Chapter 15

CLOSTRIDIUM PERFRINGENS EPSILON TOXIN

BRADLEY G. STILES, PhD*; GILLIAN BARTH, BS†; and MICHEL R. POPOFF, PhD, DVM‡

**Adjunct Professor of Biology, Biology Department, Wilson College, 1015 Philadelphia Avenue, Chambersburg, Pennsylvania 17201*
†*Veterinary Technician, Veterinary Department, Wilson College, 1015 Philadelphia Avenue, Chambersburg, Pennsylvania 17201*
‡*Chief, Anaerobic Bacteria and Toxins, Institut Pasteur, 28 Rue du Dr Roux, 75724 Paris, France*

INTRODUCTION

Clostridium perfringens is a gram-positive, spore-forming anaerobe commonly found throughout nature (ie, soil, water, gastrointestinal tracts of humans and animals, etc). This bacillus is one of the most "toxic" bacteria described to date, producing at least seventeen different "major" and "minor" protein toxins.[1,2] Other pathogenic species of *Clostridium* synthesize the most potent protein toxins known, such as tetanus and botulinum neurotoxins. Unlike a number of other bacterial pathogens (ie, *Listeria*, *Rickettsia*, *Salmonella*, *Shigella*, and *Yersinia* species), current understanding of *C perfringens* pathogenesis during various diseases does not include invasion, and subsequent replication, in eukaryotic cells.

C perfringens was first isolated in 1892 by William Welch and George Nuttall at Johns Hopkins University in Baltimore following an autopsy of a cancer patient. Of note was a rather profuse, unusually explosive formation of gas bubbles within the cadaver's blood vessels and organs only 8 hours after death. Gas is a common byproduct of anaerobic growth by clostridial species, explaining the term "gas gangrene" during severe myonecrosis induced by *C perfringens*. Over time and throughout the literature, *C perfringens* has also been known as *Bacillus aerogenes capsulatus*, *Bacillus welchii*, and *Clostridium welchii*. Many ill-effects induced by *C perfringens* in humans and animals are linked to protein toxins. Below are succinct descriptions of the classically defined major (typing) toxins with a particular emphasis on epsilon, which has been targeted recently as a select agent by various agencies within the United States and other countries.

HISTORY

Protein toxins are considered important virulence factors for *C perfringens*, and have thus received much attention by various laboratories throughout the world. For many bacterial pathogens, toxins possessing diverse modes of action play critical roles in survival that include nutrient gathering and thwarting the host's immune system. There are two primary modes of action described for the four major (typing) toxins produced by *C perfringens*: 1) increasing permeability of cell membranes (ie, alpha, beta, and epsilon toxins) resulting in ion imbalances and general leakiness; and 2) destroying the actin cytoskeleton (ie, iota toxin).[2] In either scenario, the end result elicited by any of these toxins is the same: cell dysfunction and death. Multiple studies by many groups reveal that *C perfringens* possesses highly evolved tactics, involving offensive (ie, secreted protein toxins plus enzymes) and defensive (ie, protein toxins plus spores) tools for surviving, and then thriving, in harshly diverse environments. *C perfringens* consists of five toxin types: A, B, C, D, and E (Table 15-1), based on the production of one or more protein toxins.[1,2]

Each of these toxins is lethal, dermonecrotic, and associated with a wide range of diseases that include a rapid, life-threatening myonecrosis (gas gangrene) and various enteric illnesses in both animals as well as humans (Table 15-2). Historically, for diagnostic purposes these typing toxins would be neutralized in the laboratory by type-specific antisera in mouse-lethal and guinea-pig dermonecrotic assays. The toxin source would consist of culture filtrate from *C perfringens* isolated from a patient.[3] Today, rapid genetic methods involving multiplex polymerase chain reactions are more commonly used by diagnostic laboratories around the world for typing *C perfringens*.[4,5] This technique, although rapid and accurate, suggests the presence of a toxin gene but indicates neither production nor relative quantities of a biologically-active protein. Rapid quantitation of epsilon toxin protein in complex matrices (eg, milk and serum) is also possible using a novel, mass spectrometry technique; however, this does not determine whether the toxin is biologically active.[6]

