

Research on SV 40 Exposure and the Development of Cancer

**Statement of James Goedert, M.D.
Chief, Viral Epidemiology Branch
Division of Cancer Epidemiology and Genetics
National Cancer Institute
National Institutes of Health
Department of Health and Human Services**

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Thank you, Mr. Chairman, for inviting the National Cancer Institute (NCI), an agency of the National Institutes of Health, Department of Health and Human Services, to testify before the Subcommittee today. I am James Goedert, M.D., Captain, US Public Health Service, and Chief of NCI's Viral Epidemiology Branch (VEB) in the Division of the Cancer Epidemiology and Genetics.

Our Branch conducts population-based epidemiology research to clarify the relationship of infectious agents, especially viruses, to human cancer and other conditions. Viruses may cause or increase the risk of cancer through several mechanisms. These include allowing uncontrolled cell division, blocking DNA repair, and altering the immune system. While some viruses have been known to be related to cancer for many years, new infectious carcinogenic agents continue to be found. The Branch utilizes the principles of both infectious and chronic disease epidemiology, supported by collaborative statistical modeling and laboratory investigations.

Some cancer-associated viruses appear to rarely cause cancer among exposed persons. Other viruses substantially increase the risk for cancer and the burden of this disease in the population. Knowing which cancers are associated with the different viruses can help promote targeted cancer screening, early detection, and treatment. If cancer is more common in people with exposure to the virus, then that suggests that the virus might cause the cancer. Similarly, if people with cancer are more likely to have been infected with the virus than healthy people, this also suggests that the virus could cause cancer.

Like all scientific research, individual epidemiologic studies cannot provide a definitive answer about the relationship between an exposure and the development of cancer. Rather, epidemiologic and other scientific research studies build a body of evidence -- supported over the years by larger and more rigorous research studies -- that ultimately convince the scientific community, and the policy-makers of state and Federal governments, of the existence of such a relationship. At this time, our opinion is that the body of evidence is inconclusive as to the role of SV40 in the development of cancer.

SV40 in Early Vaccines

Simian virus 40 (SV40) is a virus that infects several species of monkeys and typically does not cause disease in them. The virus was discovered in 1960 in rhesus macaque monkey kidney cells that were used in the production of the original Salk and Sabin polio vaccines (1). Since the mass immunization program for polio began in 1955, before the discovery of the virus, contaminated vaccine lots were inadvertently used for the first few years of the program.

When reports appeared in 1961 that injecting SV40 into hamsters could cause tumors (2-5), the United States (U.S.) government instituted a screening program requiring that all new lots of poliovirus vaccine be free of SV40 because of concerns about possible adverse effects on human health. Already-produced vaccine may have been used through 1962, but U.S. polio vaccine has been free of SV40 since 1963 (6). Published results of testing confirm that no SV40 has been found in U.S. polio vaccine lots tested after 1972 (47). The polio vaccine currently used in the U.S. is produced under carefully regulated conditions designed and enforced by the Food and Drug Administration to ensure that contamination with SV40 does not occur. As a result of the earlier contamination, however, it is estimated that more than 10 million to 30 million people vaccinated in the United States from 1955 through early 1963 were inadvertently exposed to live SV40 (6).

SV40 in Animals

In laboratory animal studies, the SV40 virus has been found to cause malignancies (mesothelioma, ependymoma, osteosarcoma, and leukemia and lymphoma) in newborn rodents, particularly hamsters, exposed to high levels of the virus (50). However, the question of whether SV40 causes human cancer is unsettled, as published data are contradictory.

Follow-up of Vaccine Recipients Shows No Excess of Cancer

Over the last four decades, an intense research effort has been made to determine whether the exposure to SV40 through polio vaccination has caused cancer in people. Up through the early 1990's, epidemiologic studies involving decades of observations and millions of people in the U.S. and Europe have failed to detect an increased cancer risk in those likely to have been exposed to the virus. These include a long-term Swedish study, which followed 700,000 people who received SV40-contaminated vaccine (7), a German study with 22 years of follow-up of 886,000 persons who received the contaminated vaccine as infants (8), a 20-year study of 1,000 people in the United States inoculated during the first days of life with contaminated vaccines (9), a 30-year follow-up of approximately 10 percent of the entire U.S. population (using data from the National Cancer Institute's Surveillance, Epidemiology, and End Results registry) (10), and a 40-year follow-up study of 1.8 million recipients of a widely contaminated Danish polio vaccine (38) .

