

# Case–Control Study of Simian Virus 40 and Non-Hodgkin Lymphoma in the United States

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**Background:** Recent studies have reported detection of simian virus 40 (SV40) DNA in tumor tissues from 15%–43% of U.S. non-Hodgkin lymphoma (NHL) patients. SV40 accidentally contaminated U.S. poliovirus vaccines that were widely administered from 1955 through 1962. However, epidemiologic data linking SV40 with NHL are lacking. **Methods:** We obtained serum samples from 724 incident NHL case patients and 622 control subjects from a population-based U.S. case–control study. SV40 serostatus was analyzed by two independent laboratories (designated A and B) using similar virus-like particle (VLP) enzyme immunoassays. Associations with serostatus were assessed with logistic regression, adjusting for sex, race, birth year, and study site. VLPs for the human polyomaviruses BK and JC were used in competitive inhibition experiments to assess the specificity of SV40 reactivity. Statistical tests were two-sided. **Results:** SV40 antibody results from the two laboratories were correlated ( $R = 0.59$ ;  $P < .001$ ). Laboratories A and B detected SV40 seropositivity in 7.2% and 9.8% of NHL case patients, respectively, and in 10.5% and 9.6% of control subjects, respectively. SV40 seropositivity was not associated with increased NHL risk (laboratory A: adjusted odds ratio [OR] = 0.68, 95% confidence interval [CI] = 0.46 to 1.00; laboratory B: adjusted OR = 1.02, 95% CI = 0.71 to 1.47). SV40 seropositivity was not associated with NHLs of any specific histology or site. Among subjects born before 1963, 1.0%–1.6% showed SV40-specific reactivity, i.e., SV40 reactivity confirmed in competitive inhibition experiments, whereas (based on limited data) none born subsequently demonstrated SV40-specific reactivity. **Conclusions:** In persons born before 1963, the presence of SV40-specific antibodies, although rare, could reflect exposure to SV40-contaminated vaccines. Nevertheless, NHL risk was unrelated to serologic evidence of SV40 exposure or infection. [J Natl Cancer Inst 2004;96:1368–74]

Non-Hodgkin lymphoma (NHL) incidence has increased dramatically in recent decades in the United States. The etiology of NHL is largely unknown, but viruses, including human immunodeficiency virus and Epstein–Barr virus, play a role in some cases (1). Several groups have detected DNA from simian virus 40 (SV40), a macaque polyomavirus, in the tumor tissue of 15%–43% of U.S. NHL patients (2–5). Although a source for the SV40 DNA sequences detected in NHL tissue has not been identified, SV40 infections could conceivably have occurred through injection of poliovirus vaccine from 1955 through 1962, when SV40 was a frequent contaminant of poliovirus vaccines grown in monkey kidney tissue. Tens of millions of people in the United States, mostly children, were exposed to SV40 through large-scale vaccination campaigns during these years. Following

the discovery of SV40 contamination and changes in vaccine production, vaccine lots released in 1963 and later were free of this virus (6).

On its face, a link between SV40 and NHL is biologically plausible because SV40 causes leukemia and lymphoma in laboratory rodents (7). Tumors could arise through the actions of an SV40-encoded protein (T antigen) that can inactivate cellular tumor suppressor proteins p53 and pRb (8). Nevertheless, the failure of other groups to detect SV40 DNA in NHL samples (9,10) and the wide range of other human cancers in which SV40 DNA has reportedly been detected (11) complicate interpretation of these data. Indeed, results of recent laboratory work suggest that SV40 cannot infect human lymphocytes (12). In addition, epidemiologic evidence that would support SV40 as an etiologic agent in NHL is lacking. The prevalence of SV40 infection in humans is unknown. Also arguing against SV40 as a cause of NHL are the results of retrospective cohort studies of individuals exposed to SV40-contaminated poliovirus vaccines, which have revealed them to be at the same risk of NHL as individuals not exposed to contaminated vaccine (13–15).

Recently developed enzyme immunoassays (EIAs) that detect antibodies against SV40 virus-like particles (VLPs) provide a valuable tool for epidemiologic studies of the association between SV40 and human malignancies (16,17). SV40 VLPs are empty capsids generated by spontaneous self-assembly of the major viral capsid protein, VP1. VLPs structurally resemble native virions and retain many immunologic properties, including display of surface-exposed B-lymphocyte epitopes, which are commonly viral type specific, neutralizing antigens. An important strength of the VLP EIA is that it can be used in competitive inhibition experiments to examine whether measured antibody reactivity is specific for SV40 or instead represents cross-reactivity to the SV40-related human polyomaviruses BK and JC (16,17).

