Treatment of Enterohemorrhagic *E. coli* (EHEC) infection and Hemolytic Uremic Syndrome (HUS)

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Abstract

This article provides an overview of a specialised group of *E. coli*, originally known as verotoxigenic *E. coli* (VTEC) that cause severe colonic disease and renal failure. Their pathogenicity derives from virulence factors which enable the bacteria to colonise the colon and deliver extremely powerful toxins known as verotoxins (VT) or Shiga toxins (Stx) to the systemic circulation. The article is timely because of the recent devastating *E. coli* O104:H4 epidemic in Europe which showed how helpless we are in terms of offering effective therapies. By examining the sources and distribution of these bacteria, and how they cause disease we will be in better shape to prevent and treat the inevitable future cases of sporadic disease and victims of common-source outbreaks. Developments in terms of treatment are discussed.
Introduction

The association of verotoxigenic *Escherichia coli* (VTEC) with human disease goes back over 30 years. [1,2,3] The occurrence of outbreaks due to a VTEC in the U.S.A. in 1982 [4] focussed the world’s attention onto these pathogens. Since the discovery of verocytotoxin, [1,3] and the paper by Karmali *et al.*[5] of cases of post-diarrheal hemolytic uremic syndrome (D⁺-HUS) caused by VTEC otherwise known as shiga-toxigenic *Escherichia coli* (STEC), a large body of knowledge has accumulated, yet despite this information, successful treatment of these infections has remained elusive.

Sources and pathogenesis of VTEC infection

Sources of VTEC

Gut colonisation of farm animals, especially ruminants, like cattle, sheep and goats is the likely origin of VTEC/STEC. From these sources derive a variety of vehicles of transmission to humans including many different foods of animal or plant origin, and water used for swimming and drinking and for growing edible plants. Human faecal contamination of food and seeds could also play a role, especially in developing countries.[6]

The O157 clone *versus the rest (non-O157 strains)*

The development of a specific medium for the isolation of VTEC belonging to the O157 clone [7] instigated laboratories around the world to seek these organisms in suspected cases of diarrhoea (D), bloody diarrhoea (BD) and HUS. The paradigm that one seeks, then one tends to find is apposite here, but the corollary that rare finds, are not an incentive to seek also comes into play. Thus the fact that non-O157 VTEC had
originally been reported and continued to be reported, albeit only by dedicated microbiologists, tended to be ignored by most researchers in the field. No attention appears to have been given to the generally observed fact that there is a widespread diversity of *E. coli* serotypes in the human intestine at any one time [8] and this has also been found in animals especially cattle.[9] More specific studies in animals in which tests for all VTEC were undertaken have revealed a number of important points, which continue to be ignored by the majority of scientists. These studies [10-14] revealed that there is a great diversity of VTEC serotypes in faeces of cattle and sheep and that strains of VTEC O157, if found at all were in a tiny minority. These and other studies [15] also revealed that there was a strong host specificity among VTEC serotypes, with cattle-related serotypes, being rarely found in sheep and *vice versa*. Moreover, it was not just the VTEC O157 clone that was rare, when all VTEC were sought, but other highly virulent clones such as VTEC O111. Although this was the most common VTEC isolated from patients in Australia, it was very rarely isolated from cattle. [16] Most ruminant faeces contain a variety of VTEC serotypes, but that some, such as O157 and O111, though rarely present and then in only small numbers, are particularly virulent. However, an increasing number of other serotypes can also be involved and one study of an outbreak has shown that the more VTEC serotypes, with which a patient is infected, the worse the clinical condition [17], though the main VTEC serotype was O111. This outbreak also yielded from a few patients VTEC O157 and it was noted that if VTEC O157 had been the only ones sought, then this outbreak would have been labelled “an O157 outbreak” [18]. While isolations of VTEC O111 from cattle are rare, non-VTEC, which are otherwise indistinguishable from the VTEC appear abundant especially in the faeces of sick cattle [19,20] and Bielaszewska et al. [20] noted the presence in the faeces of patients of VTEC and
non-VTEC serotypes, which were otherwise identical.

Detailed studies [21] have shown that the shiga toxins can be subdivided into a series of subtypes and that these are also host specific. Thus there is a “double host specificity” among VTEC. Some clones are specific to cattle, while others are specific to sheep and the toxin subtypes these strains carry are specific to the VTEC types found in these mammalian hosts. Thus by not looking for all VTEC serotyeps during an outbreak, a great deal of epidemiological information is lost and indication of the source animal is not identified.

