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Simian virus 40 tumor antigen expression and immunophenotypic profile of AIDS-related non-Hodgkin's lymphoma

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Abstract

Simian virus 40 (SV40) is associated with some systemic non-Hodgkin's lymphomas (NHL) among HIV-positive patients, based on assays for viral DNA sequences. To investigate the possible production of the viral transforming protein, we examined age-matched case–control specimens from patients with HIV/AIDS for the expression of SV40 large tumor antigen (T-ag). Masked specimens initially examined by polymerase chain reaction (PCR) for polyomavirus and herpesvirus DNA sequences were assessed for the expression of SV40 T-ag and phenotypic lymphocyte markers by immunohistochemistry (IHC). Fifty-five systemic NHL and 25 nonmalignant lymphoid and malignant nonlymphoid tissue control cases from two HIV community programs in Texas and New Jersey were scored for IHC positivity without knowledge of the PCR results. IHC showed expression of SV40 T-ag among B-cell lymphomas, whereas none of the control tissue samples were positive for T-ag (12/55, 22% vs. 0/25, 0%; $P = 0.01$). SV40 T-ag expression was detected 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. SV40 T-ag was demonstrated in both primary and recurrent tumors from one patient. A germinal center B-cell-like (GCB) profile was more frequently expressed by SV40-positive tumors than in Epstein–Barr virus (EBV)-related lymphomas (10/12, 83% vs. 6/13, 46%; $P = 0.05$), whereas a non-GCB phenotype was more frequent in EBV-positive than in SV40-positive lymphomas (7/13, 54% vs. 2/12, 17%; $P = 0.05$). This study shows that SV40 gene expression occurs in a fraction of cells in some B-cell lymphomas among patients with HIV/AIDS.

Keywords: Simian virus 40; T-ag protein; Non-Hodgkin's lymphoma; AIDS-related lymphoma; Lymphoma subgroups

Introduction

Immunodeficiency is recognized to increase the risk of some types of cancer, especially malignancies etiologically linked to DNA tumor viruses (Butel, 2000; Mueller, 1999). Immunosuppression related with progression of HIV infection substantially increases the risk of some neoplasias, including non-Hodgkin's lymphoma (NHL) (Butel, 2000; Centers for Disease Control and Prevention, 1992; Engels and Goedert, 2005; Fisher and Fisher, 2004; International Agency for Research on Cancer, 1996; Mueller, 1999). NHL is a common malignancy in HIV-infected patients and can be divided into systemic and primary central nervous system lymphomas (Fisher and Fisher, 2004; Mueller, 1999). Some NHL in HIV-infected patients have been

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attributed to deficient immune surveillance of herpesviruses, such as Epstein–Barr virus (EBV) and human herpesvirus 8 (HHV8), or perhaps to chronic antigenic stimulation and defective immune regulation (Butel, 2000; Centers for Disease Control and Prevention, 1992; International Agency for Research on Cancer, 1996; Mueller, 1999). In addition, different studies have demonstrated that SV40 large tumor antigen (T-ag) DNA sequences are significantly associated with NHL (with positivity rates ranging from 11% to 43%) in both HIV-infected and HIV-uninfected patients (David et al., 2001; Martini et al., 1998; Nakatsuka et al., 2003; Rizzo et al., 1999; Shivapurkar et al., 2002, 2004; Vilchez et al., 2002b, 2002c).

The discovery of polyomavirus SV40 and its introduction into the human population is related, at least in part, to the development and worldwide dissemination of early forms of the poliovirus vaccine (Butel and Lednicky, 1999; Rollison and Shah, 2001; Stratton et al., 2003; Vilchez et al., 2003b; Vilchez and Butel, 2004). These early poliovirus vaccines were prepared using primary cell cultures derived from rhesus monkeys, which are naturally infected with SV40. Live SV40 survived the inactivation treatments used to prepare the inactivated vaccines; SV40 was also present in live, attenuated poliovirus vaccines (Proceedings of the Second International Conference on Live Poliovirus Vaccines, 1960). These data led the Institute of Medicine of the National Academies to conclude that the biological evidence was of moderate strength that some SV40 infections in the human population today are related to exposure from early forms of the poliovirus vaccine and that SV40 exposure could lead to cancer in humans under natural conditions (Stratton et al., 2003).

