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CASE REPORT

EBV$^+$ Lymphoepithelial Carcinoma of the Parotid Gland in Mexican Mestizo Patients with Chronic Autoimmune Diseases

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Lymphoepithelial carcinomas of the salivary gland are rare tumors constantly associated with Epstein-Barr virus (EBV) and mainly identified in Asiatic and Greenlander population. Four cases have been described in Caucasians, only two with EBV infection. We describe two cases of parotid gland lymphoepithelial carcinomas in Mexican mestizo women in which chronic latent EBV infection was documented by immunohistochemistry and *in situ* hybridization. One patient had primary Sjögren's syndrome and the other systemic lupus erythematosus of six and three years of evolution, respectively. Epithelial neoplastic cells showed latency pattern II (LMP1$^+$, EBNA-2$^-$, EBER$^+$) with a dense inflammatory infiltrate composed mainly by CD8$^+$ T lymphocytes. Follow-up excluded nasopharyngeal involvement in both patients. This report expands the ethnic groups in which salivary lymphoepithelial carcinomas associated with chronic latent EBV infection have been described, and illustrates for the first time its association with autoimmune diseases in two women living in a region non-endemic for this unusual neoplasm. (Pathology Oncology Research Vol 12, No 1, 41–45)

Key words: lymphoepithelial carcinoma, Epstein-Barr virus, autoimmune disease, salivary glands

Introduction

Lymphoepithelial carcinoma (LEC) constitutes approximately 0.4% of malignant salivary gland tumors, it is frequently associated with Epstein-Barr virus (EBV) infection, and most of the cases have been identified in Alaskan Eskimos/Inuits and in southeastern Chinese populations.[2,5-7,10,11,15,16] An oncogene of EBV, latent membrane protein 1 (LMP1), exhibiting a 30-bp deletion in the C-terminus of the LMP1 gene identical to that observed in nasopharyngeal carcinomas (NPC), suggests a common carcinogenic pathway between salivary gland LECs and NPCs.[9]

Four cases of salivary gland LECs have been reported in non-Eskimo/Greenlander Caucasian patients with parotid tumors, two without evidence of EBV infection,[6] and two with EBV-positive tumors lacking LMP1 expression from Greece[9] and Poland.[3] As far as we know, no case of salivary LECs associated with EBV infection has been reported in Latin America or in Mexican mestizo patients (*Table 1*).

Autoimmune damage or cytotoxic/immunosuppressive therapy administered to patients with autoimmune diseases has been associated with malignancy. A three-fold increase in non-Hodgkin's lymphoma (NHL) has been observed in a population-based cohort of systemic lupus erythematosus (SLE) in Sweden,[4] but no solid tumor is clearly associated to SLE, and when it occurs it is probably by chance.[1] Sjögren's syndrome (SS) has also been related with NHL but its association with salivary gland tumors has not been demonstrated.[1,17]

The impact of EBV infection in autoimmune settings is unknown, and even though the immune system of these patients appears to be capable of containing EBV infection,[13] the blockade of a ubiquitin-dependent intracellular processing and presentation pathway of target viral antigens to CD8$^+$ cells by Epstein-Barr nuclear antigen protein-1 (EBNA-1), could be responsible for growth advantage.[12] To the authors' knowledge, there are no reports of salivary gland LECs associated with autoimmune diseases.

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Table 1. **Salivary gland lymphoepithelial carcinoma and its association with EBV infection**

			EBV study		
			Immunohistochemistry		*ISH*
	Race	*No. of cases*	*LMP1*	*EBNA2*	*EBER*
Hamilton-Dutoit et al. 1991[6]	Eskimos	11	NA	NA	+
	Caucasian Dane, Faroese	2	NA	NA	–
Chan et al. 1994[5]	Chinese	2	NA	NA	+
Leung et al. 1995[11]	Chinese	8	+	NA	+
Tsai et al. 1996[16]	Chinese	6	NA	NA	+
Kotsianti et al. 1996[9]	Caucasian Greek	1	–	NA	+
Kuo et al. 1997[10]	Chinese	9	NA	NA	+
Bialas, et al. 2002[3]	Caucasian Polish	1	–	NA	+
Saku, et al. 2003[15]	Russia-Asia area	143	NA	NA	+
Present study	Mexican	2	+	–	+

