Simian Virus 40 Contaminated Vaccine: Correlation with Present Prevalence of Lymphomas

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ABSTRACT
Simian Virus 40 (SV40) is a DNA virus associated with selected human cancers at varying frequencies. Recently, SV40 has been numerously implicated of having a role in lymphoma genesis. This review evaluated the correlation of past SV40 contaminated vaccine and present SV40 prevalence in lymphoproliferative disorders, using epidemiological and laboratory research on present accusations on SV40 as a lymphoma virus, sustaining that present lymphoma prevalence is not caused by early contaminated vaccine because environmental and natural factors are also implicated. It suggested in vitro infection of human lymphocyte cells with SV40 virus to verify productive infection which serve as a model for studying interactions of SV40 with human lymphocytes and understand the possibility of its playing a role in lymphoproliferative disorders.
KEY WORDS: DNA tumor virus, Tag, Lymphomas.

INTRODUCTION
SV40 is a nonenveloped DNA tumor virus of the papovavirus class originating from rhesus macaque. The virion is about 45 nm, an icosahedral particle, with a density of 1.34–1.35 g/cm³. The viral genome is a circular, double-stranded DNA molecule, broken up into three functional domains: (i) early viral coding regions (large and small T-antigen); (ii) late viral coding regions (VP1/2/3 and agnoprotein); and (iii) noncoding regulatory elements [1]. SV40 has been used as a paradigm to study polyomaviruses [2]. Initially delivered to about 30 million people in contaminated polio virus vaccines in the 1950s and 60s in the US, Europe and some African countries [3].

When the reports appeared in 1961 that injection of SV40 into hamsters could induce tumors [4, 5], the United States government and other infected part of Europe instituted a screening program requiring all new lots of poliovirus vaccine be free of SV40 because of concerns about possible adverse effects on human health, although earlier lots were not withdrawn from the mass immunization program. The viral contamination occurred as a result of the early vaccines were prepared in primary cultures of kidney cells derived from rhesus monkeys, which are naturally permissive host with SV40 [6].

The recognitions of SV40 virus capability to induce specific tumors in rodents, mainly brain tumors, sarcomas, mesotheliomas and neoplasms of the hematopoietic system, such as lymphocytic leukemia, histiocytic lymphomas and, rarely, B-cell lymphomas [4, 5, 7, 8], and as a potent oncogenic agent in lymphoma and other human malignancies call for urgent attention. This work evaluates the effectiveness of 1951-1963 vaccine to SV40 present prevalence incidence with Lymphomas.

EPIDEMIOLOGY AND LABORATORY RESEARCH
To date, hundreds of molecular and epidemiologic studies aimed at investigating whether SV40 infects humans, its potential mode of transmission and its putative role in human tumors, have been published [9]. Epidemiology studies involving decades of observations in the United States and Europe have failed to detect an increased cancer risk in those likely to have been exposed to the virus. These research studies include a German study with 22 years of follow-up of 886,000 persons who received the contaminated vaccine as infants [10], a 20-year study of 1,000 people in the United States inoculated during the first week of life with contaminated vaccines [10]. More recently, Studies
such as [11] did not detect SV40 in 29 samples and [12] in 9 samples of their respective Turkish mesotheliomas. Additionally, The Centers for Disease Control and Prevention were unable to collect or finds any evidence from their studies that SV40-contaminated vaccine lots cause cancer [13].

Increasing number of laboratories, using an extremely sensitive molecular biology technique, the polymerase chain reaction (PCR), have been able to detect traces of the SV40 virus in some rare human tumors including pleural mesothelioma, osteosarcoma, ependymoma, choroid plexus tumors of the brain, and recently non-Hodgkin's lymphoma [14, 15, 16].

The subsequent findings of the transforming and oncogenic activities of the SV40 viral large T (Tag) and small t (tag) antigens have prompted investigations into the potential of SV40 to induce cancer in humans.[7, 17]. Studies have observed that this SV40- encoded protein (T antigen) can inactivate cellular tumor suppressor proteins p53 and pRb which give rise to tumors in humans (18). Nevertheless not all research studies were able to detect SV40 DNA from their samples (19) and this has reportedly pose complication in interpretation of data [20].

Although further exploration of the relationship between poliovirus vaccine exposure and the incidence of SV40 virus oncogenic nature in certain human cancers showed that incidence rate remained stable or decreased from 1975 through 1977 in the groups most heavily exposed to SV40-contaminated poliovirus vaccine with only few cases of mesothelioma occurring mainly in age groups unlikely to have ever received any poliovirus vaccine [21].