In the present work, we examined the possible association between SV40 serostatus and NHL in a population-based case–

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control study. We measured SV40 antibody reactivity in NHL case patients and control subjects by using two similar SV40 VLP EIAs carried out independently in two laboratories. We sought to determine whether NHL was associated with an increased prevalence of SV40 exposure or infection, as indicated by these assays.

## PATIENTS AND METHODS

### Study Description

The National Cancer Institute (NCI) Surveillance, Epidemiology, and End Results (SEER<sup>1</sup>) case-control study of NHL has been described previously (18). In brief, between July 1998 and June 2000, subjects were enrolled from four U.S. SEER registry areas: Iowa State and metropolitan Detroit, Los Angeles, and Seattle. Eligible case patients (aged 20–74 years) were sampled from individuals prospectively identified with incident NHL. Eligible control subjects were selected from the general population in the four registry areas, stratified on age in 5-year intervals, sex, and race; controls were identified using random-digit dialing (ages 20–64 years) or Medicare eligibility files (ages 65–74 years). Individuals identified by themselves or their physician as being human immunodeficiency virus infected were excluded. Study participants provided written informed consent. The study was approved by the institutional review boards at the NCI and the participating registries.

The NCI SEER case-control study of NHL includes 1321 case patients and 1057 control subjects who completed a computer-assisted personal interview and provided a blood or buccal cell sample. In the present analysis, we evaluated the SV40 serostatus in all subjects with available blood samples, i.e., 724 case patients (54.8%) and 622 control subjects (58.9%). NHLs were classified histologically and by topographic site using abstracted pathology reports provided to the registries. Histologic diagnoses were coded according to the *International Classification of Diseases for Oncology* (2nd edition) (19), updated to include categories in the Revised European-American Lymphoma classification (20).

### Laboratory Methods

Sera were independently tested in the laboratories of Drs. Viscidi and Galloway, referred to as laboratories A and B, respectively. These laboratories evaluated sera from case patients and control subjects concurrently with sera (diluted 1:10) from 19 SV40-infected macaques that had previously demonstrated strong reactivity in an SV40 VLP EIA (16). Coded serum samples were shipped to laboratories A and B from a central repository, and personnel at both laboratories were blinded with respect to human versus macaque and case versus control status of specimens.

SV40, BK, and JC VLPs were generated by each laboratory from VP1 proteins expressed in insect cells as described previously (16,21). EIA plates (laboratory A, PolySorp [Nunc, Naperville, IL]; laboratory B, Immulon 2 [Thermo LabSystems, Franklin, MA]) were incubated overnight with VLPs (laboratory A, 30 ng/well; laboratory B, 100 ng/well). Serum specimens (laboratory A, diluted 1:400; laboratory B, diluted 1:100) were then tested in duplicate, according to each laboratory's EIA protocol (16,17).

In laboratory B, specimens exhibiting a coefficient of variation greater than 20% in EIA optical density (OD) results were retested. Although retesting was not done in laboratory A, 95% of subjects were either SV40 seronegative or SV40 seropositive in both duplicates using the cutoff defined below, and exclusion of the 5% of samples with discordant duplicates did not affect the results (data not shown). JC EIA results were not available in laboratory B because of technical difficulties with VLP production.

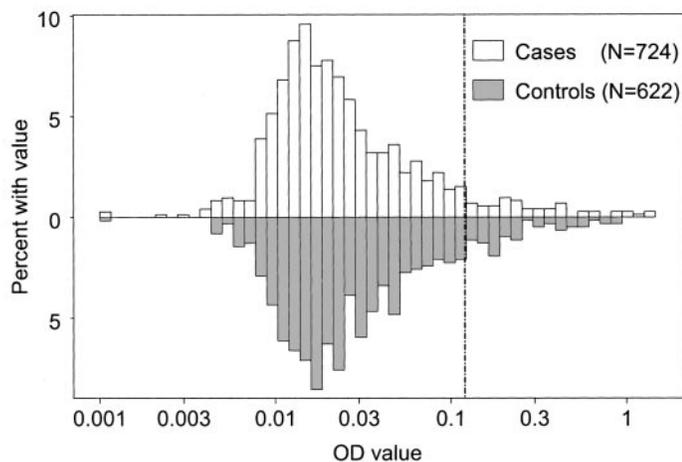
In laboratory A, the geometric mean of the duplicate OD values for each specimen was calculated. In laboratory B (16), sera were also tested against EIA wells with no antigen, and results were reported as log-difference values (16), defined as  $\ln(\text{arithmetic mean OD for specimen in wells with VLP}) - \ln(\text{arithmetic mean OD for specimen in wells with no VLP})$ . SV40 VLP EIA results were largely unimodal, with most individuals demonstrating low-level reactivity, although in each laboratory some subjects had somewhat higher reactivity (Fig. 1). We chose cutoffs for SV40 VLP EIA positivity (laboratory A, 0.12 OD units; laboratory B, 0.70 log-difference units) that were similar to cutoffs used previously (16,17,21). As shown in Fig. 1, these cutoffs separate the main body of subjects with little reactivity from those with stronger reactivity. Using slightly different cutoffs (0.10 or 0.14 OD units in laboratory A, 0.60 or 0.80 log-difference units in laboratory B) did not substantively affect results (data not shown). The same cutoffs were used for BK and JC VLP EIAs as were used for the SV40 VLP EIA.