SV40 is Variably Detected in Human Tumors

The issue of SV40 and cancer has surfaced in the last few years when some laboratories, using an extremely sensitive molecular biology technique, the polymerase chain reaction

(PCR), found traces of SV40 DNA in some rare human tumors including pleural mesothelioma (a cancer of the lining of the lung), osteosarcoma (a type of bone cancer), ependymoma and choroid plexus tumors of the brain, and recently non-Hodgkin lymphoma (12-29). Other studies reported that SV40 T-antigen, a viral protein, binds to human tumor suppressor proteins such as p53 and RB (30-32), suggesting a possible carcinogenic mechanism. Not all studies, however, have found that SV40 can be detected in human cancer (33-37, 39-46). When detected, SV40 has been found at very low levels (40), raising questions about the biological role that SV40 could play and suggesting that reported detection could be a laboratory artifact. Finally, some studies have found SV40 in a wide range of other tumors and normal tissues (16), which raises further questions about the biological interpretation of positive findings.

Laboratory Studies Result in Controversy and Uncertainty

In order to resolve why some laboratories detect traces of SV40 in mesothelioma while others do not, an International SV40 Working Group, which included the majority of laboratories studying SV40 in human tissues, was formed in 1997. Nine laboratories from the working group agreed to participate in a study, funded and organized by NCI. Under a tightly reasoned, thoroughly vetted, and tightly enforced research protocol (Appendix 1), each group was given 25 paired-duplicate samples of human mesotheliomas, a single set of 25 normal lung tissue samples, and positive and negative control samples. All the samples were masked (prepared and labeled so that the human tumors and controls could not be distinguished). Each laboratory used one or more assays for detecting SV40, many of which had been used to detect SV40 previously. The results (36) showed that none of the mesothelioma specimens was consistently positive for SV40 across all laboratories. New methods that can be used widely and easily to reliably detect the presence of SV40 DNA in human tissues are needed.

Recent and Ongoing Research

Since the early 1990's, the NCI and other investigators continue to evaluate the possible link between SV40 infection and human cancers. Our Branch is monitoring populations known to have been exposed to SV40-contaminated vaccines, and some of our recently completed and ongoing studies are described below. Additional extramural grant-supported studies funded by NCI to evaluate the possible relationship of SV40 to cancer are underway (<http://researchportfolio.cancer.gov/>). Other institutes at the National Institutes of Health are also funding a wide range of studies related to SV40 (<https://www-commons.cit.nih.gov/crisp>).

- *Mesothelioma in the U.S.* (37) Using data from the Surveillance, Epidemiology and End Results (SEER) population, which is a 10% sample of the entire U.S. population, NCI examined the incidence of mesothelioma of the lining of the lung (the pleura), with a particular focus on individuals' ages during the 1955-1963 interval when poliovirus vaccines were contaminated with SV40. The rate of pleural mesothelioma was highest among men over age 75, who were least likely to have received SV40-contaminated vaccine and most likely to have been

exposed to asbestos, a known cause of mesothelioma. In middle age individuals, between 25 and 54 years of age, who were infants or children during 1955-1963 and most likely to have received SV40-contaminated poliovirus vaccine, mesothelioma rates have been low and even decreasing. Females, although equally exposed to SV40-contaminated vaccines during childhood, had much lower mesothelioma rates, probably because they have been much less exposed to asbestos. We concluded that after almost 40 years of follow-up, U.S. cancer incidence data have not shown an increased incidence of pleural mesothelioma among the age groups that were exposed to SV40-contaminated poliovirus vaccine.

