Newly tested compound makes Gram negative bacteria less virulent

6 November 2008

(PhysOrg.com) -- A newly tested compound appears to inhibit certain mechanisms that make Gram-negative bacteria virulent disease agents.

Gram-negative bacteria -- so called because they resist staining in a diagnostic test invented by Hans Gram -- are responsible for many serious infectious diseases worldwide, including cholera, trachoma, salmonella, the plague, hemorrhagic E. coli disease, helicobacter (a major cause of stomach ulcers), Legionnaires disease, typhus, and dozens of others.

Although they cause different illnesses, Gram-negative bacteria share certain disease-inducing strategies. For example, among pestiferous bacteria there are at least six systems for secreting proteins that make them more vicious in their attack.

Dr. Samuel Miller, director of the Northwest Regional Center of Excellence for Biodefense and Emerging Infectious Diseases Research, noted, "These virulence secretion system are an appealing target for the development of new therapeutic agents because they are essential for a wide array of animal and plant infectious diseases." Miller is a University of Washington (UW) professor of microbiology, genome sciences, and medicine, Division of Allergy and Infectious Diseases.

One such therapeutic target, he said, was the assembly system for cell surface structures that stick to host cells and those that push harmful proteins into the host cell. One of these structures, the needle complex, is like a molecular syringe. The bacteria inject poisons directly into host cells, rather than pouring the toxin nearby. The authors noted that these needles are evolutionarily related to flagella, the whipping tails that propel motile bacteria.

Miller and his team rapidly screened libraries of more than 90,000 small biological and chemical molecules in search for natural or synthetic compounds that could inhibit this bacterial virulence mechanism, known as Type III secretion. From their short list, they tested and found one small molecule that broadly inhibits both the Type III system as well as another virulent protein secretion system, Type II.

These two secretion systems are behind the cell damage and resulting inflammation in plague, gastroenteritis, Gram-negative bacterial pneumonia, dysentery, enteric fever, tularemia, trachoma and endometritis in people, and account for several crop-damaging infections in plants. The small molecule, a 2-imino-5-arylidene thiazolidinone, was dubbed simply Compound 1.

In results reported in the Oct. 15 Cell Press journal *Cell Host & Microbe*, the authors found that Salmonella typhimurium grown in the presence of Compound 1 had less of the proteins required for the Needle Complex, the "syringe" that injects toxins.

"This indicates that either the formation or the assembly of the Needle Complex is the likely target of Compound 1," the authors noted. Even though the Needle Complex and flagella are related, Compound 1 didn't interfere with the secretion or function of proteins that form flagella. The bacteria were as motile in the presence of Compound 1 as they were in its absence.

Taken together, these two findings suggested to the researchers that Compound 1 targeted a protein that the outer membrane of the bacterium produces, named secretin. If this were the case, other secretion systems could possibly be inhibited and perhaps other outer ring proteins could be blocked. An example is Type 11 secretion system, which transports several substances that are toxic to mammals, as well as proteins that degrade materials inside and outside the host cells.

One of these degraders is elastase, formed by the pathogen Pseudomonas aeruginosa to produce eye ulcers, skin infections, and pneumonia. When this pathogen was grown in the presence of Compound 1, its elastase activity dipped. Moreover, the bacteria twitched less and consequently didn't spread far. Compound 1 was also shown to lower the secretion of virulence proteins from a bacterial cousin of the causative agent of tularemia, and restrained Salmonella typhimurium from killing infection-fighting blood cells known as macrophages. Compound 1 itself caused no harm to the macrophages. Compound 1 also appears to inhibit a couple of virulence secretion systems in a plague-like bacterium that lowers the host's immune response. Tobacco plants undergo tissue collapse in a hypersensitivity response to a Pseudomonas bacterial plant pathogen. Compound 1 appeared to quiet this response as well.

The researchers synthesized a more active, more soluble form of compound 1, referred to as compound 2, that had comparable anti-virulence effects.

"Thiazolidinones appear in a diverse array of drug discovery programs," Miller said, "and are under investigation as anticancer agents, anti-parasite agents, and antifungal agents. This recent study suggests that compounds with a broad spectrum of activity against Gram-negative bacterial secretion systems could be developed to prevent and treat bacterial diseases of global significance."

The development of pharmaceuticals that act on previously unutilized targets is critical, the authors said, because antibiotic resistance is increasing worldwide and adequate therapies are lacking both for diseases that were previously under control and for new bacterial illnesses.

Attacking bacterial virulence mechanisms is a strategy that could decrease the likelihood for drug resistance because it doesn't try to stop bacterial growth. It might also have fewer side effects because it doesn't harm helpful bacteria in the gut and working elsewhere in the body.

Provided by University of Washington