VIROLOGY

Monkey Virus Link to Cancer Grows Stronger

A virus that contaminated early batches of polio vaccine was deemed safe decades ago, but it keeps turning up in tumors. Now researchers are figuring out how the virus might imperil cells and are searching for ways to stop it

SV40 Timeline

1955
Jonas Salk introduces polio vaccine. Vaccine is injectible and contains dead poliovirus.

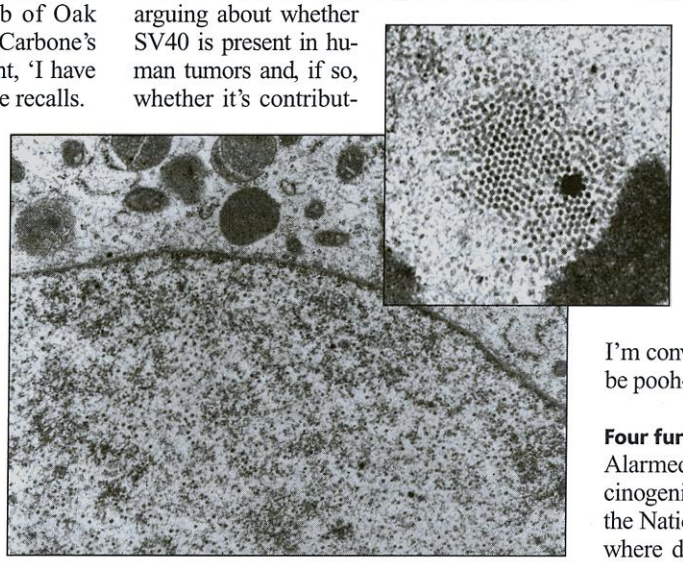
1960
Monkey kidney extracts used to make vaccine are shown to cause four rare types of tumors in hamsters.

The old man had been trying to tell Michele Carbone something for months. He'd buttonholed the molecular virologist on the last day of a busy conference, but they kept getting interrupted. He'd sent a pile of journal articles from the 1950s to Carbone's office at Loyola University Medical Center in Chicago, but they sat unread on Carbone's desk. He'd called and called again. Carbone finally relented when he realized that the 89-year-old physician, named Herbert Ratner, lived in the Chicago suburb of Oak Park, less than a kilometer from Carbone's house. "At a certain point I thought, 'I have to go' " see what he wants, Carbone recalls.

At the conference where he first met Ratner, held in 1997 at the National Institutes of Health (NIH) in Bethesda, Maryland, Carbone and colleagues reported finding traces of a monkey virus called SV40 in a rare form of cancer of tissue surrounding the lung. SV40 had been spotted in 1960 in monkey tissue used to make the polio vaccine and was soon found to cause four types of tumors in hamsters, sparking widespread alarm. By 1963, when the polio vaccine supply had been screened to ensure that it was free of the virus, more than 98 million people in the United States and hundreds of millions worldwide had been exposed to potentially contaminated vaccine. Researchers have been arguing ever since about whether that SV40 exposure increased their risk of cancer.

Carbone wanted to know whether the SV40 he'd found in tumors could have come from the polio vaccine. But a 3-year search among manufacturers, government officials, and researchers for possibly contaminated samples of vaccine had come up empty. Over tea at Ratner's home on a spring afternoon, Ratner explained that he had been director of public health for the town of Oak Park in 1955, when the polio vaccine was first released. Fearing that the new vaccine was unsafe, he refused to inject local children. Newspapers vilified him, but he stuck to his guns. Carbone thanked Ratner and headed for the door. "He said, 'No, no, wait, I have something for you,' " Carbone recalls. Ratner returned from his basement with vials of polio vaccine produced in 1954. He told Carbone that he had kept it in his refrigerator for 42 years, hoping all along that someone would test it and prove him right.

The 1997 NIH conference, dubbed a consensus conference, produced nothing of the kind. Believers and skeptics continued arguing about whether SV40 is present in human tumors and, if so, whether it's contributing to cancer in people. Skeptics pointed to a lack of epidemiological evidence linking pre-1963 polio vaccination to cancer and to other evidence suggesting that laboratory artifacts were responsible for some SV40 sightings in human tumors. Believers, meanwhile, cited decades of tissue-culture and animal research showing the virus's carcinogenic powers.



Infiltrator. Hexagonal SV40 particles (magnified in inset) dot the inside of a mesothelial cell's nucleus.

The battle continues today, but on new ground. Although most experts agree that SV40 infection is not a widespread public health problem, it clearly can cause cancer when given to newborn animals. Researchers still don't know whether the virus disproportionately infects cancer patients. Studies in the past 5 years have led many—but by no means all—to accept that SV40 genetic sequences are present in the same four types of human cancers that the virus causes in hamsters. In humans, however, it's not clear whether the virus helps cause cancer or just happens to infect cancer cells.

