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Original contribution

Kaposi sarcoma-associated herpesvirus in non-Hodgkin lymphoma and reactive lymphadenopathy in Uganda

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Summary Kaposi sarcoma-associated herpesvirus (KSHV) causes Kaposi sarcoma and is also associated with primary effusion lymphoma, a subset of diffuse large B-cell lymphomas, and multicentric Castleman disease. Because KSHV infection is endemic in sub-Saharan Africa, we sought to identify cases of KSHV-positive non-Hodgkin lymphomas (NHLs) and reactive lymphadenopathy in this region. One hundred forty-four cases (80 NHLs, 64 reactive lymph nodes) from the major pathology laboratory in Uganda were reviewed. One NHL was KSHV-positive, as indicated by staining for the viral latent nuclear antigen. This NHL was a diffuse large B-cell lymphoma in a 5-year-old boy. The tumor was also Epstein-Barr virus–positive. In addition, 2 reactive lymph nodes, both classified histologically as follicular involution, stained KSHV latent nuclear antigen-positive and thus most likely represent multicentric Castleman disease. In all 3 KSHV-positive cases, a minority of cells expressed KSHV viral interleukin 6, a biologically active cytokine homolog. In conclusion, we show that KSHV is rarely associated with lymphoproliferative disorders in sub-Saharan Africa. We describe the first case of a KSHV-positive NHL from this region; this case is also the first reported pediatric lymphoma associated with KSHV infection.

1. Introduction

Kaposi sarcoma-associated herpesvirus (KSHV; also known as human herpesvirus 8) causes Kaposi sarcoma (KS). Accumulating evidence indicates that KSHV also plays a pathogenic role in some types of non-Hodgkin lymphoma (NHL). KSHV is found in the tumor cells of primary effusion lymphomas (PELs) [1] that arise in the serous cavities of pleura, peritoneum, or pericardium. Histologically, PEL is composed of large, pleomorphic, immunoblastic-appearing cells [2]. In most cases, the cells are also infected with Epstein-Barr virus (EBV) [2]. In addition, KSHV is found in a subset of cases of multicentric Castleman disease (MCD) [3], an atypical lymphoproliferative disorder [4], and in plasmablastic NHLs that arise during MCD [5,6]. PEL and KSHV-positive MCD are strongly associated with human immunodeficiency virus

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(HIV) infection and acquired immunodeficiency syndrome (AIDS), although these lymphoid proliferations are uncommon even in that setting [7,8].

Emerging evidence also points to KSHV involvement in a subset of diffuse large B-cell lymphomas (DLBCLs) other than PEL and MCD-associated plasmablastic NHLs, particularly in HIV-infected individuals. The risk of immunoblastic NHL is especially high in individuals with AIDS who also have KS [9]. KSHV-positive NHLs, predominantly immunoblastic and plasmablastic, have been described, in contrast with PEL, to not involve the body cavities [10-12]. In these solid tumors, as in PEL, latent KSHV infection is present within each tumor cell, as demonstrated by staining for KSHV latent nuclear antigen (LANA), and many are also EBV-positive. In addition, a minority of the tumor cells in these cases also express viral interleukin 6 (v-IL6), suggesting at least partial lytic viral replication in a subset of cells with subsequent stimulation of tumor cell growth by this homolog of human IL6.

Although KSHV infection is somewhat uncommon in North America and western Europe, infection is endemic in sub-Saharan Africa [13]. In sub-Saharan Africa, KSHV infection frequently occurs in childhood and reaches a prevalence of 30% to 60% by 15 years of age [14,15]. In addition, HIV, a possible cofactor for KSHV-induced lymphoid proliferations, is also common. In the present study, we examined lymphoid specimens submitted to the Department of Pathology of Makerere University Medical School, Kampala, Uganda, which was established in the late 1940s to provide free diagnostic histologic services to all hospitals in Uganda. We sought to characterize the role of KSHV in both benign and malignant lymphoid proliferations in a region that is endemic for KSHV.

