Reoviruses Yield to Gene Rearrangement

For the first time, molecular biologists have edited the genetic code within infectious agents called reoviruses, members of a virus family that kills millions of Third World children each year. The accomplishment opens the door to the development of vaccines against such scourges as viral gastroenteritis – a fatal diarrhea that strikes youngsters in developing countries – and veterinary diseases caused by related viruses.

Reoviruses are ubiquitous in nature, infecting plants, animals and insects. They are the only viruses whose genes reside on twin strands of RNA. (Other viruses have DNA as their genetic material or only a single strand of RNA.) In recent years, molecular biologists have learned to add and delete specific genes from every other major class of virus. This has enabled scientists to discern the functions of individual genes and sometimes to create subtly mutated viruses that cause no disease but trigger a protective immune response in humans when administered as a vaccine. Inexplicably, however, reoviruses have proved resistant to traditional genetic engineering techniques.

Now Wolfgang K. Joklik, Michael R. Roner and Lisa A. Sutphin at the Duke University Medical Center in Durham, N.C., have teased these stubborn organisms to yield their genetic machinery to human will.

"This is a very important accomplishment. It really opens up a whole new field of RNA virus genetics," says molecular biologist Bernard N. Fields at Harvard University Medical School in Boston. "It will be enormously important to studying the mechanisms of RNA virus infection."

Under normal conditions, an RNA virus latches onto a host cell and injects into that cell its RNA, which both replicates itself and directs the creation of new viral coats. Viral RNA strands must package themselves in these fresh protein jackets before leaving the host cell to infect other cells. The Duke team had already become adept at rewriting genetic sequences within reoviral RNA itself. But they and others had repeatedly failed to coax this altered genetic material into cells where it could clothe itself in the protein jackets needed to complete its life cycle.

The Duke researchers hypothesized that the binding of normal reoviruses to a host cell's membrane might make that cell more vulnerable to infection by naked, genetically altered reovirus RNA. They confirm this in the December 1990 VIROLOGY, reporting that when mammalian cells are under viral attack, engineered reovirus RNA can sneak into those cells and make fully jacketed, genetically altered reoviruses.

The new work also shows that the surface-bound viruses contribute no genetic material in the process; rather, their mere presence on the host cell's outer membrane seems to change some aspect of the cellular environment in a way that's favorable to reovirus RNA infection and replication.

Exactly how intact viruses aid in the process of RNA infection remains a mystery, Joklik says. But with the ability to create intact, gene-altered reoviruses, scientists can now learn the precise functions of each reoviral gene by knocking out individual genes and observing what's lacking, structurally or functionally, in offspring viruses.

Ultimately, Joklik says, this should allow vaccine manufacturers to create reoviruses that can spur production of antireovirus antibodies in humans or animals without causing disease.

The reovirus feat comes on the heels of similar work by Peter Palese, a microbiologist at the Mount Sinai School of Medicine in New York City. Last year Palese and his colleagues created the first gene-altered influenza virus, a single-stranded RNA virus. That work may not only lead to a live influenza vaccine more effective than the current one, which is made from killed influenza viruses, but may also allow scientists to insert genes from other disease-causing organisms into influenza RNA sequences.

Influenza infection triggers unusually high levels of IgA, an immune protein that concentrates in the mucosal surfaces of respiratory, gastrointestinal and genitourinary tracts, Palese notes. Thus a vaccine against such diseases as strep throat or gonorrhea, which infect these mucosal locations, may work best if packaged in an influenza shell, he speculates.

Joklik says he and his Duke colleagues will test their gene-insertion techniques later this month on reoviruses more dangerous than the innocuous strains they've tinkered with so far. The most deadly members of the reovirus family, called rotaviruses, cause a devastating form of diarrhea and annually kill 4.6 million children under 5 years of age in Asia, Latin America and Africa, the researchers say. Other reoviruses, called orbiviruses, pose a significant veterinary threat, causing an equine disease called African horse sickness and bluetongue disease in sheep and cattle. – *R. Weiss*

Diagnostic duo highlights heart damage

The FDA has approved the use of a new chemical combo to reveal blood-starved cardiac tissue during a heart attack. Called technetium-99m sestamibi, the imaging "dye" consists of a radioactive tracer (technetium-99m) bound to a heart-seeking chemical (sestamibi).

Du Pont Merck

Blood flow patterns in healthy (left) and damaged (right) hearts. Technetium-highlighted areas indicate regions receiving adequate blood flow.

By enabling clinicians to record sharper pictures delineating healthy and damaged areas of the heart, the compound should help physicians assess the need for surgery and evaluate the effectiveness of clot-busting drugs and other heart attack treatments, says Daniel S. Berman of the University of California, Los Angeles. Physicians might also use it to identify outwardly healthy people with blood-flow blockages that place them at risk of sudden heart attack, he suggests. Berman, a nuclear cardiologist, conducted some of the clinical investigations that led to last week's FDA approval.

Developed by Du Pont Merck Pharmaceutical Co., technetium-99m sestamibi homes in on heart tissue when injected into the bloodstream, binding only to cells that are receiving sufficient blood flow. Because it sticks to the heart cells for about four hours, physicians have plenty of time to stabilize the patient's condition before taking pictures with a gamma-ray imaging camera, Berman says.

A similar technetium imaging agent, developed by Bristol-Myers Squibb Co., also won FDA approval last month. Until now, the radioisotope thallium has been used as the primary tracer for heart damage, but thallium requires physicians to obtain images within half an hour after the injection or lose the chance to size up the initial damage, Berman says.

– *K.A. Fackelmann*