

Null Results in Brief

No Association between Polyomaviruses and Primary Central Nervous System Lymphomas of HIV-Seronegative and HIV-Positive Patients

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Introduction

Primary central nervous system lymphoma (PCNSL) is a rare but often rapidly fatal form of non-Hodgkin's lymphoma. Several studies have reported that the incidence of PCNSL has dramatically increased in the past two decades (1). The reasons of this increase are unknown. Although the EBV is probably involved in the development of immune suppression-related PCNSL (2), a pathogenetic model for the immunocompetent forms is still lacking. Above all, no information is available on the possible role of other viruses in the development of human PCNSL.

The oncogenic and neurotropic polyomaviruses JC, BK, and SV40 are associated with human normal tissues and tumors and neurologic diseases (3). The ability of SV40 to infect humans is well documented (3). In addition, circulating B-lymphocytes of both healthy donors and cancer patients may harbor SV40 DNA (3). We have found recently that more than half of EBV-immortalized lymphoblastoid B-cell lines carry specific DNA and RNA sequences of SV40, suggesting the possibility that SV40 might cooperate with EBV in B-cell immortalization (4). The possibility that polyomaviruses might be associated with the development of human PCNSL has been never investigated extensively. On these grounds, we carried out a study aimed at verifying whether footprints of the SV40, BK, and JC polyomaviruses could be detected in a relatively large cohort of human PCNSL of both HIV-seronegative and HIV-infected patients.

Subjects and Methods

Study Population. Forty-seven adult patients seen at the San Raffaele H Scientific Institute from 1988 to 2002 with histologically proven diagnosis of non-Hodgkin's lymphoma, localized exclusively in the CNS, cranial nerves, or meninges, were included in the study. A HIV infection was detected in 31 of the 47 patients. The main clinicopathologic characteristics of both subgroups of patients, according to the presence of a concomitant HIV infection, are summarized in Table 1. All the original histologic slides were referred to central pathology review and classified according to morphologic and immunophenotypic criteria of the WHO classification. Fifty-three mesothelioma samples from the Department of Pathology, University of Udine, were analyzed as control groups. The difference in the prevalence of polyomavirus sequences between study group and controls was assessed by the χ^2 test or Fisher exact test for categorical variables according to the sample size. All the probability values were two sided, with an overall significance level of 0.05.

Extraction of DNA, Oligonucleotides, and PCR Amplifications. Each sample obtained from tissue block was cut at 10 μ m thick sections. DNA extraction was carried out with the commercial kit QIAmp as indicated by the supplier (Qiagen, Milan, Italy). To verify whether cross-contaminations occurred, salmon sperm DNA and mock specimens lacking DNA were extracted simultaneously with those samples under analysis. DNA extracted from samples was first assessed for suitability for PCR analysis by a control reaction designed to amplify β -globin gene sequences. DNA samples were then investigated for SV40, BK, and JC polyomavirus Tag DNA sequences. Oligonucleotides employed in the PCR and nested PCR, annealing temperatures, product sizes, PCR reagents, and conditions were described elsewhere (4, 5).

Results and Discussion

None of the HIV-related and HIV-unrelated PCNSL from our series (47 patients) carried SV40 or human

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Note: F. Martini and R. Dolcetti contributed equally to this work.

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Table 1. Patients' characteristics and extension of disease