**Globalization and food distribution**

It must be said that globalization of food presents a great opportunity for VTEC to spread quickly to large sections of the population. Global food distribution carries an inherent risk and presents great difficulties in controlling food-borne pathogens and in identifying sources of outbreaks, as was recently witnessed in Europe. On this basis, much can be recommended for locally produced food, not only for microbiological reasons but also economically in money terms and carbon emissions.

**Pathogenesis**

VTEC/ STEC/EHEC belong to clones of zoonotic E. coli of different O-serogroups that have evolved and acquired specific virulence factors that enable the bacteria to colonize and infect the human colon usually without invasion of the blood stream.[22] STEC/VTEC/EHEC cause bloody diarrhoea (BD), severe colitis and haemolytic uremic syndrome (HUS). These bacteria are known as Enterohemorrhagic E. coli (EHEC) when infection is associated with severe colonic and/or renal disease. The
production of Vero/Shiga toxins have been considered the basis for their pathogenicity, however, other toxins such as subtilase cytotoxin (SubAB)[23] and cytolethal distending toxin [24] and StcE, an inhibitor of C1 esterase probably play a role. [25(g4)]

The recent outbreak of foodborne *Escherichia coli* O104:H4 in Europe has once again drawn attention to shiga-toxin producing (STEC) or enterohemorrhagic *E. coli* (EHEC) infections together with their devastating complications of renal failure (through hemolytic uremic syndrome (HUS)), and stroke from intravascular coagulopathy and vasculopathy or thrombotic microangiopathy. The unusual virulence and lethality of the O104:H4 strain is the result of genetic admixture of virulence factors including enteroaggregative properties and multiple antibiotic resistance and is a lesson in microbial evolution and the genomic plasticity of *E. coli* [26]. The O104:H4 strain is now known as an entero-aggregative and -hemorrhagic *E. coli* (EAHEC).

We have recently observed the combined properties of enteroaggregative ability (providing strong attachment via fimbriae and colonisation of the colonic epithelium) with Shiga toxin (Stx) production in the novel and highly lethal European *E. coli* O104:H4 strain. It has since been shown that this strain belonged to an enteroaggregative *E. coli* lineage that had acquired genes for Shiga toxin 2 and antibiotic resistance [27]. Pathogenesis of HUS disease remains incompletely understood; remarkably, during HUS serum Stx is undetectable. It seems polymorphonuclear leukocytes (PMN) are key players in delivering Stx to critical sites such as the kidneys. The extent of renal damage in children with STEC-
associated HUS may relate to the concentration of Stx present on circulating PMN.

[28] Brigotti et al. [28] showed paradoxical effects of concentration of Stx; patients with high amounts of Stx on PMN showed preserved or slightly impaired renal function (incomplete form of HUS), whereas cases with low amounts of PMN-Stx usually present with acute renal failure. Equally paradoxical, high amounts of PMN-Stx induce a reduced release of cytokines by the renal endothelium, with congruent lower degree of inflammation, while low toxin PMN amounts trigger a cytokine cascade, provoking inflammation with consequent tissue damage. The microvasculature plays an important role in pathogenesis: D+ HUS is associated with platelet thrombi in the microvasculature of almost all vascular beds; [29]. Plasma from HUS patients induces apoptosis of cultured microvascular endothelial cells from most organs. [30] Involved in the pathogenesis of D+ HUS are two key events: altered Von Willebrand factor (VWF) activity (e.g. as seen with ADAMTS13 deficiency) and site-specific activation and/or apoptosis of microvascular endothelial cells. A deficiency in ADAMTS13 (a disintegrin and metalloproteinase with thrombospondin motif-13), that mediates proteolytic processing of newly released pro-adhesive ultra-large VWF multimers from endothelial cells is also thought to play a role in D+ HUS coagulopathy. [31] Targeting the interruption of these processes gives hope for potential novel treatment modalities.