- *Cancer incidence in Denmark.* (38) In Denmark, inactivated poliovirus vaccine was first administered in April 1955, a few weeks after vaccination campaigns began in the U.S. Because of the urgency of the epidemic, a concerted effort was mounted to administer poliovirus vaccine to a large proportion of the population, and Denmark maintained a high level of vaccination through the early 1960s. NCI and Danish investigators recently examined cancer incidence in Denmark as a function of birth year and calendar year, which served to identify exposure to early poliovirus vaccine. Importantly, review of 1960s Danish records identified widespread SV40-contamination of previously utilized Danish poliovirus vaccine, which, unlike in the U.S, was grown in pooled kidney tissue from dozens of monkeys. A further strength of this study was the high quality of Denmark's nation-wide data on cancer incidence, which go back to 1943. Overall cancer incidence was actually lower in SV40-exposed individuals (age-adjusted relative risks 0.86, 95%CI 0.81-0.91, and 0.79, 95%CI 0.75-0.84, for those exposed as infants or children, respectively, compared with those unexposed). No increased risk was seen for specific outcomes, including mesothelioma, bone tumors, brain tumors, and non-Hodgkin's lymphoma (NHL).
- *Brain tumors in northern India.*(39) A study involving a population uniquely exposed to SV40 - people living in northern India - was published recently. It is unclear whether humans can be infected with SV40 but, if this occurs, human infection might be especially common in northern India, where contacts between humans and SV40-infected monkeys frequently occur. NCI researchers and collaborators tested for the presence of SV40 in 47 archived samples of choroid plexus tumors and ependymomas, which are rare human brain tumors reportedly linked with SV40 (15, 17), from the All India Institute of Medical Sciences in northern India. Non-malignant brain tissues were included as negative controls, and laboratory workers were masked to the case-control status of specimens. A further strength of the study was the use of real-time polymerase chain reaction to quantify SV40 and cellular DNA detected in specimens. Investigators did not find

SV40 in any of the tumors. Given the PCR assay's sensitivity, SV40 would have been detected if it was present in at least one copy per 10 cells.

- *Case-control study of non-Hodgkin's lymphoma in Spain. (45)* In a case/control study in Spain involving 520 lymphoma cases and 587 controls, researchers tested blood samples from cases and controls for the presence of antibodies to SV40. If SV40 circulates in human populations and is implicated in lymphomas, SV40 serum antibodies might be detected at high levels in lymphoma cases. However, the researchers found no increased antibody levels to SV40 detected in lymphoma cases vs. the controls. Overall, SV40 antibody levels were low in both cases and controls. Additional testing suggested that a large part of these antibodies may be antibodies to the human virus BK, and not to SV40. Because the DNA of the SV40 virus is nearly 70 percent identical to the BK virus, it is difficult to distinguish between antibodies to the two viruses. Most humans carry antibodies to BK in their blood, since the virus commonly infects humans as children. BK, however, is not associated with any disease in healthy people
- *Polio vaccination history in brain tumor patients. (46)* In another case/control study involving 782 brain tumor cases and 799 controls (46), the risk of developing glioma, meningioma, or acoustic neuroma was not associated with having reported receiving either injected or oral poliovirus vaccine during the time period (1955-1963) when vaccines were contaminated with SV40.
- *Follow-up of recipients of U.S. Army's adenovirus vaccine (48)* To eliminate severe outbreaks of respiratory illness in basic training camps, the U.S. Army administered an inactivated adenovirus vaccine, grown in monkey kidney tissue, to entering service personnel in 1960-61. Evidence is compelling that this vaccine was widely contaminated with live SV40. Adenovirus grows extremely poorly in monkey kidney tissue without the presence of SV40 as a "helper virus." This situation is unlike poliovirus vaccine contamination, which in the U.S. did not occur uniformly, because SV40 was not a necessary cofactor for poliovirus replication *in vitro*. As with poliovirus vaccine, formalin-inactivation did not completely inactivate contaminating SV40.

NCI investigators are conducting a retrospective cohort study of Army servicemen from this era. Cases of mesothelioma, brain tumors, and non-Hodgkin's lymphoma in military veterans will be linked to military service records to determine which individuals entered Army service on a date that corresponded to the Army's use of this vaccine. An additional advantage of the study design is the attained age of the men who entered the Army in 1959-61; by

the 1990s, they would have reached an age when mesothelioma incidence becomes appreciable. Results from this study should be available soon.

