

SEPTEMBER 02, 1999

## AIDS Virus Fragment Delivers Protein Payload

A team of Howard Hughes Medical Institute (HHMI) researchers has used a piece of the AIDS virus to deliver a fully functional protein inside a cell, creating a new way to deliver therapeutic proteins.

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"This demonstration means two things," said Steven Dowdy, an HHMI investigator at Washington University School of Medicine in St. Louis, who developed the technique with postdoctoral fellows Steven R. Schwarze, Alan Ho and Adamina Vocero-Akbani. "First, it introduces a new way to study diseases and do experiments that no one has ever been able to do before. And second, it has the potential to open a new field called protein therapy."

Damage to proteins causes many human diseases, including cancer and genetically inherited disorders as sickle-cell anemia and phenylketonuria. Inserting a working version of a damaged protein into affected cells could be a promising therapy for such illnesses. Until now, however, researchers had been unable to coax bulky biomolecules through the densely packed fatty molecules of the cell membrane.

In the September 3, 1999, issue of the journal *Science*, Dowdy and his colleagues report that their way around this obstacle is to use a fragment of a protein from the human immunodeficiency virus (HIV). When hooked to a large protein, this harmless piece of HIV broaches, or transduces, the cell membrane.

"We call this transduction the parting of the Red Sea," said Dowdy. "It's as if the HIV fragment interacts somehow with the lipid bilayer of the membrane, opens it up, inserts the protein, and then seals it back up. We can't see

anything leaking out from the cell."

The fragment used by Dowdy's team comes from the HIV protein TAT, which the virus normally uses to facilitate gene transcription, not to infect host cells. That the TAT fragment can penetrate a cell at all is a biochemical quirk, says Dowdy, which was first reported by researchers in 1988. As a result, it is unlikely that the HIV fragment poses any danger of triggering disease, he said.

To demonstrate that the technique works, the HHMI team hooked the 11-amino-acid TAT fragment to a peptide tagged with a compound called fluorescein that emits a green glow when illuminated by fluorescent light. The luminous peptide-fluorescein compound is four times the size of the largest compound that can enter cells naturally. Nevertheless, cells in the spleen, liver, and muscle glowed green just 20 minutes after Dowdy's team injected the proteins into the abdomens of mice.

Even the brain tissue had a solid green color, indicating that the injected proteins were able to cross the blood-brain barrier. The blood-brain barrier is a layer of tightly packed cells designed to keep most large molecules including fluorescein out of the central nervous system.

In a second experiment designed to see whether the technique delivers functional proteins, Dowdy's group used the TAT fragment to attempt to ferry an even larger protein an enzyme called beta-galactosidase into mouse cells. Beta-galactosidase helps break down complex sugars, including a stain called X-Gal that turns blue when it is broken down by beta-galactosidase.

"We held up the tissue samples from the mice and said 'Wow,'" says Dowdy. "All of the tissues were blue, indicating that the enzyme was working inside the cells."

Dowdy's group was also surprised by the rapidity of the transduction. "The evidence suggests the cells are transduced within an hour, and probably within 30 minutes," explained Dowdy. Crossing the blood-brain barrier seemed to take longer, but even this difficult task was complete within four hours.

In designing drugs, pharmaceutical companies must consider what they call the "bioavailability wall." Any therapeutic agent too large or too attracted to water cannot penetrate the fat-based cell membrane.

The limits imposed by the bioavailability wall are stringent. For example, the cell membrane will not transduce proteins larger than about six amino acids.

Yet even small human proteins contain about 150 amino acids. Beta-galactosidase contains over 1,000 amino acids and is nearly 200 times the size limit set by the bioavailability wall.

A molecular oncologist, Dowdy plans to use the technique to study cancer. He is particularly interested in two tumor-suppressor proteins, p16 and RB, whose genetic pathway is mutated in almost 90 percent of cancers.

"There are already knockout mice that are missing genes for p16 and RB, and we know these mice are predisposed to develop early cancers," he said. "But now we can transduce those tumor suppressor proteins back into mice and then withdraw the proteins at various times to find out when they are really critical. We'll be able to ask very specific questions about the earliest steps that lead to cancer."