Part of the problem is that none of the tests to detect the virus are universally trusted. Some teams are working on better tests, which could help confirm or refute virus sightings in tumors and reveal if and how far SV40 is spreading in people. Many cancer patients with SV40-laden tumors were not vaccinated with the early polio vaccine, and researchers aren't sure how they picked up the virus.

Despite all the uncertainties, circumstantial evidence implicating the virus has piled up, particularly in the past year. Some groups are testing ways to block SV40 infection in an attempt to prevent or treat cancer. Skeptics of the SV40-cancer link remain, but more than a few are starting to come around. "I used to think it was all artifact," says cancer biologist Denise Galloway of the Fred Hutchinson Cancer Research Center in Seattle. "I wouldn't say I'm convinced, but I don't think it deserves to be pooh-poohed anymore."

Four furies

Alarmed by the early reports of SV40's carcinogenic effects in hamsters, researchers at the National Cancer Institute (NCI) and elsewhere did a series of epidemiological studies, beginning in the 1960s and 1970s, that seemed to dispel the worry about human risk. None found any increase in cancer risk in people who had received potentially tainted vaccines as infants or children. Studies in the United States and Europe tracked thousands of people for up to 20 years. However, the studies were not powerful enough to rule out a rise in rare cancers, says pediatric oncologist Bob Garcea, now at the University of Colorado School of Medicine in Denver.

The case was reopened in 1992, when Garcea, then at Harvard Medical School, and colleagues stumbled onto SV40 DNA in childhood brain tumors while searching for traces of two related human viruses. Soon after, Garcea, Carbone, and colleagues spotted SV40 DNA sequences in a bone cancer called osteosarcoma. Then Carbone and Harvey Pass, now at Karmanos Cancer Institute of Wayne State University in Detroit, spotted it in mesothelioma, a rare and uniformly fatal

CREDITS: M. CARBONE

SV40 is discovered in monkey kidney extracts.

SV40 is shown to cause four rare tumors in hamsters.

U.S. orders vaccine-makers to eliminate SV40 from Salk vaccine.

Albert Sabin introduces oral polio vaccine; it is not thought to contain SV40.

NCI study: Children who received infected vaccine face no increased cancer risk.

NCI study: SV40-exposed children, now teenagers, face no increased cancer risk.

SV40 is spotted in childhood brain tumors.

SV40 is spotted in rare bone and lung cancers.

cancer of the tissue lining the lungs. That meant the virus had shown up in three of the four types of cancer that it caused in hamsters.

But other researchers looked for the virus and didn't find it. For instance, cancer epidemiologist Howard Strickler, then at NCI, and Keerti Shah of the Johns Hopkins Bloomberg School of Public Health in Baltimore found no sign of SV40 in osteosarcoma and mesothelioma. At the 1997 consensus conference, Strickler and other skeptics said the virus sightings could have come from contamination by lab strains of SV40, which are used widely by cancer researchers.

To resolve the dispute, two researchers arranged multilab studies on mesothelioma—and came to opposite conclusions. One, led by Strickler, involved nine different labs. It found traces of SV40 in a small fraction of mesothelioma samples—but the virus was just as likely to appear in control samples of lung tissue. Strickler, who's now at Albert Einstein College of Medicine in New York City, says the study casts doubt on a role for SV40 in mesothelioma. He points out that when SV40 DNA is found in tumors, it's scarce—too scarce to be causing cancer. Furthermore, the proportion of tumors with SV40 varies greatly from one study to another, which he says casts doubt on the reliability of the screening methods used.

Meanwhile, molecular geneticist Joseph Testa of the Fox Chase Cancer Center in Philadelphia and colleagues reported in 1998 that nine of 12 mesothelioma samples turned up positive when tested in all four participating labs. Testa says the results mean that lab contamination was not to blame, but Strickler disagrees, pointing out that the researchers did not test normal tissue to make sure the assay was working properly.

Since then, several studies have replicated Testa's positive findings using other methods. Marc Ramael's team at St. Elisabeth General Hospital in Herentals, Belgium, used fluorescent tags to spot SV40 DNA and protein inside mesothelioma cells in preserved tumor tissue. And molecular biologist Adi Gazdar of the University of Texas Southwestern Medical Center in Dallas and colleagues found SV40 in 50% of the mesotheliomas they dissected from preserved human tissue. In both cases, no SV40 showed up in nearby normal lung tissue, ruling out contamination, Gazdar says. At first, "I was skeptical of the whole thing," Gazdar says, but "there's no question that the sequences are present."