Bacterial gut pathogens target the follicle-associated epithelium overlying Peyer's patches. The microorganisms breech the intestinal barrier via M cells and are captured by mucosal macrophages. [32] Etienne-Mesmin et al. showed STEC/EHEC are able to interact in vivo with murine Peyer’s patches and translocate through the mucosa. The bacteria are taken up by macrophages and produce Stx within these cells and
induce apoptosis and Stx release. The uptake of EHEC by M cells and underlying macrophages in the Peyer’s patch may be a critical step in Stx translocation and release. This could also represent a new therapeutic target.[32]

**Current Treatment Strategies**[g5]

Because of acute renal failure and its consequential perturbation of fluid and electrolyte balance and the disruption of the clotting cascade with thrombocytopenia, with the risk of stroke, together with the need to prevent further effects of toxin, and termination of Complement complex formation, these must be managed and addressed urgently with institution of general supportive measures, antiplatelet and thrombolytic agents and thrombin inhibitors, selective use of antimicrobials, probiotics, toxin neutralizers (synthetic & natural binders, antibodies, etc); and antibodies against key pathogenetic pathway elements to interrupt pathological processes (e.g. inhibition of terminal Complement complex formation). Targeting PMNs carrying Stx could be a productive strategy for future research as could possible gene therapy.

**General supportive measures**[g7]: Fluid balance and attention to the volume and sodium content of intravenous fluids administered early in the disease have been shown to reduce the risk of developing oligoanuric HUS after *E coli* O157:H7 infections. Isotonic intravenous maintenance solutions may show superiority over hypotonic fluids.[33]. Acute renal replacement therapy (ARRT) e.g. peritoneal
dialysis or hemodialysis has been shown to improve outcomes. Children with D+HUS and acute kidney injury given early peritoneal dialysis (PD).

**Acute renal replacement therapy (dialysis, plasmapharesis)**: may have improved outcomes without risk of bleeding in patients with low platelet counts. The procedure seems safe especially in cases with very low platelet counts. No bleeding episodes were recorded.[34] Alternatively hemodialysis is often necessary. Antihypertensive therapy for hypertension when appropriate is also necessary. There seems to be a beneficial role for plasma infusion [35] and plasma exchange [36], however, benefit from apheresis remains uncertain.[37]

**Managing haematological issues and coagulopathy**

Monitoring of hemoglobin, hematocrit and platelet count is essential. Monitoring hemolysis with LDH and haptoglobin is also helpful. Anaemia resulting from hemolysis needs correction with transfusions of whole blood or packed red cells. Platelet transfusions may be required.

**Preventing the further effects of toxin**

a) **Antimicrobials: to use or to avoid?**

Because of the potential for undesirable release of VT/Stx by dying and dead bacterial cells, antibiotics are usually avoided.[38] In addition, the risk of endotoxin release could add to the patient’s already potentially lethal burden. *In vitro* sub-inhibitory concentrations of antibiotics may increase production and release of VT/stx.[39]. The quinolone ciprofloxacin but not fosfomycin causes Shiga toxin-encoding bacteriophage induction and enhanced Shiga toxin (Stx) production from *E. coli*
O157:H7 in vitro and in vivo in a mouse model. [40] Fosfomycin showed evidence of better outcomes in a mouse-model of STEC infection and was recommended for human studies. [41] Similar results were observed in a gnotobiotic piglet model. [42] However, a meta-analysis suggested that antibiotic therapy of E. coli O157:H7 infections might not be harmful but called for a randomised controlled trial. Pooled prospective data showed no benefit of antibiotics. [43] The basis for inferred advantageous effects of antibiotics possibly arose from information relating to only a single study purportedly connecting fosfomycin given at various time points with a reduced risk of HUS. [44] The validity of the study has been questioned on the basis that the meta-analysis mischaracterized fosfomycin as being superior to no antibiotics. [45] 

While many doctors in Japan still use antibiotics including fosfomycin in patients with definite or possible enteric STEC infections the prevailing consensus elsewhere indicates antibiotics should be avoided. [22]

b) Lumenal Toxin neutralizers (synthetic & natural binders, antibodies, etc): [g10]

