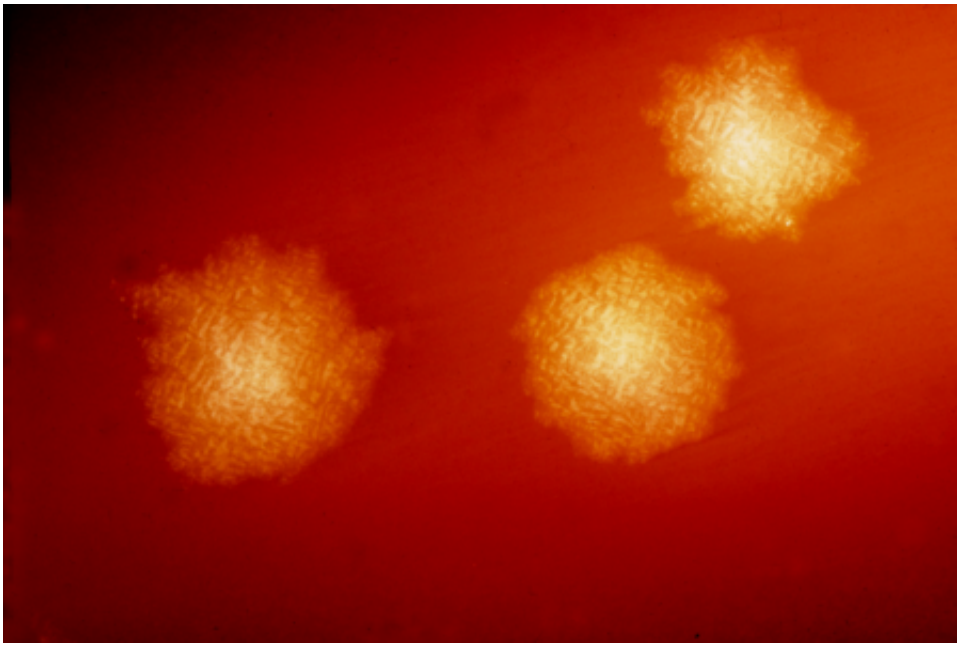
Medical Xpress

Researchers reveal how dangerous intestinal toxin enters cells

September 29 2016, by Erin Tornatore



This photograph depicts Clostridium difficile colonies after 48hrs growth on a blood agar plate; Magnified 4.8X. C. difficile, an anaerobic gram-positive rod, is the most frequently identified cause of antibiotic-associated diarrhea (AAD). It accounts for approximately 15–25% of all episodes of AAD. Credit: CDC

Researchers have identified a key target in fighting the emerging, life-threatening gastrointestinal infection *Clostridium difficile*. The work, led by Min Dong, PhD, and Liang Tao, PhD, of Boston Children's Hospital, reveals how the bug's most potent toxin gets into cells, the first step in developing treatments against it. Findings are published today in *Nature*.

Clostridium difficile, also called "C. diff," causes severe diarrhea and intestinal inflammation. It can be hard to eradicate and has become a leading cause of death from GI illness. C. diff tops the CDC's list of urgent drug-resistant threats, causing half a million infections a year in the U.S. alone. The infection is most a threat in hospitals and long-term care facilities and in people on long-term antibiotic treatment.

"Antibiotics clear out the normal intestinal bacteria and create a space for C. diff to colonize and grow in the colon," explains Dong, an assistant professor in the Department of Urology at Boston Children's Hospital and Harvard Medical School's Department of Microbiology and Immunobiology.

Dong and Tao, together with researchers at University of Massachusetts Medical School at Worcester, found that a key C. diff toxin enters cells through a receptor called Frizzled—ironically, also known to be the entryway for signals that help keep the colon healthy.

Identifying the Frizzled receptor

C diff produces a variety of toxins, the best known being toxins A and B. Dong, the study's senior investigator, chose to focus on toxin B, which has been shown to cause disease on its own, even when toxin A is absent.

"We knew that these toxins target and disrupt colonic epithelial cells, and that they may hijack a cell surface protein as its landing pad or receptor," says Dong. "Yet the specific receptor for toxin B had remained elusive."

Frizzled was identified through a novel approach. Tao, a postdoctoral fellow in the Dong Lab, teamed up with Paul Meraner, MD, a fellow in the lab of Abraham Brass, MD, PhD, at UMass Medical School. Together, they first conducted genome-wide screening, using the newly

developed CRISPR/CAS9 gene editing technology, to mutate genes in cultured human cells.

"CAS9 technology originates from bacteria, so it was gratifying to use CAS9 to learn about one of the worst types of bacteria in the hospital," says Brass. "As a GI doctor, I've seen too much hardship caused by C. diff and look forward to the day when we beat it."

Tao and Meraner now had plates of cells, each cell with a mutation disabling a different gene. "We then added toxin B to these plates and looked for cells that were resistant," says Tao.

Most cells were killed by the toxin, but not all. After four rounds of screening, the researchers had a population of cells that were unharmed. Those, they reasoned, had a mutation that protected them from the toxin. But in which gene?

The researchers turned to next-generation sequencing, which revealed a number of mutations in the surviving cells, "among them a cell surface protein called Frizzled," says Tao.

Following up on this lead, Tao first demonstrated that toxin B indeed uses Frizzled as its receptor to invade cultured human cells. Next, together with Ji Miao, PhD and David Breault, MD, PhD, in Boston Children's Division of Endocrinology, Tao used colonic stem cells to create "organoids"—3-D cultures of intestinal tissue in miniature. When the gene for Frizzled was deleted, the organoids became resistant to the toxin; those that retained the Frizzled gene showed obvious damage.

Finally, Tao worked with Jie Zhang, PhD, a senior scientist in the Dong lab, to do studies in mice, which gave parallel results.

Potential C. diff therapeutic strategies

Interestingly, Frizzled is also the receptor for "good" signals critical to repairing and maintaining tissue in the colon, via a well-known pathway known as Wnt.

"Stem cells in the colon rely on Wnt signaling to keep them functioning and generating new cells," explains Dong. "Toxin B and Wnt signals compete to bind to the Frizzled receptor, suggesting that toxin B may directly inhibit Wnt signaling in cells."

To confirm this, Tao and Dong worked with Wnt experts Xinjun Zhang, PhD, and Xi He, PhD, at Boston Children's F.M. Kirby Neurobiology Center. Together, they demonstrated that toxin B strongly inhibits Wnt signaling.

"This finding suggested that toxin B can be particularly detrimental to colonic stem cells," says Dong. "It would be important to restore Wnt activity and maintain the health of stem cells in combating these toxins."

Tao and Jie Zhang identified one potential strategy to protect the colon: they found that a recombinant fragment of Frizzled protein can be utilized to "soak up" toxin B. Another potential approach would be to stimulate Wnt signaling directly.

Cancer treatment?

The Wnt pathway is also known to be important in cancer cells. Pharmaceutical companies are trying to develop antibodies that would block Frizzled. Dong and colleagues speculate that a fragment of the C. diff toxin—one that isn't toxic—could also be a research tool to study Wnt signaling and or even possibly a cancer therapy.

More information: Liang Tao et al. Frizzled proteins are colonic

epithelial receptors for C. difficile toxin B, *Nature* (2016). DOI: 10.1038/nature19799

Provided by Children's Hospital Boston