ANDY ORDWAY

Doctoral student Brooke Jude, left, and microbiologist Ron Taylor study the action of the cholera bacterium.

One step closer to a vaccine against cholera

The media's coverage of avian flu has made Americans all too familiar with the word pandemic—the worldwide spread of a disease. Yet other pandemics are every bit as troublesome but get much less attention.

In a paper for the December 8 issue of the prestigious journal *Nature*, investigators in DMS's Department of Microbiology and Immunology—Professor Ronald Taylor, Ph.D., and graduate students Thomas Kirn and Brooke Jude—provided vital clues regarding the process by which the bacterium *Vibrio cholerae* is transmitted to humans. *V. cholerae* causes cholera, a severe diarrheal disease that kills thousands of people worldwide every year. A cholera pandemic that began in 1961 continues today; the World Health Organization says the disease is now present in places that hadn't seen a case for 100 years.

Toxin: Humans typically get infected with *V. cholerae* by drinking contaminated water or eating food taken from or washed in contaminated water. Once ingested, the bacteria attach to the surface of the small intestine and, after a short incubation period, produce a toxin that triggers massive loss of water and salts into the gut. The resulting diarrhea causes dehydration and electrolyte imbalances that lead to death within three days in 60% of untreated patients. Rehydration with a balanced salt solution can save most patients but is largely unavailable in the rural, developing areas where cholera thrives.

Taylor's lab has been studying how the bacterium colonizes the gut, using a cellular system to test the way different genetic mutants of *V. cholerae* bind to the epithelial cells that line the gut. Bacteria with deficient binding were then analyzed to determine the identity of the disrupted gene. Of particular interest was a mutation in a gene encoding a protein that binds to chitin, a tough carbohydrate which forms, among other things, the exoskeleton of aquatic arthropods.

"Most of the cholera bacteria are bound to zooplankton or phytoplankton in the aquatic environment," says Taylor. "They're not just swimming around. In India, removing plankton by straining water through a sari that has been folded four times is used as an effective way of preventing cholera."

Why should a defect in a gene that determines attachment to chitin also result in deficient binding to gut epithelium? Although chitin is not found in the gut, Taylor suspected the chitin binding protein was actually linking to a subunit of chitin known as GlcNAc, which is also common in the gut. Thus the same protein could participate in binding to both chitin and gut.

Gut: To test this hypothesis, Taylor's team produced a strain of *V. cholerae* lacking the gene that produces GlcNAc binding protein. These mutants bound to both chitin and gut epithelium much less often than wild-type *V. cholerae* did. In a control experiment, binding was partially restored when the gene was reintroduced. And in a mouse model, 10 times fewer mutant bacteria than wild-type bacteria attached to the gut.

Next, antibodies against GlcNAc binding protein were mixed with native *V. cholerae* before it was administered to mice. The addition of the antibody, which inactivated the protein, significantly increased the survival of the infected mice—indicating that bacterial binding to the gut is a major determinant of the progression of infection.

Why does *V. cholerae* colonize the human gut at all? The answer is uncertain, but Taylor has an idea: "*V. cholerae* is an environmental organism. Going into the human gut allows the organism to boost its concentration to manyfold the concentrations that can be achieved in the aquatic environment. This may be essential for its survival."

Two more studies are under way. "First," says Taylor, "we're preparing vaccines against a variety of potential cholera-binding proteins in rabbits and testing their effectiveness in preventing cholera infection in mice. Second, in conjunction with a group at Massachusetts General Hospital, we are testing the possibility of using a skin patch to deliver potential immunogens . . . to humans."

Effective: This could be the first step toward developing an effective vaccine for cholera—a goal that has eluded medical science to date. Taylor has no doubt about the importance of the work. "If we want world balance and sanity," he says, "we need to have healthy populations." JOSEPH E. MELTON, PH.D.