Recently, geographical research teams have reported different incidence of SV40 positivity in human cancers due to new genetic and molecular production techniques. However, the frequency of tumor-associated virus detection differs by geographic regions. Research works such as epidemiology study involved analyzing cancer incidence data from Denmark following exposure to SV40-contaminated poliovirus vaccine [22]. A vigorous vaccination campaign in Denmark, beginning in the spring of 1955 and extending through the early 1960s, resulted in nearly 100 percent vaccination rate in targeted groups, particularly children and young adults. In addition, there is good evidence that most, if not all, of the poliovirus vaccine lots used during this time contained live SV40.

The comparing of cancer risk in children whose parents received pre-1963 polio vaccine with cancer risk in children whose mothers did not receive vaccine by Eagle research study [23] postulated whether early-life transmission of infection from a mother to her child might be related to the later development of childhood cancer. The investigation was on mothers of 50 of these children who developed cancer and mothers of 200 children without cancer by highly specific virus like particles (VLP) assay and plaque neutralization assay. It was observed that incidence of cancer of the nervous system and the blood (mainly leukemia) was 2.5 times higher in children whose mothers received pre-1963 vaccine than in those whose mothers did not, although the type of cancers varied and did not correspond to the types in which SV40 virus has been detected. Brain tumor was also detected in a child whose mother had not received the contaminated vaccine. The different occurring types of cancer in this study showed that SV40 contaminated vaccine could not be responsible for the cancer in these children because there were few cases of the types of cancer hypothesized to be linked to SV40.

Emri and De Rienzo [11, 12] detected SV40 in two of two Italian and four of 11 American mesothelioma biopsies analyzed in parallel respectively. Leithner [24] found SV40 in three of three tumors from the USA and Italy but did not detect SV40 in eight mesothelioma and 24 bone tumors from Australia. Butel [25] using a specific plague reduction assay analysed serum samples from periodic surveillance programmes in Hungary and the Czech Republic for antibodies to SV40. The prevalence of antibodies was between 1.3 and 8.7% in Hungary and from 1.0 to 4.0% in the Czech Republic. Females of these two countries had a higher rate of antibodies than males, reaching in certain age groups 15.6% in Hungary and 8.3% in the Czech Republic.

Recently, no SV40 was detected by James [26] from 43, 30, 17, and 19 mesothelioma cases from Johannesburg-South Africa, Wales-United kingdom, New York and Croatia respectively, while Comar and Pancaldi [27, 28] detected SV40 from 15.8% of 19 mesothelioma cases and 16% of 148 buffy coats of healthy donor respectively from Northeast Italy. Still in Northeastern Italy, Campello [29] detected SV40 sequences from 6 adenocarcinoma specimens with a viral load ranging from 6.2x10³ to 9x10³ copies per reaction.

The above data show that the geographical detection of SV40 in different studies may draw conflicting conclusions regarding a viral association with a particular cancer which I guess, depends
solely on the sources of samples analyzed, the procedures followed during sample preparation, and the specificity and sensitivity of test assays. However, it is noteworthy that till date SV40 status in some geographical areas of populations is yet to be known [24, 30].

SV40 IN NON-MALIGNANT SPECIES AND IN HUMAN
SV40 virus has been identified as a tumor virus in both human and non malignant species and its oncogenic capacity of infections has been well established in laboratory animal models [6,15, 31]. The latent period of tumor development in hamsters infected with SV40 ranges from 3 months to more than a year. The frequency of tumor development is usually over 90% in animals infected as newborns but is reduced in older animals. These data suggest that the age at the time of infection, the route of infection, and the duration of infection may be factors influencing the development of malignancies by SV40. The neoplasias induced by SV40 in animal models include primary brain cancers, malignant mesotheliomas, bone tumors, and systemic lymphomas [3, 15, 31].

Lymphomas are the most common malignancy during SV40 infection. In hamsters inoculated intravenously with SV40, systemic lymphomas developed among 72% of the animals, compared to none in the control group [32]. The lymphomas were of B-cell origin. Following intravenous inoculation, about one-third of the animals developed more than one histologic type of neoplasm, with osteosarcomas being most common after lymphomas. Following intracardiac inoculation, malignant mesotheliomas and osteosarcomas developed in addition to lymphomas [33]. It was observed that the etiologic role of the virus in those cancers was supported because SV40 T-ag was expressed in all malignant cells. Animals with tumors developed antibody against SV40 T-ag, and neutralization of SV40 with specific antibody before virus inoculation prevented cancer development [32]. Probably, knowledge of these models prompted past, as well as recent investigators to consider the role of polyomavirus SV40 infections in some human malignancies.