To evaluate the specificity of the measured SV40 reactivity, each laboratory performed competitive inhibition experiments for a subset of the human and macaque samples that were SV40 seropositive in that laboratory. Specifically, under masked conditions, each laboratory evaluated samples from SV40-seropositive macaques ( $n = 10$  in laboratory A,  $n = 9$  in laboratory B) and SV40-seropositive humans ( $n = 40$  and  $n = 35$ , respectively). The human samples evaluated included those from all SV40-seropositive, BK-seronegative individuals and those from a random sample of SV40-seropositive, BK-seropositive individuals (stratified in laboratory A by JC serostatus). Approximately equal numbers of case patients and control subjects were included in these analyses. Because the laboratories differed in which sera were judged seropositive, and because of the random sampling, the sera evaluated in competitive inhibition experiments differed between the two laboratories (five macaque and nine human samples were evaluated in both laboratories).

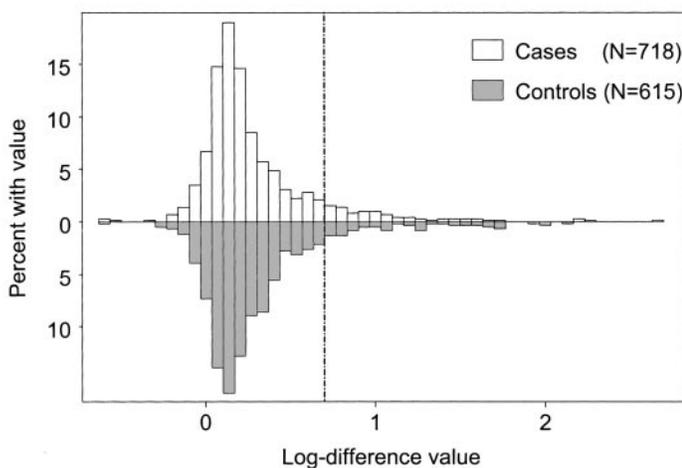
Detailed competitive inhibition methods for each laboratory are available on request. Briefly, sera (laboratory A: diluted 1:200, 1:400, or 1:800, depending on the initial SV40 EIA OD; laboratory B: diluted 1:100) were preincubated with SV40, BK, or JC VLPs at final concentrations that had previously been determined to result in maximal inhibition of SV40 VLP EIA reactivity (laboratory A: 4  $\mu\text{g/mL}$ ; laboratory B: 3  $\mu\text{g/mL}$ ). These preincubated sera and, in parallel, sera without VLP, were then added to SV40 VLP EIA plates and tested as described above.

SV40 reactivity was considered to be competitively inhibited (i.e., blocked or absorbed) by a VLP if, in the SV40 VLP EIA, the OD for serum preincubated with that VLP was less than 50% of the OD for serum without VLP. A cutoff of 50% is intuitively reasonable, because a reduction of 50% or more in the EIA OD

## Laboratory A



## Laboratory B



**Fig. 1.** Simian virus 40 (SV40) antibody reactivity measured by virus-like particle enzyme immunoassay. Data are presented as separate histograms for non-Hodgkin lymphoma case patients and control subjects; histograms are joined at their bases so that histogram for control subjects is inverted. Specimens were tested independently in laboratories A (**upper panel**) and B (**lower panel**). The cutoff points defining an SV40-seropositive result in each laboratory are indicated (laboratory A: 0.12 optical density [OD] units; laboratory B: 0.70 log-difference value). For laboratory A, the histogram was constructed and presented using log-transformed OD values. Test results from laboratory B were not available for six cases and seven controls.