Strategies using ligand mimics of the receptor for Stx - Gb3, binding to Stx in the gastrointestinal tract with the intention of preventing the spread of toxin to extraintestinal sites have been proposed, but in clinical practice the damage has already been done before these ligands could be of benefit. Only one clinical trial has been conducted (alas unsuccessfully) with one agent - Synsorb PK which bore out this fact. [46] Other molecular mimics of globotriose (Gb3) or globotetraose (Gb4) such as a constructed recombinant bacteria displaying surface Gb3 or GB4 have been proposed. [47] In terms of treatment of human STEC disease such a construct could not be used because the E. coli host strain has been derived from a clinical isolate and
additionally, the expression plasmid contained a kanamycin-resistance gene. Other Stx neutralizers are water-soluble neutralizers designed to suppress Stx cytotoxicity in the circulation. Nishikawa’s group [48] have developed linear polymers bearing clustered trisaccharides of globotriaosylceramide (Gb3) referred to as “Super Twig” as per oral Shiga toxin (Stx) neutralizers. Clinical trials are awaited [g11]. Intralumenal neutralizers might be effective in reducing systemic levels of toxin but because the toxin is not found in serum, studies examining the effect of neutralizers on the toxic effects of toxin-associated with polymorphonuclear leukocytes would be a first step. Antibodies: [g12] Neutralizing Shiga toxin-specific antibodies are potentially useful as therapeutic agents. The toxins are AB toxins with active and binding elements. Obvious targets for antibody neutralisation would include the binding component and/or the toxin’s active regions. Monoclonal antibodies targeting the A subunit epitopes of Stx1 have been shown to be highly protective, when administered to lethally-treated animals.[49] Orally administered immunoglobulin has been used therapeutically for a number of gastrointestinal infections (e.g. rotavirus – Gastrogard-R. [50] Patients with diarrhoea caused by diarrheagenic E. coli, specifically STEC and E. coli-expressing intimin and HEC-haemolysin were treated by administration of pooled bovine colostrum, rich in antibodies to Shiga toxin and enterohemorrhagic E. coli-hemolysin, in a placebo-controlled, double-blind study. Symptom resolution and fecal excretion of infecting strains were assessed. Stool frequencies in the group treated with bovine colostrum were significantly reduced compared with those in the placebo group, however, no effect of colostrum therapy on the carriage of the pathogens or on complications of the infection could be demonstrated. Prospective studies in larger numbers of children with STEC identified early in illness could determine the effectiveness of
bovine colostrum therapy.[51] Antibody to *E. coli* lipopolysaccharide (LPS) also has the potential of therapeutic use through its blocking effect on adherence of STEC to the human intestinal epithelial (Henle 407) cell line.[52] Likewise, human trials would be needed to show clinical effectiveness.

c) **Other toxin binders/neutralizers:** Most of these agents function to bind to the toxin directly and inhibit the binding to its receptor present on the target cells. Nishikawa’s group [53] reviewed other neutralizers that do not act on receptor binding but disrupt intracellular transport of the toxin effectively neutralising the toxin. Such novel Stx neutralizers offer a new therapeutic modality against STEC/EHEC infections.

Watanabe-Takahashi et al. [53] developed an oral Stx2-inhibitor known as Ac-PPP-tet. that completely inhibited fluid accumulation in the rabbit ileum caused by the direct injection of Stx2. The Ac-PPP-tet appears to form a complex with Stx2 within intestinal epithelial cells and seems to block intracellular transport of Stx2 from the Golgi to the endoplasmic reticulum, a route essential for Stx2 cytotoxicity. [53]

d) **Systemically-applied (intravenous)Toxin binders:** Stearns-Kurosawa et al. [54] evaluated whether intravenous administration of a cell-permeable peptide (TVP) that binds to Stx2 would reduce disease severity and rescue juvenile baboons from a lethal Stx2 dose (50 ng/kg). It was shown that TVP (5 mg/kg) delivered intravenously and simultaneously with toxin or at 6 or 24 h after toxin with daily 1 mg/kg supplements up to day 4 prevented acute kidney injury and delayed and reduced blood urea and creatinine levels and increased survival. Delayed administration of the peptide
significantly reduced thrombocytopenia, but had no effect on anemia. This cell-
permeable agent shows promise in counteracting Stx2 lethality in a baboon model;
outcomes of human trials are awaited.

**Blockers of bacterial and host cell interaction - Probiotics**

Gut pathogens display surface molecules enabling the organism to bind to host cell
receptors. Similarly bacterial toxins require host cell receptors for binding and cell
entry. To block microbe and host cell interaction “designer” probiotics have been
developed. [g13] The harmless recombinant bacteria express molecules that mimic
host cell receptors on their surface, thereby deceiving the pathogen into attaching to
the probiotic rather than the host cell receptor. The probiotic bacteria must be able to
survive the tube[g14] journey encountering digestive enzymes and other adverse
conditions. It has been shown that an *E. coli* expressing large amounts of the
glycolipid receptor, globotriaosyl ceramide also known as globotriose Gb3[g15] (the
host receptor for the binding portion of all Stx types associated with human disease
(Stx1, Stx2, Stx2c, and Stx2d) is able to bind these toxins with extreme avidity and
prevent disease. This approach is unlikely to be helpful in patients already exhibiting
disease effects of Stx, however, family members who have been co-exposed with
index cases and could be incubating the infection could benefit from this approach.
Supernatant of cultures of *Bifidobacterium longum* HY8001 is designed to inhibit the
effect of VT/Stx through interference of B subunit of VTs in binding to Gb3. [55]

