

DECEMBER 11, 1999

Researchers Pinpoint Septic Shock Gene

A team of HHMI researchers and their colleagues have discovered that malfunctions in a protein required for maintaining the body's early-warning system for virulent bacterial infections set the stage for septic shock.

Septic shock, an often-fatal consequence of widespread bacterial infection, kills some 20,000 people in the United States and one million people worldwide each year. According to Bruce Beutler, an HHMI investigator at the University of Texas Southwestern Medical Center at Dallas, the finding could lead to a genetic test to identify people who might be more susceptible to septic shock. Identifying such individuals in advance would allow physicians to treat patients preemptively with antibiotics to stop a potentially devastating infection. The discovery might also lead to drugs capable of stopping the dangerous immune reaction that produces septic shock.

Beutler and his colleagues published their results in the December 11, 1998, issue of the journal *Science*.

Septic shock can occur when Gram-negative bacteria, which include various species of *Salmonella*, infect a person. These bacteria shed molecules called lipopolysaccharides (LPS), collectively known as endotoxin, into the bloodstream of the infected individual. Once in the bloodstream, endotoxin triggers a class of exquisitely sensitive immune system cells to secrete chemicals that alert the immune system to begin attacking the bacteria.

This early-response system is entirely dependent on the newly found gene. Mutations in the gene permit bacteria to grow unchecked, and when the immune system does react, it does so violently and releases large amounts of immune substances into the bloodstream. This cascade, in turn, causes a plunge in blood pressure, the formation of blood clots and spontaneous bleeding the hallmarks of septic shock. There is no effective treatment for septic shock, and about half of all cases end in death.

Beutler and his colleagues made their discovery while studying a strain of mouse that reacts normally to endotoxin and one that has a defective

response. Earlier genetic studies had traced the mouse's defective response to endotoxin to a gene in an area on chromosome 4 that was named *Lps*.

Using a variety of genetic techniques, Beutler's team searched the *Lps* region and narrowed down the location of the mutant gene. Then, in a monumental effort, the researchers mapped the target region, which contained more than 2.6 million bases.

"It was a huge area," said Beutler. "In fact I think it may very well be that this is the largest critical region ever tackled in a mouse, and we had to find a single gene in all of that."

The researchers finally narrowed their search to a mouse gene called *Tlr4*, for "toll-like receptor 4." This gene is expressed in immune system cells, and belongs to a family of genes that includes the gene for the cell-surface receptor for interleukin-1.

By comparing the detailed structure of normal *Tlr4* genes with the version found in the mutant mice, the scientists discovered that only a single base pair differed between the two genes.

Further work showed that the mouse *Tlr4* gene was similar to a gene on human chromosome 9 that presumably fulfills the same function as the mouse gene. Both the human and mouse genes appear to encode a cell-surface receptor that binds to endotoxin and sends a chemical signal to the inside of an immune cell. This signal triggers a series of biochemical processes that activate the immune cell and allow it to begin battling the invading bacteria.

The new finding may also help solve a fundamental mystery of the immune system, said Beutler. "Nobody has understood how the signal from endotoxin gets across the cell membrane," he said. "Endotoxin has been used in literally hundreds of thousands of publications as a stimulus for immune cells such as lymphocytes and macrophages, but nobody has ever known how it signals, until now."

Beutler's co-authors of the *Science* paper are Alexander Poltorak, Xiaolong He, Irina Smirnova, Mu-Ya Liu, Christophe Van Huffel, Xin Du, Dale Birdwell, Erica Alejos, Maria Silva, and Betsy Layton, all of HHMI; Chris Galanos and Marina Freudenberg, of the Max-Planck Institut für Immunobiologie in Freiburg, Germany, and Paola Ricciardi-Castagnoli of the Cellular and Molecular Pharmacology Center in Milan, Italy.