory chemokines in epithelial cells, which was accompanied by an influx of PMN (10). Transmigration of PMN from the basolateral to apical area of an intestinal epithelial cell line significantly increased the movement of Stx1 and Stx2 in the opposite direction (31). Additionally, PMN recruitment in the intestine may also increase the risk of HUS by inducing the Stx2 prophage *in vivo* and augmenting Stx2 production, mainly through the production of H_2O_2 (48).

Patients also evidence a marked inflammatory response as demonstrated by systemically (blood) and locally (urine) increased levels of various inflammatory mediators, including interleukins (IL), chemokines, soluble adhesion molecules, growth factors, and acute phase response proteins. Additionally, they also show markers of endothelial injury, activation of the coagulation cascade, and inhibition of fibrinolysis (49). It has been suggested that the degree of the prothrombotic activation early in infection could be decisive in the course of the disease (50) (Fig. 2).

The activation of PMN is evidenced by a high peripheral blood PMN count at presentation, which is the poor prognosis factor most consistently reported, and increased levels of serum elastase and IL-8. Besides, the severity of renal impairment has been correlated with the degree of PMN degranulation (51). Recently, it has been demonstrated that Stx2 induces *in vivo* an oxidative imbalance, evidenced by renal glutathione depletion and increased lipid membrane peroxidation. The increased reactive oxygen species production by neutrophils may be one of the major sources of oxidative stress during Stx intoxication (52). Platelet activation is a hallmark of HUS. Thrombocytopenia is caused by consumption of platelets, probably after activation and aggregation (4). Platelet-derived products such as β-thromboglobulin, platelet factor 4, and soluble P-selectin were found to be elevated during acute HUS (49,50). Furthermore, changes in platelet ultramorphology and increased platelet-derived microvesicles were found in these patients, indicating platelet activation (53). The activation of both PMN and platelets will potentiate the inflammatory process and may enhance the primary Stx-induced endothelial damage (Fig. 2).

Activated monocytes may also contribute to Stx toxic effects by the secretion of several chemokines and cytokines (tumor necrosis factor-alpha (TNF-α), IL-1β, IL-8, regulated on activation normal T cell expressed and secreted (RANTES), tissue factor (TF)) which increase endothelial susceptibility to Stx. An increased percentage of the subset CD16+inflammatory monocytes have been reported in peripheral monocytes from HUS patients (10). The tissue-specific recruitment of leukocytes mediated by the interaction between chemokines and their specific receptors may contribute to renal injury in HUS (40,54,55) (Fig. 2).

Recent experimental *in vitro* data showed that unusual high concentrations of Stx2 (10–0.5 ug) was able to activate complement through the alternative pathway and was found to bind to factor H (FH). No apparent cleavage or destruction of FH was visible, and cofactor activity in fluid phase was unaffected, but delayed for surface-attached FH (56). On the other hand, other groups have studied the role of complement in EHEC-induced HUS, in patients and mouse models, but could not find any association (57,58). In conclusion, the uncontrolled complement activation with a pathogenic role is still pending of demonstration in patients with typical STEC-HUS.

In summary, although Stx-induced endothelial injury is the primary pathogenic event, multiple bacterial and inflammatory host components may define the course of STEC infection.

The Available Therapies to Treat the Disease Are Not Fully Effective

To counteract the disease, patients require supportive care to maintain fluid and electrolytes levels, monitor and support kidney function and dialysis to replace renal function. The 2011 outbreak in Germany also highlighted the lack of specific therapy against HUS. Although clinical features showed some differences compared to HUS secondary to classical EHEC strains, particularly that clinicians were confronted with a large number of mainly adult patients (3), the major limitation was the absence of specific anti-Stx2 therapy. Prior to the outbreak, no standardized causative treatment existed for STEC-HUS, and randomized clinical trials approving any therapeutic concept to be beneficial beyond best supportive therapy were missing (6). Most of them required renal replacement therapy, and a large proportion of them received different therapies on the basis of theoretical considerations and preceding observations, but without evidence for the effectiveness of such "best guess" strategies, including plasmapheresis, glucocorticoids, and a subset received Eculizumab, an antibody against the C5a component of complement, and a few patients underwent immune-adsorption (6,59). Taken together, each one of them was questionable and might even be adverse. Although the use of the anti-C5 antibody was prompted mainly by only one report describing its use in three children with typical HUS (60), its efficacy has not been demonstrated. Moreover, while in atypical HUS Eculizumab is pathophysiologically rational, in STEC-HUS there is no evidence enough of complement involvement and caution should be maximal because antibiotic prophylaxis is mandatory in patients receiving Eculizumab.