would suggest that the majority of reactivity could be explained by antibodies that recognize the competing VLP. Further support for the use of this cutoff to identify specific reactivity came from results of preliminary competitive inhibition experiments, which showed that BK and JC reactivity in humans was reduced by 50% or more by the corresponding VLP but not by the heterologous human polyomavirus VLP or SV40 VLP (data not shown). SV40-specific reactivity was then defined as SV40 reactivity that was competitively inhibited by SV40 VLP but not by BK or JC VLPs. Results for initially SV40-seropositive samples that were seronegative when retested in the competitive inhibition experiments were considered not evaluable ( $n = 7$  in each laboratory).

## Statistical Methods

We used logistic regression to examine the association between NHL and SV40 seropositivity, adjusting for potential confounding by sex, race, birth year, and study site. In these models, we adjusted for birth year in categories that incorporated information on whether subjects could have been exposed to SV40-contaminated poliovirus vaccine (in use in 1955–1962) and for the age at which this exposure would have first occurred: subjects born in 1963–1980 (unexposed to contaminated vaccine), in 1955–1962 (exposed as infants), in 1950–1954 (exposed as young children), in 1945–1949 (exposed as older children), or in 1923–1944 (exposed in adolescence or later). Because subject recruitment into the NCI SEER case-control study of NHL occurred in a narrow time window, subject age and birth year were almost perfectly correlated ( $R = -0.999$ ) so that results of logistic regression models that included an adjustment for age instead of birth year were identical to those of models that adjusted for birth year (data not shown). Polytomous logistic regression was used in analyses for specific NHL subtypes defined by histology, site, or prior treatment status. We also used the chi-square test and Fisher's exact test (when expected counts in contingency tables were  $<5$ ) to compare proportions across groups, the  $\kappa$  statistic to assess assay agreement between laboratories, the Student's  $t$  test and Wilcoxon rank sum test to compare continuous measures between two groups, and the Spearman correlation coefficient to compare continuous measures. All tests and confidence intervals were two-sided.

## RESULTS

### Demographic Characteristics

As a result of frequency matching of control subjects to case patients, the case patients and control subjects in the NCI SEER case-control study of NHL were demographically similar, although there were minor but statistically significant differences in age and race (data not shown). The participants in the present analysis (724 case patients and 622 control subjects with available sera; Table 1) resembled those who were excluded due to missing sera. Among participants in the present analysis, case patients and control subjects were similar in terms of sex, study center, and education level. As seen among all subjects in the NCI SEER case-control study of NHL, participants in the present analysis differed by race, with control subjects more likely than case patients to be African American (8.4% versus 5.5%), although the difference was not statistically significant ( $P = .08$ ). Case patients were also slightly younger than control subjects (mean ages were 56.7 and 58.1 years, respectively;  $P = .04$ ) and, correspondingly, were born later in calendar time (Table 1). However, most subjects in both groups were born before 1963 (93.6% of case patients and 92.6% of control subjects;  $P = .45$ ).

### SV40 VLP Reactivity in Case Patients and Control Subjects

SV40 antibody results in laboratories A and B were correlated (Spearman  $R = 0.59$ ;  $P < .001$ ). Within each laboratory, the distribution of SV40 reactivity was similar for case patients and control subjects (Fig. 1). SV40 seropositivity was observed in

**Table 1.** Study subjects

Category	Case patients (N = 724)	Control subjects (N = 622)	<i>P</i> *
Sex, n (%)			.82
Male	399 (55.1)	339 (54.5)	
Female	325 (44.9)	283 (45.5)	
Age in years at diagnosis (case patients) or selection (control subjects), n (%)			.04
20–34	38 (5.3)	39 (6.3)	
35–44	96 (13.3)	60 (9.7)	
45–54	155 (21.4)	115 (18.5)	
55–64	195 (26.9)	137 (22.0)	
65–74	240 (33.2)	271 (43.6)	
Mean age in years (SD) <sup>†</sup>	56.7 (12.4)	58.1 (12.6)	
Year of birth, n (%)			.03
1923–1944	441 (60.9)	416 (66.9)	
1945–1949	78 (10.8)	60 (9.7)	
1950–1954	75 (10.4)	52 (8.4)	
1955–1962	84 (11.6)	48 (7.7)	
1963–1980	46 (6.4)	46 (7.4)	
Mean birth year (SD)	1941 (12.5)	1940 (12.4)	
Race, n (%)			.08
White	635 (87.7)	536 (86.2)	
African American	40 (5.5)	52 (8.4)	
Other	49 (6.8)	34 (5.5)	
Center, n (%)			.71
Detroit	76 (10.5)	66 (10.6)	
Iowa	248 (34.3)	212 (34.1)	
Los Angeles	167 (23.1)	129 (20.7)	
Seattle	233 (32.2)	215 (34.6)	
Years of education, n (%)			.31
0–11	62 (8.6)	54 (8.7)	
12–15	463 (64.0)	374 (60.1)	
≥16	199 (27.5)	194 (31.2)	