Unlike the other typing toxins, alpha is, by definition, produced by all *C perfringens* and has played a significant role in military casualties over time. Type A strains are most commonly found in the environment and cause gas gangrene.[1,7–11] Alpha toxin facilitates gas gangrene due to *C perfringens* infection, an omnipresent threat to soldiers wounded on the battlefield.[7,8] Deep penetrative wounds, soiled by dirt that contains

TABLE 15-1

TOXIN TYPES OF *CLOSTRIDIUM PERFRINGENS*

Major (typing) Toxins	Toxin Type				
	A	B	C	D	E
Alpha	√	√	√	√	√
Beta		√	√		
Epsilon		√		√	
Iota					√

TABLE 15-2

***CLOSTRIDIUM PERFRINGENS* TOXIN TYPES AND DISEASES**

Toxin Type	Disease/Intoxication
A	Myonecrosis (gas gangrene), necrotic enteritis of fowl and piglets, human food poisoning, antibiotic-associated diarrhea
B	Dysentery in lambs; hemorrhagic enteritis in calves, foals, and sheep
C	Necrotizing enteritis in humans (known as pigbel, darmbrand, or fire-belly), pigs, calves, goats, and foals; enterotoxemia in sheep (struck)
D	Enterotoxemia in lambs (pulpy kidney or overeating disease) and calves; enterocolitis in goats and cattle
E	Calf and lamb enterotoxemia

various clostridial species (especially *C perfringens*), are often to blame for quickly advancing disease in the buttocks, thigh, shoulder, upper extremity, and leg (in order of decreasing prevalence).[7,10,11] The fatality rate from gas gangrene was 50% during World War II; it was especially high when fighting occurred in cultivated land (commonly fertilized with animal feces), as opposed to desert (eg, Tunisia).[10,11] The threat of gangrene from *C perfringens* or other clostridial species[11] due to wound contamination in the field or nonsterile operating conditions was particularly prevalent before 1900 and resulted in many amputations and deaths that can be avoided in modern times. If administered promptly after disease onset, medical countermeasures, such as extensive surgical debridement, various antibiotics (eg, beta-lactams, clindamycin, metronidazole), and hyperbaric oxygen provide effective treatment for most cases of *C perfringens*-induced gangrene. Antitoxin (historically, polyclonal antibodies of equine origin) administration is also another possible therapy that targets alpha toxin and mitigates myonecrosis.[7,10,12] Vaccine studies from various groups using the carboxy-terminal (cell binding) domain of alpha toxin show prophylactic protection against either toxin-induced lethality or bacterial challenge in a mouse gangrene model.[13,14]

Biochemically, alpha toxin is a zinc-containing phospholipase C (43 kDa) composed of two structural domains that destroys eukaryotic cell membranes.[15,16] The amino-terminal domain contains a catalytic site and ganglioside (GM1a) binding motif, the latter being similar to that found on another clostridial toxin studied by the biodefense community: *Clostridium botulinum* neurotoxin.[17] Interaction of GM1a by alpha toxin promotes clustering and activation of tyrosine kinase A involved in signal transduction.[17] The carboxy-terminal domain of alpha toxin binds to phospholipids on cell membranes.

In comparison to the alpha toxin and due to recent national and international biodefense concerns, *C perfringens* epsilon toxin has received much more government attention (ie, funding and regulated oversight) over the past 15 years as a potential agent used in biowarfare and bioterrorism.[18] Epsilon is the most potent of all *C perfringens* toxins as determined by a very low LD_{50} (toxin amount necessary to kill 50% of the subject population; murine intravenous assay), ranking behind only the *C botulinum* and *C tetani* neurotoxins among all clostridial toxins. In the very recent past, the Centers for Disease Control and Prevention placed epsilon toxin on the Category B list of select agents, along with bacterial diseases (eg, brucellosis, glanders, and typhus) plus other protein toxins (eg, ricin and staphylococcal enterotoxin B). Additionally, epsilon toxin represented a potential agroterrorism threat and was deemed a select agent by the United States Department of Agriculture. However, when the select agents list was modified in December 2012, *C perfringens* epsilon toxin was removed.[19]

In France, but not throughout Europe, epsilon toxin is still classified as a potential biological weapon and requires special authorization from a federal agency (Agence Nationale de Securite du Medicament) before being approved for laboratory work. There are varying opinions around the world regarding *C perfringens* epsilon toxin, its potential nefarious applications, and imposed level of government regulations. Such nonconsensus among allies affects sustained funding and effective collaborations between investigators, laboratories, and nations.