Antibiotics are in fact contraindicated in EHEC infections (4,61). At antimicrobial levels above those required to inhibit bacterial replication, several antibiotics such as mitomycin C and quinolones, including ciprofloxacin, produce DNA damage

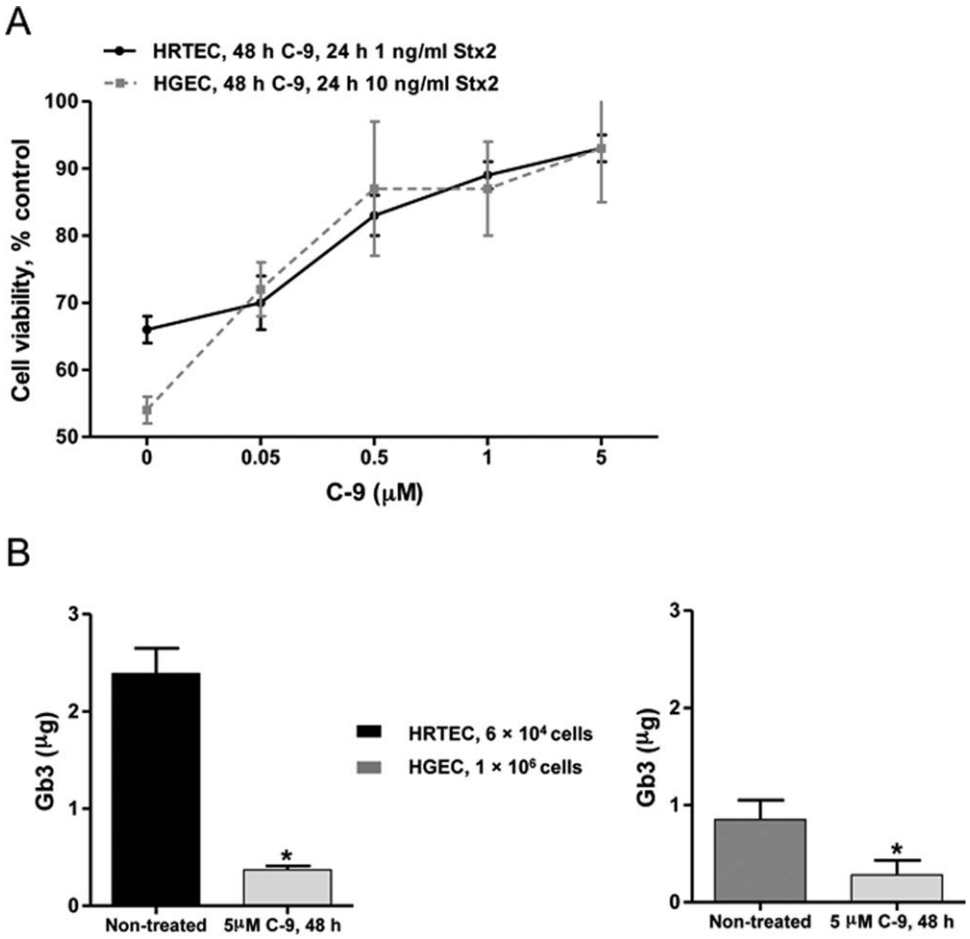

Fig 3

*Effects of C-9 on cell viability (A) and Gb3 expression (B) in human renal tubular epithelial (HRTEC) and glomerular endothelial (HGEC) cells. Both cell cultures were pre-incubated with C-9 (Genzyme Corp, USA) at the stated concentration for 48 h and then treated with Stx2 (1 ng/mL for HRTEC and 10 ng/mL for HGEC) for 24 h. After incubation, cell viability was assayed by neutral red uptake. Gb3 content was determined by mass spectrometer for HRTEC and by an enzymatic fluorometric method for HGEC. Data are reported as means ± SEM (*n = 3–4). Statistical significance was set at *$P < 0.05$.*

and therefore induce the SOS response with the unwanted secondary effect of simultaneously triggering phage production and *stx2* gene expression (3,62). Via these effects antibiotics may increase the risk of progression of an EHEC infection into HUS (4). A recent study reported, in contrast, that antibiotics such as meropenem, azithromycin, rifaximin, and tigecycline did not influence the phage and toxin levels of O104:H4 strain *in vitro* (62). However, further studies in animal models, as well as careful analyses of clinical outcomes in patients who were treated with these antibiotics, are necessary to conclude if this is a general or a particular recommendation.

New Therapeutic Approaches

In recent years, new therapeutic strategies have been developed to achieve an effective treatment for Stx-mediated HUS although these approaches have been only demonstrated in animal models.

