Lymphoepithelioma-like Carcinoma of the Uterine Cervix

A Clinicopathologic Study of 4 Cases Not Associated With Epstein-Barr Virus, Human Papillomavirus, or Simian Virus 40

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• ***Context.*—It has been proposed that Epstein-Barr virus (EBV) plays a role in the etiology of lymphoepithelioma-like carcinoma (LELC) in diverse anatomic locations. In contrast to Asian women, Western women have a low prevalence of LELC of the uterine cervix, and EBV genomes have not been identified.**

***Objective.*—To assess the presence of EBV in LELC of the uterine cervix in 4 white Western women.**

***Design.*—We collected 4 cases of LELC of the uterine cervix between 1990 and 2000. We performed histologic and immunohistochemical analyses of formalin-fixed, paraffin-embedded tumor samples. We amplified tumor DNA with polymerase chain reaction to detect EBV, human papillomavirus, and simian virus 40 DNAs.**

***Results.*—Immunohistochemically, tumor cells were positive for cytokeratins and showed strong expression of p53 and MIB-1. Staining for the oncoprotein c-Erb-B2 was focally positive, and staining for Bcl-2 and progesterone receptors was negative. Only one case showed focal nuclear staining for estrogen receptors. All cases had a dense infiltrate of mature lymphocytes expressing T-cell antigens CD45RO, CD3, and CD8. Polymerase chain reaction analysis did not detect EBV, human papillomavirus, or simian virus 40 DNA sequences in any of the 4 cases. One case had positive serologic results for anti-EBV antibodies, indicating a mild or chronic infection.**

***Conclusions.*—LELC of the uterine cervix shows the immunohistochemical profile of an aggressive tumor in spite of its good prognosis, in which CD8 cytotoxic suppressor lymphocytes could play an important role. Based on our results, the role of EBV, human papillomavirus, or simian virus 40 in the pathogenesis of LELC of the uterine cervix in Western women remains unclear.**

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Lymphoepithelioma-like carcinoma (LELC) of the uterine cervix is a fairly common tumor in Asian countries, whereas only a small number of cases have been reported in Western countries.[1–7] Epstein-Barr virus (EBV) has been proposed as the main etiologic factor in LELC of the uterine cervix in Asian patients[8] and probably plays an oncogenic role because EBV genomes have often been identified. In contrast, none of the reported cases of LELC of the uterine cervix in Western patients have been suitable for the identification of EBV genomes via in situ hybridization[3,6,9,10] or polymerase chain reaction (PCR)-based methods.[6,7,10]

Here we present a clinicopathologic and immunohistochemical study of 4 cases of LELC of the uterine cervix (one previously reported) occurring in white Western women. The results of PCR analysis of the tumor tissue were negative for EBV, simian virus 40 (SV40), and human papillomavirus (HPV) genomes, suggesting that the role of these viruses in the etiology of LELC of the uterine cervix seems unremarkable in Western patients.

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MATERIALS AND METHODS

We collected 4 cases of LELC of the uterine cervix in white Western women between 1990 and 2000. Clinical and follow-up data were available in all cases.

We performed histologic, immunohistochemical, and PCR analyses on formalin-fixed, paraffin-embedded tumor samples from surgically obtained specimens.

For histopathology, 4 μm–thick sections were stained with hematoxylin-eosin. Immunohistochemistry was performed following standard avidin-biotin immunoperoxidase procedures after antigen retrieval by autoclaving. The antibody panel used comprised primary antibodies to epithelia: cytokeratin (AE1/AE3), cytokeratin (CK) 13, CK7, carcinoembryonic antigen; to lymphoid cells: CD45RO, CD45 (leukocyte common antigen), CD3, CD4, CD8, CD20, CD56, CD57; to receptors for estrogen (clone 1D5) and progesterone (clone PgR-636); to oncoproteins: Bcl-2, c-Erb-B2 (HER-2/*neu*); and also to p53 (clone DO-7) and MIB-1. Except for the AE1/AE3 antibody from Menarini (Florence, Italy) and the CD4 and CD8 antibodies from Novocastra Laboratories Ltd (Newcastle upon Tyne, England), the rest of the antibodies used were from Dako Corporation (Glostrup, Denmark).

We analyzed genomic DNA isolated from paraffin sections by standard protocols with PCR-based methods with specific primers for detection of EBV, HPV, and SV40 DNAs.