LMP1 (latent membrane protein 1), EBNA2 (Epstein-Barr nuclear antigen 2), EBER (Epstein-Barr encoded RNA), ISH (in situ hybridization), NA (not available)

Materials and Methods

Patient 1. A 56-year-old woman with primary Sjögren's syndrome since 1987 developed a 2-cm, hard, painless mass on her left parotid gland in 1993. She was under steroid therapy, and a year later she noted progressive growth and a 4 cm lesion attached to mandibular structures. A fine-needle aspiration biopsy (FNAB) disclosed a biphasic neoplasm with large epithelial syncytial cells and lymphocytes (*Figure 1*). A CT-scan demonstrated solid left parotid gland enlargement. No abnormalities were found in nasopharynx or paranasal sinuses. Surgical excision of the left parotid gland and adjacent nodes showed a white-gray multilobulated 2.5 cm tumor, and the patient was submitted to radiotherapy in 1996. After a 9-year follow-up she developed lymphocytic thyroiditis with hypothyroidism and diabetes mellitus but no local recurrences or metastases.

Patient 2. A 28-year-old woman with a three-year history of SLE irregularly treated with prednisone (12.5 mg/day), chloroquine (1x1), methotrexate (4xweek) and thalidomide (1x1). She was admitted with sixth and seventh cranial nerve palsy as well as a painless mass on her left parotid gland attached to the soft tissues. The CT-scan showed a 15 cm tumor with defined borders, affecting the deep parotid lobule. MR imaging documented involvement of the superficial parotid lobule as well as the deep muscles and lymph nodes. The nasopharynx and paranasal sinuses were normal, and a FNAB depicted a poorly differentiated carcinoma (*Figure 1*). An incisional biopsy was performed. The patient was treated with three cycles of chemotherapy (5-fluorouracil, cisplatin, adriamycin), and a 70% reduction of tumor volume was observed, but multiple lung metastases were identified and five additional cycles of radiotherapy were administered. Eight months before her last appointment she was admitted with fever, trigeminal and conjunctive herpes, requiring intravenous acyclovir. She had active SLE, parotid and lung tumor activity, and died after 18 months of follow-up.

Immunohistochemistry and in situ hybridization

Immunohistochemical analysis using the ABC complex method was performed against LMP1 (1:50), EBNA-2 (1:50), cytokeratin (CK) 7 (1:50), CK-20 (1:50), EMA (1:100), CD3 (1:75), CD4 (pre diluted), CD8 (1:50), CD20 (1:100), and CD68 (1:50). All antibodies were obtained from DAKO (Glostrup, Denmark), except for anti-CD4 (Ventana Medical Systems, Tucson, AZ), and staining was performed in NexES automated system (Ventana). Procedures were performed using positive control and negative control was run omitting primary antibody.

In situ hybridization for EBER1/2 RNA was performed on 6-mm-thick formalin-fixed tissue using PNA ISH Detection Kit and FITC-conjugated Epstein-Barr virus (EBER) PNA probe (DAKO). Briefly, slides were deparaffinized in sequential baths of xylene and ethanol, then digested with proteinase K for 30 minutes, rinsed in pure water and air dried after a rapid immersion in 96% ethanol. Once dried, 1 or 2 drops of EBER PNA probe was applied, and slides were incubated for 1.5 h at 55°C with plastic coverslips. Slides were immersed in stringent wash solution for 25 min and incubated with anti-FITC/AP for 30 min at 37°C. Slides were washed with TBS and pure water and then incubated with color devel-

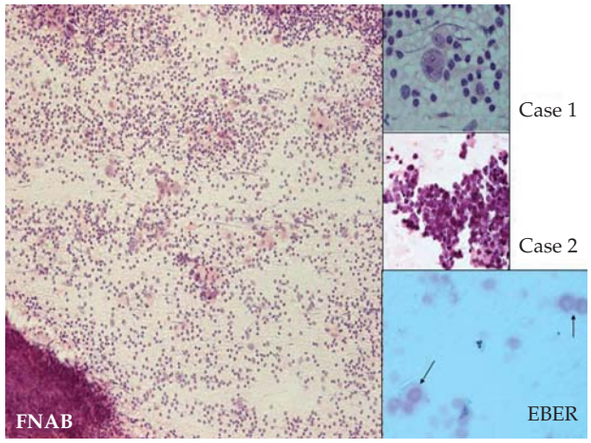

Figure 1. *Fine-needle aspiration biopsy with scattered epithelial cells admixed with mature lymphocytes. Case 1: non-cohesive epithelial cells with larger nuclei and prominent nucleoli. Case 2: cohesive homogeneous groups of neoplastic cells. Both aspirates showed nuclear EBER signals in the epithelial component by in situ hybridization (arrows).*

oping reagent for 60 min. Slides were washed with tap water, counterstained with red (FNAB) and light green, air-dried and mounted with Immuno-mount (Shandon, Pittsburgh, PA).

