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Case report

Immunophenotypic analysis of tumor infiltrating lymphocytes in Epstein-Barr virus-negative lymphoepithelial carcinoma of the oral cavity: Report of a case

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ABSTRACT

Lymphoepithelial carcinoma (LEC) is a rare subtype of undifferentiated carcinoma with prominent lymphoid stroma consistently associated with Epstein-Barr virus (EBV) in Asian populations. We encountered a case of LEC of the floor of the mouth. In the present LEC, the EBV genome was not detected in tumor cells or tumor-infiltrating lymphocytes (TILs) by in situ hybridization. The invasive pattern of lymphoid infiltration in the present LEC was strongly characteristic. The TILs in this LEC were predominantly $CD8^+$ and $CD45RO^+$ T cells that invaded into and around tumor nests and islands. Moderate numbers of $PD\text{-}1^+$ and $FoxP3^+$ cells were also dispersed both within and between the tumor nests and islands. A profuse infiltrate of $CD4^+$ T cells as well as some $CD45RO^+$ T cells and a very few $CD20^+$ B cells was mostly restricted to the tumor stroma. Membranous expression of PD-L1 on the tumor cells was not found in the entire lesion of the present LEC. These findings suggest that TILs in the present EBV-negative LEC might behave as tumor-suppressive immune cells in response to the invasion and proliferation of LEC.

Introduction

LEC is a tumor with morphological characteristics identical to those of undifferentiated nasopharyngeal carcinoma characterized by accumulation and infiltration of non-neoplastic lymphocytic monocytes and/or macrophages [1,2]. In situ hybridization for Epstein-Barr encoded RNA (EBER) is positive in the vast majority of cases in the oral cavity [3]. Local control can be achieved for LEC of the oral cavity in a high percentage of cases even in the presence of regional lymph node metastasis [1]. These favorable clinical characteristics of LEC led to us to hypothesize the presence of a local host immune response to the host's own cancer cell antigens or EBV-related antigens. Although infiltrating lymphocyte subsets have been analyzed by immunohistochemistry in EBV-positive gastric and nasopharyngeal cancers [5–8], little is known about the distinctive morphological features and immunophenotypic differences of LEC of the oral cavity.

In the present study, a panel of monoclonal antibodies was used to determine the distinctive immunophenotypic features of tumor-infiltrating lymphocytes (TILs) in order to clarify the presence or absence of host immune responses to tumor-specific or −related antigens possibly shared on the EBV-negative tumor cell surface.

Case report

An 82-year-old Japanese man was referred to the Oral Surgery Outpatient Clinic of Sapporo Medical University Hospital for hemostasis of bleeding from a tumor of the floor of the month. For 3 months prior to presentation, he had been experiencing repeated bleeding and hemostasis within the tumor. He had a past medical history of cerebral infarction (paresis of his right side), carotid artery stenosis (carotid artery stenting), thoracic aortic aneurysm, and old myocardial infarction, which had occurred as 4 separate incidents about 22 years earlier. Physical examination revealed the presence of an ulcerative 60 × 45 mm indurative swelling in the floor of the mouth that had invaded the tongue

☆ AsianAOMS: Asian Association of Oral and Maxillofacial Surgeons; ASOMP: Asian Society of Oral and Maxillofacial Pathology; JSOP: Japanese Society of Oral Pathology; JSOMS: Japanese Society of Oral and Maxillofacial Surgeons; JSOM: Japanese Society of Oral Medicine; JAMI: Japanese Academy of Maxillofacial Implants.

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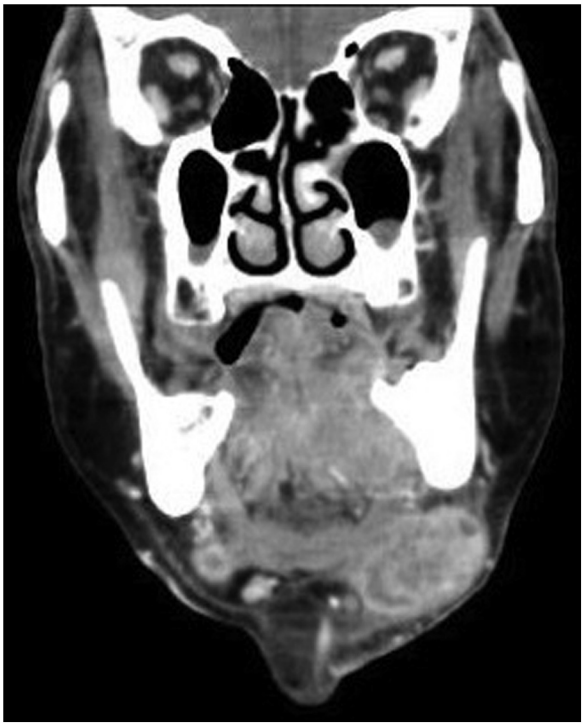

Fig. 1. Frontal view of a CT scan showing the tumor occupying the floor of the mouth and extending to the tongue and mandible, with remarkable enlargement of bilateral metastatic lymph nodes.

