Markers of Past Infection with Simian Virus 40 (SV40) and Risk of Incident Non-Hodgkin Lymphoma in a Maryland Cohort

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Abstract

Simian virus 40 (SV40) genome sequences have been detected in human non-Hodgkin lymphoma (NHL) tissues, and past infection with SV40 may be a risk factor for NHL. We conducted a population-based nested case-control study to investigate the association between serum antibodies to SV40 and incident NHL. Two research serum banks were established in Washington County, MD, with >45,000 volunteers contributing blood samples collected in 1974 and 1989. Incident cases of NHL diagnosed through 2002 (n = 170) were identified among participants by linkage to population-based cancer registries. Two controls were matched to each case (n = 340) on age, sex, and date of blood draw. Circulating immunoglobulin G antibodies to SV40 were measured using virus-like particle (VLP) ELISA. Positive samples were tested for cross-reactivity with JC virus (JCV) and BK virus (BKV) through competitive inhibition assays. Associations between SV40 antibody seropositivity and NHL were estimated using conditional logistic regression. Whereas SV40 antibodies were detected by VLP ELISA in 15% of cases and 10% of controls (matched odds ratio (OR), 1.97; 95% confidence interval (95% CI), 1.03-3.76), the SV40 reactivity of 85% of the SV40 antibody-positive sera was decreased by adsorption with BKV and/or JCV VLPs. Antibodies specific for SV40 (not cross-reactive) were identified in only 1.8% of cases and 1.6% of controls (OR, 1.51; 95% CI, 0.41-5.52). Our findings suggest that past infection with SV40 is not associated with an increased risk of developing NHL. (Cancer Epidemiol Biomarkers Prev 2005;14(6):1448–52)

Introduction

The incidence of non-Hodgkin lymphoma (NHL) has nearly doubled in the United States since the early 1970s (1). Viral infections have been considered as potential risk factors for NHL (2-4), including infection with simian virus 40 (SV40) (5). SV40 is a natural polyomavirus infection of the rhesus macaque. Millions of Americans were potentially exposed to SV40 through accidentally contaminated polio vaccines distributed in the United States between 1955 and 1963, after which time, polio vaccines were free of SV40 (6). Levels of SV40 contamination varied across polio vaccine lots, and it is unclear how many individuals were actually exposed and infected with SV40. SV40 has been shown to be oncogenic in several laboratory models (7) and can produce B-cell lymphomas in Syrian golden hamsters (8). The T antigen, a protein coded by the virus, complexes with and subsequently inactivates tumor suppressor proteins p53 and pRb.

The evidence linking SV40 infection to NHL is inconsistent. Among nine case series which investigated SV40 in tumor tissues, six reported the presence of SV40 sequences in 10% to 43% of cases (9-14), but three failed to detect SV40 sequences from NHL tissues (15-17). Findings from two cohort studies were negative: no increases in NHL incidence rates were observed among HIV-positive individuals in the United States born in years during which the polio vaccine was contaminated with SV40 compared with individuals born later (18) or in a study of national incidence rates of NHL in Denmark (19). A population-based case-control study of NHL in San Francisco found no associations with self-reported history of polio vaccination, regardless of the year of vaccination or age at vaccination (20). A hospital-based case-control study conducted among U.S. Army Veterans observed no associations between past exposure to SV40-contaminated adenovirus vaccine and NHL, brain tumors, or mesothelioma (21). None of these four studies incorporated biomarkers of SV40 exposure. A hospital-based case-control study of NHL in Spain observed an inverse association between B-cell lymphomas and the presence of serum antibodies to SV40 (5.9% positive in cases versus 9.5% controls; ref. 22). The inactivated polio vaccine was not used universally in Spain (22); thus, the magnitude of opportunity for SV40 exposure in this study population is unclear. A recent population-based case-control study in the United States observed no increased risk of NHL associated with serum antibodies to SV40 measured after NHL diagnosis (23).

