EPSILON TOXIN

Epsilon toxin is secreted by toxinotype B and D strains of *Clostridium perfringens*, a Gram positive, anaerobic, spore-forming bacteria present widely in nature from soil to the gastrointestinal tract of healthy humans and animals.[1,2] Epsilon toxin belongs to the heptameric β-pore-forming toxin family which includes aerolysin and *C. septicum* alpha toxin, and which is characterized by the formation of a pore through the plasma membrane of eukaryotic cells, altering the cell permeability, resulting in potassium ions and fluid leakage, and causing edema and cell death by necrosis.[1-3]

Epsilon toxin producing strains are most commonly isolated from farm animals, particularly sheep, goats, and cattle, but rarely from humans.[4-7] However, there is a growing body of evidence that indicates epsilon toxin may be a potential trigger for human multiple sclerosis (MS), an inflammatory disease of the central nervous system characterized by disruption of blood brain barrier (BBB) and demyelination of the myelin sheath that insulates the neurons.[8]

There are five types of *C. perfringens*, designated from A through E. Each type produces a unique spectrum of toxins: type A produces alpha toxin; type B produces alpha, beta and epsilon toxins; type C produces alpha and beta toxins; type D produces alpha and epsilon toxins; and type E produces alpha and iota toxins. In addition to these major toxins, they also produce at least 12 other secondary toxins with diverse modes of action, such as beta2, gamma, delta, theta, and lambda toxins. Based on a large number of toxins produced and its abundance in nature, *C. perfringens* is responsible for diverse pathologies in humans and animals resulting from gastrointestinal or wound contamination, including food poisoning, enteritis, necrotic enteritis, enterotoxemia, gangrene and puerperal septicemia.[1-3]

Epsilon toxin and other toxin genes are located in large plasmids and variable regions of *C. perfringens* chromosome.[9,10] The toxin is synthesized as a single protein containing a signal peptide (32 N-terminal amino acids). The secreted protein (~33 kDa) is poorly active and is called a prototoxin. Proteases produced by *C. perfringens* or present in the host lumen remove the signal peptide along with 29 C-terminal amino acids to convert the prototoxin to active toxin with a reduction in size (~28.6 kDa). Activation significantly decreases the pI value from 8.02 to 5.36 and most likely creates a conformational change.[11,12] Once activated, the toxin can oligomerize to a heptameric form consisting of a β-barrel of 14 amphipathic β strands capable of creating a pore through the plasma membrane of eukaryotic cells.[13-15] Epsilon toxin receptors are yet to be identified, however, lipid rafts associated myelin and lymphocyte protein (MAL) on the membrane have been proposed to be the receptors for epsilon toxins.[16,17]

Overgrowth of *C. perfringens* and high epsilon toxin production induces an increase in the permeability of the intestinal mucosa, mediating the passage of toxin into the blood, disseminating and accumulating particularly in the kidney and brain. In experimental mouse and rat intestinal loops, epsilon toxin at a concentration of 10^3 mouse lethal doses/mL and higher causes an accumulation of fluid in the intestinal lumen, a decrease in transepithelial electrical resistance and an increase in the passage of macromolecules across the intestinal barrier.[18,19] Hemorrhage, suffusion and edema which have been observed in various tissues from naturally or experimentally intoxicated animals, suggest that epsilon toxin targets endothelial cells and alters the integrity of the vascular barrier. Epsilon toxin efficiently increases the vascular permeability of rat mesentery microvessels or skin vessels after intradermal injection.[20,21] In kidney, epsilon toxin causes interstitial hemorrhage between tubules and degeneration of proximal and distal epithelium (pulpy kidney disease in lamb) suggesting that kidney is one of the target organs for epsilon toxin.[22]

An important pathology of epsilon toxin is its ability to cross the blood–brain barrier (BBB), reaching the CNS and accumulating in the brain by binding to specific cells with high affinity.[23] The toxin is able to alter the integrity of BBB resulting in prominent lesions consisting of perivascular edema described in mice, rats, sheep and calves. In the acute state, the brain lesions develop foci of necrosis and hemorrhage.[24-27] A direct and rapid epsilon toxin effect in the brain involves the stimulation of glutamate release from glutametargic neurons abundant in the CNS, which is probably the main cause of the neurological symptoms of excitation (convulsions) observed in epsilon toxin dependant enterotoxemia in sheep. Epsilon toxin has also been shown to induce the release of other neurotransmitters such as dopamine. In that respect, epsilon toxin can be used as a reagent to stimulate glutametargic neurons where many bacterial neurotoxins inhibit the release of neurotransmitters.[21,28] Epsilon toxin has been reported to be used as delivery vehicle to facilitate the transport of drugs through the BBB for the treatment of experimental malignant brain tumors in mice.[29]

Recent studies strongly support the notion that epsilon toxin is responsible for triggering MS. First, *C. perfringens* type B that produces epsilon toxin has been isolated from a patient who was experiencing a flare-up of her MS.[8] Second, epsilon toxin is known to bind and kill the brain's endothelium cells and oligodendrocytes (myelin producing cells), the same cells that die in MS lesions.[30] Third, epsilon toxin has been found to bind to the retinal vasculature and kill meningeal cells;

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meningeal inflammation and subpial cortical lesions are known pathologies associated with MS.[30] These findings are very important for future MS research, and epsilon toxin will be a valuable reagent and tool to understand the pathophysiology of MS.

Epsilon toxin is much more potent (100X) than aerolysin and *C. septicum* alpha toxin. The lethal dose by intraperitoneal route in mice is 70 ng/kg for epsilon toxin and for comparison is 1.2 ng/kg for botulinum neurotoxin A. For this reason, epsilon toxin is classified as a category B bio-threat agent that has potential for malice.[31] In that respect, active/inactive/mutated epsilon toxin has potential for vaccine development and in biodefense research.

Active epsilon toxin (native) from List Labs is lyophilized and highly pure in nature. **This product is intended for research purposes only and is not for use in humans. For further information, please contact List Biological Laboratories, Inc.**

Product No.	Description	Size
126A	Epsilon toxin (native)	0.1 mg

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