Results

Fine-needle aspiration biopsy showed scant epithelial cells surrounded by mature lymphocytes (*Figure 1*). Both tumors were composed of epithelial neoplastic cells with large vesicular nuclei and eosinophilic cytoplasm forming trabeculae, sheets and anastomosing islands surrounded by lymphocytes, histiocytes and plasma cells (*Figure 2*). In the case associated with SS, abundant epithelioid histiocytes in a granulomatous pattern were observed, and in that with SLE a comedo-like necrosis was found focally. There was no evidence of squamous or glandular differentiation (*Figure 2*).

The epithelial origin of the neoplastic cells was demonstrated by its diffuse expression of epithelial membrane antigen (*Figure 3*), but no staining for CK-7/CK-20 was observed. The inflammatory infiltrate was composed of mature T and B cells positive for CD3, CD20, CD4 and CD8. CD8-positive T cells alternated with the epithelial neoplastic cells, while the CD4-positive T cells surrounded them. Scattered groups of histiocytes expressing CD68 were also observed (*Figure 3*).

More than 90% of the nuclei of neoplastic cells showed strong EBER-1/2 signal in both tumors, without staining the inflammatory infiltrate or residual salivary tissue (*Figure 2*). LMP1 was expressed in more than 70% of epithelial neoplastic cells in both patients (*Figure* 2), but no signal was observed using EBNA-2 antibody (not shown).

Discussion

Salivary LECs have been described mainly in Eskimo and Asiatic populations and only in four Caucasians, two of such tumors were associated with chronic EBV infection (*Table 1*). In addition to the occurrence in Mexican mestizo patients, a new ethnic group in which these tumors are recognized, the carcinomas here described were affect-

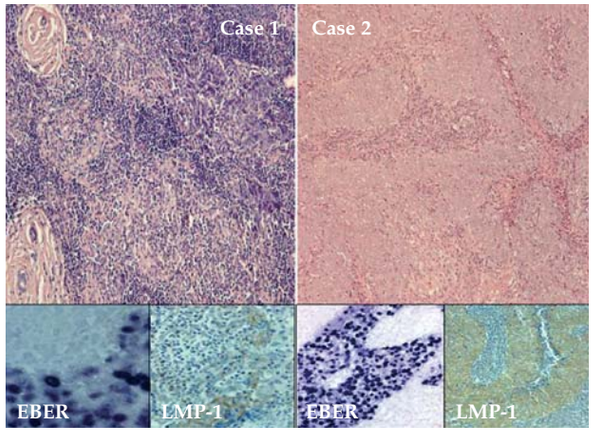

Figure 2. *Parotid lymphoepithelial carcinoma showing epithelial neoplastic cells surrounded by inflammatory infiltrate. Residual salivary ducts can be observed in case 1. A membranous expression of latent membrane protein (LMP1) and strong nuclear EBER signals can be observed only in epithelial neoplastic cells, and confirm the presence of EBV in both tumors.*

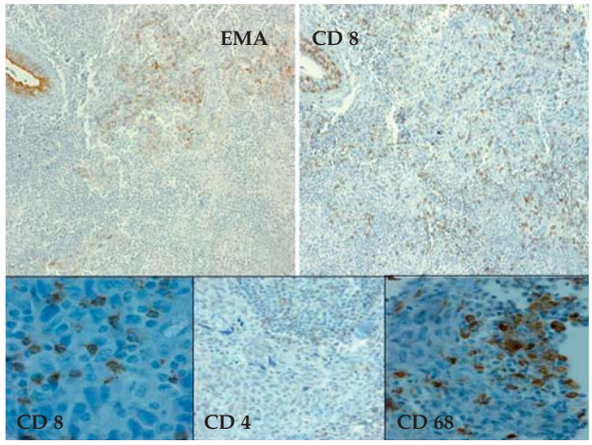

Figure 3. *Inflammatory infiltrate in salivary lymphoepithelial carcinoma. Most of the lymphocytes were cytotoxic CD8⁺ T cells in close contact with epithelial neoplastic cells and damaging residual salivary ducts. Lymphocytes surrounding groups of neoplastic cells were CD4⁺. Abundant CD68⁺ histiocytes were observed in the inflammatory background and between neoplastic cells. Epithelial origin of the neoplastic cells is demonstrated with the strong epithelial membrane antigen (EMA) expression.*

