

SHORT REPORT

LACK OF SEROLOGICAL EVIDENCE FOR AN ASSOCIATION BETWEEN SIMIAN VIRUS 40 AND LYMPHOMA

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Recent studies have implicated simian virus 40 (SV40) in non-Hodgkin's lymphomas based on detection of SV40 DNA sequences. We employed a virus-like-particle (VLP)-based enzyme immunoassay for antibodies to SV40 to test sera from 520 lymphoma cases and 587 controls in Spain. The SV40 seroprevalence was 9.5% in controls and 5.9% in cases. Antibody levels of the positive sera were low. There was no association of SV40 seropositivity with any subtype of lymphoma. VLPs of the human BK virus substantially inhibited the SV40 reactivity of human sera. There was no serological evidence of widespread SV40 infection and no association of SV40 seropositivity with human lymphomas in Spain.

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Recent reports of the detection of simian virus 40 (SV40) DNA sequences in non-Hodgkin's lymphomas^{1,2} add to a growing list of human cancers that may be associated with SV40 DNA positivity.³ SV40 is a natural infection of Asiatic macaques to which millions of people were inadvertently exposed through contaminated poliovirus vaccines administered between 1955 and 1963. These vaccines were used in Spain but not applied universally.⁴ Furthermore, the detection of SV40 in cancers in persons born after 1963 suggests that the virus may have established itself in human populations and is transmitted by person-to-person spread. Previous reports have largely relied on detection of viral DNA as evidence of SV40 infection. If SV40 circulates in human populations and is implicated in lymphomas, one might expect to detect serum antibodies to SV40 in lymphoma cases. We have employed a newly developed enzyme immunoassay (EIA) for SV40 antibodies⁵ in a seroepidemiological study to evaluate the association between SV40 infection and lymphomas.

Study subjects were recruited at 4 centers in Spain: Barcelona, Tortosa, Reus and Madrid. Cases were consecutive patients newly diagnosed with a lymphoid malignancy between 1998 and 2002. The diagnosis of lymphoma was verified by histology or cytopathology. Cases were categorized according to WHO Classification for Neoplastic Diseases of the Lymphoid Tissues. Controls were hospitalized patients matched to the cases by 5-year age group, gender and study center. Interviews were conducted to collect data on demographic, medical and family history, and environmental exposures. Cases and controls provided a blood sample. Informed consent was obtained from all subjects and the Institutional Review Boards of the participating centers approved the study.

Of 703 eligible cases, 520 (74.0%) were included in the seroepidemiological study. Reasons for exclusion were refusal to participate ($n = 28$), death before the interview ($n = 25$), absence of blood sample ($n = 125$) and absence of interview ($n = 5$). Of 655 eligible controls, 587 (89.6%) were included in the study. Reasons for exclusion were refusal to participate ($n = 23$) and absence of a blood sample ($n = 45$).

A recombinant baculovirus expressing VP1, the major capsid protein of SV40, was constructed and insect cells were infected with the virus. Virus-like-particles (VLPs) were purified from infected insect cell lysates by ultracentrifugation and column chromatography methods. For the enzyme immunoassay, microtiter wells were sensitized with 30 ng of VLP protein per well. The serum dilution (1:400) was left to react for 1 hr at 37°C. Antigen-bound immunoglobulin was detected with peroxidase-conjugated antibodies against human IgG (Zymed, San Francisco, CA). After 30 min at room temperature, color development was initiated by the addition of 2,2'-azino-di-(3-ethylbenzthiazoline-6-sulfonate) hydrogen peroxide solution. The reaction was stopped after 20 min and absorbance was measured at 405 nm.

