Simian Virus 40 and Human Disease

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(See the article by Engels et al., on pages 2065–9.)

The inadvertent and unrecognized presence of simian virus 40 (SV40) in the commercial inactivated Salk poliovaccines administered between 1955 and 1963 resulted in the potential exposure of millions of individuals, in the United States and elsewhere, to this polyomavirus of the rhesus macaque [1]. Many of the monkey-kidney cultures used to prepare poliovirus pools were infected with the indigenous SV40. Soon after its discovery in 1960 [2], SV40 was found to be oncogenic in laboratory animals [3]. Therefore, the possibility that SV40 may cause human disease, particularly cancers, has been a topic of interest since the 1960s [4]. This debate has intensified during the past decade because several groups of investigators, using polymerase chain reaction (PCR) amplification methodology, have detected SV40 genomic sequences in a number of human cancers. These investigators have suggested that the virus contributes to the development of mesothelioma, osteosarcoma, pediatric and adult brain tumors, and non-Hodgkin lymphomas [5–8]. The reported presence of SV40 in tumors in individuals born after 1963 would seem to imply that SV40 is now established as a human infection circulating in communities via person-to-person contact [8]. Other investigators have been skeptical of these claims [9–12]; several groups have not been able to detect SV40 sequences in the aforementioned tumors [13–20], and epidemiologic studies have not revealed an increased risk of these cancers in populations exposed to SV40-contaminated poliovaccines or adenovirus vaccines [21–23]. In addition to a large number of scientific publications, the controversy has spawned a report by the Institute of Medicine [24], a book in the popular press [25], and litigations. The evidence for the pathogenicity of SV40 in humans can be conveniently examined in 3 parts: (1) the nature of the human response after exposure to SV40, (2) the evidence that infection with SV40 has become established in humans, and (3) the evidence that infection with SV40 contributes to the development of human cancers.

HUMAN RESPONSE TO SV40

That individuals exposed to SV40 may develop a transient infection was documented during the 1960s. Morris et al. [26] found that, after intranasal inoculation of live SV40 (which was an inadvertent contaminant of an experimental respiratory syncytial virus vaccine), ~60% of the volunteers developed neutralizing antibodies to the virus. The antibody titers in the positive sera were low: small amounts of infectious virus were recovered from throat swabs of some of the individuals 7 or 11 days after inoculation; virus was not recovered from rectal swabs. After oral administration of SV40 (which was a contaminant of the experimental lots of the Sabin attenuated poliovaccine) to children, small amounts of virus were recovered intermittently, for up to 5 weeks, from the stools of some of the volunteers, but none of the children developed an antibody response [27, 28]. Both the serological study of American zoo workers that is reported by Engels et al. [29] in this issue of the Journal and an earlier study of employees of monkey-export firms in India [30] suggest that infection with SV40 may also be acquired by contact with (presumably) naturally infected primates. Engels et al. [29] were able to show (1) that the prevalence of SV40 antibody in “nonhuman-primate zoo workers” (i.e., “those currently working specifically with either nonhuman primates or a larger class of animals including nonhuman primates and those in senior administrative positions, which were assumed to be filled by individuals with extensive animal-handling experience”) was higher than that in “other zoo workers” (i.e., “those currently working with classes of animals not including nonhuman primates and those performing maintenance, clerical, or visitor service”) and (2) that the SV40-reactive antibodies in the sera of the nonhuman-primate zoo workers were more likely to be SV40 specific (see below) than were those in the sera of the other zoo workers.
IS SV40 ESTABLISHED AS A HUMAN INFECTION?

Although SV40 may produce transient infection in exposed individuals, the results of serological and virological studies indicate that SV40 has not become established in the human population. It is very likely that the low levels of SV40-reactive antibodies described in human sera in many previous investigations [31–33] are the result of cross-reactivity between SV40 and the widely prevalent human polyomaviruses BKV and JCV [34]. Sera of rhesus macaques naturally infected with SV40 react strongly with SV40 virusslike particles (VLPs), but they also react—to a lesser degree but unambiguously—with BKV VLPs and with JCV VLPs [35, 36]. Rhesus serum’s reactivity with BKV VLPs and with JCV VLPs is decreased by preadsorption with both SV40 VLPs and either BKV VLPs or JCV VLPs. Conversely, the low-level SV40 reactivity of human sera was clearly correlated with reactivity to BKV and JCV and, whenever measured, was significantly decreased by preadsorption with either BKV VLPs or JCV VLPs [35–38]. Investigators who have tested human sera against all 3 viruses—SV40, BKV, and JCV—have concluded that the serological evidence does not support the notion of widespread prevalence of SV40 in humans. In their study of 2054 serum samples collected in England and Wales, Knowles et al. [39] found 79 samples with neutralizing antibodies against SV40, but only 1 of these was negative for both antibody against BKV and antibody against JCV. They concluded that “[t]here is no serological evidence that SV40 entered the human population during the past 80 years” (p. 115). Carter et al. [36] reported that 46 of 699 serum samples from the United States were reactive against SV40 in VLP-based ELISA but that “none of these samples could be confirmed as having authentic SV40 antibodies following pre-adsorption with JCV or BKV VLPs” (p. 1522) and that their data “do not provide support for SV40 being a prevalent human pathogen” (p. 1522). Sanjose et al. [37] tested 1107 serum samples from Spain with SV40 VLPs and stated that “[t]here was no serological evidence for widespread circulation of SV40...in Spain” (p. 522).

