Persistent Infections: The Role of Viruses

At one time or another, practically every chronic degenerative disease of unknown origin has been linked to a viral cause. A partial listing of the diseases includes cancer, rheumatoid arthritis, systemic lupus erythematosus, and a number of slowly progressing but fatal neurological conditions. Multiple sclerosis and subacute sclerosing panencephalitis are examples of the latter. Investigators also think that certain forms of the mental deterioration sometimes associated with old age could have a viral cause. However, direct efforts to isolate the putative causative agent from the tissues of diseased individuals have only rarely succeeded. Even then, the results have often proved difficult to confirm and therefore controversial.

Because of the problems with the direct approach and because of epidemiological and other evidence that the viruses linked to the diseases may persist for a long time in the affected individual, often without producing symptoms for many years, investigators have turned to in vitro systems of cultured cells to study the mechanisms by which a virus may establish and maintain a long-term infection without killing the infected cells. The hope is that a better understanding of these mechanisms will provide the clues that permit investigators to uncover hidden viruses that may contribute to human disease.

Several mechanisms, which are not necessarily mutually exclusive, have been proposed to explain the development of persistent infections in cultured cells. One involves the action of defective viral (DI) particles that interfere with the reproduction of the parent virus. Another focuses on the selection of temperature-sensitive viral mutants that reproduce poorly at normal body temperatures and may cause latent infections. And, according to a third (there are others which will not be considered in this article), information from the viral genome may be sequestered in a noninfectious form in the affected cell. There is evidence that all of these may occur in animal disease but as yet no direct support for their participation in human disease.

Seven years ago, Alice Huang of Harvard Medical School and David Baltimore of the Massachusetts Institute of Technology suggested that DI particles, which have been described for nearly all classes of animal viruses, might be involved in the etiology of persistent virus diseases of humans and animals. Since then evidence has accumulated that a number of viruses establish persistent infections of cultured cells only in the presence of DI particles; otherwise, they kill the cells.

One of the best studied of these viruses is vesicular stomatitis virus (VSV), a large RNA virus that usually infects animals but occasionally produces a flulike disease in humans. According to John Holland, Luis Villarreal, and their colleagues at the University of California at San Diego, VSV that has been rigorously purified to remove DI particles rapidly kills infected hamster cells. In the presence of large quantities of the particles the virus produces a persistent infection. The San Diego group has maintained one such persistently infected cell line for over 3½ years.

Like other DI particles, the defective VSV particles have lost a portion of their genome. They can replicate only in the presence of intact virus because the latter is needed to supply missing information or enzymes. Huang has found that during successive rounds of virus reproduction in cultured cells, the concentration of complete virions peaks before that of the DI particles. As the particles accumulate, they suppress formation of standard virus. In order to establish a persistent infection, the DI particles must reach a concentration at which they suppress standard virus formation sufficiently to prevent all the cells from being killed.

Mechanism of Suppression

The mechanism by which defective VSV particles suppress the reproduction of infectious virus and prevent cell-killing is still unclear. However, both Huang and Holland have evidence that the particles interfere with replication of the viral genome. A possible explanation of the interference is that the RNA's of the particles and the intact virus are in competition for the polymerizing enzyme that makes copies of the RNA's, with the particle RNA winning the competition. Whatever the mechanism, the outcome is that the replication of the virus is reduced sufficiently so that the infected cells are not destroyed as they would normally be. The outcome of infection depends on the cell type as well as on the virus. Numerous investigators have noted that it is easy to establish a persistent viral infection in one kind of cell and difficult or impossible to achieve the same result in another.

One way to resolve the unanswered questions about the mechanism by which defective VSV particles, which are deletion mutants, are formed is to determine the structures and nucleotide sequences of their RNA genomes. This kind of information may also help to clear up the uncertainties about the way the particles suppress reproduction of the standard virus. Several investigators, including those in the laboratories of Huang and Holland, already have determined at least partial structures for the RNA's. The data from both laboratories show that large sequences of nucleotides have been lost from the particle genome, but that there have also been additions of some nucleotide segments. Jacques Perreault and Ron Leavitt, who work with Holland, have found that the nucleotide sequences at both ends of the particle DNA are identical to those at the ends of intact viral RNA. Thus, the deletions appear to occur in the interior of the genome, but it is still too early to develop a complete picture of how the DI particle RNA is derived from that of the intact virus.

Vesicular stomatitis virus is not the only RNA virus that may require the presence of DI particles to produce persistent infections of cultured cells. Similar results have been found for reovirus by Angus Graham and his colleagues at McGill University and for lymphocytic choriomeningitis virus by Fritz Lehmann-Grubbe at the University of Hamburg and by Raymond Welsh and Michael Oldstone at the Scripps Clinic and Research Foundation. Holland's group also has evidence that DI particles produced in cultured cells infected with rabies, measles, mumps, and influenza virus may help establish persistent infections by these viruses.

Further evidence supporting Huang and Baltimore's hypothesis comes from work with animal models. Injection of VSV or reovirus directly into the brains of test animals usually produces a rapidly fatal brain infection. Both Holland and Graham have evidence that simultaneous injection of the corresponding DI particles can moderate the severity and prolong the course of the ensuing disease. According to Holland, when they inject mice with normally fatal low doses of VSV, together with DI particles, the animals survive the brain infection. Defective particles administered with high doses of VSV also produce a lengthened disease course; the animals die after a week or more of a wasting paralytic disease instead of succumbing within 2 to 3 days. Viral assays show that virus replication in the brains of the animals is

reduced in the presence of the particles.