To investigate the association between past SV40 infection and the subsequent risk of NHL, we conducted a population-based case-control study of serum antibodies to SV40 and incident NHL, nested within two community-based cohort studies in Washington County, MD.
Materials and Methods

Study Population. A research serum bank was established in Washington County, MD, with 23,951 specimens collected from county residents in August through November, 1974. This campaign was entitled “CLUE” for the slogan, “Give us a clue to heart disease and cancer.” A second CLUE campaign was conducted between May and November 1989 with the collection of 25,079 blood specimens from county residents. Participants completed a brief baseline questionnaire at the time of blood donation. Serum (1974) and plasma (1989) from participants were stored at −70°C to −80°C. All participants consented to the use of their stored blood in future research studies. The protocol for the current study was approved by the Committee on Human Research, the institutional review board at the Johns Hopkins Bloomberg School of Public Health.

Cases of NHL occurring among CLUE cohort members through 2002 were identified by linkage to the Washington County Registry [International Classification of Diseases, Ninth Revision (ICD-9) code 200 or 202], which has been maintained since 1958, linkage to the Maryland State Cancer Registry since 1992, and periodic review of death certificates. Cases were defined as participants of CLUE I, CLUE II, or both, who were county residents at the times of both blood donation and subsequent diagnosis with NHL, where NHL was their first cancer diagnosis with the possible exceptions of nonmelanoma skin cancer or cervical cancer in situ. NHL subtypes were classified using ICD-9/ICD-10 morphology codes: diffuse large B-cell lymphomas (ICD 9680/3, 9682/3, 9684/3, and 9680), follicular B-cell lymphomas (ICD 9675/3, 9693/3, 9691/3, 9695/3, 9696/3, 9698/3, and 9690), T-cell lymphomas (ICD 9700/3), others, others (ICD 9590/3, 9591/3, 9592/3, 9593/3, 9595/3, 9670/3, 9672/3, 9763/3, 9866/3, 9867/3, 9694/3, 9699/3, 9711/3, 9823/3, 9940/3, 9533/1, 9693/3, and 9685/3). Cases (n = 172) were identified. Two cases were excluded due to inadequate amounts of available serum.

Two controls were matched to each case on sex, age, race within 1 year, date of blood draw within 2 weeks, freeze/thaw status of the serum, and participation in CLUE I, CLUE II, or both. Controls were residents of Washington County at the time of blood donation and not known to have died or developed cancer (except for possibly nonmelanoma skin cancer or cervical cancer in situ) as of the date of diagnosis of the case. Matching criteria were relaxed in certain cases to achieve a match: seven controls were up to 2 years older than their matched cases, and for four case-control pairs, date of blood draw differed as much as 1 month. Participants in this study include 74 NHL cases and 120 controls selected for a previous case-control study of pesticide exposure conducted within these cohorts in 1994 (24).

Laboratory Methods. Virus-like particles (VLP) were purified from insect cells infected with recombinant baculoviruses expressing the VP1 major capsid protein of SV40, as previously described (25). For ELISA, Polysorp microwell plates (Nunc, Naperville, IL) were sensitized with 20 to 30 ng of VLP protein per well. The serum dilution (1:400) was left to react for 1 hour at 37°C. Absorbance values of >0.1 were considered positive by ELISA. The sensitivity and specificity of the ELISA for SV40 detection in humans is unknown because there are no human reference standards, but the assay demonstrates 100% sensitivity and 100% specificity for SV40 infection in macaques (25).
stratified by NHL subtype, combining diffuse large B-cell lymphoma with follicular B-cell lymphoma, after initial analyses revealed homogenous risk estimates for these subtypes. One case of T-cell lymphoma was identified and combined with the other/unknown group. Analyses were also stratified by two categories of time between blood draw and diagnosis (<11 and ≥11 years), defined by the median value in the cases. All statistical tests were two sided. Analyses were conducted using SAS, version 8 (SAS Institute, Inc., Cary, NC).