For EBV DNA, we analyzed 2 divergent gene loci, the EBV nuclear antigen 2 and 3C loci, according to the methods of Lin

Clinicopathologic Data*							
Case No.	Age, y (Parity)†	FIGO Stage	Tumor Gross Appearance (Size, cm)	Follow-up Status	Treatment	Previous Pathology	EBV Serology
1	74 (10)	IB1 N0	Polypoid (1)	Free (7 y)	Residual cervix; surgery	Previous hysterectomy at 45 y of age (unknown reasons)	Not stated
2	58 (3)	IB1 N2	Infiltrating (2.5)	Fee (5 y)	Radical hysterectomy with bilateral lymphadenectomy	. . .	Not stated
3	77 (3)	II N0	Polypoid (2.2)	Free (2 y)	Radical hysterectomy with bilateral lymphadenectomy	. . .	EBV VCA IgG+ (at 1:512) EBV VCA IgM− Anti-EBNA+ (at 1:40)
4	67 (4)	IB1 N2	Polypoid (2)	Recurrence (at 2 y)	Radical hysterectomy with bilateral lymphadenectomy	Previous nephrectomy (tuberculosis)	EBV VCA IgG− EBV VCA IgM− Anti-EBNA−

* FIGO indicates Fédération Internationale de Gynécologie et d'Obstétrique; EBV, Epstein-Barr virus; EBNA, EBV nuclear antigen; Ig, immunoglobulin; and VCA, viral capsid antigen.
† No. of live births.

et al[11] and Sample et al.[12] Nasopharyngeal LELCs known to be positive for EBV were used as positive controls.

We undertook detection of HPV DNA following previous protocols using a pair of consensus primers, GP5+/GP6+[13] and MY09/MY11.[14] We also used an additional pair of β-globin primers to provide an internal positive control.

We screened for SV40-like DNA with primers that amplify a 574–base pair region of SV40 large T antigen, which encompasses the Rb-pocket binding domain, as previously described.[15,16] We used bone sarcomas known to be positive for SV40 as positive controls.

In 2 patients (cases 3 and 4), we also performed serologic tests for antibodies of the immunoglobulin (Ig) G and IgM classes against EBV viral capsid antigen, antibodies against early antigen nuclear antigen, and antibodies against EBV nuclear antigen.

RESULTS

Clinicopathologic data of our patients are summarized in the Table. Our patients had disease of FIGO (Fédération Internationale de Gynécologie et d'Obstétrique) stages I and II. Although one of the patients had a recurrence 2 years after the initial treatment, all of them were free of disease at the time of the study. Macroscopically, 3 of the 4 tumors were polypoid (Figure 1). Microscopically, all tumors had similar histology, showing the characteristic microscopic appearance of LELC: tumor cells growing in sheets and nests, surrounded by a dense inflammatory infiltrate that was composed mainly of small lymphocytes with occasional plasma cells. The tumor cells were undifferentiated, with scant cytoplasm and poorly defined margins. The nuclei were large, with finely granular or clear chromatin and variably prominent nucleoli. Mitotic activity in cases 1 through 4 was 10, 12, 8, and 11 mitoses per 10 high-power fields, respectively. Except in case 2, the overlying mucosa (squamous or glandular) was ulcerated and infiltrated by the tumor, but showed no evidence of koilocytosis, dysplasia, or carcinoma in situ.

Immunohistochemically, tumor cells were uniformly reactive for cytokeratin (AE1/AE3) (Figure 2). We observed strong and predominant staining (>75% of tumor cells) for CK13 in 3 cases (cases 1, 2, and 3) and for CK7 in 2 cases (cases 1 and 4). Only occasional and isolated tumor cells showed staining for carcinoembryonic antigen. Staining for progesterone receptors was negative in all cases, and only case 3 showed focal nuclear staining for estrogen receptors.

The lymphoid infiltrate was predominantly composed of cells expressing the T-cell antigens CD45RO, CD3, and CD8 (Figure 3). In contrast, staining for CD4, CD5, CD15, CD30, CD56, and CD57 was consistently negative.

All cases showed positive nuclear staining in more than 75% of the tumor cells for MIB-1 and p53 (Figure 4). Staining for oncoprotein c-Erb-B2 was focally positive (>10% of cells stained) in cases 3 and 4; staining for Bcl-2 was positive in some lymphocytes but not in tumor cells.

We performed EBV serologic tests in 2 patients; only one patient (case 3) showed a postinfection serologic profile: the results of a viral capsid antigen IgG test were positive at a titer of 1:512, and the results of an anti–EBV nuclear antigen test were positive at a titer of 1:40.