Figure 1 displays OD histograms of SV40 VLP EIA neutralizing antibody negative and positive macaque sera (*a,b*) and human sera from controls and cases (*c,d*). The neutralizing antibody negative macaque sera gave uniformly low reactivity, with a median OD value of 0.017 (interquartile range [IQR] 0.023–0.13). In contrast, high levels of reactivity were recorded among the antibody positive macaque sera, with a median OD value of 1.43 (IQR = 1.24–1.52). Reactivity of sera from human subjects was tightly clustered at the low end of the OD scale, with a median OD value of 0.030 (IQR = 0.022–0.047) for cases and 0.030 (IQR = 0.022–0.053) for controls. The difference in seroreactivity between cases and controls was not statistically significant (*t*-test, $p = 0.76$).

We chose a cut-point for seropositivity in humans as the approximate midpoint between the highest OD value of the antibody negative macaque sera and the lowest OD value of the antibody positive macaque sera (≥ 0.130 OD units) but all the results presented below were consistent at higher and lower cut-points. SV40 seropositivity was not associated with gender, age, history of

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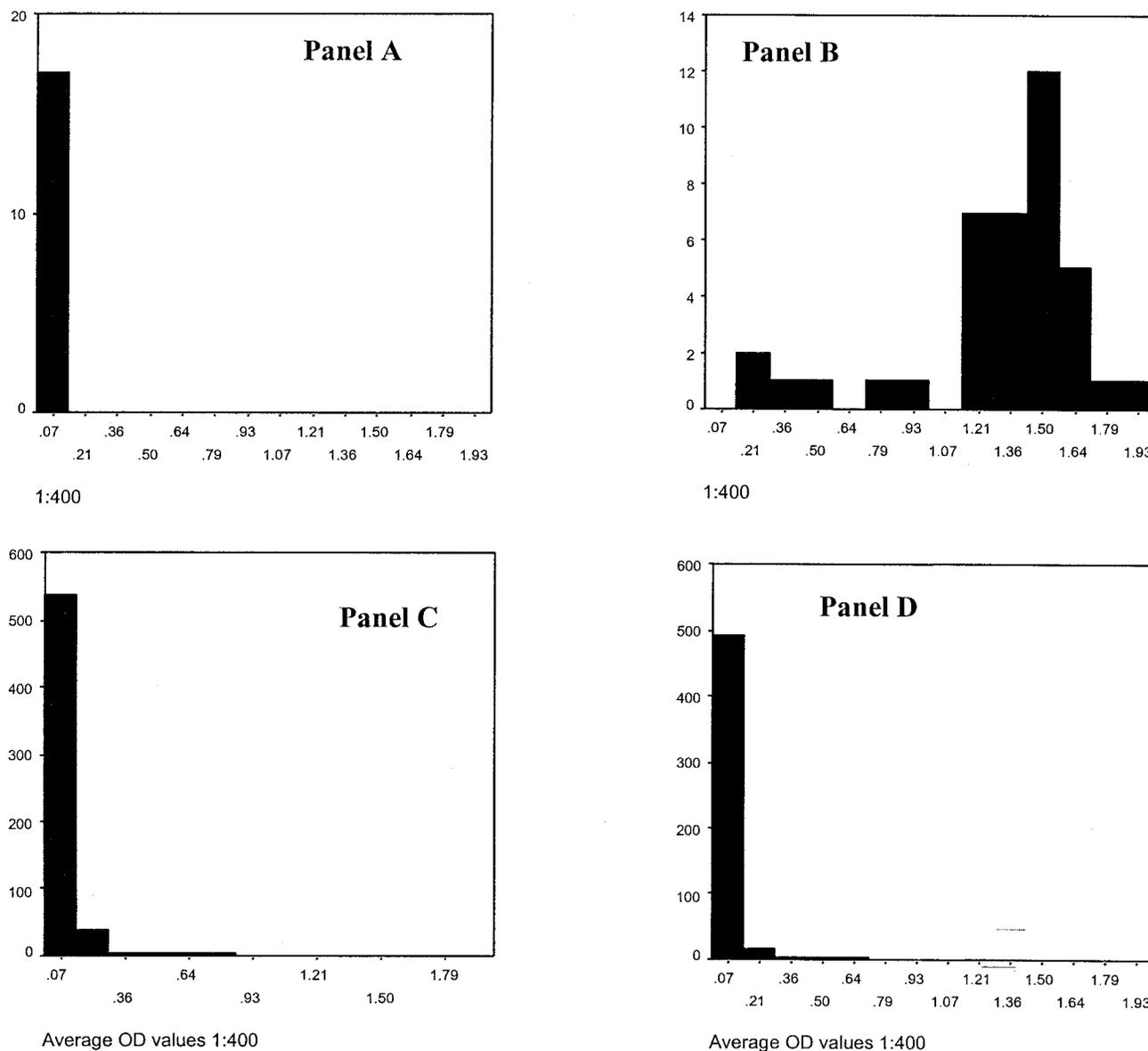


