New study sheds light on how intracellular pathogens trigger the immune system

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(PhysOrg.com) -- Disease-causing microbes like the food-borne bacterium Listeria monocytogenes specialize in invading and replicating inside their animal hosts' own cells, making them particularly tricky to defeat. Now, a new study led by biologists at the University of California, Berkeley, has identified a molecular alarm system in which the intracellular pathogen sends out signals that kick the immune response into gear.

The findings, to be reported the week of July 14 in the journal *Proceedings of the National Academy of Sciences,* shed light on how the cells recognize and destroy the pathogenic bugs living within their walls, and may even provide new targets for the research and development of new vaccines and drugs.

The pathogens' signals come from multidrug resistance transporters (MDRs), membrane proteins used by a wide variety of organisms to pump out a broad range of molecules from their systems. Similar transporters have been linked in other studies to the development of resistance to multiple drugs that are toxic to the pathogen. This study is the first to connect multidrug resistance transporters directly to stimulation of the immune system, although the nature of the molecules that the bacteria are spitting out remains unclear.

"For the MDRs to work, the pathogen needs to be alive, so this study actually shows how the immune system can tell the difference between a living, harmful microbe and one that is dead," said the study's principal

investigator, Daniel Portnoy, a UC Berkeley professor with joint appointments in the Department of Molecular and Cell Biology and the School of Public Health, and associate director of the Berkeley Center for Emerging and Neglected Diseases. "This is important because you don't want the immune system to overreact to non-threats, which is what happens in autoimmune disorders such as inflammatory bowel disease, asthma and multiple sclerosis."

The Listeria bacterium makes headlines when it contaminates deli meats, raw cheeses, cole slaw and other foods. According to the Centers for Disease Control and Prevention, Listeria causes some 2,500 infections and 500 deaths each year, and at greatest risk are people who have weakened immune systems or are pregnant.

The bacteria first trick immune cells into swallowing them, where they become encased in bubbles called vacuoles. The bacteria become dangerous when they break out of these bubbles into the cells' internal fluid, or cytosol, to multiply and spread the infection. The role of MDRs is not clearly known, but the results of this study plainly show that one particular MDR transporter is necessary for the host to respond to the infection, the authors said. In addition, overexpression of this or other related MDRs leads to an enhanced host immune response.

"The only way the bug molecule enters into the cytosol is if the bacterium is virulent," said Portnoy, who is also a member of UC Berkeley's Health Sciences Initiative. "We know that there are different immune system receptors in different compartments of a cell, but until this paper, it was not understood exactly how the cytosolic surveillance system was triggered. Our findings suggest that the molecules pumped out by the pathogen while it's in the cellular fluid help the immune system gauge whether a bacterium is a threat based upon its location inside the cell."

The researchers isolated the role of multidrug resistance transporters by manipulating specific genes in the bacteria that controlled their expression and then measuring how increased or decreased activity by the transporter proteins impacted levels of interferon beta, a protein produced by the immune system that rally more disease-fighting cells when infections are detected.

They found that greater MDR expression led to greater stimulation of the immune system, as measured by interferon beta levels.

Strains of Listeria with higher levels of MDR expression increased interferon beta levels up to 20-fold compared with unmodified, wild-type Listeria in cell cultures, the study found. Tests in mice infected with those same mutant strains of Listeria had bacterial loads that were 20 times lower in their livers, although the researchers could not attribute the decreased levels solely to the higher levels of interferon beta.

"This paper raises the classic issue of the tug-of-war in the evolution of the host and the pathogen; it's a never-ending arms race," said Gregory Crimmins, UC Berkeley graduate student in molecular and cell biology who, along with former UC Berkeley post-doctoral researcher Anat Herskovits, was the study's co-lead author.

The study results could provide clues to the actions of other intracellular pathogens, such as the bacteria responsible for tuberculosis and Legionnaires' disease, since they also activate similar immune mechanisms, the researchers said.

Crimmins noted that better understanding of how the class of interferons in this study is triggered could have implications for a variety of diseases. "Type I interferons have wide-ranging effects on the immune system, and are used to treat multiple sclerosis, hepatitis C and some types of cancer," he said. "The strains generated in this study may

provide novel insight into the role of Type I interferons in coordinating the host immune response."

"By understanding the pathways of innate immunity, we can better understand acquired immunity, and that is important for vaccine development," added Portnoy. "The concept of making safe but fully effective vaccines is still a challenge, especially for intracellular pathogens."

Portnoy pointed out that weakened Listeria is already being used to develop cancer vaccines by Anza Pharmaceuticals, a Concord-based biopharmaceutical company with which he consults.

Provided by UC Berkeley