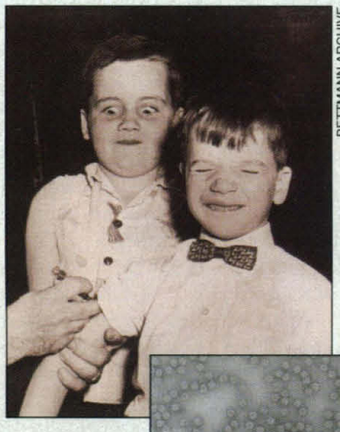
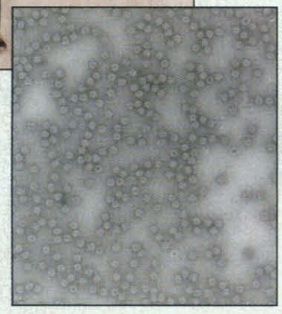
CANCER RESEARCH

Monkey Virus DNA Found In Rare Human Cancers

When Keerti Shah walked into the Natcher Auditorium at the National Institutes of Health (NIH) last week, he felt as if he were stepping back in time. The topic—whether a monkey virus called simian virus 40 (SV40) causes cancer in humans—was one he had first addressed more than 30 years earlier. Now, the issue had popped up again.



BETTMANN ARCHIVE



ROBERT GARCEA/UCHSC

Déjà vu. New results reopen the possibility that a polyomavirus found in some 1950s polio vaccines causes cancers.

In 1960, researchers discovered that SV40 had inadvertently contaminated some batches of polio vaccine. A year later, it was proven capable of causing cancer in hamsters, raising alarms that some of the 98 million people in the United States who had been vaccinated during the late 1950s were at risk. Epidemiological and experimental studies done by Shah, a virologist at the Johns Hopkins School of Public Health in Baltimore, and others at that time apparently laid that worry to rest: There was no evidence of increased cancer risk in people who had received the suspect vaccines.

But several research teams have recently found what seems to be SV40 DNA in human tumors, including a bone cancer, certain brain cancers, and mesothelioma, a connective-tissue cancer previously linked to asbestos. Although a few other teams haven't been able to confirm them, the findings raised the specter that the contaminated polio vaccine could be causing long-term consequences. Shah and about 250 other researchers met at NIH to discuss that worrisome possibility.

After 2 days of heated discussions of new data, they reached the same conclusion of 3 decades ago: New epidemiological studies indicate that the SV40 in those early polio vaccines is not a public health threat. "I don't think there's any evidence at this time to be concerned about," says the U.S. Food and Drug Administration's Andrew Lewis, who organized the workshop with epidemiologist Howard Strickler of the National Cancer Institute.

That doesn't mean all the questions have been answered, however. Some evidence suggests that the virus does infect humans and may have existed in the human population even before the polio vaccine. Researchers want to verify this, as well as try to confirm the SV40 DNA sightings in the cancers. Assuming that they do, it's possible that SV40 may have contributed in some unknown way to the development of a small number of cancers. If so—and that's a very big if given the uncertainties—then infection by this virus might help explain, for example, the mesotheliomas not linked to known asbestos exposure. In that event, vaccines against these tumors might be possible, notes pediatric oncologist Robert Garcea of the Children's Hospital in Denver.

The data that led to the workshop first began to appear in 1992. At the Dana-Farber Cancer Institute in Boston, Garcea and Daniel Bergsagel were following up on the 1985 discovery that transgenic mice carrying the SV40 genome developed choroid plexus brain tumors. Thinking that SV40 doesn't infect humans, they decided to see whether brain tumors from some of their human pediatric patients show signs of infection by the JC and BK polyomaviruses, which are closely related to SV40 and are found in human populations. When they used PCR, a very sensitive technique for detecting nucleic acids, to look for viral DNA in the tumors, they instead found SV40 DNA in half of 20 choroid plexus tumors and in 10 of 11 ependymoma brain tumors.

