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Novel insights into the early histopathogenesis of immunodeficiency-associated Burkitt lymphoma: A case report of Burkitt microlymphoma arising within HIV lymphadenitis

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Abstract

Aims—To report a case detailing the early histopathogenesis of Burkitt lymphoma (BL), Epstein-Barr virus (EBV) positive, in the clinical setting of HIV infection.

Methods and Results—We describe a 57 year-old woman with newly diagnosed HIV infection who presented with unilateral localized axillary lymphadenopathy. Histopathological, immunohistochemical, and flow cytometric analyses were performed and showed EBV-positive nests of Burkitt cells, including rare nests also positive for cytomegalovirus, exclusively located within hyperplastic monocytoïd B-cell areas of HIV lymphadenitis. Due to positron emission tomography/computed tomography findings of persistent localized lymphadenopathy with markedly increased fludeoxyglucose avidity a core needle biopsy was performed. Histopathological, immunohistochemical, fluorescence in situ hybridization, and flow cytometric analyses confirmed BL.

Conclusions—Early histopathogenesis of HIV-associated BL occurred as nests of EBV-positive Burkitt cells within prominent monocytoïd B-cell areas in our case. The results equip the pathologist to identify a very subtle Burkitt microlymphoma which would allow for prompt and appropriate chemotherapeutic intervention and may promote research into the possible relationship between monocytoïd B cells and BL.

Keywords

Burkitt lymphoma; human immunodeficiency virus; Epstein-Barr virus; Cytomegalovirus; monocytoïd

INTRODUCTION

Compared with immunocompetent people, people living with human immunodeficiency virus (HIV)/acquired immune deficiency syndrome (AIDS) (PLWHA) have a marked increase risk of developing lymphoma, particularly aggressive non-Hodgkin lymphomas.

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The most common lymphomas include Burkitt lymphoma (BL), diffuse large B-cell lymphoma, plasmablastic lymphoma, primary effusion lymphoma, and classical Hodgkin lymphoma. Of these, BL occurs early in HIV infection when CD4 counts are relatively high ($>200 \times 10^6/L$). The majority of HIV-associated BL cases show plasmacytoid differentiation which have a higher association with Epstein-Barr virus (EBV) than cases with classical or atypical morphology.¹ The other lymphoma types occur later in HIV infection, including rare cases of large B-cell lymphoma arising in human herpes virus 8 (HHV8)-associated multicentric Castleman disease (HHV8 MCD) in people with profound immunodeficiency. As the majority of HIV-associated lymphomas are aggressive, knowledge of the early histopathogenesis of these lymphomas is scarce and limited to cases associated with HHV8 MCD.²⁻⁸

Dupin et al. in 2000 described 4 cases of HHV8 MCD involving the spleen where plasmablasts in 3 of the 4 cases coalesced to form confluent clusters of plasmablasts, termed plasmablastic microlymphoma. However, no microlymphomas were identified in any of the lymph node specimens available in 3 of the 4 cases. Furthermore, only 1 of the 4 cases demonstrated overt HHV8 positive plasmablastic lymphoma involving a different site than the one which showed plasmablastic microlymphoma. Other than these cases of plasmablastic microlymphoma associated with HHV8 MCD, no cases have been reported that describe the early histopathogenesis of other de novo aggressive B-cell lymphomas. Herein we describe a case of Burkitt microlymphoma (BmL) involving a lymph node of a 57 year old female with newly diagnosed HIV infection. The patient subsequently developed BL within 2 and 4 months after the initiation of combination antiretroviral therapy (CART) and initial biopsy, respectively.

MATERIALS AND METHODS

Patient Data, Morphologic, Immunophenotypic, Cytogenetic, and Molecular Studies
Clinical, radiologic, and laboratory findings were reviewed in the electronic medical record charts. Histopathologic diagnoses were re-reviewed and confirmed by three senior hematopathologists. Immunohistochemical (IHC) studies were performed on 5- μ M-thick formalin-fixed paraffin-embedded tissue sections with antigen retrieval and staining using BenchMarkXT (Ventana/Roche, Tucson, AZ) or Leica BOND-III (Leica Biosystems, Buffalo Grove, IL). Slides were stained using the following monoclonal antibodies from Ventana unless otherwise specified: CD3 (2GV6), CD20 (L26), CD10 (SP67), BCL2 (124), BCL6 (GI191E/A8; Cell Marque, Rocklin, CA), MUM1 (MRQ43; Cell Marque), C-MYC (Y69), Ki67 (MIB-1; Dako, Carpinteria, CA), CD43 (L60), kappa and lambda light chain (polyclonal; Dako), EBV latent membrane protein 1 (LMP1) (CS1-4; Dako), cytomegalovirus (CMV) (CCH2+DDG9; Dako), TdT (SEN28; Leica), and HHV8 (13B10; Cell Marque). EBV-encoded RNA (EBER) in situ hybridization study was performed using 2,4-dinitrophenyl probe for EBER 1 followed by iVIEW Blue Plus detection kit from Ventana. Flow cytometric analysis using the following antibodies from BD Biosciences (San Jose, CA), Beckman Coulter (BC) (Miami, FL), or Dako (Carpinteria, CA), and acquisition using standardized settings on Canto-10 flow cytometers (BD Biosciences) was performed using the following eight color antibody combinations as described by Wu et al.⁹: T cell tube: CD2-BV241 (BD, RPA2.10), CD5-BV510 (BD, UCHT2), CD7-FITC (BC, 8H8.1),

