

## Brief Report

DEVELOPMENT OF KAPOSI'S SARCOMA  
IN A SURGICAL WOUND

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**K**APOSI'S sarcoma is the most common neoplasm in patients with the acquired immunodeficiency syndrome (AIDS) and is diagnostic of the progression of human immunodeficiency virus (HIV) infection to AIDS.<sup>1</sup> The lesion usually appears first on the skin but may also involve visceral organs. Kaposi's sarcoma-associated herpesvirus, also called human herpesvirus 8 (HHV-8), is linked to the development of Kaposi's sarcoma.<sup>2</sup> The pathogenesis of Kaposi's sarcoma in patients with AIDS is unclear, but certain cytokines and growth factors play a part.<sup>3</sup>

In this report, I describe the development of Kaposi's sarcoma of the labial oral mucosa after minor oral surgery in an HIV-infected patient. The tumor was localized to a site of previous iatrogenic trauma, a finding suggestive of Koebner's phenomenon.<sup>4</sup>

## CASE REPORT

A 38-year-old man with HIV infection (CD4 cell count, 621 per cubic millimeter; viral load, 189,000 copies per milliliter) underwent evaluation at the dental clinic for salivary-gland disease, including biopsy of labial minor salivary glands. On previous visits to the dental clinic, he had been found to have xerostomia and minimal-to-moderate enlargement of both parotid glands — features of HIV-associated salivary-gland disease. The patient's medical history included systemic lupus erythematosus, hypertension, hepatitis C, cholelithiasis, hepatomegaly, anal condyloma, and arthritis. Intolerance of various antiretroviral medications made control of the HIV infection difficult. He had no known drug allergies and was taking hydroxychloroquine, prochlorperazine, triamterene-hydrochlorothiazide, ramipril, and gabapentin and using a fentanyl transdermal patch. He had smoked one to two packs of cigarettes a day for 25 years and had used injection drugs 20 years earlier.

After the patient had given informed consent, a throat washing with phosphate-buffered saline solution was obtained, a sample of venous blood was collected, and biopsy of minor salivary glands was performed. These procedures were well tolerated. Approximately six days after the biopsy, the patient returned because of a vascular fungating mass, 3 cm by 3 cm, at the site of the incision (Fig. 1A). The lower lip was swollen and painful, and there was discharge from the site. An infected hematoma was suspected. The patient was given a seven-day course of antibiotics and was asked to

return in one week. On his return, the lesion was much larger and pedunculated. Pain around the lower lip radiated into the jaw and down the neck. There was bilateral, shotty cervical adenopathy. The lesion was excised, and the wound sutured. Examination of the biopsy specimen showed a proliferation of atypical spindle cells, which formed vascular channels. These findings were highly suggestive of Kaposi's sarcoma, but an organizing hematoma could not be ruled out.

Within six days, the oral lesion had recurred, causing the surgical wound to dehisce. A new lesion on the chin was continuous with the oral lesion. The chin lesion was dome-shaped and crusted. A biopsy specimen revealed ulceration, with multiple multinucleated cells and a cytopathic effect consistent with a herpesvirus infection. The lesion continued to grow, the pain and swelling intensified, and bleeding required cautery. A small, brown-to-purplish lesion that was irregularly shaped and slightly raised appeared subsequently on the scrotum; biopsy was not performed. The patient received radiation therapy to the lower lip and chin with local electron fields at a dose of 40 Gy delivered in two fractions. The lesion resolved within six weeks after the initiation of radiation therapy (Fig. 1B). The scrotal lesion also resolved. Over the next two years, there were no additional lesions.

## METHODS

## Preparation of Specimens

Biopsy specimens were immediately frozen and stored at  $-80^{\circ}\text{C}$ . A portion of each specimen was fixed in formalin and prepared for staining with hematoxylin and eosin and light-microscopical examination. Lymphocytes were separated from peripheral blood through a Ficoll step gradient. Throat washings were obtained by having the patient gargle with 10 ml of sterile phosphate-buffered saline, and pellets were collected by centrifugation.

## Nucleic Acid Isolation

Frozen tissue was pulverized and dissolved in 4 M guanidine isothiocyanate, and DNA was isolated through a cesium chloride step gradient as previously described.<sup>5</sup> Total DNA was obtained from purified lymphocytes and throat-washing pellets after proteinase K digestion, phenol-chloroform extraction, and ethanol precipitation.

