Killer toxin's punch lies below the belt

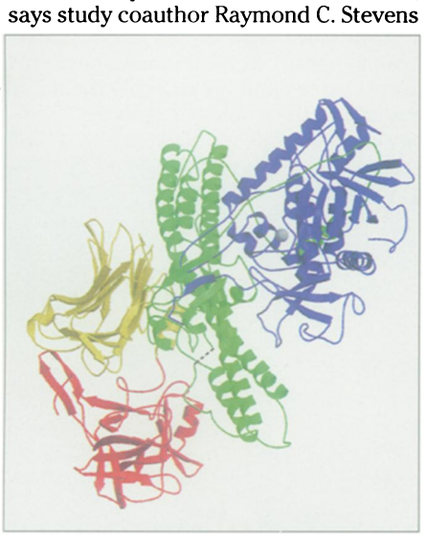
A newly determined three-dimensional structure of the toxic protein that causes botulism shows a surprising twist: A belt of amino acids protects the lethal part of the toxin. Researchers searching for an antidote against the muscle-paralyzing disease may need to revise their strategies.

Although botulism is contracted most often through contaminated food, terrorists have seized upon the toxin as a biological weapon. Produced by the bacterium *Clostridium botulinum,* the toxin kills by shutting down the muscles needed for breathing. A fatal dose is just 100 billionths of a gram, making the toxin one of the most lethal poisons known.

Bibhuti R. DasGupta of the University of Wisconsin-Madison and his colleagues enlisted the help of researchers at the Lawrence Berkeley (Calif.) National Laboratory to identify the positions of the 1,285 amino acids in the protein. Using X-ray crystallography, they examined one of seven types of the toxin, each produced by a different strain of the bacterium. The structure they deciphered confirms some earlier findings about the toxin, but it also reveals an unexpected feature, the researchers report in the October NATURE STRUCTURAL BIOLOGY.

Previous results had shown that the toxin consists of three parts, each playing a separate role in shutting down the nerve cells, or neurons, that control muscles. After one part binds to receptors on a neuron, a second opens up a pore in the cell. A third portion then passes through the pore, breaks away from the rest of the toxin, and interferes with the nerve-signal transmitter called acetylcholine. The new images show what these three parts, or domains, look like, says study coauthor Raymond C. Stevens of Lawrence Berkeley.

Stevens



The three-dimensional structure of the botulinum toxin. One part of the protein (red and yellow) binds to receptors on the target nerve cells. A belt-shape portion (green) opens up a pore in the cells, and the toxic fragment (blue) enters.

Surprisingly, the second domain of the toxin loops around the third domain like a belt, hiding the lethal piece of the protein, Stevens says. The belt conceals the third portion until it enters the cell, making the toxin "like a Trojan horse."

"We had no inkling that the loop was there," says Frank Lebeda of the Army Medical Research Institute of Infectious Diseases at Ft. Detrick in Frederick, Md. Drugs designed to lock onto and disable the toxic part of the protein will have to move the belt out of the way or be small enough to squeeze by.

Currently, no good inhibitors to the toxin exist. Patients are put on artificial respiration and closely monitored for weeks or months until their immune systems clear the toxin. One compound that can be used to treat botulism (SN: 8/2/86, p. 76) "only indirectly alleviates some of the symptoms," Lebeda says. "It temporarily reverses the paralysis."

Interestingly, botulinum toxin itself is being used as a treatment for certain neuromuscular disorders (SN: 1/19/98, p. 42). Injected in tiny, harmless amounts, it quiets muscle spasms.

With the shapes of the six other types of toxin still unknown, Eric A. Johnson of the University of Wisconsin-Madison's Food Research Institute comments that DasGupta's study is "a seminal piece of work, but it's just the beginning." —*C. Wu*

Exposure to smoke yields fetal mutations

Cancer can take years to develop, in large part because many steps must occur for a tumor to arise. Typically, some environmental factor causes a gene to mutate, leading to other genetic irregularities, which in the worst-case scenario result in uncontrolled cell growth. For the disease to strike a young child, this sequence of events must start very early in development, perhaps in the womb.

Some studies have associated pregnant mothers' exposure to tobacco smoke with the incidence of childhood cases of leukemia and lymphoma. A new study of blood taken from the umbilical cords of newborns now may provide a biological basis for such a link—even in a mother who doesn't smoke but lives or works with someone who does.

The results, reported in the October NATURE MEDICINE, are the first to demonstrate smoke-induced genetic mutations in the womb. Infants born to nonsmoking mothers who were exposed to secondary cigarette smoke during pregnancy had more cancer-related mutations in a gene called *HPRT* than did newborns of unexposed mothers, says study coauthor Barry A. Finette, a pediatrician at the University of Vermont College of Medicine in Burlington.

Finette and his colleagues have shown that "the DNA mechanism of repair is damaged in these kids," says Steven R. Myers, a toxicologist at the University of Louisville (Ky.) School of Medicine. "This is a very good piece of work."

The gene *HPRT* is named for the enzyme it encodes, hypoxanthine-guanine phosphoribosyltransferase. This gene is an excellent indicator of mutations that can arise in a cell when outside influences—in this case, chemicals derived from tobacco smoke in the mother—apparently cause the DNA chain to break and reform haphazardly, Finette says. In particular, certain *HPRT* mutations point to misguided action by a combination of enzymes called V(D)J recombinase, or V(D)J.

"V(D)J recombinase is a critical enzyme system for rearranging DNA," Finette says. "It cuts DNA, removes pieces, and puts the other [strand] ends together."

This enzyme cluster is essential to the immune system. As children grow, they encounter viruses, bacteria, and foreign substances, and V(D)J is instrumental in reshaping DNA to encode immune system compounds that fend off the potential invaders. A recognizable type of *HPRT* mutation tips off scientists that this vital V(D)J activity has been disrupted.

Previous research uncovered evidence of V(D)J running amok in childhood leukemia and lymphoma. Because pediatric tumors occur early, the genetic changes associated with them may start in the womb with disruption of V(D)J activity, Finette and his colleagues suspect.

Searching for evidence of V(D)J irregularities, the investigators studied umbilical cord blood taken from 24 newborns, half of whose nonsmoking mothers were exposed to smoke during pregnancy. In the babies of the exposed mothers, they found a wide variety of mutations, including deletions of parts of the DNA chain that contain the *HPRT* gene. In these babies, 18 of 35 mutations analyzed were of the deletion variety. Of these, 12 were the result of V(D)J rearrangements of the *HPRT* gene. Of 30 mutations in the nonexposed infants, 20 were deletion mutations but only 6 were caused by V(D)J.

Researchers are now striving to determine how the body makes V(D)J. They hope to clarify its mechanism of action and why it can act on the wrong genes.

"If [the *HPRT* finding] is confirmed, this will add to the credibility of the epidemiological findings linking prebirth exposure to carcinogens with childhood leukemia," says Sholom Wacholder of the National Cancer Institute in Bethesda, Md. He says that future research will require more information detailing where mothers encountered the smoke and in what amounts. — *N. Seppa*