DESCRIPTION OF THE EPSILON TOXIN

Natural Occurrence

The epsilon toxin is naturally produced by types B and D *C perfringens* that are involved in animal (cattle, goat, and sheep) enterotoxemias that often prove widespread, rapidly fatal, and economically damaging for the agriculture industry.[1,20] Although *C perfringens* is considered normal intestinal flora in ruminants, types B and especially D can cause severe, life-threatening illness in a "naïve" digestive system shortly after

birth, or following a diet change involving higher carbohydrate levels (particularly starch) among older animals. If there is little microbial competition within the gastrointestinal tract or an overly nutrient-rich diet is suddenly consumed, resident *C perfringens* types B and D can rapidly proliferate in the intestines and concomitantly produce life-threatening levels of toxins that include epsilon. Those who study *C perfringens* and disease naturally associate the epsilon toxin with veterinary issues. In fact, neither the epsilon toxin nor *C perfringens* types B and D infections are commonly linked to human disease, which may make the toxin ideal for nefarious use as a biological weapon against humans: a bioterrorist event employing epsilon toxin against humans could be very difficult to diagnose and treat because there is no "natural" precedent and classically trained physicians do not anticipate human illness linked to *C perfringens* epsilon toxin.

Chemical and Physical Properties

C perfringens epsilon toxin is a plasmid-borne, 311 amino acid (32.9 kDa) protein secreted as a protoxin, activated subsequently by extracellular proteases (trypsin and chymotrypsin) that remove amino-terminal (14 amino acids) and carboxy-terminal (29 amino acids) peptides.[20] The nascent protoxin contains a typical leader sequence (32 amino-terminal residues) that normally facilitates protein secretion from the bacterium into the environment. The toxin is resistant to inactivation by serine-type proteases commonly found throughout nature, including those in the gastrointestinal tracts of various mammals.

The crystal structure of epsilon toxin (Figure 15-1) reveals an elongated beta-sheet (100 Å × 20 Å × 20 Å) containing three domains, which shares conformation with other bacterial (aerolysin family) pore-forming toxins, including *Aeromonas hydrophila* aerolysin, *C perfringens* enterotoxin, and *Clostridium septicum* alpha toxin.[21,22] The putative roles for each domain of epsilon toxin include receptor binding (domain I: amino terminus), channel formation (domain II: central region), and monomer-monomer interaction (domain III: carboxy terminus).[2,21,23] Loss of a carboxy-terminal peptide from epsilon toxin seems primarily responsible for monomer-monomer interactions and subsequent homoheptamer formation.[23]

Proteolysis is a common method of activating many bacterial toxins, and for epsilon this process induces conformational changes that facilitate homooligomerization on the external surface of a eukaryotic cell. Essentially, proteolytic activation causes "protein priming" that enables the toxin to quickly act after binding to diverse target cells of neuronal, renal, and endothelial origins (described in detail below). Additionally, proteolysis of the amino- and carboxy-termini on the epsilon protoxin leads to a more acidic isoelectric point (5.4 versus 8.0), which may play a role in receptor interactions.[20,24] For enteric-produced toxins requiring proteolysis, the proteases synthesized by resident bacteria (including *C perfringens* lambda toxin)[25] and host[23] are bountiful. Recent evidence suggests that epsilon toxin can be activated intracellularly (in select strains), remains in *C perfringens* until stationary or death phase, and is finally released into the environment following autolysis.[26] This unique protease and a further understanding of its novel activation mode in clostridia (and possibly in other secreted toxin-producing pathogens) remains elusive.

Mechanism of Action

The mode of action for epsilon toxin involves pore formation in eukaryotic cell membranes facilitated by detergent-resistant membrane fractions, also known