Analysis of tumor genomic DNA by PCR methods failed to show the presence of EBV (Figure 5), HPV, and SV40-like sequences in any of the 4 cases studied.

COMMENT

LELC of the uterine cervix is an infrequent neoplasm in Western women. To our knowledge, only 11 cases (ours not included) have been reported,[1–7] and although these tumors apparently occur in Western women, race has been explicitly stated for only 4 cases: 2 black[4,7] and 2 white.[1,3]

Low FIGO stage at diagnosis is the rule in LELC of the uterine cervix.[8,17] In cases previously reported in Western women, only one patient had FIGO stage IIIb disease, with a survival of 17 months.[2] The prognosis of LELC of the uterine cervix seems to be better than that of cervical squamous cell carcinoma.[8,17] In our cases, 3 patients were free of disease at a follow-up of 8 years, even though one patient had lymph node metastasis. The diagnosis of tumors in early stages and their low tendency to metastasize to lymph nodes have been related to a more favorable outcome,[17] although larger clinical studies are necessary to verify such observations.

Older patient age, higher parity, lower FIGO stage, predominant exophytic growth, and apparently good prognosis are characteristics of LELC. These characteristics were predominant in our patients. LELC of the uterine cervix generally appears in adults, without large differences in age at diagnosis between Western patients (mean age, 42.3 years; range, 21–58 years) and Asian patients (mean age, 43.5 years).[8,17,18] Our patients (mean age, 69.2 years; range, 58–77 years) are among the oldest reported.