Garcea was astonished by these results because there was little evidence that SV40 infects humans. Because SV40 is commonly used in research, he worried that PCR had picked up unexpected contamination—a nagging problem with the technique. So, he sent his samples to Janet Butel, a longtime SV40 specialist at Baylor College of Medicine in Houston, without telling her which were positive for SV40. She and Baylor's John Lednicky, also using PCR, confirmed the Boston group's results. Furthermore, her team was able to grow virus from one fresh tumor sample and, from its sequence, verified that it was natural SV40 and not one of the strains used in her laboratory for research.

The list of cancers containing SV40 DNA also began to expand. At NIH in 1994, Michele Carbone, now at Loyola Medical Center in Maywood, Illinois, linked the virus to mesotheliomas. He had determined that SV40 causes mesotheliomas in hamsters and wanted to see whether the virus had anything to do with human cases not obviously linked to asbestos. In studies of about 120 tumor samples, including 48 mesotheliomas, he and his colleagues found that 60% of the mesothelioma samples tested positive for SV40-like DNA. They sequenced DNA from three of those just to be sure it was SV40 genetic material. "We were very surprised by this," says Carbone. "Up to then, mesotheliomas were thought to be only asbestos-related."

Back in Boston, Garcea and his colleagues found SV40 DNA in more than half of 18 osteosarcomas, a relatively rare bone cancer. Since then, Garcea, working with Illinois's Carbone, has extended that finding. In a blinded survey of 345 tumor samples, including more than 150 from bone tumors, they found evidence of SV40 in what turned out to be 40 of 126 osteosarcomas and 14 of 34 other bone tumors.

Those findings revived concerns about SV40, especially because the virus causes the same types of cancers in rodents. The FDA's Lewis, along with Strickler; Arthur Levine, a molecular biologist at the National Institute of Child Health and Human Development; and officials at the Centers for Disease Control and Prevention in Atlanta decided to get together all the researchers involved to hash out the findings and "try to arrive at the scientific truth," says Levine.

The epidemiological data presented at the meeting were encouraging. Strickler compared cancer incidence and mortality in people who were likely to have received the contaminated vaccine as infants with those in the next closest 5-year age group. He looked at data for all cancers as well as those for mesotheliomas, osteosarcomas, brain cancers, and a few more common cancers. His conclusion: The probable exposure to the contaminated vaccine "hasn't resulted in any increases in cancers that are detectable." A similar study in Sweden also yielded no indication that the contaminated vaccine had raised cancer incidences.

While encouraging, those results leave unanswered the question of what role, if any, SV40 might have played in the tumors in which its DNA was detected. Several other groups from the United States, France, Italy,

and the United Kingdom have now added to the earlier results, reporting at the meeting that they, too, have found evidence of SV40 in some of the tumors studied, most strikingly in about a third of the mesotheliomas tested.

But not everyone has been able to confirm the sightings. For example, working with Shah, Strickler and his colleagues tested 50 mesothelioma tumors for SV40 DNA but found none. "We've tried to replicate exactly what [the other researchers] did, and we do not get the same results," adds Ethel-Michele de Villers, who also failed to detect SV40 in 32 tumors other than mesotheliomas with her colleagues at the German Cancer Center in Heidelberg. "We've heard compelling data from both sides—from excellent labs," commented Levine.

The reason for the discrepancy is unclear. The positive results might have come from sequences from the JC and BK viruses. But that seems unlikely because several teams sequenced enough of the DNA to tell whether it came from SV40. Contamination by lab strains of SV40 also seems improbable given the large number of sightings. "It's unlikely that all the positive results can be written off as contamination," says retrovirologist Robin Weiss of London's Institute of Cancer Research.

Assuming that the sightings are authentic, the next step will be to try to pin down a biological connection between SV40 and human cancer. "Just because some of us detect [SV40] in tumors doesn't mean it's causing the tumors," Baylor's Lednicky cautions. Proving that means coming up with results that everyone agrees are valid.

The answer might be important in view of evidence that SV40 exists in human populations, apparently even predating the contaminated vaccine. Over the years, several teams have screened blood samples taken before the vaccine became available, or surveyed isolated populations who have never gotten polio vaccines, for antibodies against SV40 proteins. They find these antibodies in 5% to 12% of those studied. However, those studies did not completely rule out the possibility that the antibodies were detecting JC or BK proteins. "We don't necessarily have good exposure data," Strickler says. "So, it's hard to tell how to interpret the [antibody] findings."

