New bacterial signaling molecule could lead to improved vaccines

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(PhysOrg.com) -- Many disease-causing microbes carry pumps that expel antibiotics, making the bugs hard to kill with standard drugs.

Ironically, these same pumps could be the bugs' Achilles heel.

University of California, Berkeley, scientists have found that the molecular pumps in Listeria bacteria, and perhaps in other pathogens, also expel small signaling molecules that stimulate a strong immune response in the cells they infect. A robust immune response, involving mobilization of killer cells and a host of other defenses, is needed to kill bad microbes before they can do damage

The surprising find that bacteria pump out a totally new and highly immunogenic molecule suggests that it may be possible to improve vaccines that use live or disabled bacteria to activate the immune system. These vaccine-grade bacteria could be engineered to ramp up production of the signaling molecule or ramp up the number of pumps, for example.

"We think this could translate directly into better vaccines," said Daniel Portnoy, UC Berkeley professor of molecular and cell biology and of public health and associate faculty director of the campus's Center for Emerging and Neglected Diseases. "We can certainly get Listeria bacteria to make more of this molecule; we already have a mutant that does that."

The researchers report their discovery, funded in part by the American

Recovery and Reinvestment Act (ARRA) through the National Institute of Allergy and Infectious Diseases of the National Institutes of Health, in the May 28 issue of the journal *Science*.

For more than 20 years, Portnoy has been studying Listeria monocytogenes bacteria, which cause the serious foodborne illness listeriosis, to understand how they generate an unusually strong immune response in host cells. What he learns could help boost the effectiveness of a Listeria vaccine, but he also hopes to use the bacteria's powerful immunogenicity to boost the effectiveness of vaccines for other diseases and even for cancer. Several years ago, in collaboration with investigators at Aduro Biotech, he successfully inserted cancer antigens into Listeria to stimulate both an "innate" immune response and a potent "acquired" immune response, in effect delivering a one-two punch to cancer cells.

So far, Portnoy has found that the Listeria bacteria have to be alive and able to enter the cell's guts, the cytosol, in order to stimulate a strong innate immune response. Once the bacteria are detected, the cell responds with the release of Type I interferon, a molecule that actives immune cells and other defenses, and is currently used to increase the effectiveness of treatments for cancer and viral infections.

The mystery was what live Listeria secrete to stimulate the production of interferon by the host cell.

"We think this is the magic molecule we have been searching for for years," Portnoy said. "This is the molecule Listeria use to activate that so-called cytosolic surveillance pathway that leads to interferon."

He and post-doctoral chemist Joshua Woodward discovered the small molecule, cyclic-di-AMP, by studying mutant Listeria that, when infecting immune cells called macrophages, overproduce interferon.

Based on previous work in Portnoy's lab, they knew that these mutants actually make an unusually large number of pumps, which are known as multidrug efflux pumps because they typically can rid bacteria of a variety of drugs.

Woodward collected fluid from cultures of mutant bacteria and, to determine its constituents using mass spectrometry, enlisted the help of Anthony Iavarone, a research scientist with the California Institute for Quantitative Biosciences (QB3) at UC Berkeley.

Of the molecules in the fluid, only one stimulated interferon production in macrophages. Woodward and Iavarone identified it as cyclic-di-AMP, a molecule discovered only two years ago in the bacteria Bacillus subitlis and thought to be involved in the production of spores. Its role in Listeria is unknown.

"We actually think that the bacteria are probably secreting it all of the time, and that the molecule only gains access to the cytosol when Listeria breaks open that vacuole and the molecule tells the host that the bacteria has also gotten in," Woodward said.

A search through the genomes of other pathogens shows that not only Listeria, but also the Staphylococci, Streptococci, Mycobacteria, Mycoplasma and Chlamydia bacteria, have the gene for cyclic-di-AMP. The gene is also found in Archaea, indicating that cyclic-di-AMP is an ancient signaling molecule.

"Most intracellular pathogens like Listeria turn on the same interferon response, but we don't know how the other ones do it yet," Portnoy said. "Maybe they all have small molecules we haven't seen. We don't know how general this discovery is."

Bacteria engineered to make altered amounts of cyclic-di-AMP may be

more potent vaccines, he said.

The next step, Woodward said, is to discover how cyclic-di-AMP actually turns on interferon. For this, he and Portnoy plan to use mutant strains of Listeria in which the cyclic-di-AMP gene can be turned on and off at will.

"By studying innate immunity, we discovered an essential bacterial molecule that somehow got missed over 50 years of basic bacteriology research," Portnoy said. "We've uncovered a whole new area for our field."

Provided by University of California - Berkeley