Results

Baseline characteristics are presented for NHL cases and matched controls in Table 1. All but one case was White, and there were more females than males, reflecting the race and sex distributions in the underlying CLUE cohorts. No statistically significant differences between cases and controls were observed for education, smoking status, or body mass index (Table 1).

SV40 antibody levels in the human sera were low; the median absorbance value of the SV40-positive human sera was 0.23 compared with 1.86 for the macaque sera. Based on the dichotomous absorbance cut point of 0.1, SV40 antibodies were detected using the ELISA assay in 15% of cases and 10% of controls (matched OR, 1.97; 95% CI, 1.03-3.76; Table 2). This association was particularly strong for B-cell lymphomas (OR, 2.85; 95% CI, 1.32-6.17), whereas SV40 antibodies were not associated with lymphomas of other/unknown subtypes (OR, 0.92; 95% CI, 0.30-2.83). There was no dose-response relationship between SV40 antibody levels and overall risk of NHL (Table 2).

The median time between blood draw and NHL diagnosis was 10.8 years (SD = 7.7 years). Results were similar for cases diagnosed 11 years before or 11 years after the time of blood draw (Table 3). Only one case diagnosed within 11 years of blood draw and three cases diagnosed ≥11 years after blood draw had SV40-specific antibodies, and no statistically significantly associations were observed with NHL for either of these groups (Table 3). Sixteen cases were diagnosed within 2 years of blood draw, none of which were positive for SV40-specific antibodies, and exclusion of these cases did not appreciably change the results (data not shown). Similar results by time to diagnosis were obtained when cases of other/unknown subtypes were excluded (data not shown).

Discussion

Individuals positive for SV40 antibodies based on the ELISA assay alone had an increased risk for developing NHL, particularly B-cell NHL (OR, 2.85; 95% CI, 1.32-6.17), but further investigation showed that the association was due to cross-reactivity with JCV and/or BKV. Presence of antibodies specific to SV40, as measured from serum collected years before diagnosis, was not associated with the subsequent development of NHL.

This is the first prospective study of SV40 antibodies and the development of NHL. In contrast to analyses of NHL incidence rates by birth cohorts, the measurement of antibodies as biomarkers of past SV40 infection provides exposure data at the individual level. However, antibodies themselves are limited in their specificity for SV40 infection status. If individuals were exposed to inactivated SV40 through the polio vaccine, they could have produced antibodies to SV40 capsid proteins in the absence of active infection. In this case, these individuals would be misclassified as having past SV40 infection. Measurement of antibodies to the T antigen might clarify this issue, because only those with active infection could develop T antibodies. However, only 4 of 217 NHL case samples and 7 of 434 control samples had antibodies specific to SV40 in this study. Therefore, it is unlikely that a true association between SV40 infection and NHL is masked by nondifferential misclassification of exposed, noninfected individuals as infected in this small subset. The small number of participants who had SV40-specific antibodies limited the interpretation of results stratified by NHL subtype.

SV40 antibodies were measured in this study using VLP-based ELISA assays, which were previously validated against the plaque neutralization assay for detection of SV40 antibodies in SV40-infected macaque sera (25). Recent data suggest that the low level of SV40-neutralizing antibodies detected in human sera are largely the result of cross-reactivity with BKV and JCV antibodies (28). Therefore, we used competitive inhibition or "blocking" assays to determine the specificity of SV40 antibodies initially detected by...
ELISA, an approach used also by other investigators (23, 26). Consistent with previous studies, SV40 antibodies detected by ELISA in the present study were highly cross-reactive with JCV and BKV VLPs, and the actual prevalence of SV40-specific antibodies was low (2%).

Whereas the effect of long-term storage on SV40 antibodies in human sera has not been documented, there is no reason to suspect freezing would differentially affect antibodies to SV40 versus antibodies to JCV and BKV, which were highly prevalent among study samples. No significant differences in SV40 antibody levels were observed between samples collected in 1974 and 1989. Finally, even if freezing had a small effect on antibody levels, no bias would be introduced into the results, because cases and controls were matched on the number of freeze/thaw cycles of the sera.