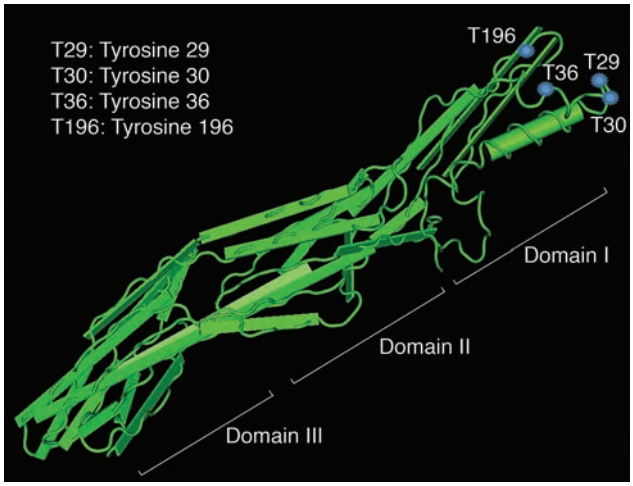

Figure 15-1. Crystal structure of *Clostridium perfringens* epsilon toxin. Three domains exist and are putatively involved in (I) receptor binding (amino terminus), (II) channel formation, and (III) monomer-monomer interactions (carboxy terminus). Designated amino acids (tyrosines 29, 30, 36, and 196) facilitate receptor (hepatitis A virus cellular receptor 1) binding, and when individually replaced with glutamic acid, yield nontoxic variants of epsilon toxin.
Data sources: (1) Cole AR, Gibert M, Popoff MR, Moss DS, Titball RW, Basak AK. *Clostridium perfringens* epsilon-toxin shows structural similarity to the pore-forming toxin aerolysin. *Nat Struct Mol Biol*. 2004;11:797–798. (2) Ivie SE, McClain MS. Identification of amino acids important for binding of *Clostridium perfringens* epsilon toxin to host cells and to HAVCR1. *Biochemistry*. 2012;51:7588–7595. (3) Madej T, Addess KJ, Fong JH, et al. MMDB: 3D structures and macromolecular interactions. *Nucleic Acids Res*. 2012;40:D461–464.

as lipid rafts, that concentrate toxin monomers into homoheptamers.[27,28] These cholesterol-rich membrane domains play important roles in many diseases elicited by bacteria, associated toxins, and viruses.[29] Furthermore, caveolins 1 and 2 found in these domains are bound directly by epsilon toxin and necessary for toxin oligomerization.[30] Epsilon toxin oligomers, critical for biological activity:

- form within 15 minutes at 37°C on Madin-Darby canine kidney cells (MDCK);
- are more stable at 37°C versus 4°C;
- promote potassium efflux; and
- are internalized from the cell surface, forming vacuoles in the late endosomes and lysosomes.[31,32]

Sialidases, also known as neuraminidases, are produced by *C perfringens* and enhance binding of the bacterium as well as epsilon toxicity to select cultured cells.[33] For example, in the presence of bacterial-derived sialidase, there is increased adherence of *C perfringens* to a human colorectal epithelial line (Caco-2), but not to MDCK or Vero (monkey kidney) cells. Contact between the bacterium and certain cell lines like Caco-2 leads to gene upregulation and increased activity for *C perfringens*-derived sialidase. Increased toxin binding to target cells following sialidase activity has been described previously for another enteric toxin-producing pathogen, *Vibrio cholerae*.[34]

In concert with disrupted cell membranes facilitating free passage of 1 kDa-sized molecules,[35] secondary effects of epsilon toxin involve cytoskeletal dysfunction that becomes lethal for an intoxicated cell.[36] Additionally, the integrity of a cell monolayer is readily disrupted by epsilon toxin,[27] providing another clue toward understanding subsequent dysfunction of the vascular endothelium, edema, and crossing of the blood-brain barrier.[37] The ill effects induced by epsilon toxin upon the circulatory system are quite substantial, with albumin-sized molecules (~ 65 kDa) subsequently transiting from the blood stream into the brain.[38]

CLINICAL SIGNS AND SYMPTOMS

Although epsilon toxin can be found in the heart, lungs, liver, and stomach following intoxication, it noticeably accumulates in kidneys, causing what veterinarians classically refer to as "pulpy kidney disease."[1,2,20,39–41] Another indicator that kidneys are a primary target of epsilon toxin is that the few susceptible cell lines discovered to date are of kidney descent from dog, mouse, and human.[20] Postmortem results from lambs and mice given epsilon toxin show similar results in the kidneys that include congestion, interstitial hemorrhage, and degenerated epithelium within the proximal tubules. Toxin accumulating in the kidney may represent a natural defense mechanism by the host to prevent lethal concentrations in the brain.[41] During lamb enterotoxemia, glucose excretion into the urine following epsilon toxin exposure may be a result of liver-released glycogen.[42]