Researchers clearly still have plenty of work to do before they can totally exonerate SV40 as a cause of human cancer. "The main thing is to verify the observations and standardize the techniques," says Garcea. Toward that end, Strickler says his institute is beginning to prepare a panel of samples to be sent out to various labs for testing. And the FDA scientists say they plan to work on developing better PCR procedures and antibody screens. Says Shah: "Unlike mad cow disease, where you don't know what to look for, there's so much intellectual power behind this virus [that] we should be able to find out very quickly what is going on."

–Elizabeth Pennisi

ENVIRONMENT

Mixed Reviews for Habitat Plan

For years, the northern spotted owl has been the focus of a bitter struggle between environmentalists and loggers over the old-growth forests of the Pacific Northwest. Now, it is about to become the poster species of an ambitious new plan for preserving biodiversity.

Last week, federal and state officials signed a 70-year land management agreement to protect a host of species, including the infamous owl, on 650,000 hectares of land managed by the Washington state Department of Natural Resources. Said Secretary of the Interior Bruce Babbitt at the signing ceremony, "We are creating a conservation mosaic across Washington's magnificent forests that will lead to survival, indeed, recovery, of aquatic species and wildlife now endangered or in peril, while offering long-term certainty for rural timber economies."

Based on recommendations from a scientific team from the U.S. Fish and Wildlife Service, the National Marine Fisheries Service, and other federal and state agencies, the "habitat conservation plan," or HCP, sets aside prime land—including old-growth forests, buffer strips along streams, and caves, cliff sides, and other specialized habitats—for the region's endangered species while opening other areas to timber harvesting. The plan also allows for the "taking," or harming or killing, of several endangered species during logging operations as long as it does not send the species spiraling into extinction.

The Washington plan is the first statewide, multispecies HCP, and, like other HCPs around the country, it is controversial. As might be expected, it has drawn fire from timber interests. Says Jim Geisinger, president of the Northwest Forestry Association in Portland, Oregon, "When you look at the percentage of the land base that would be made off limits to logging, we believe it will be very difficult to maintain [the plan's projected annual harvest of 655 million board feet]."

Other critics question the plan's ecological underpinnings. Although it is supposed to protect over 285 species, from the grizzly bear and Rocky Mountain elk to the golden paintbrush and Oregon silverspot butterfly, the biologists who worked on the plan looked primarily on the habitat requirements of a handful of species, including the northern spotted owl, the marbled murrelet, and several species of salmon and steelhead trout, according to Martin Raphael of the Forest Services Laboratory in Olympia, Washington, who worked on the plan. The idea is that if these relatively well-studied "indicator" species are protected, the rest will be preserved, too. Laura Hood of the Defenders of Wildlife, an environmental organization in Washington, D.C., counters that this approach promises far more than it delivers: "They should admit that [the plan] covers just a few species."

JOE MCDONALD/ANIMALS, ANIMALS

VIREO

Protected. Northern spotted owl *(top)* and marbled murrelet *(above)*.

Some biologists also question the ecological wisdom of the plan's "no surprises" clause. This provision guarantees that once a deal is struck, the federal government cannot come back and require the state to spend more money on preserving habitat, even if additional species become threatened or already-included species fail to thrive. "That clause could be dangerously inflexible," contends Tim Cullinan, staff scientist at the Washington field office of the National Audubon Society in Olympia.

All ecosystems change over time, whether as a result of hurricanes, fires, disease outbreaks, or invasion by exotic species, points out Gary Meffe, a senior ecologist at the Savannah River Ecology Lab in Aiken, South Carolina. He argues that "['No surprises' language] does not reflect ecological reality and rejects the best scientific knowledge. ... This is a political, not a scientific, perspective."

Still, some ecologists and environmentalists say the HCP is a big step in the right direction. "Washington state has been living hand-to-mouth off its forests," says Cullinan. The HCP may help it build a more sustainable future.

–Jim Kling

Jim Kling is a free-lance writer in Bellingham, Washington.