All study participants were born before 1960; thus, all were potentially exposed to SV40-contaminated polio vaccine. By 1961, an estimated 62% of individuals under the age of 60 received polio vaccine, with 88% coverage among those 20 years old and younger (29). In the present study, no significant differences were observed in SV40 antibody seropositivity between those who were younger or older than age 20 in 1961 (data not shown). Human transmission of SV40 has been proposed as another route of exposure for individuals born after 1963 (30). However, if SV40 infection is truly associated with NHL, then SV40 antibody levels should be higher among NHL cases than controls in a case-control study, regardless of the route of SV40 infection.

Cohort participants who moved out of Maryland would have been lost to follow-up, as incident cases were ascertained only within the state. However, the population of Washington County is increasing, and NHL is a relatively rare cancer; thus, few cases are likely to have been missed. Additionally, those who moved out of state had the same opportunity for SV40 exposure than those who remained in the state, because all were potentially vaccinated for polio. Limited information was available about the timing and route of exposure to SV40 in those who moved out of state.

Table 2. SV40 antibody seroprevalence and risk of incident NHL: Washington County, MD (1975-2003)

<table>
<thead>
<tr>
<th>Method of measurement: SV40 antibody (Ab) status</th>
<th>Cases*, n (%)</th>
<th>Controls*, n (%)</th>
<th>Matched OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ELISA: SV40 Ab-negative</td>
<td>184 (84.8)</td>
<td>393 (90.6)</td>
<td>1.00</td>
</tr>
<tr>
<td>SV40 Ab-positive</td>
<td>33 (15.2)</td>
<td>41 (9.5)</td>
<td>1.97 (1.03-3.76)</td>
</tr>
<tr>
<td>SV40 Ab-positive, low reactivity†</td>
<td>9 (4.1)</td>
<td>13 (3.0)</td>
<td>1.74 (0.67-4.51)</td>
</tr>
<tr>
<td>SV40 Ab-positive, middle reactivity</td>
<td>17 (7.8)</td>
<td>14 (3.2)</td>
<td>3.30 (1.38-7.91)</td>
</tr>
<tr>
<td>SV40 Ab-positive, high reactivity</td>
<td>7 (3.2)</td>
<td>14 (3.2)</td>
<td>1.16 (0.43-3.13)</td>
</tr>
<tr>
<td>ELISA following blocking assays:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SV40-negative Ab†</td>
<td>186 (86.9)</td>
<td>396 (91.2)</td>
<td>1.00</td>
</tr>
<tr>
<td>Cross-reactive Ab</td>
<td>27 (12.6)</td>
<td>31 (7.1)</td>
<td>2.07 (1.03-4.16)</td>
</tr>
<tr>
<td>SV40-specific Ab</td>
<td>4 (1.8)</td>
<td>7 (1.6)</td>
<td>1.51 (0.41-5.52)</td>
</tr>
<tr>
<td>B-cell NHL only:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ELISA: SV40 Ab-negative</td>
<td>119 (83.2)</td>
<td>264 (92.3)</td>
<td>1.00</td>
</tr>
<tr>
<td>SV40 Ab-positive</td>
<td>24 (16.8)</td>
<td>22 (7.7)</td>
<td>2.85 (1.32-6.17)</td>
</tr>
<tr>
<td>ELISA following blocking assays:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SV40-negative Ab</td>
<td>119 (83.2)</td>
<td>267 (93.4)</td>
<td>1.00</td>
</tr>
<tr>
<td>Cross-reactive Ab</td>
<td>21 (14.7)</td>
<td>16 (5.6)</td>
<td>3.50 (1.45-8.46)</td>
</tr>
<tr>
<td>SV40-specific Ab</td>
<td>3 (2.1)</td>
<td>3 (1.0)</td>
<td>3.22 (0.67-15.54)</td>
</tr>
</tbody>
</table>

Abbreviation: Ab, antibody.
*Cases (n = 170) contributed 217 blood samples (47 donated blood in both 1979 and 1989); 340 controls contributed 434 blood samples (94 donated blood in both 1979 and 1989).
†Three levels of reactivity were determined based on the distribution of ELISA absorbance values among controls who were positive (absorbance values > 0.1).
‡Includes two cases (other/unknown NHL) and three controls (matched to B-cell NHL cases) with samples that tested positive for SV40 antibodies by ELISA but were not inhibited by SV40, JCV, or BKV in blocking assays.