\**P* values were derived using the chi-square test, except for age and birth year, where the two-sample Student's *t* test was used.

<sup>†</sup>SD = standard deviation.

7.2% and 9.8% of case patients in laboratories A and B, respectively, and in 10.5% and 9.6% of control subjects in laboratories A and B, respectively. The two laboratories exhibited good agreement regarding which subjects were classified as SV40 seropositive ( $\kappa = 0.62$ ;  $P < .001$ ).

By comparison, 19 of 19 (100%) and 15 of 16 (94%) samples from SV40-infected macaques evaluated concurrently with human samples were SV40 seropositive in laboratories A and B, respectively (results for three macaques were missing from laboratory B because of discordant results on duplicate testing). In addition, even though sera from macaques had been diluted 10-fold compared with human sera, SV40 reactivity was stronger in SV40-seropositive macaques than in SV40-seropositive humans (laboratory A: median ODs for macaques = 0.65 and for humans = 0.22 [ $P < .001$ ]; laboratory B: median log difference for macaques = 1.89 and for humans = 1.01 [ $P < .001$ ]).

Most of the human sera were BK seropositive (64.6% in laboratory A, 71.0% in laboratory B) and JC seropositive (53.8% in laboratory A; not analyzed in laboratory B). Among humans, SV40 reactivity was correlated with BK reactivity (laboratory A: Spearman  $R = 0.42$  [ $P < .001$ ]; laboratory B: Spearman  $R = 0.21$  [ $P < .001$ ]) and with JC reactivity (laboratory A: Spearman  $R = 0.28$  [ $P < .001$ ]), but BK and JC reactivity were not correlated (laboratory A: Spearman  $R = -0.04$  [ $P = .19$ ]). Only one

macaque was weakly BK seropositive (in laboratory A only, with OD = 0.15), and none was JC seropositive.

Case–control comparisons are presented in Table 2. Overall, SV40 seropositivity as measured by laboratory A was slightly inversely associated with NHL risk (adjusted odds ratio [OR] = 0.68, 95% confidence interval [CI] = 0.46 to 1.00), whereas in laboratory B there was no association (OR = 1.02, 95% CI = 0.71 to 1.47). No associations were observed between SV40 serostatus and NHLs of specific histology, site, or prior treatment status (Table 2).

Among control subjects, SV40 seropositivity was more common in persons born before 1963 than in those born in 1963 or later, although the differences were not statistically significant: 10.9% versus 4.4% (laboratory A,  $P = .21$ ) and 9.8% versus 6.5% (laboratory B,  $P = .61$ ). Among case patients, SV40 seropositivity was also more common in those born before 1963 than in those born in 1963 or later in data from laboratory B (10.0% versus 6.5%;  $P = .61$ ), but the reverse was true in the data from laboratory A (7.1% versus 8.7%;  $P = .56$ ). Again, neither difference was statistically significant.

### Competitive Inhibition Studies

Competitive inhibition results are shown in Table 3. In the seropositive macaque samples, SV40 reactivity was consistently blocked by SV40 VLP but not by BK or JC VLPs. Among humans, by contrast, SV40 reactivity was frequently blocked by BK or JC VLPs. This inhibition was largely a function of BK and JC serostatus, with SV40 reactivity being most commonly inhibited by BK or JC VLPs in humans who were BK or JC seropositive (Table 3). Of further note, in laboratory B, SV40 reactivity was not blocked by SV40 VLPs in 12 (43%) of the 28 humans evaluated (Table 3).

