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RESEARCH NEWS

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Streptococcus Infects Humans by Thwarting Blood Clotting

Streptococcal bacteria may infect humans by using a bacterial enzyme to "hijack" the blood-clotting system, according to new research by Howard Hughes Medical Institute scientists.

In studies published in the August 27, 2004, issue of the journal *Science* , the researchers establish that the enzyme streptokinase is responsible for the pathogen's ability to infect humans while exhibiting little activity against other mammals.

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— David Ginsburg

The scientists genetically altered strains of mice to make the animals susceptible to infection by streptococcus. They say their strategy outlines a new path for developing animal models for human-specific microbes. The research is also likely to open the way to new understanding of the factors that enable bacteria to evolve host specificity, the researchers said.

Howard Hughes Medical Institute investigator David Ginsburg led the research team, which included lead author Hongmin Sun and colleagues at the University of Michigan and Lund University in Sweden.

"Understanding why bacteria in general are so species-specific has been a major problem for a long time," said Ginsburg. "And this species-specificity had greatly hindered our ability to develop an animal model for human-specific bacteria such as Group A streptococci, which are an important human pathogen."

Ginsburg said that Hongmin Sun's achievement of constructing a transgenic mouse susceptible to streptococcus infection represents a major step not only in understanding infection by that bacterium, but in opening the way to

similar studies of other bacteria.

In infecting its human host, the group A streptococcus secretes its own streptokinase, which activates the human form of the enzyme, plasminogen. Plasminogen, in turn, dissolves blood clots by degrading the protein, fibrin. A major question was what role streptokinase played in the bacterium's overall pathogenicity, said Ginsburg.

To develop the “humanized” mouse that would be vulnerable to bacterial streptokinase, Sun attached the gene for human plasminogen to a regulatory DNA sequence that normally activates the gene for a mouse blood protein, albumin. This protein is produced in large amounts in the animal. The result was a transgenic mouse that made significant amounts of human plasminogen.

To show that the human plasminogen was functional in the mice, Sun crossed the transgenic mice with another strain in which their own plasminogen genes had been deleted. This cross essentially restored plasminogen function in the resulting mice. In test-tube experiments, Sun also demonstrated that streptokinase acted on the human plasminogen from the transgenic mice to dissolve blood clots just as if it were acting on a human clot.

“The critical experiment, though, was when Hongmin infected the skin of these transgenic mice with the group A streptococcus bacteria,” said Ginsburg. “She found that the bacteria were much, much more toxic to these mice than the normal mice. This fit with the idea that streptokinase was an important component of the pathogenicity of strep.

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In further experiments, the researchers found that when they removed the streptokinase gene from group A streptococci bacteria, there was little difference in their infectivity between normal and the transgenic mice.

Such studies have led Ginsburg and his colleagues to theorize that streptokinase “hijacks” the human clot-forming system for the bacteria's own infective ends. “The theory is that the bacteria cause a local infection and begin to grow. Many of the bacterial products, as well as our immune cells, trigger the human clotting system, which evolved in part as a defense against such infection,” said Ginsburg. “This system produces clots in the blood vessels around the infection, closing the highways that the bacteria would use to spread. However, the bacterial streptokinase bypasses this system causing the blood clot to dissolve so the bacteria can spread.”

Sure enough, when the researchers bypassed the clotting defense by injecting the streptococcus directly into the bloodstream of both normal and transgenic mice, they both showed similar susceptibility to infection. In another experiment to demonstrate the defensive importance of the clotting system, the researchers administered a substance derived from snake venom that

degrades another clotting protein, fibrinogen, discovering that the treatment greatly increased the mice's mortality from this streptococcus infection.

Streptokinase's importance to group A streptococci may generalize to many other human-specific bacteria that have evolved their own distinctive plasminogen-activating enzymes, said Ginsburg. Also, he said, the findings highlight the evolutionary arms race between bacteria and humans.

“Clearly, if we could mutate our plasminogen so it still worked, yet was resistant to a bacterial streptokinase, it would give us an advantage,” said Ginsburg. “But then the bacteria could mutate their streptokinase to keep up. So, you can see how one bacterial species and one host get locked in this evolutionary dance and would evolve apart from other host-bacterial pairs—ending up with a multitude of variants of streptococci, one for each host.

“This evolutionary mechanism probably functions for many other pathogenicity factors, not just streptokinase, and probably underlies the species-specificity of all kinds of infectious organisms,” said Ginsburg.

Such findings also hint that subtle variations in plasminogen genes among humans could partially explain differences in susceptibility to certain infection in different people. Thus, he said, his laboratory is exploring the genetic variations in the blood-clotting system that might affect risk factors for infection. “Although this is speculation at this point, it might ultimately be possible to tailor treatment of infections to the pattern of genetic variability in clotting genes or other pathogenicity factors,” said Ginsburg.