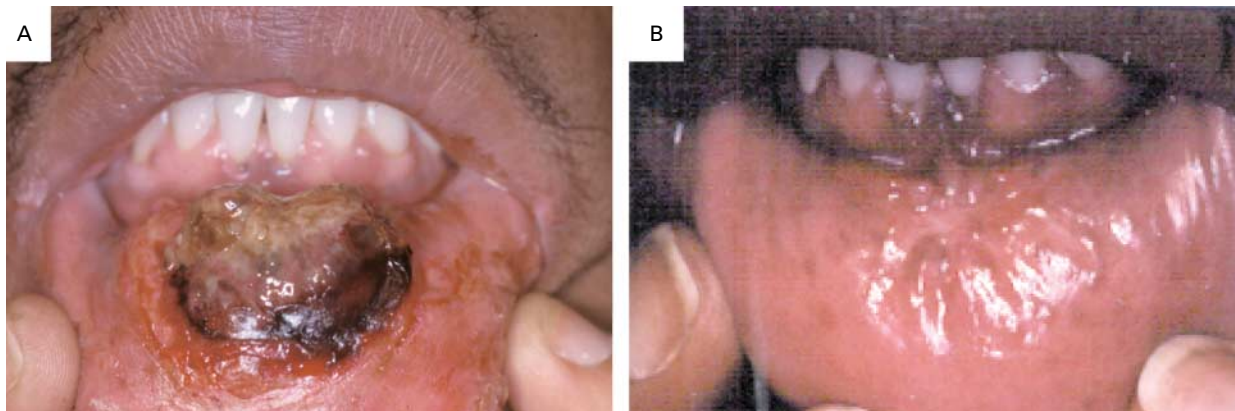
## Polymerase-Chain-Reaction Assay

Single-stranded DNA oligonucleotides were synthesized for use as polymerase-chain-reaction (PCR) primers. Amplified sequences included KS330 Bam<sub>233</sub>, which represents the HHV-8 minor capsid sequence. PCR products were separated by electrophoresis on a 1.5 percent agarose gel.

## Immunocytochemical Studies

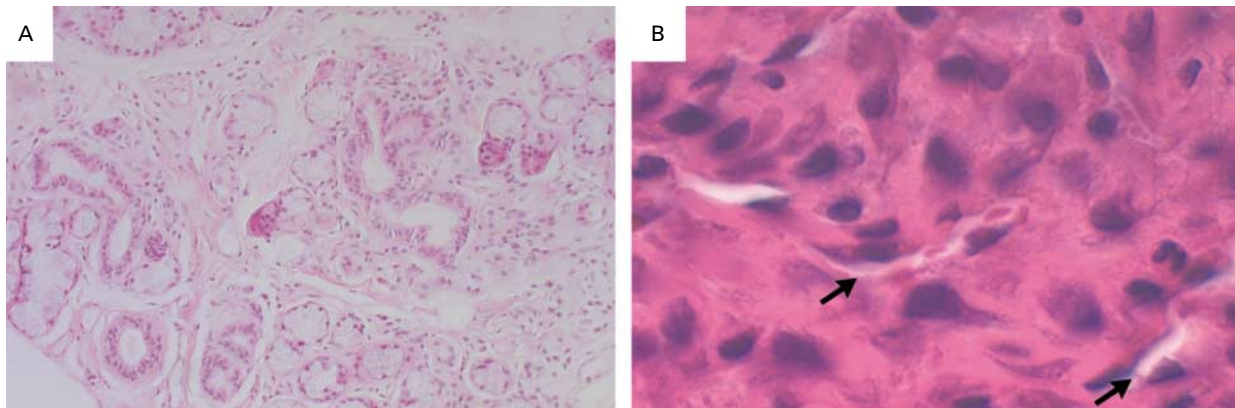
Biopsy specimens were placed in neutral buffered formalin, embedded in paraffin, and sectioned serially at a thickness of 4  $\mu\text{m}$ . Frozen sections were fixed in methanol and acetone. Formalin-fixed tissue sections were adhered to poly-L-lysine-coated slides, deparaffinized, and washed with phosphate-buffered saline. The slides were incubated in 3 percent hydrogen peroxide and blocked with a blocking agent (Dako). The slides were then incubated with primary antibodies for HHV-8 latency-associated nuclear antigen (Advanced Biotechnologies), interferon- $\gamma$ , CD45, interleukin-6, vascular endothelial factor (Santa Cruz Biotechnology), basic fibroblast growth factor, or HHV-8 early antigen (open reading frame 59) (Advanced Biotechnologies). Either fluorescein-conjugated secondary antibodies or labeled streptavidin-biotin plus peroxidase (Dako) was used according to the manufacturer's specifications. Vectashield mounting medium (Vector Laboratories) was used after the application of 3,3'-diaminobenzidine-chromagen

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**Figure 1.** Development and Resolution of a Kaposi's Sarcoma Lesion after Oral Surgery in a Patient with Human Immunodeficiency Virus Infection.

An aggressive angiogenic lesion developed six days after biopsy of a labial minor salivary gland (Panel A). Six weeks after the initiation of radiation therapy, the lesion had resolved (Panel B).