Microscopically, sheets of undifferentiated cells with in-

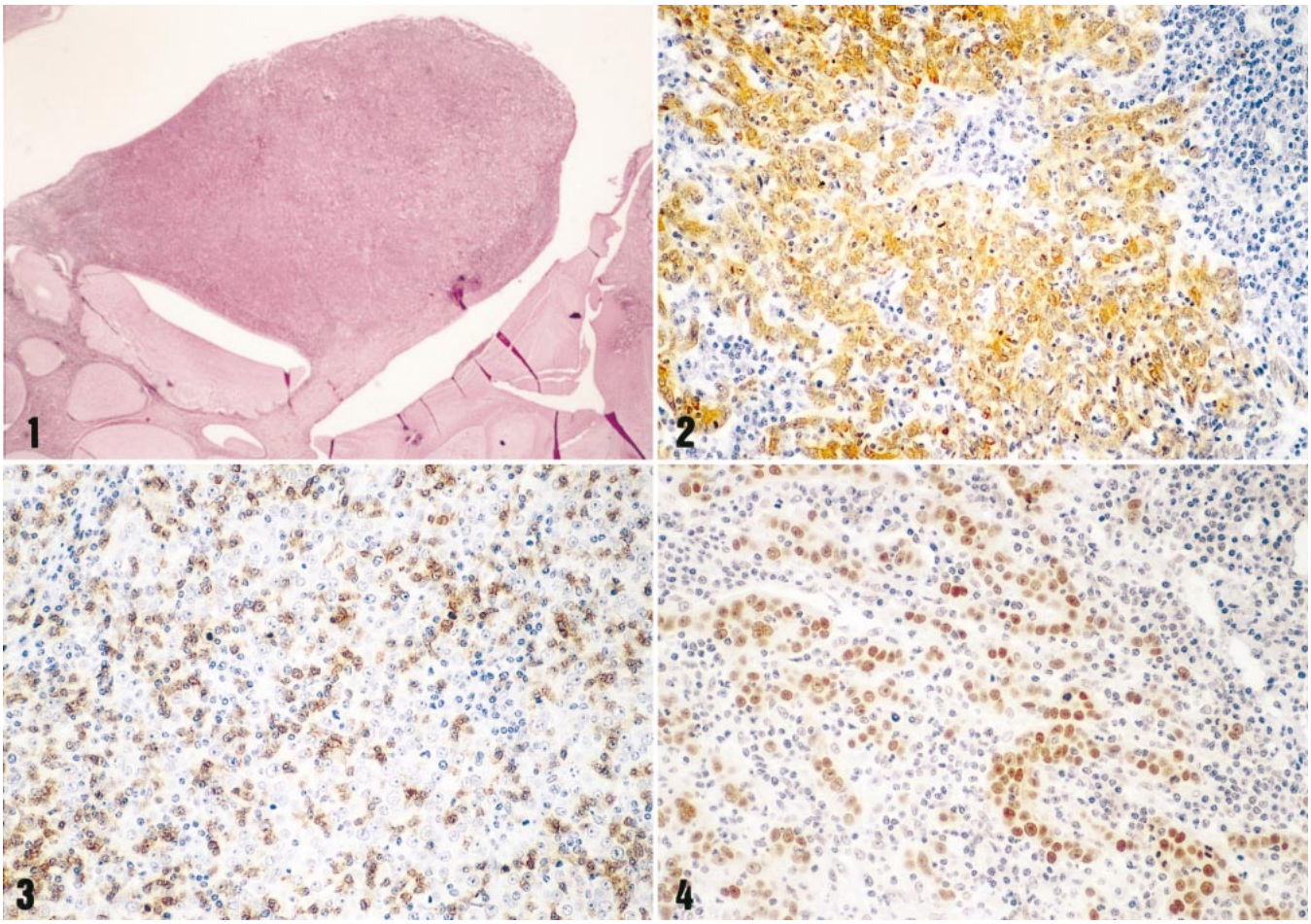

Figure 1. *A polypoid endocervical tumor, which does not deeply infiltrate the endocervical stroma (hematoxylin-eosin, original magnification ×40).*

Figure 2. *Positive cytokeratin (AE1/AE3) staining of the cytoplasm of tumor cells (immunoperoxidase, original magnification ×100).*

Figure 3. *The cell membranes of most lymphoid cells stain for CD8 (immunoperoxidase, original magnification ×100).*

Figure 4. *Positive p53 nuclear staining in tumor cells (immunoperoxidase, original magnification ×100).*

distinct cellular borders growing in a dense lymphoid stroma provide the clue in the differential diagnosis of LELC from poorly differentiated squamous cell carcinoma and from glassy cell carcinoma of the uterine cervix with a prominent lymphoid stroma.

Immunohistochemically, all tumor cells in the present study were positive for cytokeratin (AE1/AE3). At the same time, the vast majority of tumor cells expressed CK13 and to a lesser degree CK7,[19] and were only focally positive for carcinoembryonic antigen. Reactions for estrogen and progesterone receptors, Bcl-2, and c-Erb-B2 were negative or only focally positive, although we found strong immunohistochemical reactivity to p53 and MIB-1. These results differ from those of the only reported case of LELC of the uterine cervix in which immunohistochemistry was used to study expression of c-Erb-B2 and p53[5]; cells in that study were negative and nonreactive for both of these antigens. Immunohistochemical overexpression of p53 protein and strong reactivity to antibody MIB-1 have been reported in LELC of the vagina[20] and larynx.[21] Immunohistochemical p53 overexpression is generally observed in undifferentiated nasopharyngeal carcinoma,[22] in the absence of *p53* gene mutations,[23] with an inverse immunohistochemical correlation between the expression of p53 and Bcl-2 proteins.[24]

The immunohistochemical profile of our cases is suggestive of an aggressive neoplasm: we found positive immunostaining for proliferation marker MIB-1 and tumor suppressor gene p53 protein (which is expected to be positive in high-grade tumors) and negative immunostaining for Bcl-2 and for both estrogen and progesterone receptors (which are most frequently present in well-differentiated tumors). This apparent immunohistochemical aggressiveness contrasts with the longer survival of patients, although it may be partly explained by the low FIGO stages found in our patients. Similar discordant results between immunohistochemical markers and survival have been noted in familial medullary breast cancer related to the *BRCA1* gene.[25]

Marked peritumoral and intratumoral lymphocytic infiltration is a histologic feature of LELC of the uterine cervix. As noted in our tumors, a predominant lymphoid T-cell population (CD45RO, CD3) has been observed in LELC of the cervix,[26] vagina,[20] stomach,[27] and lung[28]; practically all lymphoid T cells were suppressor/cytotoxic CD8 cells. We did not study immunohistochemical reac-

