Stanford investigators decipher how dangerous food-borne pathogen evades body's defenses

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Scientists at the Stanford University School of Medicine have pushed into place another piece of the puzzle of how *Listeria monocytogenes*, a dangerous food-borne pathogen, slips through the intestine's defenses and causes disease.

Listeria monocytogenes is a hardy, if unwelcome, brunch guest. This bacterial strain thrives in salty or cold environments where prissier pests might perish: cold cuts, smoked salmon, soft cheeses and many a refrigerator. Abundant in the environment, it seldom causes disease in humans — but when it does, it's deadly. Responsible for a mere 0.02 percent of food-borne illness, Listeria accounts for more than one in four deaths due to food-borne infections in the United States, according to the Centers for Disease Control and Prevention.

Manuel Amieva, MD, PhD, assistant professor of pediatrics and of microbiology and immunology, is the senior author of a study that describes the way Listeria grabs onto molecular handles on cells in the small intestine and then switches on those cells' own uptake systems to hitch a ride inside. The study will be published online May 13 in PLoS Pathogens. Mickey Pentecost, PhD, a recent graduate from Amieva's laboratory, is the first author.

We think of the small intestine as a long, smooth, hollow tube through which torrents of nutrient-rich food flow. But close inspection shows

that the gut's inner surface lining, known as the intestinal epithelium, is anything but smooth. In fact, it's much more like the Rocky Mountains than the Great Plains. The gut's serrated character vastly increases its surface area, which in turn increases nutrient absorption.

The microscopic mountains of the intestine's rugged surface terrain are called villi. Amieva and his colleagues have previously shown that cells at the very tips of villi are particularly vulnerable to infection by Listeria. But gaining that unauthorized access is no mean feat. The cells of the intestinal epithelium are tightly stitched together by molecules on their cell surfaces that connect them to each other.

The tight junctions formed in this way effectively seal off the intestine from the microbes that course through our digestive tracts. And luckily so: Even normally benign bugs — there are trillions of them in our gut — would cause serious problems if they could squeeze through the intestinal wall and enter the bloodstream.

Listeria and other invasive organisms manage to get around that seal, said Amieva. "You get a new intestinal epithelial lining every week," he said. "The cells at the tips of the villi are constantly dying and being shed, at the rate of 10 billion a day. When a cell dies it is shed into the hollow space of the gut. To avoid leaving holes, the surrounding cells quickly move closer together and re-assemble tight junctions. But in the process, the normally hidden molecules that dot the sides of the cells abutting the hole — like the sides of teeth adjacent to one that just fell out — get transiently exposed."

And therein lies a tale. As it turns out, Listeria exploits these exposed spots by doing a sophisticated two-step.

Like many other microbes, Listeria manufactures a couple of hooks (Listeria's are known as Internalin A and Internalin B) that grab onto

specific molecules protruding from cells of the intestinal lining. Internalin A's targeted molecule, E-cadherin, is a key player in forging the junctions between adjacent cells of the gut epithelium. The target for Internalin B is C-Met, a receptor for a growth factor (growth factors are external molecules that can drastically change a cell's behavior).

Oddly, though, both E-cadherin and C-Met are typically located along the normally inaccessible sides of villus cells. This was puzzling, said Amieva. "E-cadherin and C-Met would seem to be unlikely attachment sites. Why on earth would a microbe pick such hard-to-reach sites to grab onto?"

Amieva's group had also previously shown that Internalin A is able to find E-cadherin only at the special remodeling junctions that occur as cells are being shed from the epithelial lining. The new study throws light on the subtle role played by Internalin B once Internalin A locks onto E-cadherin.

The group discovered that by activating C-Met, Internalin B hastens the uptake of cell-surface molecules, including E-cadherin, at villus tips where cells have been shed. Thus, Internalin A lets the bacteria find a hidden door, while Internalin B presses the button to the elevator on which Listeria rides into the cell's business offices.

When the investigators compared, in a mouse model, the infectivity of a Listeria variant containing a functional Internalin B versus that of an engineered strain missing the molecule, they found that the bugs retaining Internalin B function were the best at being bad.

"The mutant bacteria lacking Internalin B adhered just as well as the bacteria that had it, but were slower to get inside the cells," Amieva said.

And when the researchers sprinkled purified Internalin B on top of a

layer of cells plated onto filters, they saw an uptick in the cells' uptake of molecules from their outer surfaces, mainly at sites of cell shedding. This showed that by activating C-Met at those sites, Internalin B can accelerate the uptake of E-cadherin, along with the bug that's clinging to this molecule.

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