Bacteria hijack host cell process, create their own food supply to become infectious

November 29 2012, by Emily Caldwell

Bacteria that cause the tick-borne disease anaplasmosis in humans create their own food supply by hijacking a process in host cells that normally should help kill the pathogenic bugs, scientists have found.

This bacterium, *Anaplasma phagocytophilum* (Ap), secretes a protein that can start this process. The protein binds with another protein produced by white blood cells, and that connection creates compartments that siphon host-cell nutrients to feed the bacteria, enabling their growth inside the white blood cells.

The finding defies conventional wisdom about most bacteria, which try to avoid this cellular process. Called autophagy, the process allows a cell to digest parts of itself to produce energy when it is experiencing starvation. But that digestive feature also is enacted by the immune system to help clear away certain intracellular pathogens, including those that cause salmonellosis or shigellosis.

The Ap bacterium, however, launches and then manipulates the autophagy process to its own advantage.

"This study shows how bacteria subvert natural processes," said Yasuko Rikihisa, professor of veterinary biosciences at Ohio State University and lead author of the research. "They are creating their own food supply through a cellular mechanism that hurts other infectious bacteria. And because this process doesn't cause inflammation, they do it very gently, becoming an insider that eventually kills the host cell."

The finding could help identify new targets for drugs to treat this infection, which is a rare but emerging infectious disease that can be lethal for the elderly and people with compromised immune systems. The current first-line treatment is the antibiotic doxycycline.

Also known as human granulocytic anaplasmosis, the disease affects more than 1,000 people per year in the United States, up from just 348 reported cases in 2003, according to the Centers for Disease Control and Prevention. It is transmitted to humans by tick bites primarily from the black-legged tick and the western black-legged tick.

The study appears online this week in the early edition of the *Proceedings of the National Academy of Sciences*.

The Ap bacterium secretes substances to perform a process resembling mating to infect host cells, primarily the granulocyte class of white blood cells that fight off invading pathogens. Rikihisa's lab previously identified a protein called Ats-1 that is secreted by Ap bacteria during this process.

In this new study, the researchers found that once inside the host cells, Ats-1 binds to another protein called Beclin 1, which is part of a system of molecules involved in the earliest stages of the autophagy process.

The scientists observed that when the two proteins bind, they create little bubbles known as vesicles. Through a series of experiments, the researchers determined that these bubbles were in fact autophagosomes – bubble-like compartments that are formed as a cell prepares to undergo autophagy.

They were able to confirm this by imaging the vesicles to determine that they had the tell-tale double membrane characteristic of autophagosomes, and by testing for the presence of other compounds

that serve as markers of the initiation of the cell-digesting process.

Under normal circumstances, autophagosomes contain the nutrients that are meant to be digested and recycled for other uses – but in this case, the bacteria take those nutrients to promote their own growth.

That Ats-1 could start this process on its own represents a rare power for a single protein.

"We believe this is the first bacterial protein that has been found to do this," said Rikihisa, also an investigator in Ohio State's Center for Microbial Interface Biology and Comprehensive Cancer Center. "Ats-1 initiates an early stage of the autophagosome, then picks up nutrients from the main body of the host cell and closes the layers."

Most of the action of bacterial growth takes place inside a special compartment that typically doesn't contain many nutrients. But after the Ap bacterial protein starts this process of producing autophagosomes that can encase nutrients, these bubbles fuse with the compartment, creating a steady supply of food at the site of bacterial replication and growth.

The researchers showed this by further imaging the bubbles to determine that an autophagy marker protein could be found both inside and outside of the bacterial growth compartments. They also showed that infected cells did not contain any lysosomes, which are cell parts that perform the actual digestion and degradation of foreign bodies during autophagy.

An experiment in mice deficient in the Beclin 1 protein showed that infection levels were much lower if mice had low levels of this protein – confirming its role in binding with Ats-1 and producing autophagosomes to promote infection. In cell cultures, the researchers also showed that when Ats-1 was overproduced, bacteria grew 10 times more effectively

than they did in cells in which an unrelated protein was overproduced.

All of this activity allows the bacteria to remain hidden from the immune system because the induction of autophagy is considered a normal cell function and it does not produce any inflammation, which would recruit infection-fighters to the scene. Instead, the Ap bacteria set themselves up comfortably inside granulocytes and steadily grow for a few days until they rupture their host cells and generate a strong immune response – which makes an infected person sick.

In one final experiment, Rikihisa and colleagues blocked autophagosome production in Ap-infected cells using an experimental drug called 3-MA. With that process blocked, bacterial growth declined dramatically.

3-MA is toxic to humans, but its effectiveness in blocking the infectious properties of Ap in cells suggests that its structure could serve as the basis for a safe small-molecule drug, Rikihisa said.

"A similar compound could be a potential treatment to inhibit bacterial growth," she said.

Clarifying the power of Ats-1 in inducing autophagy also suggests that this protein could be an important tool in further studies of this complex cell process that remains poorly understood, she added.

Provided by The Ohio State University