FIGURE 1 – SV40 seroreactivity in VLP-based enzyme immunoassay of 17 SV40 neutralizing antibody negative macaque sera (a), 39 SV40 neutralizing antibody positive macaque sera (b), 587 human control sera (c) and 520 human case sera (d). SV40 neutralizing antibodies were measured by plaque inhibition assay. Each bar = the number of sera with average OD values within the range indicated on the x-axis.

having received a full course of childhood immunizations or history of blood transfusions, or other socio-demographic, medical and environmental exposure variables (Table I). The seroprevalence was 9.5% in controls and 5.9% in cases (Table II). Among the cases with different types of lymphomas the seroprevalence ranged from 0–19.3%. The seroprevalence in diffuse large B-cell lymphoma and follicular lymphoma, 2 types where SV40 DNA sequences have been previously reported, was 2.2% and 0%, respectively. Hodgkin's lymphoma was associated with a non-statistically significant 2-fold increased risk of SV40 seropositivity.

Cross-reactivity with BK virus antibodies may account for part of the low level SV40 reactivity of human sera. The SV40 specificity of VLP seropositivity was assessed by a competitive VLP binding assay. After preincubation of 77 SV40 seropositive sera with 3 μ g/ml BK virus VLP protein for 30 min at 37°C, the median reactivity on SV40 VLP coated plates was reduced by 42% (range

29–61%) (Fig. 2). The reduction in reactivity was similar for case and control sera. These results are in agreement with Cappello, et al.⁶

The SV40 VLP-based EIA is a sensitive and specific assay for SV40 infection as demonstrated by its ability to discriminate perfectly between macaques that are seropositive and seronegative in a SV40 plaque neutralization assay. Using this EIA, we found no serological evidence for widespread circulation of SV40 in Spain and no association of SV40 infection with any histological type of human lymphoma that could not be explained by cross-reactivity with human polyomavirus.

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TABLE I – DISTRIBUTION OF NEUTRALIZING ANTIBODIES AGAINST SV40 BY LYMPHOMA CASES AND CONTROLS¹

	Controls Positive/total n (%)	Cases Positive/total n (%)
All	56/587 (9.5)	31/520 (6.0)
Study area		
Barcelona	46/488 (9.4)	26/408 (6.4)
Madrid	7/55 (12.7)	4/67 (6.0)
Tarragona	3/44 (6.8)	1/45 (2.2)
Gender ²		
Men	30/308 (9.7)	12/284 (4.2)
Women	26/279 (9.3)	19/236 (8.1)
Age ²		
<40	9/104 (8.7)	12/82 (14.6)
40–52	9/95 (9.5)	6/83 (7.2)
53–63	10/105 (9.5)	2/90 (2.2)
64–69	9/98 (9.2)	5/94 (5.3)
70–75	10/90 (11.1)	5/94 (5.3)
>74	9/95 (9.5)	1/77 (1.3)
Year of birth		
<1964	47/502 (9.4)	22/462 (4.8)
≥1964	9/84 (10.7)	9/58 (15.5)
Educational level		
Ever school	48/525 (9.1)	26/455 (5.7)
No school	8/62 (12.9)	5/65 (7.7)
Adequately vaccinated		
Yes	50/518 (9.7)	30/451 (6.7)
No	5/61 (8.2)	1/58 (1.7)
Unknown	1/6 (16.7)	0/8 (0)
More sick than schoolmates in childhood		
Yes	4/53 (7.5)	3/38 (7.9)
No	52/601 (8.6)	27/478 (5.6)
Perceived healthy childhood ²		
Yes	53/539 (9.8)	26/488 (5.3)
No	3/47 (6.4)	4/32 (12.5)
History of previous surgery		
Yes	49/487 (10.1)	26/425 (6.1)
No	7/98 (7.1)	5/95 (5.3)
Previous blood transfusion		
Yes	15/154 (9.7)	4/129 (3.1)
No	41/429 (9.6)	27/388 (7.0)
Smoking		
Yes	32/310 (10.3)	19/280 (6.8)
No	24/276 (8.7)	12/238 (5.0)