Recent advances in the development of tetravalent peptides (PPP-tet and MMA-tet) have shown that these peptides protected mice from a fatal dose of *E. coli* O157:H7 producing Stx1 and Stx2 (63,64). Recently, oral application of MMA-tet, inhibited Stx cytotoxicity with greater potency than PPP-tet (64). This MMA-tet peptide may be a promising therapeutic agent against systemic complications during STEC infections.

A novel therapeutic strategy was based on short-term inhibition of host Gb3 synthesis to reduce binding and uptake of Stx by host cells. The rate-limiting first step in the biosynthesis of Gb3 and others glycosphingolipids is the reaction catalyzed by the enzyme glucosylceramide (GL1) synthase (65,66). Different inhibitors of GL1 synthase have been identified and could inhibit glycosphingolipid synthesis in cultured cells without inhibiting cell growth or raising intracellular ceramide levels (65). Recently, it was demonstrated that C-9 (Genzyme Corp, USA), a specific inhibitor of GL1 synthase, decreased Gb3 expression levels and prevented the cytotoxic effects of Stx2 on primary cultures of human renal epithelial (HRTEC) (67) and endothelial cells (HGEC) (68) (Fig. 3). These results indicate that the Stx cytotoxicity in renal cells is associated with the

expression of the Gb3 receptor. The protective efficacy of C-9 was also shown in an experimental model of HUS in rats (69). These data suggest that the inhibition of Gb3 synthesis may be a potential treatment for protection against the pathological effects of Stx producing HUS. Furthermore, other findings have shown that small molecules inhibitors of Stx-mediated MAPKinase response like DHP-2 may prevent the Stx-induced inflammation that is thought to contribute to pathogenesis by STEC (70). Recently, the pre-treatment of infant rabbits with the drug Imatinib, a MAPKinase inhibitor resulted in a decrease of Stx-mediated heterophil infiltration suggesting that it may act as a potential therapeutic target for treating Stx-associated illnesses (71). In addition, a recent study showed that manganese interferes with the retrograde movement of Stx to the Golgi, leading to increased degradation of the toxin in lysosomes. Pretreatment of mice with this metal reduced the lethal effects of Stx, raising a promise for a future clinical use (72).

The severity of the German outbreak brought to light the lack of effective humanized monoclonal antibodies against Stx2. Highly neutralizing anti-Stx2 antibodies able to form stable immune complexes, and to clear the toxin before it interacts with receptors in tissues may be a desirable specific therapy, as for other toxin-mediated diseases. Recently, a new Stx2B subunit-based immunogen induced antibodies with high neutralizing capacity for Stx2 and its variants. Immunized mice were completely protected against i.v. Stx2-challenge and their sera protected weaned mice orally challenged with EHEC. These data demonstrate that the transferred antibodies were capable of neutralizing the toxin as it is delivered by EHEC (72). Thus, this report raises a promising candidate for vaccine or antibody development with preventive or therapeutic ends, for use in HUS endemic areas or during future outbreaks caused by pathogenic strains of Stx-producing *E. coli*.

STEC Reservoir

The main reservoir of classical EHEC is cattle, which are asymptomatic carriers of this strain. In contrast, currently available data suggest that cattle are not the reservoir for the *E. coli* O104:H4 outbreak strain. Indeed, previous data indicated that EAEC are highly adapted to humans, which would suggest that the human population is the reservoir (6). In this context, while cattle vaccination may be a preventive tool by reducing cattle shedding of classical EHEC (73), and so preventing the direct contamination of meat and dairy products and the indirect passage of the pathogen to vegetables through contaminated irrigation waters, it may not offer a significant protection for humans against others existing or new pathogenic STEC strains.

Since *E. coli* O104:H4 cannot be regarded as a zoonotic disease and the primary reservoir responsible for human exposure has not been identify yet, this outbreak remarks the concept that STEC-HUS prevention remains a challenge to farming, agriculture, food safety, public health, and scientific research alike. Troubling as this is, the most fearsome aspect of the outbreak is that it may occur again.

Final Remarks

Progress in the discovery and development of drugs to block the action of the Stx appears as the most potent and promising treatment to ameliorate HUS. New antibiotics that do not trigger a SOS response and do not lead to extensive lysis of the pathogen could also offer tools to curb the infection, as would molecules that compete with bacterial attachment to the intestinal mucosa. However, one of the main lessons of Germany outbreak is that sustained effort must be done to expand the available knowledge to understand how *E. coli* may acquire virulence factors as Stx2 becoming a new virulent and potentially lethal strain.

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