

## Loosen Up

### Bacterial toxin may lead to less painful treatments for diabetes and brain cancer

By JOHN TRAVIS

Take the nasty bacterium that causes cholera, delete the gene for its well-studied toxin, and you should end up with a harmless microbe that can immunize people against the real thing. It sounded like a good plan, recalls Alessio Fasano of the University of Maryland School of Medicine in Baltimore.

The scheme failed, however. A decade ago, when Fasano and his colleagues used a genetically stripped *Vibrio cholerae* to vaccinate 10 people, half the volunteers developed mild diarrhea. The altered bacterium obviously had some bite left in it.

The effort to understand what went wrong in that vaccine experiment has shifted the interests of Fasano and his colleagues to topics far from cholera. In 1991, they identified a second *V. cholerae* toxin that contributes to the diarrhea of the disease. Since then, they've learned that this toxin mimics a protein that the body uses to regulate the permeability of the seams between cells within the intestine, brain, heart, and other tissues.

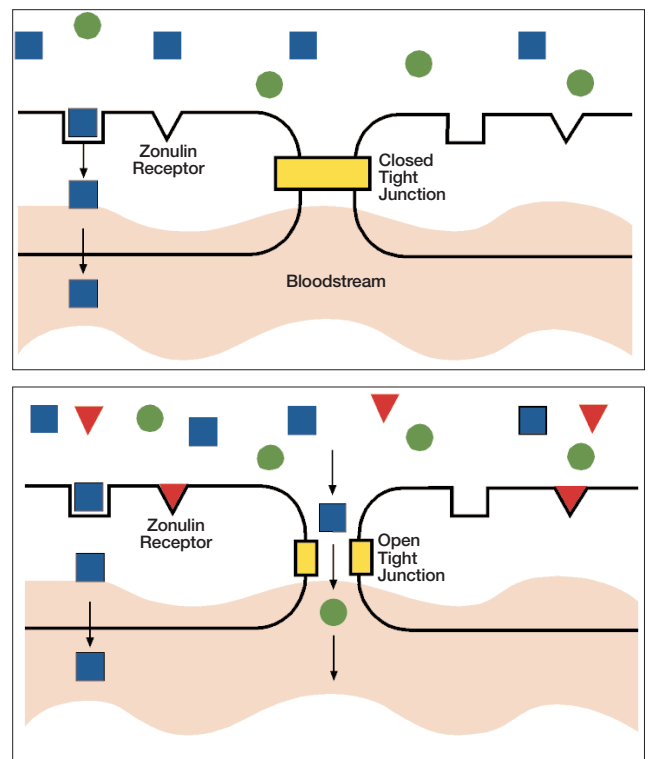
Fasano sees many uses for this human protein. By helping drugs slip out of the intestine and into the bloodstream, the protein may become a component of an insulin pill that replaces a diabetic person's periodic shots. A similar approach could enable scientists to sneak medications past the formidable blood-brain barrier.

Moreover, the discovery of the way the body normally controls tight junctions, the specialized seams between cells, may help investigators explain why the junctions sometimes aren't tight enough. Leaky junctions occur in disorders including diabetes, celiac disease, and even some brain illnesses.

"It's the typical example of scientific serendipity. We were looking for one thing and came up with something totally different," chuckles Fasano.

If the cells that line the intestine represent bricks in a wall, tight junctions are the mortar that binds them together, or so scientists long thought. More recently, as they've identified some of the proteins that make up a tight junction, investigators have begun to realize that the bricks-and-mortar analogy is misleading. "Our molecular understanding of the junction complex is just exploding now," notes Mark Donowitz of the Johns Hopkins Medical Institutions in Baltimore.

Tight junctions have proven to be far more flexible and dynamic than mortar. Although the



Tight junctions (yellow) normally seal the space between cells in the intestine (top). Then, nutrients or drugs (blue and green) can only cross into the bloodstream when the cell takes them in, using proteins on the cell surface. Zonulin (red) and the bacterial toxin Zot can trigger cells to open their tight junctions (bottom), permitting molecules to move into the bloodstream.

junctions in the intestine usually prevent proteins, water, and other molecules from passing between adjoining cells, they occasionally permit some molecules to slip through. In fact, cells can signal the tight junctions to alter their permeability, says Fasano.