The ability of *C perfringens* epsilon toxin to rapidly disrupt the blood-brain barrier, bind neuronal cells, and cause lethality is of obvious concern.[20,37,39] Among neuronal cell populations (sheep), neurons are most susceptible, followed by oligodendrocytes and astrocytes.[43] In the brain, there are signs of swelling, vacuolation, and necrosis. Edema in the rat brain following an intraperitoneal injection of epsilon toxin causes increased levels of aquaporin-4 in astrocytes, which may be the body's attempt to reduce osmotic pressure surrounding sensitive neurons.[44] Aquaporins are membrane proteins that regulate water flow in various cell types and could be a target for therapies against epsilon toxin. Necrosis of the brain following epsilon intoxication is likely due to multiple factors that include reduced blood flow, sustained hypoxic state, and direct toxicity on various cell types.

Clinical signs attributed to epsilon toxin given intravenously to calves, lambs, and young goats occur rapidly (approximately 30 minutes, depending on dose).[45,46] These animals can experience labored breathing, excited or exaggerated movements, intermittent convulsions, loss of consciousness, and ultimately death. Further signs of epsilon intoxication include elevated blood pressure and vascular permeability, lung edema, and brain congestion with edema.[20] Results from another laboratory reveal that an intravenous injection of epsilon toxin (2–4 LD_{50}, in which an LD_{50} is ~ 70 ng/kg) into mice yields seizures within 60 minutes.[47] More naturally, duodenal inoculation of goats with whole culture or supernatant of *C perfringens* type D leads to diarrhea, respiratory distress, and central nervous system dysfunction (ie, recumbency and convulsions).[48] Similar symptoms are also evident in lambs, minus the diarrhea.[49] Furthermore, in ovines (namely lambs) there can be sudden death or acute disease involving neurological manifestations that include struggling, opisthotonos, convulsions, lateral recumbency, and violent paddling. The mode of action for epsilon toxin in vivo seemingly involves ion imbalance, endothelial disruption, and edema.

A vicious cycle is established by *C perfringens* epsilon toxin in the intestinal tract via increased permeability, leading to higher circulating levels of toxin.[43]

Further studies with a wild-type D culture (sheep isolate, CN1020) given intraduodenally to sheep, goats, and mice reveal that a genetic knockout of epsilon toxin does not cause disease compared to controls.[50] Complementing this mutant with the wild-type toxin gene generates the original phenotype that causes epsilon toxin-based disease. In this thorough study, clinical signs of epsilon intoxication in mice included depression, ataxia, circling, and dyspnea. Overall, these results show that the epsilon toxin from a type D strain is critical for disease in these diverse animal models.

Different animal models show that the toxin is quite active when given intravenously or intraduodenally. However, from a biodefense perspective, critical data are lacking in the literature for epsilon toxin and effects following aerosol delivery. It is unclear what happens to nonhuman primates following epsilon intoxication. To establish a basal level of knowledge for further study and develop medical management strategies for humans, it is necessary to carry out and publish nonhuman primate studies.

MEDICAL MANAGEMENT

From a therapeutic perspective, very little has been done with *C perfringens* epsilon toxin, partly because of its natural association with animal (and not human) disease. An effective vaccine against epsilon toxin (described below) is readily available for animal use, thus obviating the need for a therapeutic in susceptible populations. There is no therapeutic agent or vaccine against epsilon toxin approved for human use at this time. Findings from different laboratories and various in vivo and in vitro studies suggest that therapy is possible. Perhaps a proteomics-based approach following epsilon toxin exposure can reveal unique, host-based targets for therapeutic intervention. This approach has recently been taken in mice given epsilon toxin intravenously, with subsequent analysis of select organs (brain plus kidney), plasma, and urine for differentially-expressed proteins.[51] However, there is much more work to be done on this front.