In a similar manner, Graham found that 75 percent of rats given intracerebral injections of reovirus plus DI particles survive whereas all of those injected with virus alone die of an acute brain inflammation. However, most of the survivors exhibit decreased growth and signs of chronic brain degeneration. These symptoms are similar to those observed in about half of the rats injected subcutaneously with standard reovirus. About 10 percent of the animals given subcutaneous injections die of encephalitis within a few days; the remaining 40 percent recover completely.

Graham says that defective reovirus particles are formed in the brains of all the rats, no matter what the route of virus administration. But the particles found in the animals that die of acute infections differ structurally from those in persistently infected rats. The reovirus genome consists of ten separate RNA segments, each constituting one gene. The only DI particles in animals with acute infections are those lacking the segment designated L_1, whereas persistently infected animals have additional DI particles with multiple gene deletions.

Some of the chronically infected brains of animals killed 100 days after virus administration contain a mixed population of several DI particles, even though no infectious virus is detected. Graham says that it is possible that a mixed population of defective particles can multiply, even in the absence of infectious virions, if each particle supplies information lacking in the others; together they would have all the information needed for replication. Such a situation could help to explain how a virus might produce long-term degenerative effects even after the infectious agent has apparently disappeared.

Although the evidence indicates that DI particles are needed for the establishment of persistent infections by at least some viruses, other factors may also be involved. One of these is the selection of temperature-sensitive viral mutants that replicate well at 31°C but poorly at the normal human body temperature of 37°C. (The temperatures of small mammals are somewhat higher than this.) Julius Youngner and his colleagues at the University of Pittsburgh School of Medicine found that the proportion of temperature-sensitive mutants formed in cultured mouse L cells rapidly increases from about 4 percent in the original VSV preparation used to infect the cells to almost 18 percent after 10 days of infection. At 63 days, 100 percent of the virus isolated from the cells is temperature-sensitive. Although establishment of the initial infection requires the presence of many DI particles, the temperature-sensitive mutants establish persistent infections in the absence of defective particles.

Youngner says that it is important to distinguish between establishment and maintenance of persistent infections when considering mechanisms. He agrees that DI particles are needed to suppress reproduction of wild-type VSV in order to establish long-term infections, but he suggests that after infection with wild-type virus, selection of temperature-sensitive mutants is involved in maintaining the infection. Other investigators have found that temperature-sensitive mutants of measles, rubella, and Western equine encephalitis viruses may be selected during persistent infections of cultured cells by the viruses.

Youngner and his colleagues have evidence that the temperature-sensitive mutants of VSV suppress replication of the wild-type virus, even at the higher temperature. This would help explain why revertants from the mutant to the wild type, which are known to occur spontaneously, do not replace the temperature-sensitive virus in the cells.

In earlier experiments, the Pittsburgh group showed that Newcastle disease virus, an RNA virus that often causes serious epidemics in birds, will persistently infect hamster, mouse, and canine cells and that temperature-sensitive mutants are rapidly selected during the infection. The presence of DI particles does not appear necessary for the establishment of this long-term infection.

Aleutian Mink Disease

Youngner points out that investigators who are attempting to isolate viruses from diseased human tissue ought to perform some of the experiments at lower temperatures than usual in case a temperature-sensitive virus is lurking in the material. Recently, David Porter and his colleagues at the University of California School of Medicine at Los Angeles were able to grow the virus that causes Aleutian mink disease (a persistent viral infection of mink) in cultured feline cells incubated at 31°C. The virus, which investigators had failed to grow in tissue culture in previous attempts, could not be isolated from cultures kept at 37°C. Although the virus does not replicate at any temperature in cultured mink cells, the agent isolated from the feline cells produces the disease when injected into mink. Porter thinks that the temperature-sensitive nature of the virus may help to decrease its virulence for mink and permit it to persist in the animals.

A third mechanism for explaining persistent infections involves the integration of all or part of the viral genome into the DNA genome of the host cell. The viral genes may be maintained there for long periods. Before the genetic information of RNA viruses can be integrated into the host cell genome it must first be transcribed into DNA by the enzyme reverse transcriptase. Some investigators have found traces of these DNA copies of RNA genome, which are called proviruses, in cells infected by certain viruses.

A recent example is the discovery by Ashley Haase of the Veterans Administration Hospital in San Francisco and Opendra Narayan of the Johns Hopkins University School of Medicine that cells from the brain of a sheep fetus infected with visna virus contain DNA copies of the RNA viral genome. Visna, a persistent disease of the central nervous system of sheep, resembles multiple sclerosis except for the fact that visna progresses steadily whereas the human disease is characterized by periods of remission and exacerbation.

The experiments of Haase and Narayan provide evidence that the provirus exists in the brain cells but that its expression is blocked. They found that only a very few of the cells containing the proviral DNA contain visna virus antigens. This is consistent with previous findings that the agent cannot be observed in or recovered from diseased brain tissue, unless the tissue is cultured for several days. The conditions of culture somehow relieve the block in virus formation.

Visna virus contains reverse transcriptase which provides an obvious mechanism for provirus synthesis. However, some investigators have suggested that even RNA viruses that lack the enzyme can produce proviruses in persistently infected cells. For example, Robert Simpson of Rutgers University found evidence of DNA copies of the RNA genome of respiratory syncitial virus in persistently infected cells and V. M. Zhdanov of the Academy of Medical Science in Russia did the same for measles virus. Since most cells do not contain reverse transcriptase, it is not clear how the proviruses are formed.

In contrast to the results of Simpson and Zhdanov, Holland and his colleagues do not detect VSV provirus in the line of infected cells that they have maintained for 3½ years. And, with Welsh and Oldstone, Holland was unable to find evidence for provirus in cells persistently infected with measles, mumps, rabies, influenza, and lymphocytic choriomeningitis viruses. Because of the great diversity of virus and cell characteristics, there may well be several mechanisms for establishing persistent viral infections.

—JEAN L. MARX