We defined SV40 reactivity as SV40 specific if it was inhibited competitively (i.e., blocked by at least 50%) by SV40 VLP but not by BK or JC VLPs. With this definition, the prevalence of SV40-specific reactivity did not differ between case patients and control subjects. Specifically, in laboratory A, four of 17 SV40-seropositive case patients and four of 18 SV40-seropositive control subjects demonstrated SV40-specific reactivity (24% versus 22%;  $P = 1.00$ ). In laboratory B, two of 12 SV40-seropositive case patients and two of 14 SV40-seropositive control subjects demonstrated SV40-specific reactivity (17% versus 14%;  $P = 1.00$ ).

Thirty-three of the 35 subjects analyzed by laboratory A were born before 1963, as were 25 of the 28 subjects analyzed by laboratory B. Among the subjects born before 1963, eight (24%) of 33 (laboratory A) and four (16%) of 25 (laboratory B) showed SV40-specific reactivity. We weighted these results, using as weights the fractions with which we sampled subjects for competitive inhibition assays (based on BK and JC VLP serostatus). We thereby estimated that, overall, 1.0%–1.6% of subjects born before 1963 would demonstrate SV40-specific reactivity. By comparison, only eight subjects born in 1963 or later were SV40 seropositive in either laboratory. Four of these individuals were evaluated in competitive inhibition assays (two in laboratory A, three in laboratory B; one was evaluated in both laboratories). None of the four showed SV40-specific reactivity. Because of the small number of SV40-seropositive subjects born in 1963 or later, we could not formally test for differences in SV40-specific reactivity by birth year.

**Table 2.** Associations between case-control status and SV40 serostatus\*

Category	Laboratory A serostatus		Laboratory B serostatus†	
	SV40 seropositive/total (%)	OR‡ (95% CI)	SV40 seropositive/total (%)	OR‡ (95% CI)
Control subjects	65/622 (10.5)	1.00	59/615 (9.6)	1.00
Case patients	52/724 (7.2)	0.68 (0.46–1.00)	70/718 (9.8)	1.02 (0.71–1.47)
Cases, by NHL histologic subtype				
Follicular	15/199 (7.5)	0.73 (0.40–1.33)	19/196 (9.7)	1.05 (0.61–1.83)
Diffuse large B cell	16/214 (7.5)	0.74 (0.42–1.32)	20/212 (9.4)	1.02 (0.59–1.75)
T cell	2/47 (4.3)	0.33 (0.08–1.41)	3/47 (6.4)	0.60 (0.18–2.01)
Other/unknown§	19/264 (7.2)	0.67 (0.39–1.15)	28/263 (10.7)	1.09 (0.67–1.75)
Cases, by NHL site				
Nodal	29/481 (6.0)	0.57 (0.36–0.90)	39/478 (8.2)	0.85 (0.55–1.30)
Extranodal	23/243 (9.5)	0.90 (0.54–1.49)	31/240 (12.9)	1.37 (0.85–2.18)
Cases, by NHL treatment				
No prior treatment	10/121 (8.3)	0.74 (0.37–1.50)	9/120 (7.5)	0.71 (0.34–1.48)
Prior treatment or unknown treatment status	42/603 (7.0)	0.67 (0.44–1.00)	61/598 (10.2)	1.09 (0.74–1.59)

\*SV40 = simian virus 40; OR = odds ratio; CI = confidence interval; NHL = non-Hodgkin lymphoma.

†SV40 serostatus results were not evaluable for seven control subjects and six case patients tested in laboratory B because the results of duplicate tests were discordant.

‡Odds ratios are adjusted for sex, race, birth year (1923–44, 1945–49, 1950–54, 1955–62, 1963–80), and study site.

§Other/unknown NHLs include those histologically classified as small lymphocytic (n = 78), lymphoplasmacytic (n = 13), mantle zone (n = 30), Burkitt (n = 8), marginal zone (n = 21), mucosa-associated lymphoid tissue (n = 43), and not otherwise specified (n = 71).

||Extranodal NHLs include 14 cases with unknown site.

## DISCUSSION

In this population-based case-control study of incident NHL in the United States, we found an overall prevalence of SV40 reactivity of 7%–10% among case patients and 10%–11% among control subjects. After accounting for the cross-reactivity of SV40 antibodies with BK and JC viruses, we estimated that the prevalence of SV40-specific reactivity in case patients and control subjects combined was less than 2%. Importantly, SV40 seroprevalence was very similar in control subjects, in case patients overall, and in case patients subdivided by NHL histology, site of origin, or treatment status. Thus, we found no evidence that SV40-seropositive individuals were at increased risk for NHL. Our findings are in accordance with negative results from a prior case-control study in Spain (17). Together with follow-up studies of recipients of SV40-contaminated poliovirus vaccines (13–15), these data argue against SV40 as a cause of NHL.