- *Case-control study of childhood cancer.* NCI investigators are conducting a case-control study of childhood cancer that should be informative with respect to the role of SV40 in human cancer. In the U.S. during the 1950s and 1960s, pregnant women were frequently given inactivated poliovirus vaccine, potentially leading to infection of their children with SV40 *in utero* or soon after birth. Given the carcinogenic potential of SV40 in newborn laboratory animals, follow-up of children whose mothers were vaccinated during pregnancy represents a unique means of determining whether SV40 causes human cancer.

To pursue this line of inquiry, NCI has organized a study of SV40 and childhood cancer nested in the Collaborative Perinatal Project (CPP) cohort study. CPP enrolled pregnant women and their subsequently-born children in 1959-66 at 12 U.S. university medical centers. The cohort comprises 54,796 children born to 44,621 mothers. Enrolled mothers had study visits scheduled as part of their prenatal care, and detailed data on vaccinations during pregnancy reveal that 22.5% of CPP children were exposed *in utero* to pre-1963 poliovirus vaccine, 17.0% were exposed *in utero* to 1963+ poliovirus vaccine, and 60.5% of children were unexposed.

Through age 8 years, 52 CPP children developed cancer (18 neural tumors, 22 hematologic malignancies, 12 miscellaneous tumors). In a nested case-control study, paired sera (from early and late in pregnancy) have been selected from the 50 mothers of these children with available specimens and from 200 CPP control mothers. These sera are being evaluated for SV40 antibodies using an SV40 plaque neutralization assay and a virus-like particle-based (VLP) enzyme immunoassay.

- *Case-control study of non-Hodgkin's lymphoma in the U.S.* The possibility that SV40 causes a substantial fraction of non-Hodgkin's lymphoma in the U.S. was recently raised by two studies reporting the molecular detection of SV40 DNA in 40-50% of tissues. However, confirmatory evidence of SV40 infection (e.g., SV40 antibody) in non-Hodgkin's lymphoma cases was lacking, and SV40 was detected in tissues other than non-Hodgkin's lymphoma in one of the studies. In addition, these studies could not provide an estimate of the relative risk associated with SV40 infection.

NCI and laboratory collaborators at two institutions are pursuing this question further using samples collected in a case-control study of non-Hodgkin's lymphoma in the U.S. This study includes approximately 800 HIV-uninfected

non-Hodgkin's lymphoma cases and 700 age-matched population controls from the NCI-Surveillance Epidemiology and End Results (SEER) Case-Control Study of non-Hodgkin's lymphoma SV40 and BK serostatus will be assessed using VLP assays. Strengths of the U.S.-based study include the widespread exposures of the U.S. population to SV40-contaminated poliovirus vaccine and the representative nature of the non-Hodgkin's lymphoma cases (sampled consecutively at four SEER registry sites) and population-based controls. This study will help answer whether SV40 infection is more common in persons with non-Hodgkin's lymphoma than controls and thus provide evidence on whether SV40 might cause non-Hodgkin's lymphoma. Importantly, finding low SV40 seroprevalence in cases (i.e., substantially less than 40%, reported previously) would argue against SV40 as a cause of non-Hodgkin's lymphoma.