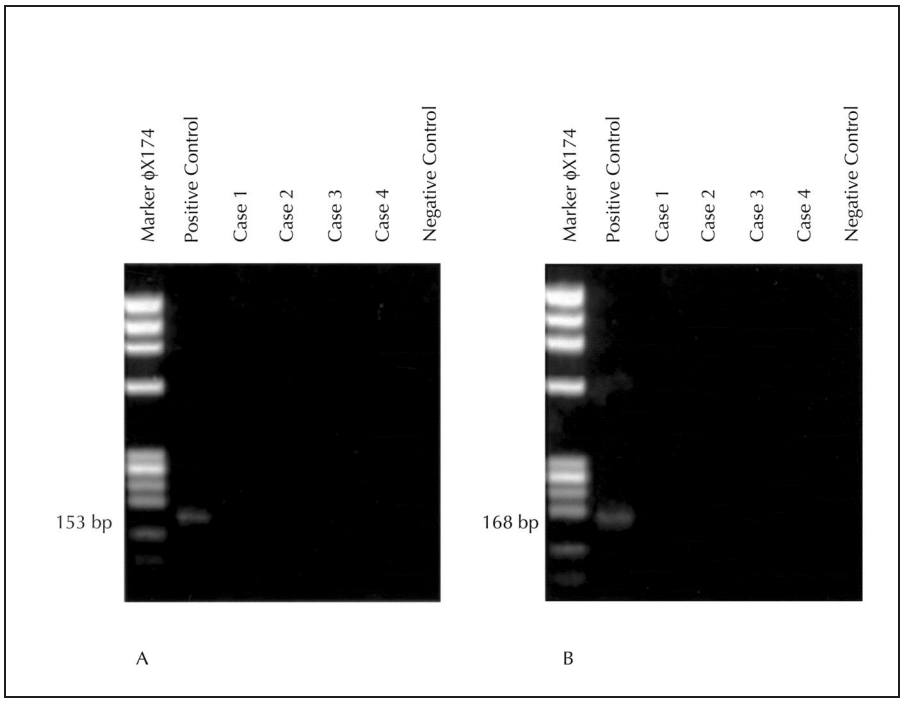

Figure 5. *Agarose gel of Epstein-Barr virus (EBV) polymerase chain reaction (PCR) products. A, PCR with primers EB1 and EB2 for EBV nuclear antigen 2 sequences. B, PCR with primers EB3 and EB4 for EBV nuclear antigen 3C sequences. Amplified fragments of 153 and 168 base pair (bp), respectively (arrows).*

tivity for T-cell–restricted intracellular antigen (TIA-1) or granzyme B, which defines a subset of cytotoxic CD8 T cells in either active or resting state. An increased number of intratumoral lymphoid CD8 T cells may inhibit lymph node or hematogenous metastasis of tumor cells infected by EBV, probably resulting in a better prognosis.[27,28] Cytotoxic CD8 T lymphocytes have also been identified in the lymphoid stroma of certain non–EBV-associated neoplasms, such as colorectal cancer[29] and medullary breast carcinoma[30]; their presence is considered an active mechanism in the host versus tumor response, leading to an improved prognosis.

Epstein-Barr virus has been implicated in the etiology of squamous cell carcinoma and LELC from diverse anatomic locations in both Asian and Western patients.[31] Studies of LELC of the uterine cervix[2,6,7,9,10,26,32] in Western patients by PCR or in situ hybridization have consistently shown an absence of the EBV genome, including one patient with serologic tests revealing a previous mild or chronic infection by EBV.[7] Thus, we found no evidence of any etiologic relationship between LELC of the uterine cervix and EBV, reinforcing the hypothesis that LELC of the uterine cervix in Western patients is not related to EBV and suggesting that the presence of this virus is merely related to a racial or geographic influence.[33]

A study of the presence of HPV in Taiwanese patients with LELC of the uterine cervix showed that HPV-16 could be detected in 20% (3 of 15) of the patients studied.[8] Other authors[34] found HPV-16 and -18 in 2 cases of LELC of the cervix in European women. In our cases, we were unable to detect the presence of HPV by PCR with 2 widely used pairs of consensus primers, GP5+/GP6+[11] and MY09/MY11.[12] Previous studies of Western patients have also failed to find HPV by PCR[7] and by in situ hybridization.[26]

DNA sequences similar to those of SV40 have been found in certain human tumors.[15,16,35] SV40 early region sequences have also been detected in sperm fluids from healthy individuals,[36] suggesting that sexual transmission may contribute to the spread of the virus. In the context of an ongoing study wherein SV40-like sequences are being studied in bone sarcomas,[37] we included DNA from our 4 cases with LELC and from 5 additional cases of squamous cell carcinoma of the uterine cervix; however, we again failed to show the presence of SV40-like sequences.

In summary, and according to our negative results, it appears that the presence of EBV, HPV, or SV40 does not play any etiologic role in LELC of the uterine cervix in Western patients.

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