Recently, SV40 went four for four. Researchers found the virus in humans with non-Hodgkin's lymphoma, a cancer found in SV40-infected hamsters. Teams led by Gazdar and Janet Butel of Baylor College of Medicine in Houston tested hundreds of human tumors. As both groups reported in the 9 March issue of *The Lancet*, they found SV40 DNA in about four in 10 samples, but not in normal lymph tissue or blood cells. The results still need to be confirmed in other studies, Butel says, but the virus "might account for a large fraction of non-Hodgkin's lymphoma."

Virus hunting

When Carbone's team analyzed Ratner's old polio vaccine, they found that it was contaminated with a variant of SV40, the genome of which differed from that of common lab strains of the virus. The distinctive strain has now been found again, in three non-Hodgkin's lymphoma patients, Butel's team reported in its recent *Lancet* paper. The patients' SV40 and that extracted from Ratner's vaccine have an identical molecular fingerprint, a matching DNA sequence in a stretch of an SV40 gene that varies from strain to strain. "This is the ultimate proof you'd want that at least some of this virus [infecting cancer patients] came from the polio vaccine," Carbone says.

Carrier. SV40 from rhesus monkeys contaminated early batches of polio vaccine.

Butel and colleagues didn't emphasize the match when they reported their findings. "I don't want to be responsible for scaring people so that they are afraid to use the polio vaccine," Butel says. She points to all the good the vaccine has done: It has practically eradicated the dread disease in much of the world, saving tens of thousands from paralysis and saving thousands of lives. And the polio vaccine now used in the United States is grown in cultured monkey kidney cells uninfected by SV40.

Still, the largest human exposure to SV40 probably came from contaminated vaccines between 1955 and 1963, many researchers say. But SV40 DNA has turned up in people who never received possibly contaminated vaccine. Some skeptics see this as evidence of an alternative source, such as an endemic strain of SV40 in the population, possibly introduced through monkey bites. Those who point to the vaccine link say that SV40 from contaminated vaccines has spread from person to person. SV40 can replicate in people, Butel and others say, and it can also be excreted in feces and urine. "It seems to me that there's no way around arguing [that there has been] transmission of the virus," says Galloway of Fred Hutchinson Cancer Research Center.

It's been difficult to track the virus's progress, however, because there's no widely trusted test to quickly detect antibodies against SV40 in human blood. Existing tests are either too slow to screen a large number of samples, or they have trouble telling SV40 from two of its cousins, JC virus and BK virus, both of which are common in people. Galloway is "very optimistic" that an as-yet-unpublished assay her group has developed, which distinguishes BK from JC, can be modified to distinguish both from SV40. A reliable test could confirm or refute disputed SV40 sightings and reveal whether cancer patients have more SV40 infections than healthy people, Galloway says.

Even if SV40 infection is found in cancer patients, that doesn't mean it causes cancer. Epidemiological studies continue to show no increased risk of cancer in people exposed to the polio vaccine, Strickler says. A 1998 study he co-authored, for example, examined data on millions of patients from NCI's national cancer registry and found no rise in the incidence of cancer—rare or common—in people who have been exposed to contaminated vaccine.

Even where the virus seems to be present in tumors, says Strickler, it does not infect ev-

CREDIT: WOLFGANG KOEHLER/CORBIS

NCI researchers and others screen tumors, find no SV40.

NIH-sponsored SV40 consensus conference: Debate is still unresolved.

Multilaboratory study confirms SV40 in mesothelioma.

NCI: Still no increased cancer risk after more than 3 decades.

Second consensus conference: SV40 is present in mesothelioma and probably other tumors; it may cause cancer.

SV40 is spotted in non-Hodgkin's lymphoma in two large studies.

ery cancer cell, raising the question of how the virus could cause cancer in cells it doesn't infect. And unlike known cancer-causing viruses, which usually infect a single tissue, SV40 seems to turn up in a wide variety of tumor types. "It's hard to see how a virus can go to all these tissues and produce all these cancers," Shah of Johns Hopkins says.

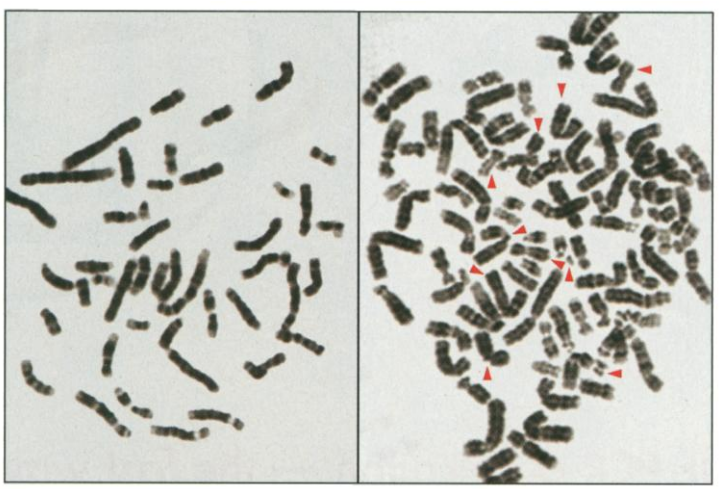
Proponents counter that some of the putative SV40-induced cancers are so rare that a link to polio vaccination wouldn't reach statistical significance even in huge epidemiological studies. They also say that if SV40 is indeed spreading through the population, studies based on polio vaccine exposure wouldn't have a reliable measure of who's been exposed. Those who suspect a causal role say the scarcity of DNA can be explained if SV40 causes cancer via a temporary infection that is later undetectable. Alternatively, the virus might release cancer-promoting molecules that act at a distance.