The majority of studies of SV40 in lymphomas among HIV-infected patients have measured viral DNA sequences (David et al., 2001; Martini et al., 1998; Nakatsuka et al., 2003; Rizzo et al., 1999; Shivapurkar et al., 2002; Vilchez et al., 2002b, 2002c), but the presence of SV40 T-ag DNA does not necessarily indicate synthesis of the viral transforming protein. As virus-caused tumors are predicted to exhibit the production of a viral transforming protein in at least some cells (Butel, 2000), this marker was sought in AIDS-related cancers. A study from Italy reported the detection of SV40 T-ag staining in 17% of SV40 DNA-positive lymphomas from HIV-infected and -uninfected patients (Martini et al., 1998). In contrast, a study of lymphoma specimens from France and Canada reported negative results for T-ag staining, although there was no analysis for SV40 DNA (Brousset et al., 2004). The small number of T-ag-positive specimens on the one hand and the lack of testing for viral DNA sequences on the other made it difficult from those studies to evaluate the pattern of expression of SV40 T-ag. It is recognized that the NHL category includes several subgroups of lymphomas based on gene expression profiles (Rosenwald et al., 2002) and lymphocyte marker protein expression (Hans et al., 2004). No information is available about the phenotypes of SV40-positive NHL. We report here, in an age-matched, masked, case–control study of systemic NHL from patients with HIV/AIDS, an examination of SV40 DNA-positive and -negative lymphomas to determine the expression of SV40 T-ag and the phenotypic lymphocyte markers of those opportunistic malignancies.

Results

Polymerase chain reaction and DNA sequence analysis of AIDS-related NHL and control samples

Polymerase chain reaction (PCR) analysis using primers directed against the N-terminus of polyomavirus T-ags (PYVfor/PYVrev) was carried out as described in Materials and methods. SV40 T-ag DNA sequences were identified in 12 (22%) of the 55 NHL cases (Table 1). Sequence analysis of amplified products obtained from lymphoma samples using universal polyomavirus large T-ag primers revealed the DNA sequences to be identical to that of the SV40 T-ag gene. The NHL-associated sequences were proven to be from SV40 rather than from JC or BK viruses by nucleotide polymorphisms and the diagnostic absence of a 9 base pair insert in this region of the large T-ag gene amplicon (Fig. 1). All the control specimens (normal lymph node, lung, spleen, and liver and Kaposi sarcomas) were negative for SV40 DNA (Table 1). SV40 sequences were detected significantly more often in lymphomas than in tissue control cases (12/55, 22% vs. 0/25, 0%; $P = 0.01$). Herpesviruses EBV and HHV8 DNA sequences were detected in 16 (29%) and 0

Table 1
Morphology and viral DNA sequences among AIDS-related systemic non-Hodgkin's lymphoma and control tissues from HIV-positive patients[a]

Type of sample	No. of samples	EBV DNA positive, *n* (%)	SV40 DNA positive, *n* (%)
Lymphomas			
Diffuse large B-cell	43	13 (31)	12 (28)
Burkitt	5	3 (60)	0 (0)
Malignant lymphoma	3	0 (0)	0 (0)
Follicular	1	0 (0)	0 (0)
T-cell	3	0 (0)	0 (0)
Total lymphomas	55[b]	16 (29)	12 (22)[c]
Control tissues			
Kaposi sarcoma	5	0	0 (0)
Lymph nodes	10	6	0 (0)
Lung	3	0	0 (0)
Spleen	4	2	0 (0)
Liver	3	0	0 (0)
Total control tissues	25	8	0 (0)

[a] Assayed for viral DNA by PCR using primers directed against EBV LMP2a and a conserved region of polyomavirus large T-ag. SV40-specific products were identified by sequence analysis.
[b] Viral DNA results from 26 of the samples were included in earlier reports (Vilchez et al., 2002b, 2002c).
[c] The 12 SV40 DNA-positive lymphomas were found to express SV40 T-antigen (this report).