Long-ignored enzyme turns out to be key to killing infectious bacteria

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New research shows that an enzyme that has long been considered relatively useless to the immune response instead has an important role in setting up immune cells to kill infection-causing bacteria.

Ohio State University scientists have determined that this enzyme, called caspase-11 in mice, enables components in immune cells to fuse and degrade the bacteria that cause Legionnaires' disease, a type of pneumonia. Without that fusion and degradation, these bacteria thrive, grow or replicate and cause illness. Whether the effect is the same in other bacteria remains unknown.

The parallel enzyme in humans is a combination of caspases 4 and 5. The researchers determined that *Legionella pneumophila* bacteria somehow suppress activation of these two enzymes in human cells. But if the enzymes are added back into immune cells, they set off the same fusion events - those also seen in mice - that will kill the bacteria.

The findings could lead to the development of non-antibiotic drugs designed to fight certain bacterial infections by activating these caspases. Those most vulnerable to Legionnaires' disease include the elderly, smokers and people with chronic diseases or compromised immune systems, including patients with cancer and AIDS.

"If there were a therapeutic way to express, deliver or induce caspase 4 and 5, humans wouldn't get *Legionella* infection. Imagine if you just replenish cells with these caspases, working around *Legionella*'s tricky

way to suppress them, and then the infection would not happen. That would be huge," said Amal Amer, assistant professor of microbial infection and immunity and internal medicine at Ohio State and senior author of the study.

The research appears online and is scheduled for future print publication in the journal *Immunity*.

Amer said these findings represent a paradigm shift in caspase research. For years, studies have suggested that a different enzyme, caspase-1, was required to set up cells to kill bacteria. Most published research suggested that caspase-11 was necessary only to activate caspase-1.

A common way to test an enzyme's role and effectiveness is to see what happens to cells when the enzyme is not present. A mouse strain genetically altered so it would not have caspase-1 genes served as the basis for many studies that appeared to confirm caspase-1's importance to the immune response against a range of bacterial, viral and fungal infections.

It turns out, however, that those same mice also did not have caspase-11 because the genes responsible for the two enzymes are located very close to each other. So studies that pointed to caspase-1 as the critical enzyme in clearing away pathogens did not take into account any role that caspase-11 might have played.

"Scientists should go back and test whether it was really caspase-1 or caspase-11 clearing the bacteria, viruses or fungi," said Amer, also an investigator in Ohio State's Center for Microbial Interface Biology and Davis Heart and Lung Research Institute. In fact, Amer is among the scientists who have published studies about caspase-1.

She noted that caspase-1 has been considered an attractive drug target

because of these many studies. However, driving up activation of caspase-1 to clear bacteria can have a troubling side effect - this enzyme also causes extensive inflammation. Caspase-11 appears to have no such effect, making it a potentially more desirable drug target for pharmaceutical companies, she said.

Amer and colleagues demonstrated how caspase-11 helps clear *Legionella* bacteria in a series of experiments using mouse and human cell cultures and mice genetically altered so they wouldn't produce caspase-11.

As immune cells known as macrophages recognize these bacteria, they consume the bacterial cells and place them inside a compartment called a phagosome. Under normal circumstances, this phagosome will fuse with another cell component called a lysosome. When the two compartments join, the lysosome breaks the unwanted infectious bacteria into pieces.

The research showed that caspase-11 in mice, and caspase 4 and 5 together in human cells, made that fusion occur, and did so in an unexpected way. These types of enzymes influence activation of proteins, and typically do so by snipping a compound in a specific way to generate the protein needed by a cell. But caspase-11 instead activates one of its target proteins, called actin, by adding a phosphorous group to it - a process called phosphorylation.

"If we put pathogenic bacteria in a normal cell, they may or may not succeed according to the pathogen, but in a cell that doesn't have casapse-11, the bacteria are going to survive," Amer said. "Without caspase-11, the phagosome doesn't fuse with the lysosome, and *Legionella* survives, replicates and causes infection."

She added that in human cells, the *Legionella* bacteria somehow suppressed the activation of caspases 4 and 5 - the researchers don't yet

know how the bacteria pull off this stunt. But when they added the two caspases back to immune cell cultures containing *Legionella* bacteria, the bacteria no longer survived.

Amer specializes in *Legionella* research, but she also tested whether Salmonella bacteria can suppress caspases 4 and 5 in human cells. "Salmonella does not do that. It's a very peculiar trick for *Legionella*," she said.

She also noted that caspase-11 is activated only when a pathogenic bacterium enters a cell, and is not needed to clear bacteria that do not cause infection.

"That's exciting, because it means that either caspase-11 can differentiate according to the type of bacteria present, or that two different bacteria, pathogenic and nonpathogenic, enter a cell from completely different pathways - one that engages caspase-11 and another that doesn't," Amer said. "We don't know yet which scenario applies, but that finding provides more evidence that caspase-11 is required to clear certain pathogenic bacteria."

She also noted that caspase-1 can generate the fusion of lysosomes and phagosomes to degrade pathogenic bacteria "to a small degree," but that her research shows that "caspase-11 is the big player."

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