- *AIDS-associated non-Hodgkin's lymphoma.* (49) Recent reports of the detection of SV40 DNA sequences in 40% of AIDS-associated non-Hodgkin's lymphomas prompted NCI investigators to examine whether, among individuals with AIDS, those exposed to SV40-contaminated poliovirus as children had an increased risk for non-Hodgkin's lymphoma. Non-Hodgkin's lymphoma incidence was estimated for two cohorts with AIDS: persons born in 1958-61 (exposed to SV40-contaminated poliovirus vaccine as infants) or born in 1964-67 (born after the vaccine was cleared of SV40 and thus unexposed). Non-Hodgkin's lymphoma incidence was marginally higher in the exposed cohort (unadjusted relative risk 1.15, 95%CI 0.99-1.34, vs. unexposed cohort). Notably, however, the exposed cohort developed AIDS slightly earlier than the unexposed cohort (mean year of onset 1992 vs. 1993). Due to the temporal evolution of the U.S. AIDS epidemic, the two cohorts thus differed in composition, with the exposed cohort having more males and whites, who are known to be at increased risk of non-Hodgkin's lymphoma irrespective of AIDS or vaccination status, than the unexposed cohort. Also, exposed individuals were, on average, five years older at AIDS onset than unexposed individuals. With adjustment for these differences, non-Hodgkin's lymphoma incidence was identical in exposed and unexposed individuals (relative risk 0.97, 95%CI 0.79-1.20).
- *SV40 infection in primate workers.* Evaluation of persons with occupational exposure to SV40, i.e., exposure to macaques, would be valuable in documenting whether SV40 infection can occur in humans. Rhesus macaques are universally infected with SV40 by adulthood, and cynomolgus macaques are readily infected through rhesus contacts in captivity. Humans working with monkeys could become infected with SV40 via bites, scratches, or exposure to contaminated urine.

With collaborators at the Centers for Disease Control and Prevention (CDC) and

Johns Hopkins, NCI is undertaking a pilot study to examine whether workers in primate centers and zoos in North America display serologic evidence for SV40 infection. Investigators will determine whether SV40 seroprevalence is higher in workers exposed to primates than controls. Additionally, SV40-seropositive subjects will be further characterized, with regards to the specificity of SV40 antibody reactivity (i.e., evaluation of BK virus reactivity) and SV40 antibody titer. Evidence for SV40 infections will prompt additional studies that would include more detailed exposure and health outcome data and other types of biological specimens.

IOM Report

The Institute of Medicine (IOM) of the National Academy of Sciences issued a report in October 2002 (50), which concluded that scientific "evidence is inadequate to accept or reject a causal relationship between SV40-containing polio vaccines and cancer." (p. 11, Executive Summary). The committee stated that the "biological evidence is of moderate strength that SV40 exposure could lead to cancer in humans under natural conditions" and that "biological evidence is of moderate strength that SV40 exposure from the polio vaccine is related to SV40 infection in humans." (p. 11, Executive Summary)

Based on these conclusions, the Institute of Medicine made the following research recommendations: (Appendix II)

Research

The committee recommends development of sensitive and specific serologic tests for SV40.

The committee recommends the development and use of sensitive and specific standardized techniques for SV40 detection.

The committee recommends that once there is agreement in the scientific community as to the best detection methods and protocols, pre-1955 samples of human tissues should be assayed for presence or absence of SV40 in rigorous, multi-center studies.

The committee recommends further study of the transmissibility of SV40 in humans.

Until some of the technical issues are resolved, the committee does not recommend additional epidemiological studies of people potentially exposed to the contaminated polio vaccine.

Our Branch will continue to collaborate with others in multidisciplinary research fields to settle the uncertainties that remain, and to pursue new leads to clarify the relationship between SV40 and human cancer, if any.

Conclusion

As we move forward to resolve the uncertainties in this field, researchers will need to understand what the detection -- or lack of detection -- of SV40 DNA in tumors implies. In recognition of the IOM's recommendation that molecular methods for SV40 detection be standardized, future studies will need to include sufficient numbers and types of positive- and negative-control specimens and to make the status of the specimens (i.e., controls, tumors, and others) unknown to the persons performing the laboratory analyses. Valuable data may come from newly available serologic techniques, but only with rigorous study designs that mask case-control status and include sufficient number of subjects. To study whether SV40 is in the human population and, if so, its modes of transmission, epidemiologic studies could be conducted if assays, such as the new SV40 antibody techniques, are shown to be highly sensitive, specific, and reproducible.

Because of the widespread exposure to SV40 through contaminated vaccines, the question of whether SV40 causes some human cancers has substantial public health implications. However, the types of cancers claimed to be linked to SV40 have been and continue to be very rare. In addition, SV40 prevalence in the general population is unknown, and detection of SV40 in humans is controversial. We remain committed to helping resolve these questions.

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