If SV40 infection were responsible for a tumor, the probable sequence of events would include SV40 viremia, for the virus to reach the target organ, and virus multiplication at the site of the tumor, before the clonal expansion of transformed cells. These events should then lead to a higher prevalence of antibodies in these patients than in control subjects or other groups. However, previous studies have not shown that either the sera of patients with mesothelioma [40], osteosarcoma [36, 40], or lymphomas [37, 38] or the prediagnostic sera of patients with brain cancer [41] have greater reactivity to SV40 than do the sera of other groups.

There is no consistent virological evidence that SV40 circulates in the community. Large amounts of infectious human polyomaviruses BKV and JCV are present in the urine of immunocompromised patients, and urinary virus shedding is accompanied by a serological response. We did not recover SV40 sequences from any of 166 urine samples from HIV-seropositive or HIV-seronegative individuals, but we were able to identify SV40 sequences in all 17 masked urine specimens that were spiked with ~200 copies of SV40; BKV was detected in 14% of these specimens, JCV in 34% [42]. Similarly, Bofill-Mas et al. [43] did not detect SV40 sequences in any sewage samples collected in different geographic areas of Europe and in South Africa, but BKV sequences and JCV sequences were recovered from most of the same samples. These results are consistent with the negative results described above. In contrast to these negative results, there are reports of recovery of SV40 sequences from urine, peripheral-blood cells, and other tissues from normal healthy individuals, as well as from patients with renal disease [44–46]. However, fully documented and serologically confirmed human SV40 infections have not yet been described.

SV40 AND HUMAN CANCERS

The most puzzling aspect of the controversy—and the heart of the problem—has been a lack of agreement on whether authentic SV40 sequences are present in human tumors. The studies with positive results have reported small copy numbers (often estimated as being <1 copy/10^10 cells) of T antigen–coding sequences in a wide variety of unrelated tumors. Relatively few studies have attempted to identify SV40 transcripts or T protein in the tumor tissue, and the results of these studies have been inconclusive. On the other hand, employing similar or more-sensitive methods, several recent studies of mesothelioma [15–16], lymphomas [17, 18], and brain tumors [19, 20] have shown largely or completely negative results. Contamination with laboratory plasmids has now been identified as one reason for the discrepancy. At a 1997 meeting organized by the US Food and Drug Administration to examine SV40 as a possible human pathogen, Griffiths et al. [47] and Volter et al. [48] independently suggested the possibility that false-positive results may follow such contamination, because fragments of the SV40 genome—especially of the early region coding for large T antigen—have been used in the construction of hundreds of expression vectors worldwide. The reality and magnitude of this hypothesized risk have now been confirmed: in a recent study of mesothelioma, Lopez-Rios et al. [15] demonstrated conclusively that their initial observation of SV40 sequences in a majority of cases did not reflect the presence of genuine SV40 genomes but, instead, was due to contamination by SV40 sequences in a specific plasmid (i.e., pGL2) used in their laboratory; they also provided evidence that contamination by similar plasmid-derived SV40 sequences was responsible for the positive results that had been reported by another group.
of investigators. The report by Lopez-Rios et al. [15] should stimulate a reappraisal of previous studies showing positive results—and should lead to modification of the PCR primer sets employed in future studies.

CONCLUSION

Individuals exposed to SV40 by contact with the virus or with its animal hosts may contract the infection, but currently available evidence does not suggest that SV40 circulates in the community by person-to-person contact or that it contributes to the development of any human cancer. However, there is room for doubt, because the interpretation of the results of previous studies is limited by the uncertainties surrounding the assessment of exposure to SV40. Generally, individual investigations have measured viral DNA, circulating antibodies, or other markers of presumed exposure to SV40 but have rarely incorporated multiple markers of such exposure. Additionally, the presence of antibodies against SV40 T antigen has not been adequately evaluated as a marker of SV40-associated malignancy. These uncertainties in the assessment of exposure to SV40 may be clarified by future studies that (1) investigate the “full signature” of infection with SV40 [49], by using masked specimens from cancer cases and control subjects and correlating data for all markers of exposure to SV40 (e.g., genomic sequences, transcripts, antibodies to VLPs and T antigen, and T cell response [50]), and (2) incorporate strict monitoring of the assays for sensitivity, specificity, and reproducibility. A closer study of individuals with exposure to nonhuman primates (individuals similar to those described by Engels et al. [29])—and of these individuals’ families—may help define the characteristics of human SV40 infection and its transmissibility. It is necessary to determine, with certainty, whether a potentially oncogenic virus inadvertently introduced into the population during the course of administration of a highly successful vaccine does or does not cause any unanticipated adverse effect.

References

34. Shah KV, Galloway DA, Knowles WA, Viscidi


