Mechanism Leading To Life-Threatening Infection Identified By UCSD School Of Medicine Researchers

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The mechanism used by the bacteria that cause anthrax, bubonic plague and typhoid fever to avoid detection and destruction by the body's normal immune response - leading to life-threatening bacterial infections - has been identified by researchers at the University of California, San Diego (UCSD) School of Medicine.

Published in the March 18, 2004 issue of the journal *Nature*, the lab-culture research with mouse cells identifies a protein kinase called PKR that causes the death of macrophages, the large white blood cells that act as the body's first defense against pathogens. Without macrophages to detect, engulf and stop the invading bacteria, the infection goes unnoticed by the immune system and spreads.

"If we are able to develop specific inhibitors for PKR, and the drug industry can easily produce them, we may be able to control these nasty infections," said the study's senior author, Michael Karin, Ph.D., UCSD professor of pharmacology and an American Cancer Society Research Professor.

"In addition, these findings may be applicable to serious cases of the flu, where individuals also get bacterial super-infections," Karin noted. "Every year, you have tens of thousands of deaths among people infected with the flu. We believe this super-lethal type of flu is not due to the virus alone, but to a bacterial super-infection that follows the viral infection, and because of that, can lead to macrophage death."

In the UCSD study, the researchers focused on macrophages, which act like a security force traveling throughout the body, looking for invaders. The macrophages have a receptor on their cell surface, called a Toll-Like Receptor 4 (TLR4), that alerts them to the invading pathogen by placing the macrophage in an activated state, ready to do combat. In addition to their importance in the direct killing of bacterial pathogens, macrophages alert other components of the immune system to the presence of an infection and secrete proteins that recruit other types of white blood cells to join the fight against the bacterial invaders.

Three different pathogens were used to activate TLR4 on the surface of macrophages: Bacillus anthracis, which causes anthrax; Yersiniae pseudotuberculosis, a less virulent substitute for Yersiniae pestis, the causative agent of bubonic plague; and Salmonella typhimurium, a similar substitute for Salmonella typhi, which causes typhoid fever. Both Yersiniae pestis and Salmonella typhi are too virulent to use in most laboratories.

TLR4 activation normally results in signals for both survival and death of macrophages, with the survival signal almost always dominating. However, virulence factors produced by the B. anthracis, Yersiniae and Salmonella bacteria caused TLR4 to generate only the macrophage death signal.

"Instead of the macrophage being able to swallow the bacteria and recruit other white blood cells to the battle, it kills itself in a process called apoptosis," Karin said.

To find out why the toxin-TLR4 combination elicited a death signal, the Karin team used further lab tests to determine the molecular components involved in the pathogen-activated TLR4 death signal. One of these was the

dsRNA responsive kinase PKR. In subsequent experiments in mice bred with and without PKR, the team found that those without PKR retained healthy macrophages that are resistant to killing by the B. anthracis, Yersiniae and Salmonella bacteria, and could prevent bacterial infection.

In preliminary studies not yet published, the Karin team activated PKR first with a viral nucleic acid and then with bacteria. The result was an especially vigorous infection.

"This suggests that some people who have the flu and then get a secondary bacterial infection, are probably more prone to a life-threatening infection due to the bacteria acting together with the virus to kill macrophages through PKR," Karin said.

In addition to Karin, the study was conducted by the paper's first author, Li-Chung Hsu, Ph.D., and by Jin Mo Park, Ph.D., Jun-Li Luo, Ph.D., and Shin Maeda, M.D., Ph.D., UCSD Laboratory of Gene Regulation and Signal Transduction, UCSD Department of Pharmacology; Lars Eckmann, M.D. and Donald G. Guiney, M.D., UCSD Department of Medicine; and Kezhong Zhang, Ph.D. and Randel J. Kaufman, Ph.D., Howard Hughes Medical Institute and Department of Biological Chemistry, University of Michigan, Ann Arbor.

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