ing two women with autoimmune disorders of six or three years of evolution under immunosuppressive treatment, respectively. Chronic immunosuppression as a result of autoimmune diseases or medication could have deleterious effects on the cytotoxic T-lymphocyte control of EBV infection, as it is well recognized in post-transplant lymphoproliferations.[13] The contribution of the immune status, age and ethnicity appear to be important variables in the evolution of chronic EBV infection. In Mexico, latent EBV infection has been frequently observed in patients with hematological neoplasms,[14] probably reflecting an early, chronic and prevalent infection.

Latent infection with a type II pattern was recognized in the epithelial neoplastic cells in these salivary lesions; the patchy expression of LMP1, demonstrated immunohistochemically in the presented cases, is an evidence of multimeric aggregates of latent membrane protein-1 in the plasma membrane, and the small non-polyadenylated RNAs, abundantly detected in more than 90% of the nuclei, are confirmatory. As in nasopharyngeal carcinomas, where EBV nuclear protein-1 is usually not detected, these salivary tumors were also negative. The follow-up did not show nasopharyngeal involvement, strongly supporting parotid glands as the primary origin. This finding is of paramount importance due to the similar morphology between the tumors in both locations; even the reactive inflammatory infiltrate was similar showing mature T and B lymphocytes with the $CD4^+$ T cells surrounding groups of epithelial neoplastic cells admixed with $CD8^+$ T lymphocytes (*Figure 3*). The close contact of epithelial cells and $CD8^+$ T lymphocytes in both salivary LECs suggests the presence of EBV-specific cytotoxic T-lymphocyte response. This presumably cytotoxic response might be enhanced in salivary LECs, as has been reported, with important clinical impact in the management of nasopharyngeal tumors.[12]

There is no clear biological explanation for the higher risk of developing solid cancer in the setting of autoimmune diseases, however, a nearly two-fold increase in the incidence of lung cancer and epidermoid carcinoma was observed in a large cohort of SLE patients followed for more than 30 years,[4] and an increased risk for developing solid tumors among five rheumatic diseases was found in a previous report from the institution where these patients were admitted.[17] Some new light was recently added to the field with the finding of a significant increase in IFN-gamma production by EBV-specific $CD69^+/CD4^+$ T cells, and a decrease in its production by $CD69^+/CD8^+$ T cells in patients with systemic lupus erythematosus.[8] The IFN-gamma concentration changes in SLE patients were associated with a 40-fold increase in viral loads when compared with controls. These changes were not explained by disease activity or immunosuppressive medication, but depict alterations in $CD4^+$ T cells in controlling and probably in $CD8^+$ T cells in regulating viral replication in SLE patients.[8]

Residual salivary tissue showed focal atrophy and hyperplasia of ductal epithelial cells in both cases. Chronic inflammatory infiltrate with abundant histiocytes were observed in the patient with primary Sjögren´s syndrome. None of the cases had dysplastic changes or LMP1/EBER expression in the residual acini/ductal system of salivary glands. Previous reports of salivary LECs have not called attention to the presence of preneoplastic changes in the remnant tissue.[2,5-7,10,11,15,16] The absence of such changes contrasts with the frequent morphological sequence of hyperplasia, dysplasia and invasive neoplasia, observed in nasopharyngeal carcinomas.[13] As far as the authors know, the presence of EBV in the neoplastic cells does not have significance in prognosis, but a practical use of this finding could be in the study of metastatic undifferentiated non-keratinizing carcinoma in supraclavicular lymph nodes: a primary tumor in the nasopharynx, salivary glands or stomach should be suspected, but not in the breast, colon or uterine cervix where this phenotype could also be found but usually not associated with EBV.

In summary, this article expands the ethnic groups in which salivary LECs associated with chronic latent EBV infection have been described, and illustrates for the first time its association with autoimmune diseases in two women living in a region non-endemic for lymphoepithelial carcinoma.

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