The cholera toxin that his group discovered has now led the researchers to a regulator of the junction. The cholera toxin identified earlier directly increases secretions from the cells lining the intestine, resulting in diarrhea. Fasano's toxin instead opens tight junctions, enabling water and other molecules from blood to seep between the cells and into the intestine.

Drawing on the scientific name for tight junctions, the investigators dubbed the bacterial protein zonula occludens toxin, or Zot. They also identified the protein on intestinal cells to which Zot binds, triggering the loosening of the tight junctions.

Fasano and his colleagues immediately wondered if there was a human protein resembling the bacterial toxin. After all, because of their intimate and long-term association with the organisms they infect, bacteria are among the best cell biologists around. The microbes frequently make proteins that mimic some of the host's molecules.

Sure enough, the investigators found a Zotlike human protein, which they named zonulin. Zonulin, too, makes the intestine "leakier," says Fasano. To confirm that the human intestine produces this protein, the scientists obtained various tissues from a cadaver and screened them for zonulin by using antibodies that latch onto Zot. As expected, intestinal tissue contained the protein, but so did heart, brain, and a few other tissues.

This briefly perplexed the researchers, until they remembered that tight junctions join cells in many different regions of the body. Indeed, next to the intestinal lining, the best-known locale for tight junctions is the blood-brain barrier. This sheet of cells lines the blood vessels that snake through the brain, creating a barricade that protects the delicate nerve tissue from potentially damaging molecules within the bloodstream.

In the January *JOURNAL OF NEUROCHEMISTRY*, Fasano's group confirms that brain tissue contains the cell-surface protein that zonulin binds to induce a cell to open its tight junctions. Furthermore, the scientists have confirmed that in pieces of the blood-brain barrier grown in dishes, both zonulin and Zot open the tight junctions.

"We have precious little understanding of the cell biology of blood-brain-barrier tight junctions. This adds to that. It's a nice piece of work," says William M. Pardridge of the University of California, Los Angeles.

Fasano calls zonulin a key that opens a gate between cells. He notes that the human body actually has several similar keys. Zonulin made in the intestine differs by a few amino acids from the ones produced in the brain or in other tissues. The subtle alterations seem to prevent zonulin meant for one tissue from altering the tight junctions of another.

"It makes sense," says Fasano. "[The body] may have a need to make the intestine leakier but leave the blood-brain barrier unaffected, or vice-versa."

While intrigued by zonulin's potential to help elucidate the workings of tight junctions, Fasano is far more excited by the prospect of using the protein to improve human health. He envisions physicians administering zonulin or Zot to deliver drugs through the intestine into the bloodstream. This technique might eliminate the need for injecting many drugs, he says.

"We can make the intestine leakier to the point that we can allow passage of molecules not normally absorbable by the intestine. The implications of this are tremendous," says Fasano.

An obvious candidate for such a strategy is insulin, the hormone that people with diabetes take to control their blood sugar. Like many other proteins with medical uses, insulin molecules are too large to be absorbed by intestinal cells or to slip past the tight junctions. So, oral insulin stays in the digestive tract, where it gets broken apart. That's why people must inject themselves with the drug, an onerous task, especially for kids.

In a 1997 mouse study, Fasano's team found that Zot dramatically increases the intestine's ability to absorb insulin. Moreover, mice given oral-insulin doses with Zot controlled their glucose levels just as well as mice receiving insulin injections did. Recently, the same approach has controlled the blood-glucose concentrations of diabetic monkeys, adds Fasano.

Wouldn't the toxin trigger diarrhea? None of the animals experienced that side effect or suffered any other problems, says Fasano.

Water from the bloodstream does seep into the intestine, but the colon, where Zot has no effect, apparently reabsorbs it. This suggests that Zot alone can't trigger diarrhea and that other molecules made by *V. cholerae* must contribute to that symptom. Moreover, the Zot-induced change in intestinal tight-junction permeability reverses itself within a half hour, Fasano adds.

