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Anthrax Stages Two-Pronged Attack on Cells, New Research Shows

Terrible and swift as anthrax appears to its victims, the deadly toxin takes its time breaking into their cells. The entry of anthrax toxin into its cellular target is part of a carefully-planned, two-pronged attack, scientists have found. Howard Hughes Medical Institute international research scholar Gisou van der Goot has identified for the first time the cell signaling event that sets the deadly strike in motion.

In a paper published the week of Dec. 28, 2009, in the journal, *Proceedings of the National Academy of Sciences*, van der Goot and her colleagues at the Global Health Institute of the École Polytechnique Fédérale de Lausanne in Switzerland reveal how the anthrax toxin carefully times its attack, slowly assembling its component parts on the surface of a target cell before suddenly hijacking the cell's own signals. The van der Goot lab reports that the assembled toxin, moored to a receptor outside the cell, directly activates an enzyme family inside the cell, called src-like kinases. The receptors—with the anthrax toxin attached—are pulled by the kinases into the cytoplasm for digestion. But once inside, the toxin instead slices up the cell.

Scientists had known that anthrax toxin hovers outside of cells, but no one knew how it got inside the cells. Van der Goot and her colleagues identified anthrax's two step strategy. In the first stage, the bacterium, *Bacillus anthracis*, pumps out two molecules: edema factor (EF) and lethal factor (LF). But EF and LF don't become dangerous to individual cells until stage two, when the anthrax bacteria creates a protective antigen (PA). PA is the key that allows EF and LF inside the cell through two common cell surface receptors, TEM8 and CMG2. Together, those three components—PA, EF, and LF—make up the anthrax toxin.

Once docked onto one of the receptors, PA begins assembling a seven-molecule structure—called a heptamer—that ensures EF and LF are correctly positioned to get inside the cell. It also signals the src-like kinases to pull the heptamer inside, buried inside a cellular bubble called an endosome. Once EF and LF are safely in the cell, they burst into the cytoplasm and cut important cellular proteins.

This effort to assemble the heptamer explains why anthrax toxin seems to hesitate on the cell's surface before invading, van der Goot says. If PA enters the cell too early—before the assembly is complete—PA opens up the cell but EF and LF are left outside and the toxin won't work, she says.

Van der Goot is especially intrigued by the anthrax toxin's use of the host's cell receptor CMG2. Mutations in CMG2 have also been implicated in systemic hyalinosis, a rare genetic disorder that manifests itself in newborns as severe joint problems, recurrent intestinal illnesses, and pulmonary infections. The CMG2 receptor may be forced into service as an anthrax toxin receptor, van der Goot says, "but it's not its job to be the anthrax receptor. It's there to do something else." This study is the first to reveal a connection between hijacked CMG2 receptor and src-like kinases—more commonly involved with responding to epithelial growth factors—and may help scientists figure out the cause of systemic hyalinosis.

This research is just the beginning of understanding how the anthrax toxin works. "It's likely much more complex," van der Goot says. Anthrax has a long and resilient evolutionary history, she says, and it is likely to employ more than one signaling pathway to enter host cells. Her lab is pursuing other pathways that might explain how the ingested toxin moves inside the cell. They are also investigating how the cytoskeleton is co-opted into moving the deadly freight into the cell.

Van der Goot says it is unlikely that drugs that block src-like kinases will be a treatment for anthrax. Src-like kinase blockers exist but they might be unsafe for humans, she explains. They are used primarily by labs studying tumors where mutant src genes have long been identified as cancer-associated genes. "Still this is the first evidence that src-like kinases are important in anthrax," she says.