Studies have reported the localization of SV40 DNA sequences to renal tubular epithelial cell nuclei in renal biopsies of patients with focal segmental glomerulosclerosis using in situ hybridization. These studies displayed several strains of SV40 that included strain 776 and other strains with mutations in the early and late regions [9, 34]. Additionally, other different studies showed that SV40 DNA sequences from the viral regulatory region were detected and identified in the allograft of immune-compromised pediatric renal transplant recipients and in the native kidneys of a young adult lung transplant patient with polyomavirus nephropathy [35]. SV40 virus DNA sequences in PBMCs in both oncological patients and normal individuals from various populations have also been detected by different study groups [9, 28, 34]. These results demonstrate the nephrotropic and lymphotropic properties of SV40 and indicate that the kidney can serve as a reservoir for the virus in humans.

SV40 AS A LYMPHOMA VIRUS IN HUMAN
Like other cancers, lymphoma occurs when lymphocytes are in a state of uncontrolled cell growth and multiplication [36]. The T and B cells are the highly specific recognition of pathogens in the adaptive immune system and also are mostly cells that result to lymphomas with the natural killer cells [NK cells] [37]. Human T and B lymphocytes have a finite and predictable proliferative life span in culture similar to that observed in fibroblasts. In order to circumvent this problem, cells obtained from human lymphoblastic leukaemias or lymphomas are often used because of their unlimited lifespan [37]. In the United States, Lymphoma is the most common type of blood cancer, the seventh most common cancer in adults and the third most common in children. In the United States each year, some 54,000 people are diagnosed with non-Hodgkin lymphoma (NHL) and 7,000 are diagnosed Hodgkin lymphoma (HL) while in European Union over 50,000 cases of NHL are diagnosed annually [13].

Different studies have provided evidence for the detection of sequences of simian virus 40 (SV40) DNA with human lymphomas and other lymphoproliferative disorders (Table 1). Although many still cannot detect SV40 sequences in their samples [38]. The main question now is can SV40, just like in the case of mesothelioma be a causative agent or have a role to play in lymphomagenesis? Assessing for the expression of SV40 T-ag and phenotypic lymphocyte markers by immunohistochemistry in the masked specimens initially examined by polymerase chain reaction for polyomavirus and herpesvirus DNA sequences (IHC). 55 systemic NHL, 25 nonmalignant lymphoid and malignant non lymphoid tissue control cases in this study showed detection of SV40 T-ag expression only in B-cell lymphoma
specimens that contained SV40 DNA sequences. Not all lymphoma cells in a positive specimen stained for T-ag, and the reaction was lower intensity than observed in SV40 hamster tumors [39]. This study could aid in given a clue to the answer of the above question because it showed that SV40 gene expression occurs in a fraction of cells in some B-cell lymphomas among patients with HIV/AIDS. Although, evidence regarding a causal role for SV40 in human neoplastic disease depends on the malignancy under consideration. The collected evidence in some studies [26] strongly favors a causal link for SV40 virus with malignant mesotheliomas, probably most often as a cofactor with asbestos. Supportive data are not as complete for the brain tumor and lymphoma systems as for mesotheliomas. Additional information is needed to provide mechanistic foundations for the virus in divisions of those cancers before causality can be decided [40].

Table 1: Some Published Studies of SV40 DNA sequences with Lymphoproliferative disorders using different genetic and immunological assay techniques.