2. Materials and methods

2.1. Specimens

We examined formalin-fixed, paraffin-embedded tissue specimens of NHLs and benign lymphoid proliferations, with an overselection of cases classified as DLBCL, submitted between 1997 and 2001 to the Department of Pathology of Makerere University Medical School, Kampala, Uganda. Lymphomas involving the body cavities (ie, PELs) and lesions diagnosed as Hodgkin lymphoma were excluded. Basic demographic and clinical information was recorded. Before our pathologic investigations, the specimens were delinked from personal identifiers, allowing the study to be exempted from human subjects review.

2.2. Pathology evaluation

Hematoxylin and eosin–stained sections from each specimen were evaluated morphologically by 3 hematopathologists (EO, DMK, and AC). Cases with NHL were classified according to the World Health Organization classification scheme for lymphoma [16]. In instances where it was not possible to accurately classify a case in this manner because of poor morphology and/or lack of immunoreactivity, the specimen was classified as "high grade" or "low grade" based on nuclear size and mitotic rate. Benign lymphoid proliferations were classified into well-defined categories or classified as "reactive" when findings were nonspecific.

Sections from all cases were immunostained for CD20 (DAKO Cytomation, Carpinteria, CA), CD3 (NeoMarkers, Fremont, CA), HIV p24 (DAKO Cytomation), and KSHV ORF73 (LANA, Advanced Biotechnologies, Columbia, MD). Immunostaining was performed on the TechMate500TM automated immunostainer (Ventana Medical Systems, Tucson, AZ) after appropriate antigen retrieval [17]. To assist in classification, additional immunostaining was performed in selected cases, after appropriate antigen retrieval, for CD15 (Becton Dickinson, San Jose, CA); κ and λ immunoglobulin light chains, myeloperoxidase, epithelial membrane antigen (EMA); CD21, CD30, CD79a, and BCL-2 (DAKO Cytomation); BCL-1 (NeoMarkers); CD2, CD5, CD7, CD10, CD23, and CD56 (Vision Biosystems-Novacastra, Newcastle Upon Tyne, UK); OCT-2 (Santa Cruz Technology, Santa Cruz, CA); TdT (Surpertechs, Rockville, MD); and Ki-67 (Zymed Laboratories, South San Francisco, CA). Immunostaining for vIL-6 was performed in LANA-positive cases using a rabbit polyclonal vIL-6 antibody prepared by immunizing rabbits with the peptide PDVTPDVHDK, as reported by Moore et al [18].

In situ hybridization for EBV was performed using the EBV Probe ISH Kit (Vision Biosystems-Novacastra) according to the manufacturer's instructions.

3. Results

One hundred fifty-five cases (1-3 biopsies per case) were evaluated. Eleven cases were excluded because of the lack of a lymphoid proliferation or because the specimen was unreadable. In the 8 cases with more than one biopsy, the morphologic findings were similar in all tissues. Of the 144 included cases, 80 cases were classified as NHL. Most NHLs were high-grade/aggressive lymphomas, with 54 cases classified as either Burkitt NHL (n = 27), DLBCL (n = 24), or high-grade B-cell NHL (n = 4) not otherwise classifiable because of poor tissue quality. The remaining 64 cases were benign lymphoid proliferations. Of these cases, 45 showed nonspecific reactive changes, whereas the remaining 19 cases could be classified into recognized entities (Table 1).

In situ hybridization for EBV was successful in 79 of the 80 NHLs and in 63 of the 64 benign lymphoid proliferations (Table 1). EBV was present in 43 NHLs (54%), in a substantial proportion of the tumor cells in 38 (48%) cases, and in a few tumor cells in 5 (6%) cases. Of the 27 Burkitt NHLs, 26 (96%) were EBV-positive (n = 23 with many positive cells, n = 3 with only scattered positive cells). There