Table 3. SV40 antibody seroprevalence and risk of incident NHL by time from blood draw to diagnosis: Washington County, MD (1975-2003)

<table>
<thead>
<tr>
<th>Method of measurement: SV40 antibody (Ab) status</th>
<th>Cases*, n (%)</th>
<th>Controls*, n (%)</th>
<th>Matched OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood draw &lt;11 y before diagnosis:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ELISA: SV40 Ab-negative</td>
<td>93 (83.0)</td>
<td>203 (91.4)</td>
<td>1.00</td>
</tr>
<tr>
<td>SV40 Ab-positive</td>
<td>18 (16.2)</td>
<td>19 (8.6)</td>
<td>2.28 (1.08-4.81)</td>
</tr>
<tr>
<td>ELISA following blocking assays:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SV40-negative Ab</td>
<td>95 (85.6)</td>
<td>203 (91.4)</td>
<td>1.00</td>
</tr>
<tr>
<td>Cross-reactive Ab</td>
<td>15 (13.5)</td>
<td>15 (6.8)</td>
<td>2.35 (1.03-5.37)</td>
</tr>
<tr>
<td>SV40-specific Ab</td>
<td>1 (0.9)</td>
<td>4 (1.8)</td>
<td>0.55 (0.06-4.80)</td>
</tr>
<tr>
<td>Blood draw ≥11 y before diagnosis:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ELISA: SV40 Ab-negative</td>
<td>91 (85.9)</td>
<td>190 (89.6)</td>
<td>1.00</td>
</tr>
<tr>
<td>SV40 Ab-positive</td>
<td>15 (14.2)</td>
<td>22 (10.4)</td>
<td>1.63 (0.66-4.03)</td>
</tr>
<tr>
<td>ELISA following blocking assays:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SV40-negative Ab†</td>
<td>91 (85.8)</td>
<td>193 (91.0)</td>
<td>1.00</td>
</tr>
<tr>
<td>Cross-reactive Ab</td>
<td>12 (11.3)</td>
<td>16 (7.6)</td>
<td>1.99 (0.75-5.28)</td>
</tr>
<tr>
<td>SV40-specific Ab</td>
<td>3 (2.9)</td>
<td>3 (1.4)</td>
<td>3.08 (0.49-19.31)</td>
</tr>
</tbody>
</table>

Abbreviation: Ab, antibody.
*Cases (n = 170) contributed 217 blood samples (47 donated blood in both 1979 and 1989); 340 controls contributed 434 blood samples (94 donated blood in both 1979 and 1989).
†Includes two cases (<11 years prior to diagnosis) and three controls (≥11 years prior to diagnosis) with samples that tested positive for SV40 antibodies by ELISA but were not inhibited by SV40, JCV, or BKV in blocking assays.
available for other suspected risk factors for NHL at baseline, including birth order, income, and history of infections. These factors, if associated with polio vaccination, could have been negative confounders of the association between SV40 infection and NHL, assuming that polio vaccination was the main source of SV40 exposure.

Our results indicate that past SV40 infection is not associated with the development of NHL. The observed association between NHL and antibodies to SV40 that cross-react with JCV and/or BKV suggests that these polyomaviruses should be examined further as potential risk factors for NHL. Future epidemiologic studies of polyomavirus antibodies and cancer should also incorporate biomarkers of immunologic cross-reactivity with other oncogenic viruses to investigate the potential interplay between viruses in the etiology of NHL.

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References
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