and mandible and bilateral submandibular lymphadenopathy with sizes of 45 × 35 mm in the left neck and 35 × 20 mm in the right neck. A clinical diagnosis of a malignant oral tumor with bilateral regional lymph node metastases (T4aN2cMx by UICC/AJCC staging system) was made. Laboratory examination results were within normal limits except for slight anemia and a low level of serum albumin. A CT scan showed a mass in the floor of the mouth extending to the tongue with mandibular involvement (Fig. 1). Pathologic enlargement of bilateral lymph nodes was also demonstrated. An FDG-PET/CT scan disclosed no PET-positive distant metastasis or simultaneous second or third malignant tumor (cT4aN2cM0 disease). Based on these imaging features, the clinical diagnosis of a primary malignant tumor of the floor of the mouth was confirmed. An incisional biopsy of the tumor in the floor of the mouth was performed and the histopathlogic findings were compatible with LEC (Fig. 2A). Tumor cells were characterized by nests and islands of undifferentiated polygonal cells with indistinct borders and eosinophilic cytoplasm, large vesicular nuclei, and single to multiple prominent nucleoli. Several mitotic figures were also seen and there were ulcerative changes. Tumor nests were surrounded and invaded by a lymphoid infiltrate comprised predominantly of small lymphocytes. In situ hybridization was negative for EBV-encoded RNAs (EBERs) in both the tumor cells and stromal mononuclear infiltrate (Fig. 2B). Radiotherapy was suggested; however, the patient and his family requested palliative therapy for his cancer as well as for the other above-mentioned complicated diseases. The patient was treated with an HLA-A24-restricted survivin 2B peptide vaccine with interferon-α, and a stable state was achieved for the tumor for 6 months, but progressive lesions were observed from the 7th month and he died after 12 months.

Immunohistochemistry

Formalin-fixed and paraffin-embedded biopsied tissue blocks were cut into serial 4 μm slices with a microtome for immunohistochemical processing. Immunohistochemical analysis using the ENVISION$^+$ system (Dako, Japan) was performed for lymphocytes and tumor antigens as shown in Table 1. TILs in the immunohistochemical investigation were defined as lymphocytes infiltrated either in tumor parenchyma or the stroma surrounded the tumor nests. Procedures performed using positive and negative controls were run omitting the primary antibodies.

The results for immunoreactivity of each antibody are shown in Table 1. The TILs were composed of mature T and B cells positive for CD4$^+$, CD8$^+$ cytotoxic cells, CD45RO$^+$ memory cells, FoxP3$^+$ regulatory T cells, CD20$^+$ B cells, and PD-1$^+$ activated lymphocytes (Fig. 3). The TILs in this LEC were predominantly CD8$^+$, CD4$^+$, and CD45RO$^+$ T cells. CD8$^+$ T cells alternated with epithelial neoplastic cells, while the CD4$^+$ T cells almost surrounded the tumor nests. The CD45RO$^+$ and PD-1+ cells infiltrated both within and between the tumor nests and islands. Cells with nuclear expression of FoxP3 also infiltrated both within and between the tumor nests and islands. Therefore, although the lymphocytes infiltrating the tumor stroma were almost exclusively positive for CD4$^+$, very few CD20$^+$ B cells were present around tumors nests. Among the T cell population, there were more CD8$^+$ T cells than CD4$^+$ and/or CD45RO$^+$ T cells. On the other hand, some cells with membranous expression of PD-1 were also present, though widely scattered with indistinct localization like the CD8$^+$ cells, but surface expression of PD-L1 on the

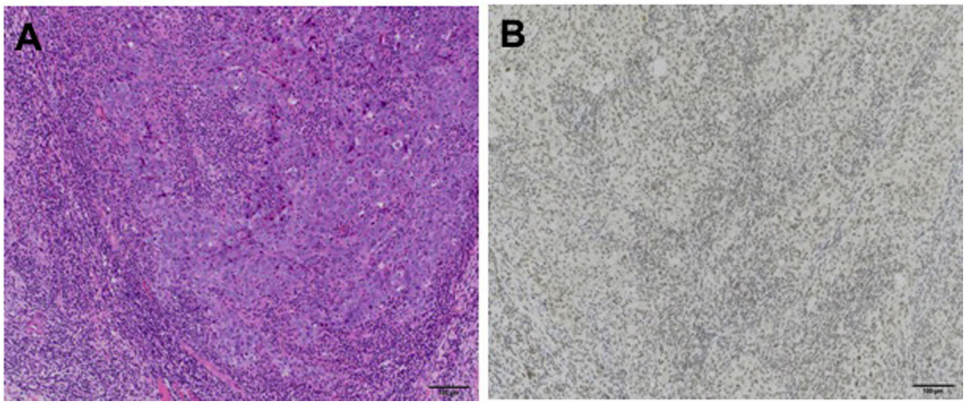

Fig. 2. Microscopic view of biopsied specimen. Tumor cells were characterized by nests and islands of undifferentiated polygonal cells with indistinct borders and eosinophilic cytoplasm, large vesicular nuclei, and single to multiple prominent nucleoli. Several mitotic figures are also seen. A substantial number of lymphocytes also infiltrate the tumor cell nests (Fig. 2. A, H&E stain, ×100). In situ hybridization was negative for EBV-encoded RNAs in both tumor cells and stromal mononuclear infiltrates (Fig. 2. B, ×100).

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