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Protein Affects Recovery from Septic Shock

Researchers have discovered that a mouse gene that helps regulate the inflammatory response can exist in slightly different forms that appear to control whether mice can stave off septic shock.

When the researchers eliminated one form of the gene in mice, the animals showed impaired recovery from septic shock--a potentially lethal systemic inflammation that occurs in humans and other mammals. The findings may help researchers better understand septic shock--and other inflammatory disorders such as atherosclerosis.

In an article published in the February 8, 2002, issue of the journal *Cell*, Howard Hughes Medical Institute investigator Stephen V. Desiderio and colleagues at The Johns Hopkins University School of Medicine reported that the Stat3 protein appears to determine how mice respond to septic shock.

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- Stephen V. Desiderio

"Stat3 has been known for some time to be an essential regulator of many processes in mammals, including systemic inflammation, or what clinicians call the acute phase response," said Desiderio. Researchers have also known that in the processing of genetic material, the *Stat3* gene can actually be "alternatively spliced" to create two different proteins, or isoforms, Stat3 α and Stat3 β .

"The difficulty has been in parsing the significance of these two isoforms," said Desiderio. "The beta isoform has generally been understood to be a minor isoform. Its structure is such that just on first principles--if you knew nothing about the context in which it worked--you might imagine that it

would suppress the activity of the more abundant alpha isoform." In particular, said Desiderio, the Stat3 β protein lacks a key component that enables it to activate transcription of its gene target. However, said Desiderio, researchers had found that the two isoforms behaved differently in different contexts in the cell, so Stat3 β wasn't simply a "dominant negative" form that shut down transcriptional activation.

"The function of Stat3 β is not readily predictable because of this context-dependence, and we didn't quite know the rules that governed its behavior," said Desiderio.

The scientists also had a more general reason for exploring how the two isoforms worked. "As it became obvious that the size of the mammalian genome is rather limited in terms of the number of protein-encoding units, the dilemma has become how mammals can achieve such greater functional complexity compared to, say, nematodes, with not many more genes," he said. "One possibility is that this complexity can be generated through a diversification of transcription factors at the level of alternative splicing. So, the Stat3 system was really the first opportunity to selectively ablate one of two alternative forms of a transcription factor at the headwaters of a regulatory circuit and see what happens."

Joo-Yeon Yoo, lead author of the *Cell* article, approached this task by developing a sophisticated method to eliminate the Stat3 β isoform in mice. Yoo's technique mutated the Stat3 gene in such a way that alternate splicing inactivated only the Stat3 β isoform.

When Yoo tested the effects of this ablation in embryonic cells from the mutated mice, she found that the absence of the Stat3 β isoform reprogrammed the expression of some 100-200 genes in the cells. When the scientists attempted to determine the effects of the ablation on the whole animals, they could find none at first.

"The first couple of months were frustrating, but we noticed that some of the mutant mice were dying prematurely," said Desiderio. "When we looked at their pathology, we found indications that they had died of septic shock, which was a bit of a surprise."

When the researchers administered the inflammation-triggering bacterial endotoxin to normal and mutant mice, they found that, while both mouse strains suffered from septic shock, the mutant mice failed to recover and died.

Further analyses led the scientists to propose that the Stat3 β isoform normally negatively regulates a set of inflammatory genes. They hypothesized that in Stat3 β 's absence, those genes become overactive. The researchers next tracked the expression of these genes as the mutant animals reacted to endotoxin, and discovered that almost all of the approximately 120 genes did, indeed, become overactive. Further genetic studies are continuing to reveal

the function of these genes. For example, one gene appears to maintain the integrity of blood vessels, said Desiderio.

"A great number of the genes are of unknown function, and a number of the genes are of known function, but might heretofore have not been appreciated as inflammatory markers," he said. "So, in the bargain in these studies, we're discovering many new genes."

The gene that codes for apolipoprotein E is one of the few genes that are downregulated in the mutant mice. Absence of apolipoprotein E can induce atherosclerosis, and the mutant mouse might be a useful model for studying how inflammation promotes atherosclerosis. Desiderio plans to collaborate with clinicians to study whether differences in the *Stat3* gene may affect the susceptibility to atherosclerosis in humans.