Cellular havoc

Evidence that SV40 acts directly on cells to cause at least one cancer, mesothelioma, has been growing. As early as 1997, teams led by Carbone and Antonio Giordano, now at Temple University in Philadelphia, showed that in animals and lab-grown human mesothelioma cells, an SV40 protein called the large T antigen turns off two tumor-suppressor proteins, Rb and p53, removing two separate brakes that keep genetically damaged cells from multiplying.

But according to 1999 work by NCI's David Schrump and colleagues, removing most of SV40's large T antigen can thwart tumor cells. The strategy restored the p53 pathway in mesothelioma cells isolated from a human tumor; in response, the tumor cells stopped growing and self-destructed. George Klein of the Karolinska Institute in Stockholm, who did pioneering work linking the Epstein-Barr virus to cancer, says that such experiments are "the strongest piece of evidence you can obtain to say that the virus is essential for the growth of the tumor," but he adds that more experiments are needed to prove a causal role.

Other studies in mesothelia show that SV40 infection induces several hallmarks of cancer. Testa's team showed in 1999 that SV40 causes dramatic chromosome rearrangements and damage. "It looks like you set a bomb off in these cells," he says. In work published in the 21 February issue of *Oncogene*, Carbone, Testa, and colleagues report that SV40 spurs activity of an enzyme called telomerase that extends the tips of chromosomes, thus allowing cells to become immortal. And in work that was presented in April at a meeting of the American Association for Cancer Research, Gazdar, Carbone, and colleagues show that SV40 infection causes cells to disable a tumor suppressor gene called *RassF1* several weeks after infection, just when cells are becoming malignant.



Like a bomb. Compared to normal chromosomes (*left*), those from a SV40-infected cell are multiplied and often abnormal (*right*, red arrows).

CREDIT: J. TESTA

Another new study, in the October 2001 issue of the *Proceedings of the National Academy of Sciences*, suggests that SV40 doesn't have to be in every cell to cause cancer. Giovanni Gaudino of the University of Piemonte Orientale in Novara, Italy, Luciano Mutti of the Maugeri Foundation in Pavia, and their colleagues showed that SV40 infection caused mesothelium cells to secrete a growth factor. When it diffuses to neighboring cells, it activates a gene that starts the cells down the road to cell division.

A clean vaccine

As evidence for SV40's ability to induce mesothelioma builds, some researchers are trying to disable the virus as a way to treat or prevent this fatal cancer. Martin Sanda and Michael Imperiale of the University of Michigan Medical School in Ann Arbor came up with an SV40 vaccine in 1999. They found that a virus called vaccinia containing a neutered SV40 T antigen held off SV40-induced mesothelioma in mice and shrunk preexisting tumors. Others have shown that injecting naked DNA encoding the SV40 T antigen accomplishes the same thing.

Pass of Wayne State University is planning a phase I clinical trial. The vaccine could eventually help treat patients whose tumors show signs of SV40 infection, and it might be given to people already at high risk of mesothelioma, such as asbestos workers, he says. NCI is looking for a company to manufacture the vaccine.

But longtime SV40 researchers claim that few other funds have been available from the government, which oversaw distribution of early batches of contaminated polio vaccine and vouched for its safety. The lack of funds means that key questions have remained unanswered for years, many SV40 researchers say. "The federal government's attitude has been, 'We don't believe this story; it's not proven; so we're not going to fund it,'" Gazdar says.

May Wong, program director for DNA viruses at NCI, acknowledges that funding has fallen far short of what SV40 researchers have sought, mostly because the work was considered "very speculative and controversial." "I don't think the government was trying to cover up anything. It was just that the data wasn't there," she says. But the stream of new reports has begun to change that, she adds: NCI recently funded Carbone and Butel. And a new NCI program to look at possible cancer-causing microbes, including SV40, is in the works.

Researchers are itching to get started on a backlog of work, including studies that track the cancer risk of SV40-infected people. They want to test whether SV40 assists other carcinogens such as asbestos and see if blocking an SV40 infection could help treat mesothelioma or other cancers. Those studies will take money and time, and the answers won't just turn up in someone's refrigerator.

–DAN FERBER