Engineered protein shows potential as a strep vaccine

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A University of California, San Diego-led research team has demonstrated that immunization with a stabilized version of a protein found on Streptococcus bacteria can provide protection against Strep infections, which afflict more than 600 million people each year and kill 400,000.

In the March 7 issue of the journal *Science*, the researchers describe, for the first time, the detailed structure of the streptococcal M protein, which is critical to the virulence of Group A Streptococcus (GAS). GAS causes a wide variety of human diseases including strep throat, rheumatic fever, and the life-threatening "flesh-eating" syndrome called necrotizing fasciitis. Studies were performed using M1 protein, which represents the version of M protein present on the most common disease-associated GAS strains.

The team also produced a variant of M1 protein that stimulates the immune system in mice, without the serious side effects caused by natural M1 protein. They say that their results should help scientists develop M1 protein-based vaccines against GAS.

"Using X-ray crystallography, we determined that M1 protein has an irregular, unstable structure," explained Partho Ghosh, a professor of chemistry and biochemistry in UCSD's Division of Physical Sciences. "We created a modified version of M1 with a more stable structure, and found that it is just as effective at eliciting an immune reaction, but safer than the original version of M1, which has serious drawbacks to its use

in a vaccine."

"Certain antibodies that are produced by the immune system against M1 protein have been shown to cross-react with normal human tissues including heart muscle, potentially triggering the serious autoimmune disease known as rheumatic fever," added Victor Nizet, professor of pediatrics and pharmacy at the UCSD School of Medicine and the Skaggs School of Pharmacy and Pharmaceutical Sciences. "M1 protein can also act as a toxin, producing clotting abnormalities and lung injury when injected into mice. Therefore our results with modified M1 provide very novel insight about the role of M proteins in invasive GAS disease and rheumatic heart disease."

Because M protein is vital for the survival of GAS in the host, various research groups have been trying to determine its structure for decades. According to Ghosh, M1 is "long and skinny," which makes it a particularly difficult protein to crystallize. Case McNamara, who solved the protein structure while he was a graduate student working with Ghosh and is the first author on the paper, spent three years optimizing the conditions to crystallize the protein and collect the required data.

In the Science article, the team reported that M1 proteins extend from the surface of Strep bacteria in sets of two that coil around on each other. However, the coils do not meet along the entire length of the protein because imperfections in the protein force the two strands to splay apart. By making small changes to the amino acid building blocks that constitute M1 protein, the researchers were able to develop a version of the protein molecules that coiled together along their entire length.

"This is a crude analogy, but if you imagine that M1 protein is a zipper, then the front half of the molecule is zipped up," said McNamara, who is currently a postdoctoral fellow at the Genomics Institute of the Novartis Research Foundation. "However, unfavorable amino acids prevent the

molecule from zipping up all the way. It was clear from the literature that mutations of these unfavorable amino acids to favorable amino acids would allow the molecule to continue to zip up."

Unstable coils provide a protein with the flexibility to perform different functions, according to Ghosh. Proteins with structures similar to the original version of M1 are found in the human body, including in heart muscle. The researchers hoped that modifying the structure of M1 would make it less likely that an M1 vaccine would trigger an autoimmune response against heart muscle.

This indeed turned out to be the case. In tests with antibodies against heart muscle, Madeleine Cunningham, a professor of microbiology and immunology at the University of Oklahoma Health Sciences Center, determined that engineered M1 was much less reactive than natural M1. In addition, Annelies Zinkernagel, a postgraduate researcher in Victor Nizet's laboratory, discovered that unlike mice injected with natural M1, mice injected with stabilized M1 did not develop lung injuries.

Therefore, the instabilities in M1 are responsible for its toxic properties and tendency to trigger autoimmune reactions. However, the instabilities in M1 were not necessary for M1 to be effective as a vaccine. When injected into mice, stabilized M1 elicited a strong immune reaction that provided protection against infection with GAS bacteria.

"Stabilized M proteins may also last longer in the body than natural M proteins, which would be beneficial for vaccine development," said Ghosh.

"However, a strep vaccine would have to cover many strains of strep, so stabilized M1 might be one of multiple stabilized M protein antigens used in a vaccine," added Nizet.

Source: University of California - San Diego