Miyamoto et al[47] show that riluzole, a benzothiazole (234 Da) used for treating human amyotrophic lateral sclerosis by preventing presynaptic glutamate release, can minimize murine seizures induced by epsilon toxin. These results were manifest after riluzole injection (16 mg/kg, intraperitoneal) given 30 minutes before toxin (140 or 280 ng/kg, intravenous), but the drug was evidently not used in subsequent experiments as a therapeutic administered after toxin. Another murine-based study shows that epsilon toxin binds preferentially to the cerebellum, particularly oligodendrocytes plus granule cells.[52] Incubation of primary-cultured granule cells with epsilon toxin causes a rapid increase in intracellular calcium levels and glutamate release. This study provides a brain cell-specific target (and assay) for therapeutic intervention (and screening of potential therapeutic molecules).[52]

Small molecule libraries have also been screened using an MDCK cell assay for therapeutic effects that counter epsilon toxin.[53] Three structurally unique inhibitors were discovered that do not prevent toxin binding or oligomerization on cells, but likely affect pore function or an unidentified cofactor important in epsilon intoxication. Two of these compounds (*N*-cycloalkylbenzamide and furo[2,3-b]quinoline) protected cells when added 10 minutes after toxin exposure, thus providing therapeutic potential in an in-vitro scenario. Such results logically lead to efficacy studies in animals, though none have been published to date.

Another therapeutic approach against epsilon toxin includes dominant-negative inhibitors, which have been successfully employed as experimental therapeutics for other oligomer-forming bacterial toxins produced by *Bacillus anthracis*, *Escherichia coli*, and *Helicobacter pylori*.[54–57] This concept involves a recombinantly modified toxin that is no longer toxic after deleting a peptide region or substituting specific amino acids. Upon integrating a dominant-negative protein into a complex with wild-type toxin monomers in solution or on a cell surface, a nonfunctional toxin oligomer is generated. Two dominant-negative inhibitors for epsilon toxin were created via cysteine substitutions of four amino acids (isoleucine 51 plus alanine 114, and valine 56 plus phenylalanine 118) that constrain the membrane insertion domain. These paired mutations facilitate an intramolecular cystine bond, oligomer dysfunction (decreased heat and detergent stability plus poor prepore-to-pore transition), and ultimately toxin inactivation in vitro.[57] When used in vitro with MDCK cells, these inhibitors dose-dependently inhibit epsilon-induced cytotoxicity at a 1-, 2-, 4-, or 8 (wild-type toxin)-to-1 (dominant-negative protein) mole mixture. Furthermore, dominant-negative molecules like those presented in this study represent potential vaccine candidates worthy of future study.

Additional therapy and prophylaxis studies show that the epsilon protoxin protects mice (ie, delays time to death) when given intravenously before activated toxin. This protective effect presumably occurs via competitive occupation of the cell surface receptor by the protoxin, primarily localized within the brain.[39]

Such data suggest that a receptor-targeted approach for prophylaxis and therapy is possible; Buxton discovered that a formalin toxoid of the protoxin (100 mg/mouse, intravenous) affords protection up to 100 minutes after intravenous exposure to epsilon toxin (0.5 mg/mouse).[58] The readout in this study was extravasation of horseradish peroxidase from the blood stream into the brain. Recent studies by Dorca-Arévalo et al report a similar binding dissociation constant (K_d ~ 4–6 nM) for the epsilon protoxin and activated toxin to MDCK cells.[59] These results evidently depend on plasma membrane integrity plus an unidentified glycoprotein. Existing literature suggests that further work with receptor antagonists as potential therapeutics against epsilon toxin has not been readily pursued by various laboratories.

Knowledge of cell surface receptors for epsilon toxin and intimate molecular interactions can be useful in formulating effective receptor-based therapies. Early studies by Nagahama and Sakurai reveal that the receptor is perhaps a heat-labile sialoglycoprotein; a pretreatment of rat synaptosome membranes with heat (70°–80°C for 10 minutes), neuraminidase, or pronase effectively reduces the binding of epsilon toxin.[60] Furthermore, this same study reveals that a snake presynaptic neurotoxin (beta-bungarotoxin) decreases epsilon toxin binding in a dose-dependent fashion, suggesting a common receptor for these very different toxins. In contrast, the presynaptic neurotoxin produced by *C botulinum* type A had no effect on epsilon toxin binding. It seems that kidney cells and synaptosomes have different receptors for epsilon toxin, as evidenced by varying results following sialidase pretreatment of cells.[33,60]

Recent studies by Ivie et al show that hepatitis A virus cellular receptor 1 (HAVCR1) acts as a receptor, or coreceptor, for epsilon toxin.[61,62] The natural role of HAVCR1 appears linked to regulatory T cells and maintaining immunological balance. This class I, integral-membrane glycoprotein contains multiple isoforms varying within a mucin-like domain that possesses multiple glycosylation sites. Domain I tyrosines (29, 30, 36, 196) found on one end of epsilon toxin (see Figure 15-1) are surface-accessible and important for binding to HAVCR1.[62] Although these data advance our understanding of epsilon toxin–receptor interactions and are potentially useful for therapeutic agent and vaccine development, additional studies are needed to clarify in further detail these cell-surface interactions.