**Figure 2.** Histologic Specimens Obtained before and after the Development of the Lesion.

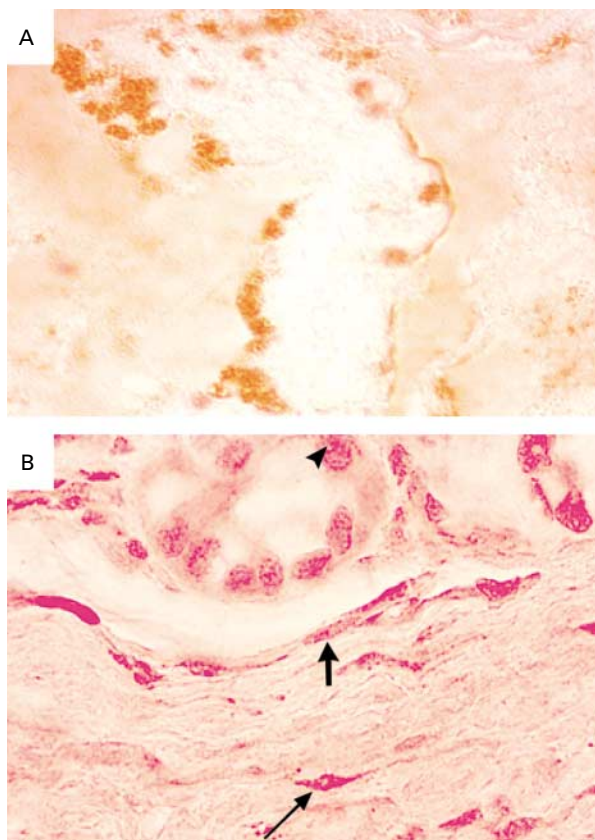
A specimen from a minor salivary gland obtained before the development of the lesion shows no abnormalities (Panel A; hematoxylin and eosin,  $\times 20$ ). A specimen of the lesion (Panel B; hematoxylin and eosin,  $\times 40$ , oil-immersion lens) shows slit-like vascular channels (arrows) caused by the proliferation of atypical spindle cells — a feature of early Kaposi's sarcoma.

solution. The slides were counterstained with hematoxylin (Sigma) and mounted with coverslips with the use of Permount (Fisher Scientific).

## RESULTS

Before the development of the labial neoplasm, HHV-8 DNA was detected in the throat washings from the patient by PCR but not in the minor salivary gland or peripheral-blood lymphocytes (data not shown). Microscopical examination of the labial minor salivary gland showed no abnormalities, and HHV-8

viral proteins were not detected (Fig. 2A). In the angiogenic lesion that developed at the site of the incision, there were slit-like vascular channels and proliferation of atypical spindle cells (Fig. 2B); inflammatory cells were also present. Staining with a monoclonal antibody specific for the HHV-8 early antigen (open reading frame 59) showed that the antigen was present in macrophages lining vessels within the lesion (Fig. 3A). Endothelial cells, spindle cells, and ductal epithelial cells were positive for latency-associated nuclear antigen (Fig. 3B), as were overlying epithelial cells and



**Figure 3.** Immunoperoxidase Staining Showing Human Herpesvirus 8 (HHV-8) in the Lesion.

In Panel A, the brown staining indicates the presence of the HHV-8 early antigen (open reading frame 59), a viral DNA-replication factor, in macrophages ( $\times 20$ ). In Panel B, the speckled pattern of staining characteristic of latency-associated nuclear antigen is present in endothelial cells lining a blood vessel (short arrow), spindle cells (long arrow), and ductal epithelial cells (arrowhead) ( $\times 40$ , oil-immersion lens).

lymphocytes in the central portion of the lesion. Interferon- $\gamma$ , interleukin-6, vascular endothelial growth factor, and basic fibroblast growth factor were all detected in the lesion (data not shown).

### DISCUSSION

In this patient with AIDS, Kaposi's sarcoma developed in the oral cavity soon after a biopsy of the lip. Although HHV-8 had not been found in the mucosa where the Kaposi's sarcoma developed, there was shedding of the virus into the saliva before the lesion developed.

The mouth may be a site of recurrent viral inocu-

lation, since HHV-8 is frequently shed in saliva in patients with AIDS.<sup>6,7</sup> Koebner's phenomenon, in which a nonspecific stimulus elicits a disease-specific reaction, has been reported in a few cases involving the development of Kaposi's sarcoma.<sup>4,8</sup> In this instance, the disease occurred in oral mucosa damaged during a biopsy.

It is likely that inflammation and Kaposi's sarcoma are linked. In this case, trauma may have precipitated inflammatory changes that recruited HHV-8 to the site; post-traumatic inflammation may have also contributed to the rapid development of this aggressive lesion. Macrophages, which participate in wound healing, produce growth and angiogenic factors and may recruit HHV-8 into tissues.<sup>9,10</sup> The findings in this case indicate that macrophages in the early lesion were productively infected with HHV-8.

In other studies, HHV-8 has not been detected in the majority of cells in early lesions,<sup>10</sup> but in this case, it was detected in the early lesion in macrophages, endothelial cells, spindle cells, epithelial cells, and lymphocytes. In addition, the early lesion expressed latent HHV-8 gene products. The latency-associated nuclear antigen encoded by open reading frame 73 of HHV-8 was detected in endothelial cells and lymphocytes and, remarkably, in epithelial cells. Together with in situ evidence of viral DNA in the overlying epithelium,<sup>11</sup> these findings suggest that epithelial cells may be a site of viral latency. In this case, both viral infection and wound healing appeared to play a part in the development of Kaposi's sarcoma.

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