The University of Maryland, which owns several patents stemming from the zonulin research, has assigned most of them to Zone Therapeutics, a firm in Rockville, Md. With the success of the animal testing, the company now plans to ask the Food and Drug Administration for permission to start testing the insulin-Zot or insulin-zonulin strategy on people.

There's certainly a strong demand for an insulin pill. When Fasano and his colleagues published their study of mice, people with diabetes besieged his office with requests for oral insulin.

The investigators believe that Zotlike compounds will also let them breach the blood-brain barrier. This barricade has frustrated drug developers for many years. "More than 98 percent of all drugs do not cross the blood-brain barrier," notes Pardridge.

Because of this obstacle, physicians targeting the brain must directly inject most drugs through the skull, a risky and infection-prone procedure. Working with mice and a tracer that mimics drugs, Fasano's group has tried something far simpler—with success.

"When we administer both Zot and a tracer, we see the tracer reaching the brain," says the researcher. Buoyed by these still unpublished results, he suggests this strategy could help physicians get chemotherapy drugs to brain tumors.

Pardridge expresses skepticism about this approach, however. "If you're going to deliver drugs by disrupting the blood-brain barrier, you're going to let in everything else in the bloodstream," he warns, pointing out that other compounds that disrupt the barrier have induced seizures in animals.

Fasano counters that those compounds irreversibly destroy the barrier, whereas Zot or zonulin would open tight junctions of the blood-brain barrier just long enough to allow transport of a drug across it. Preliminary tests with rodents support this belief, he says.

"If well used, zonulin should not cause any side effects, since the process is quick and reversible within a few minutes," asserts Fasano.

Researchers other than Pardridge also worry that Fasano is overly optimistic at this early stage in his research. They note that he hasn't yet announced the finding of the gene for zonulin or provided a detailed description of the protein in a scientific journal.

"The story sounds wonderful, [but] there's information that needs to be filled in: the precise identity of the protein, where it's made [in the body], and how it works," cautions Jeffrey B. Matthews of Beth Israel Deaconess Medical Center in Boston, who studies intestinal permeability.

Fasano says that his group has such data but waited for the University of Maryland to patent zonulin and its uses. A number of publications should appear soon, he says.

Fasano predicts that besides delivering drugs, zonulin will help explain facets of several diseases. Take celiac disease, an enigmatic intestinal disorder seemingly triggered by abnormal sensitivity to gluten, a component of wheat, barley, and other grains. People with celiac disease suffer a broad array of symptoms, including diarrhea, weight loss, and malnutrition.

Curiously, people with celiac disease also seem to face an unusually high risk of developing autoimmune disorders, such as type I, or juvenile-onset, diabetes.

Scientists have yet to develop a good explanation for what goes wrong in celiac disease, but Fasano's team may have found an important clue. It's been known for some time that intestinal tight junctions become more permeable in celiac disease, especially when the disorder flares up. "These gates are stuck open, and we don't know why," says Fasano.

He and his colleagues recently reported at a meeting of pediatricians in Agrigento, Italy, that intestinal tissue from people with acute celiac disease contains much more zonulin than does similar tissue from people free of the disease. The investigators hypothesize that gluten, for a still unknown reason, stimulates inappropriate zonulin production, leading to the condition's varied symptoms.

They also speculate that the leaky tight junctions in a person with celiac disease may allow molecules to leak from the intestine into the blood and trigger autoimmune reactions. To support this idea, Fasano has examined laboratory rats that spontaneously develop diabetes because their immune systems begin attacking insulin-producing cells in the pancreas. These rodents, he finds, always have an increase in intestinal permeability several weeks before the diabetes symptoms appear.

As scientists pay more attention to zonulin and its ability to regulate tight junctions throughout the body, they may link the protein to additional illnesses, Fasano predicts. Although no one has yet implicated zonulin in Alzheimer's disease, one of the symptoms of the disorder is a breakdown of the blood-brain barrier.

"We have also identified an important heart disease in which zonulin seems to be involved," says Fasano, hinting at a new line of investigation.

Not bad for a failed vaccine experiment. □