By contrast, variable results have been described in studies that used sensitive polymerase chain reaction (PCR) methods to detect SV40 DNA in NHL tumor tissue. Several U.S. studies found SV40 T-antigen sequences in tumor tissue from 15%–43% of NHL patients (2–5). In a Japanese study, SV40 T-antigen sequences were detected in NHL tissues from 11% of patients (22). Moreover, the investigators demonstrated the presence of SV40 sequences in three tumors by using *in situ* hybridization (22). An Italian study also found SV40 sequences in 14% of NHL specimens (23). On the other hand, two additional European studies of patients with lymphoproliferative disorders were strikingly negative (9,10). In a study carried out in Italy and Spain (9), SV40 T-antigen sequences were detected in only 3% of tissues by using two sets of PCR primers, and none of the initially positive results was confirmed using a third primer set. In a study carried out in the United Kingdom (10), SV40 T-antigen sequences were not detected in any NHL specimens using quantitative real-time PCR, despite a very low detection

limit of 10 SV40 copies in 100 ng of genomic DNA (i.e., approximately one SV40 copy per 1500 cells) .

The reasons for the variability in results from PCR-based studies are unclear. Although it is possible that results from different countries reflect unknown geographic differences in the study populations, results varied somewhat even among studies in the United States (2–5) and among studies in Italy (9,23). Differences in assay methods, such as the choice of PCR primers or reaction conditions, may have contributed to the diversity of results. Some molecular studies also suffered from methodologic problems, including a lack of masked evaluation of tumor specimens (2–5,9,22,23), lack of appropriate human tissues as negative controls (2,5,9,22,23), and the amplification of SV40 sequences from negative control tissues (3). Furthermore, the amount of SV40 DNA in PCR-positive specimens appears to have been quite low, as indicated by semiquantitative PCR (23), by the inability to confirm positive results with additional primers or direct Southern blotting (2,5,9), and by the negative results reported with a highly sensitive PCR assay (10). Because SV40 appears to be present within each cell in animal NHLs and other tumors (7,24), detection of SV40 DNA at much lower levels in humans raises the possibility of PCR contamination or other laboratory artifact (25). Overall, the accuracy and reproducibility of molecular methods for detection of SV40 DNA in human tumors remain uncertain (11), and so it is challenging to reconcile these diverse PCR-based findings with our own results.

The prevalence of SV40 infection in the U.S. general population is unknown. In this study, the measured SV40 seroprevalence of 10%–11% among adult control subjects would likely overestimate the prevalence of actual infection because of cross-reactivity with the fairly common human viruses BK and JC (16,17,26). In support of this conclusion, we found that SV40 reactivity was correlated with reactivity to BK and, to a lesser extent, JC. SV40 reactivity in most instances was blocked by BK or JC VLPs. In some subjects, SV40 reactivity was not blocked

**Table 3.** Competitive inhibition of SV40 VLP EIA reactivity by SV40, BK, and JC VLPs among SV40-seropositive humans and among macaques\*

Category of subject	No. evaluated	SV40 reactivity inhibited by:			SV40-specific reactivity†, n (%)
		SV40, n (%)	BK, n (%)	JC, n (%)	
Laboratory A‡					
Humans, by serostatus					
BK+ JC+	18	18 (100)	15 (83)	7 (39)	2 (11)
BK+ JC-	9	9 (100)	7 (78)	0 (0)	2 (22)
BK- JC+	6	6 (100)	2 (33)	2 (33)	3 (50)
BK- JC-	2	2 (100)	1 (50)	0 (0)	1 (50)
Macaques	8	8 (100)	0 (0)	0 (0)	8 (100)
Laboratory B§					
Humans, by serostatus					
BK+	21	11 (52)	12 (57)	4 (21)	1 (5)
BK-	7	5 (71)	1 (14)	2 (29)	3 (43)
Macaques	9	9 (100)	0 (0)	0 (0)	4 (100)

\*SV40 = simian virus 40; VLP = virus-like particle; EIA = enzyme immunoassay. JC serostatus was measured in laboratory A only.

†SV40-specific reactivity is SV40 EIA reactivity competitively inhibited by SV40 VLP but not by BK or JC VLPs.

‡In laboratory A, results for competitive inhibition experiments were not evaluable for five humans and two macaques due to low SV40 reactivity in sera with no competing VLP (*see* "Patients and Methods").