**Inhibition of terminal Complement complex formation:**

Based on evidence that Shiga toxin activates complement and binds factor H and
evidence for an active role of complement via the alternative pathway diarrhoea-
associated in hemolytic uremic syndrome\cite{56,57} a few anecdotal reports of successful treatment of severe Stx-associated HUS with the monoclonal antibody eculizumab have been published.\cite{58} The antibody was given intravenously at seven day intervals, twice in two patients and four times in a third patient. Neurologically, all three patients improved dramatically within 24 hours after the first eculizumab infusion. Clinical improvement was associated with rapid normalization of markers of disease activity including normalization of platelet counts, and lactate dehydrogenase levels decreasing within five days in all three patients. These initial results is extremely promising and outcomes from large-scale randomized placebo-controlled trials are awaited with optimism.

**Vaccines:**

Several vaccine strategies have been used with variable success in a number of animal models. The strategies have involved the use of recombinant virulence proteins such as Stx, Intimin and EspA \cite{59} or peptides \cite{60} or fusion proteins of A and B subunits of Stx2 and Stx1 such as Stx2Am-Stx1B \cite{61} or avirulent ghost cells of EHEC O157:H7.\cite{62} The application of live attenuated bacteria such as Salmonella as a carrier for vaccine proteins against mucosal pathogens including EHEC have obvious advantages. The study by Gu et al.\cite{63} used a live attenuated EIS-producing Salmonella vaccine in mice model. Vaccination with the recombinant Salmonella induced significant increases of EspA, intimin and Stx2 specific IgG in serum and secretory IgA in feces as well as antigen-specific T cell proliferation. \cite{63} Recombinant fimbrial proteins have been developed in a quest to protect against the STEC-related entity - piglet edema disease. Early results are mixed.\cite{64}
Asper et al.[65] examined antibodies produced in humans with HUS. In addition, the serological response against 37 cloned LEE-encoded proteins and 29 non-LEE effector proteins was measured and compared against sera from rabbits immunized with type III secreted proteins (T3SPs) from four STEC serotypes, experimentally infected cattle, and human sera from six HUS patients. Overall, proteins common to several HUS serotypes, including Tir, EspB, EspD, NleA, and EspA were highly immunogenic in vaccinated and naturally infected subjects and represent future candidates for a STEC vaccine.

DNA vaccines are a recent development in EHEC prevention; a DNA vaccine encoding the EHEC Shiga-Like Toxin 2 A₂ and B subunits was shown to confer protective immunity to Stx challenge in a mouse model.[66]

The mode of delivery (intramuscular, intranasal, per oral, intragastric, etc.) of a number of these vaccines not only affects immunogenicity but also protective effect under challenge. Vaccination with a plant-based oral vaccine protected mice against lethal systemic intoxication with Stx2 [67]. This is seen as encouraging. Clearly there is some time to go before human trials are reported but the numerous and frequent outbreaks of EHEC disease constantly remind us of the urgent need to protect the population against these emerging and often devastating zoonoses.

Future Directions and Conclusions[c16]

There remain significant barriers to successful treatment of HUS given the complexity of the pathogenesis of HUS which involves perturbation of key homeostatic pathways involving complex biochemical and physiological systems. It is unlikely
that targeting a single pathway with a treatment modality will be sufficiently successful; a multi-targeted approach would seem necessary. However, given the apparent success of eculizumab, albeit with tiny case numbers, it could offer a promising strategy for treatment. Treatment is designed to prevent the most serious complications of STEC infection (i.e. renal failure and central nervous complications, e.g. stroke, and shock) which remain far too common. It is clear that better understanding of the pathogenesis of HUS will lead to additional and possibly better targets for treatment. In terms of prevention, we should question the globalization of food distribution with its inherent dangers and its wasteful use of energy resources resulting in a giant’s carbon footprint.

**Competing interest:** none

**Author's information:** Prof. Goldwater and Dr Bettelheim have been involved in STEC/EHEC research for over 30 years. Prof. Goldwater is a senior consultant clinical microbiologist and infectious diseases physician and with his collaborators has used his professional knowledge to further develop an understanding of STEC infection in children. Dr Karl Bettelheim, now retired, introduced Prof. Goldwater to the fascinating field of *E. coli* microbiology and both have been close colleagues and research collaborators over many productive years.
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