	HIV-seronegative patients	HIV-positive patients
No. patients	16	31
Age (y)		
Median	58	34
Range	29-70	26-56
Gender, <i>n</i> (%) males	9 (56)	22 (71)
Eastern Cooperative Oncology Group-performance status,* <i>n</i> (%)		
0-1	6 (38)	0 (0)
2-3	9 (56)	8 (26)
4	1 (6)	2 (6)
5	0 (0)	21 (68)
Prior cancer, <i>n</i> (%)	1 (6)	1 (3)
Histotype,† <i>n</i> (%)		
Diffuse large B-cell	12 (75)	29 (94)
Burkitt like	2 (13)	0 (0)
Lymphoblastic	1 (6)	0 (0)
Anaplastic large T-cell Ki1	1 (6)	0 (0)
Unclassified	0 (0)	2 (6)
Systemic symptoms, <i>n</i> (%)	1 (6)	14 (45)
Lactate dehydrogenase ratio >1,‡ <i>n</i> (%)	3/11 (27)	20/25 (80)
Ocular disease,‡ <i>n</i> (%)	2/14 (14)	0/13 (0)
Positive cerebrospinal fluid cytology examination,‡ <i>n</i> (%)		
High cerebrospinal fluid protein level,‡,§ <i>n</i> (%)	5/8 (63)	11/13 (85)
Multiple lesions,‡ <i>n</i> (%)	8 (50)	12/25 (48)
Involvement of deep structures, <i>n</i> (%)	8 (50)	23 (74)

*Performance status according to the Eastern Cooperative Oncology Group score at the time of histopathologic diagnosis.

†Histotype was defined according to the REAL/WHO classification. All cases but one shared a B-cell phenotype.

‡Relationship between the number of positive cases and the total number of assessed patients.

§The cutoff for normal cerebrospinal fluid protein concentration was 45 mg/dL in patients ages ≤60 years and 60 mg/dL in patients ages >60 years.

||Involvement of deep structures of the brain that is basal ganglia and/or corpus callosum and/or brain stem and/or cerebellum.

polyomavirus DNA sequences. Similar negative results were reported recently (6) in analyzing 500 different human non-Hodgkin's lymphomas including some PCNSL. These findings indicate that probably polyomaviruses do not play a role in the onset/progression of human PCNSL. Although conducted in a retrospective manner, the findings presented herein have the advantage of being monoinstitutional; more importantly, these results were obtained in a relative large series of such a rare disease, encompassing either HIV-positive and immunocompetent individuals.

When faced with striking results, some technical issues deserve to be discussed in more detail. First, the target of our repeatedly negative PCR for polyomavirus was the Tag coding sequences (4, 5). With the same approach, 24 of the 53 mesothelioma samples analyzed as internal controls were found positive for SV40 Tag, with a prevalence value (45%; $P < 0.00001$) similar to that obtained by other laboratories (3).

Second, our previous investigations were able to detect a fraction of SV40-positive specimens in all cases

although with a wide range of prevalence rates (3). The sensitivity and specificity of our nested PCR protocol were assessed in reconstruction experiments and gave results comparable with those obtained with the single-step PCR followed by filter hybridization with specific internal oligoprobes (5).

A new wave of investigations aimed to test the presence of SV40 footprints in human lymphomas has risen since the first report by Martini et al. (5), documenting the presence of SV40 DNA in a substantial proportion of a miscellaneous group of lymphoproliferative disorders. Following that experience, other studies confirmed the association of SV40 DNA with human non-Hodgkin's lymphoma although with different frequencies. However, subsequent reports did not confirm these data (3, 6). At present, the nature of these conflicting results is not known. Our results agree with these more recent observations (6) and suggest that SV40 may not play a primary role in the development of human PCNSL. Most importantly, the present experience extends for the first time the evaluation also to other two critical polyomavirus family members such as JC and BK polyomaviruses.

Although these are the most plausible interpretations of our results, alternative explanations cannot be entirely excluded. These include the possibility that polyomavirus DNA could have been selectively degraded during the processing of tissue samples. In addition, the polyomavirus DNA could have been lost during the extraction procedure because is present in a lower amount than threshold value (5) as compared with SV40-positive mesothelioma samples. If this is the case, however, the biological significance of this finding in the light of PCNSL pathogenesis would be questionable. Finally, according to the hypothesis that the polyomaviruses may act with the "hit-and-run" mechanism (3), their sequences could be dispensable after B-cell transformation, an issue that has been suggested in a recent report (7). In conclusion, our study shows that, rather than SV40 alone, the main members of polyomavirus family are likely to be not involved in the development of human PCNSL, irrespective of patient's immunocompetence status.

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