§In laboratory B, results for competitive inhibition experiments were not evaluable for seven humans because of low SV40 reactivity in sera with no competing VLP.

||Two BK-seropositive humans and five macaques were excluded from this analysis because competitive inhibition assays were missing for JC VLP.

by SV40 VLPs. Nonspecific reactivity of this type could arise if the SV40 antibodies detected by EIA in some individuals target only epitopes that are exposed on VLPs bound to EIA plates. In contrast, VLPs added in solution for competitive inhibition experiments would have a native conformation and would absorb only antibodies directed specifically at SV40 virions. Unlike humans, macaques exhibited SV40 reactivity that was blocked consistently and only by SV40 VLP.

Nevertheless, humans inoculated experimentally with SV40 can generate low-level but SV40-specific antibody responses (27), and the SV40 reactivity documented in some of our subjects could still reflect prior SV40 exposure or infection. Among control subjects in our study, SV40 seroprevalence was marginally higher in those born before 1963 than in those born later. Incorporating data from the competitive inhibition experiments, we estimated that 1.0%–1.6% of subjects born before 1963 would demonstrate SV40-specific reactivity. Our results therefore point to the possibility that exposure to SV40-contaminated poliovirus vaccine could have led to SV40 antibody responses measurable several decades later. If so, however, the SV40 seroprevalence we observed was lower than that previously reported for individuals exposed to SV40-contaminated vaccine in the 1950s and 1960s (28–30), indicating that SV40 antibody levels in most exposed people have declined over time, presumably reflecting a lack of ongoing viral replication. It is not known whether routes of exposure other than receipt of SV40-contaminated vaccines can produce SV40 infection in humans.

The VLP EIAs used in our study are sensitive and specific (16,17,21). Because the assays detected SV40 infection in 94%–100% of infected macaques, even when sera had been diluted 10-fold, the assays should have readily detected SV40 infection in humans. It is conceivable that transient or latent SV40 infection could lead to NHL without causing a durable, detectable antibody response. However, although there are no data on SV40 antibody production in animals with SV40-induced NHL (7), animals with SV40-induced sarcoma show robust production of virus-neutralizing antibodies, and those antibody levels are correlated with tumor size (31). On this basis, and because

the VLP EIAs and neutralization assays detect similar SV40 antibody responses (16,21), it is reasonable to expect that humans with SV40-induced NHLs would mount readily detectable VLP antibody responses. It is unlikely that NHL treatment affected SV40 antibody levels because SV40 seroprevalence was similar in treated and untreated cases (Table 2). Additional evidence that NHL treatment did not affect antibody levels is provided by the observation that BK and JC antibody levels among NHL patients undergoing chemotherapy change little over time (our unpublished data). The specificity of the SV40 VLP EIAs was at least 90%, as judged from the approximately 10% SV40 seroprevalence in our controls, and we found that specificity could be increased further by incorporating data from competitive inhibition experiments.

A limitation of the present study is that we did not have adequate tumor tissue specimens available from NHL cases to perform PCR testing for SV40 DNA. A direct comparison of serology and PCR results on these case patients would have allowed a more complete evaluation of the strengths and weaknesses of both approaches. Our study's strengths include its large size, population-based sampling of case patients and control subjects, and pathologic classification of NHL cases. Although we lacked data for some potentially informative individuals (due to nonparticipation in the NCI SEER study or lack of serum sample), it is unlikely that the absence of data was related to SV40 serostatus. Thus, our results should provide an unbiased estimate of the true association between SV40 serostatus and NHL. Specimens were evaluated under masked conditions in two laboratories, adding to the validity of our data.

Based on the assumption that SV40 infection would have resulted in a measurable immune response to virion proteins, the results presented in this report do not support the idea that SV40 is a common infection of humans or a major cause of NHL. In the future, it will be of interest to develop additional serological assays for SV40 infection for studies of persons with cancer and the general population (11). Assays that measure antibody to the SV40 T antigen may be especially informative, because animals with SV40-induced cancers, including NHL, frequently make

antibodies to this oncoprotein (7,32). Continued improvements and new approaches in molecular and serological methods may provide opportunities to revisit the question of whether SV40 is a cause of cancer in humans (11).

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## NOTES

<sup>1</sup>*Editor's note:* SEER is a set of geographically defined, population-based, central cancer registries in the United States, operated by local nonprofit organizations under contract to the National Cancer Institute (NCI). Registry data are submitted electronically without personal identifiers to the NCI on a biannual basis, and the NCI makes the data available to the public for scientific research.

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