Epsilon toxin is naturally found in the veterinary arena and efficacious vaccines are commonly used in the field, as explained earlier.[63–65] Hyperimmune sera can also afford temporary, passive protection for 3 to 4 weeks in weaned lambs; however, animals showing clinical signs of epsilon intoxication are not typically afforded protection by antitoxin immunoglobulins.[66] It is also possible that a monoclonal antibody targeting a critical epitope, like the membrane insertion region of epsilon toxin, could be a more useful therapeutic, better characterized and purified, than polyclonal antibodies.[67] Immunoglobulins derived by either active or passive immunization are effective tools against *C perfringens* epsilon toxin when present before or after toxin exposure.

Historically, vaccines for humans and animals have proven remarkably effective against myriad diseases throughout the world. However, as with many veterinary vaccines, those for *C perfringens* and associated toxins are often formaldehyde toxoids consisting of various antigens from culture filtrates or whole cells. These vaccines are efficacious and cost effective for animals, but considered too crude for humans.[68] Furthermore, current veterinary vaccines containing epsilon toxoid can vary in immunogenicity and ultimately protective efficacy.[64] The vaccination regimen also varies depending on the animal species.[65] Typically, two doses are given within 2 to 6 weeks of each other using aluminum hydroxide adjuvant, followed by an annual (sheep) or quarterly (goat) boost. In animals, and perhaps in a very heterogeneous population like humans, an epsilon toxin vaccine is clearly not a "one and done" scenario for lasting, protective immunity.

Recombinantly produced epsilon toxin that is subsequently inactivated by 0.5% formaldehyde can be used as a superior cost-effective vaccine (eliciting a sustained, higher antitoxin titer) at much lower protein doses than standard epsilon toxoids.[64] Use of a more defined (purified) vaccine, as opposed to a crude culture filtrate, also affords easier quality control. The gene for epsilon toxin was first successfully cloned, sequenced, and expressed in 1992, making subsequent recombinant work possible.[69] Any human vaccine against epsilon toxin will likely be chemically or recombinantly mutated critical amino acids for receptor binding, oligomerization, or channel formation detoxified versions of the purified protein. In terms of recombinant protein, replacing histidine 106 with proline results in a nontoxic form of epsilon toxin[70] that provides vaccine-based protection against 1,000 LD_{50} of wild-type toxin given intravenously to mice. Further recombinant work could be done using data derived from earlier chemical modifications of epsilon toxin.[71] X-ray crystallography of a toxin-receptor complex would likely yield definitively useful data, leading to an even better recombinant vaccine or novel therapeutics. Medical management of epsilon intoxication, particularly in humans, is currently a wide-open field, not only for physicians but also microbiologists, biochemists, and immunologists.

SUMMARY

C perfringens is a very "toxic" bacterium, employing various proteins to promote life in (and out of) various mammals. Myriad proteins are toxins intimately linked to many *C perfringens* diseases in humans and animals; in particular, the epsilon toxin has been studied by various groups around the world and is primarily a veterinary concern. Vaccines are available for veterinary use, but an equivalent has not been generated for human use in biodefense. From a therapeutic or short-term prophylactic perspective, toxin-specific immunoglobulins should be of logical interest for human use. However, critical experiments employing characterized immunoglobulins (monoclonal and especially those humanized) are lacking in the literature. Simply put, countermeasures for epsilon intoxication in humans are currently highly experimental.

Finally, military and civilian physicians throughout time have been concerned with soiled penetrating wounds involving muscle tissue for fear of gas gangrene due to *C perfringens* (and other anaerobes). More recently, biodefense in the 21st century has targeted a *C perfringens* toxin, epsilon, as a potential nefarious agent. As the discovery of *C perfringens* over 120 years ago suggests, along with subsequent work on the various virulence factors of this extraordinarily toxic bacterium, a collaborative international effort propelled by scientific endeavor is key to discovering knowledge-based medical interventions.

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