NEWS RELEASE

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Protein Vaccine Fully Protects Mice from Lethal Aerosol Challenge with Ricin Toxin

Scientists have developed an experimental vaccine against ricin, a potential biological threat agent, which fully protected mice from aerosol challenge with lethal doses of the toxin. The study was performed at the U.S. Army Medical Research Institute of Infectious Diseases (USAMRIID).

Ricin is a toxin derived from the castor plant, which is grown throughout the world for commercial purposes. Approximately one million pounds of castor beans are used each year in the process of manufacturing castor oil.

When inhaled as a small-particle aerosol, ricin produces severe respiratory symptoms followed by respiratory failure within 72 hours. When ingested, ricin can cause severe gastrointestinal symptoms followed by vascular collapse and death.

Given its ready availability and its high level of toxicity—particularly when delivered as an aerosol—ricin is a significant potential agent of biological warfare or terrorism. Currently, there is no vaccine or therapy available for human use.

According to lead investigator Mark A. Olson, Ph.D., ricin is composed of two different protein subunits called the A-chain and the B-chain. The ricin B-chain (RTB) binds the toxin to the cell surface, an interface that is essential for the ricin A-chain (RTA) to enter the cell. Once inside the cell, RTA effectively stops new protein synthesis and causes cell death.

Previous attempts to develop a ricin vaccine suggested that isolated RTA could induce protective immunity against the toxin in animals. However, using RTA as a vaccine component was problematic because it was not stable—it failed to maintain its structural integrity when heated or placed in solution, resulting in clumping and separation. The safety and efficacy of a vaccine depend upon the stability of its formulation.

The new vaccine candidate, called RTA 1-33/44-198, is a fragment of the ricin A-chain that has been modified to eliminate the toxic enzymatic property of RTA, increase protein stability, and maintain its ability to elicit a protective immune response.

In the August online issue of Protein Engineering, Design and Selection, Olson and his team—John H. Carra, Virginia Roxas-Duncan, Robert W. Wannemacher, Leonard A. Smith, and Charles B. Millard—describe using a combination of molecular modeling and protein engineering to design the new vaccine. The team started with an extensive computer-aided analysis of the toxin structure, using a three-dimensional model provided by colleagues at the University of Texas, Austin.

"We compared ricin with other proteins of the same family," Olson explained. "We tried to figure out where the protein molecules are diverging within the family—to see what changes were made by nature so we could make the changes we needed to make."

To improve upon its stability—in effect, to make it go against its natural tendencies—Olson and his team had to change the structure of the RTA molecule. Once they had developed the necessary genetic sequences, they handed them off to Smith and others at USAMRIID for protein engineering.

"We went straight from the computer to molecular biology," explained Smith. "We had to clone and purify the proteins, and test them in animals for toxicity and protection."

RTA 1-33/44-198, the most promising vaccine candidate, was tested in three groups of ten mice each. One group received the purified protein alone; a second group received the protein plus an adjuvant called Alhydrogel; and a third group served as the control, receiving an injection of saline solution instead of the vaccine.

Purified RTA 1-33/44-198 protected 10 out of 10 mice from a whole-body aerosol challenge with lethal doses of ricin. The survival rate was the same with or without the adjuvant. All 10 animals in the unvaccinated control group died.

"Molecular modeling and protein engineering represent a significant step forward in vaccine development," said George V. Ludwig, Ph.D., acting science director for USAMRIID. "In the past, our reliance on using natural proteins and other immunogens limited our ability to make useable and producible vaccines. New techniques such as those described offer nearly unlimited possibilities."

According to Smith, the next step will be to test the new ricin vaccine in nonhuman primates. He said the team also is working to refine a scaled-up production method that is robust and reproducible. This involves optimizing the fermentation process, developing a process for purification, and refining the analytical methods for characterizing both the manufacturing process and the final product. In addition, USAMRIID is conducting pre-formulation studies to produce a formulated vaccine that will induce the optimum immune response possible in animal models.

USAMRIID, located at Fort Detrick, Maryland, is the lead medical research laboratory for the U.S. Biological Defense Research Program, and plays a key role in national defense and in infectious disease research. The Institute's mission is to conduct basic and applied research on biological threats resulting in medical solutions (such as vaccines, drugs and diagnostics) to protect the warfighter. USAMRIID is a subordinate laboratory of the U.S. Army Medical Research and Materiel Command.

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Reference: "Finding a New Vaccine in the Ricin Toxin Fold," *Protein Engineering Design and Selection*, August 2004, *in press*).

For more information on USAMRIID: www.usamriid.army.mil