¹Cut-off value for seropositivity is OD > 0.13 at dilution 1:400.–
²p-values for heterogeneity <0.20.

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TABLE II – ASSOCIATION OF SV40 VLP SEROPOSITIVITY AND HUMAN LYMPHOID NEOPLASMS

Disease category	Subjects n	Positive n (%)	OR ¹ (95% CI)
Controls	587	56 (9.5)	Reference
All lymphoid neoplasms	520	31 (5.9)	0.61 (0.38–0.95)
B-cell neoplasms	485	28 (5.6)	0.59 (0.37–0.94)
Chronic lymphocytic leukemia	110	8 (7.3)	0.75 (0.34–1.67)
Diffuse large-cell lymphoma	93	2 (2.2)	0.28 (0.07–1.16)
Plasma cell myeloma	68	2 (2.9)	0.28 (0.07–1.16)
Other B-cell lymphoma	45	4 (8.9)	0.93 (0.32–2.71)
Follicular lymphoma	38	0	NA
Marginal zone B-cell lymphoma	28	0	NA
Splenic marginal zone lymphoma	26	0	NA
Lymphoplasmacytic lymphoma	20	1 (5.0)	0.51 (0.07–3.90)
Hodgkin's lymphoma	57	11 (19.3)	2.04 (0.96–4.33)
T-cell lymphoma	35	3 (8.6)	0.76 (0.22–2.57)
Mycosides fungoids/Sezary	16	2 (12.5)	1.10(0.24–5.13)
Other T-cell lymphoma	19	1 (5.3)	0.54 (0.07–4.21)

¹The odds ratio was adjusted for age and gender. NA, not applicable.

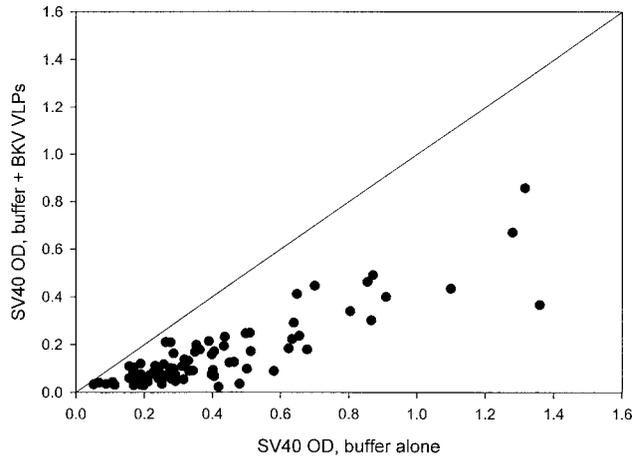


FIGURE 2 – Correlation of SV40 VLP reactivity of serum samples prepared in buffer alone or buffer + BK virus VLPs. Serum samples were preincubated for 30 min at 37°C with 3 µg/ml of BK virus VLP protein or sample dilution buffer alone and added to an SV40 VLP-coated plate.

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