<table>
<thead>
<tr>
<th>Author/Study (Year)</th>
<th>Sample</th>
<th>Technique</th>
<th>Place</th>
<th>Results/SV40 status in Lymphomas.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Martini et al, 1998</td>
<td>Human lymphoproliferative disorders obtained from human-immunodeficiency-virus (HIV)-seronegative and HIV-infected patients.</td>
<td>PCR; Filter hybridization and Immunochemistry</td>
<td>Ferrara Italy</td>
<td>1. NHL patients in (a)HIV- 11/79(13.9%) (b)HIV+ 27/61(12.5%) 2. Reactive lymphadenopathies in (a) HIV- 3/9(33.3%) (b) HIV+ 6/17(35.3%) 3. Hodgkin’s disease in (a) HIV- 7/43(16.3%) (b)HIV+ 1/12 (8.3%)</td>
</tr>
<tr>
<td>Shivapurkah et al., 2002.</td>
<td>68 Non Hodgkin Lymphoma and 31 Hodgkin Lymphoma patients</td>
<td>PCR and Southern Blot</td>
<td>USA</td>
<td>NHL patients 29/68 (43%) H L Patients 3/31(9%)</td>
</tr>
<tr>
<td>MacKenzie et al., 2003.</td>
<td>152 NHL samples, from patients in the U.K. with lymphadenopathy and lymphoid leukemia</td>
<td>PCR assay</td>
<td>United kingdom</td>
<td>0/152 (0%)</td>
</tr>
<tr>
<td>Nakatsuka et al., 2003.</td>
<td>122 cases with NHL and 3 with Hodgkin’s lymphoma</td>
<td>Immunohistochemistry, PCR, Southern Blot and In situ Hybridization</td>
<td>Japan</td>
<td>19%</td>
</tr>
<tr>
<td>Eagles et al., 2004.</td>
<td>724 incident NHL case patients and 622 control subjects from a population-based U.S case–control study</td>
<td>Enzyme immunoassays and logistic Regression, adjusting for sex, race, birth year, and study site.</td>
<td>USA</td>
<td>Laboratory A= 52/724 (7.2%) Laboratory B=70/718 (9.8%)</td>
</tr>
<tr>
<td>Janet et al., 2003</td>
<td>154 NHL patients, 186 non malignant lymphoid samples and NHL from HIV-1-positive patients</td>
<td>PCR, southern blot hybridization and DNA analysis</td>
<td>USA</td>
<td>64/154 (42%) 0/186(0%) 33% of HIV-1-positive Patients.</td>
</tr>
<tr>
<td>Dana et al., 2005</td>
<td>119 B cell NHL and184 NHL main samples</td>
<td>Elisa, logistic regression</td>
<td>Washington</td>
<td>24 (16.8%) and 33(15.2%)</td>
</tr>
<tr>
<td>Meneses et al., 2005.</td>
<td>Lymphoma and control specimens from HIV negative patients</td>
<td>PCR, Southern blot and Immunohistochemistry</td>
<td>Costa Rica</td>
<td>30/125(24%) 64% from B-cell Lymphoma 0% from Control Samples</td>
</tr>
<tr>
<td>Po-Min Chen et al., 2006.</td>
<td>.91 frozen lymph nodes from NHL patients</td>
<td>PCR, Southern blot hybridization and sequence analysis</td>
<td>Taiwan</td>
<td>.13(14.3%).</td>
</tr>
<tr>
<td>Khaled et al., 2007</td>
<td>108 diffuse large B-cell type lymphoma(DLBCL ) and 60 non tumoral samples</td>
<td>PCR assays and methylation specific PCR</td>
<td>Tunisia</td>
<td>63/108 (56%) and 4/60(6%)</td>
</tr>
<tr>
<td>Toracchio et al., 2009.</td>
<td>171 archival paraffin-embedded lymphoma specimens</td>
<td>cross-sectional study design, PCR analysis and Immunohistochemistry</td>
<td>Houston</td>
<td>Part A=10/44(23%) Part B= 4/127 (3%)</td>
</tr>
</tbody>
</table>
Furthermore, with the results of data (Table 1) of the detection of sequences of SV40 virus DNA of oncological patients (lymphomas) one can neither be against nor in favour, arguing about the possibility of SV40 being able to play a role in lymphoma diseases because some of the results [16, 23, 36] indicated no statistical association between the virus and non-Hodgkin lymphoma while others did. Although the authors were from different geographical distribution with different techniques and different number of samples from different population the results pointed out as concrete evidence of detection of SV40 virus sequences in the oncological patient samples.

CONCLUSION
All together, study data and research output giving life to this review work postulate SV40 as a transforming DNA virus with fast increase in our environment. SV40 virus cannot therefore be attributed solely to the early contaminated polio vaccines but to other factors such as environmental and natural factors. Numerous study data and research output also postulate that exposure to SV40 virus under natural conditions can lead to cancer in humans. Furthermore, looking at the rate of detection of SV40 virus DNA by different molecular and genetic assays in lymphoma samples, involvement of SV40 in proliferation of lymphomagenesis becomes undoubtable. More research work is recommended for full knowledge of SV40 virus mechanism of interaction or association with lymphocyte cells because data of the findings (Table 1) suggested that the lymphoid tissue could represent a reservoir for SV40 and may constitute the first step in understanding whether this small DNA tumor virus has a role in the pathogenesis of human lymphoproliferative disorders. Finally, each phase of lymphomagenesis should be investigated for sequences of SV40 viral load using genetic molecular assays such as real time polymerase chain reaction (RQ-PCR). I believe that this approach may allow to define the possible diagnostic/prognostic role of SV40 infection in lymphoma patients. In vitro infection of human lymphocyte cells with SV40 virions and monitored for productive infection could serve as a simple model for studying interactions of SV40 with a human lymphocytes and understand the possibility of its playing a role in lymphoproliferative disorders.

REFERENCES