CD56-PE (BC, N901), CD4-PC5.5 (BC, 13B8.2), CD3-PC7 (BC, UCHT1), CD8-APC (BD, SK1), CD45 APC-H7 (BD, 2D1). B cell tube: CD19 BV421 (BD, HIB19), CD5 BV510 (BD, UCHT2), anti-kappa-FITC (Dako, polyclonal) anti-Lambda PE (Dako, polyclonal), CD10-PC5 (BC, Alb-1), CD38-APC (BD, HB7), CD45-APC-H7 (BD, 2D1). The data was analyzed using in-lab custom software Woodlist (generous gift of Dr. Brent Wood, University of Washington, Seattle, WA). Fluorescence in situ hybridization (FISH) studies for MYC gene rearrangement and MYC-immunoglobulin heavy chain (IGH) fusion were respectively conducted using Vysis LSI MYC dual color break-apart rearrangement (Abbott Molecular, Des Plaines, IL) and Vysis LSI IGH/MYC/CEP 8 tri-color dual fusion (Abbott Molecular) probes on 5- μ M-thick formalin-fixed paraffin-embedded tissue sections following standard deparaffinization procedures. IGH gene rearrangement polymerase chain reaction (PCR) studies were performed using fluorescently-labeled Biomed-2 primers followed by detection of amplified products by capillary electrophoresis on an Applied Biosystems 3730 DNA analyzer (Life Technologies, Carlsbad, CA). Limit of detection of IGH gene rearrangement PCR studies is 5% as determined by our laboratory. This study was approved by Memorial Sloan Kettering Cancer Center's Institutional Review Board on 08/11/2015 (WA0341-14(1)).

CASE REPORT

On routine mammography a 57 year old woman was incidentally found to have right axillary lymphadenopathy. Confirmatory ultrasound showed multiple enlarged lymph nodes up to 3.7 cm in size. No enlarged left axillary lymph nodes were identified. A core needle biopsy was performed, but results were inconclusive due to the limited sample size of the specimen. Therefore, an excisional biopsy of the largest right axillary lymph node was subsequently performed. At the time of the excisional biopsy, the patient was found to be HIV positive with a viral load of $>50,000$ copies/mL, CD4 T-cell count of $237 \times 10^6/L$, and a normal complete blood count (CBC) and lactate dehydrogenase (LDH) level. Initial full body staging evaluation of the extent of HIV lymphadenopathy using computed tomography (CT) and positron emission tomography (PET) scans with administration of fludeoxyglucose (FDG) was performed 6 weeks after the excisional biopsy. The PET/CT study demonstrated FDG avid (SUV 13.7) disease involving mildly enlarged right axillary lymph nodes up to 1.3 cm in size with minimal to no suspicious FDG uptake elsewhere. After HIV genotyping, patient was started on CART 2 months after the excisional biopsy. A follow up PET/CT study performed 2 months after the initiation of CART therapy was significant for persistent and markedly increased FDG avidity (SUV 38.8) of enlarged right axillary lymph nodes up to 2.9 cm in size. A core needle biopsy of a right axillary lymph node in the area of the original excisional biopsy was obtained and showed BL 4 months after the excisional biopsy.

RESULTS

Pathologic Findings

Morphologic examination of the excisional lymph node specimen revealed an enlarged node with intact nodal architecture containing floridly hyperplastic follicles with attenuated mantle zones and prominent contiguous perisinusoidal areas composed of monocytoid

lymphoid cells (Figure 1A). Exclusively within the monocytoid areas were occasional small nests of 5–15 atypical medium sized lymphoid cells with round nuclei, finely clumped chromatin, multiple small peripheral nucleoli, and small to moderate amounts of cytoplasm morphologically consistent with Burkitt cells, including some with plasmacytoid differentiation (Figure 1A, inset and arrows). Occasional small nests within the monocytoid areas appeared to coalesce forming a focal ill-defined aggregates reminiscent of BL (Figure 1A, arrowhead). Occasional mitotic figures and apoptotic bodies were seen within the nests and focal ill-defined aggregates. IHC stains showed the Burkitt cells were positive for CD20 (Figure 1B), BCL6 (Figure 1C), CD10 (Figure 1D), C-MYC (Figure 1F), and Ki67 (Figure 1G), while negative for BCL2 (Figure 1E). Epstein-Barr virus encoded RNA (EBER) in situ hybridization was positive only in nests of Burkitt cells (Figure 1H). Rare nests were positive for CMV by immunohistochemistry (Figure 1H, inset). Burkitt cells were also positive for immunoglobulin lambda light chain and CD43, while negative for immunoglobulin kappa light chain, EBV LMP1, HHV8, CD34, and TdT. No vessels surrounding the nests of Burkitt cells were seen on CD34 IHC stain. Flow cytometric analysis detected a very small abnormal monotypic B-cell population [1.3% of total white blood cells (WBC), 2.8% of B cells] with expression of CD19 (bright), CD20, CD10, CD38 (bright), and lambda surface light chain (bright) in a prominent background of polytypic B cells (Figure 2A–D). No CD5 expression was detected in abnormal B cells. Molecular study for IGH@ gene rearrangement was performed and was negative for a clonal rearrangement. FISH study for MYC gene rearrangement using MYC break-apart probe showed split signals in 5% of interphase nuclei analyzed and results interpreted as nondiagnostic (cut off for normal variation of test is 10%). FISH study for MYC-IGH fusion using MYC-IGH fusion probe did not detect any fusion signals in 100 interphase nuclei analyzed. Both FISH analyses were performed in hyperplastic monocytoid B-cell areas containing occasional nests of Burkitt cells based on corresponding H&E and C-MYC IHC stains. However, no significant signals were detected for either a MYC gene rearrangement or MYC-IGH fusion. Thus, based on the overall findings, specifically: (1) nests composed of atypical medium sized lymphoid cells consistent with Burkitt cells by morphology and immunohistochemistry (IHC); (2) EBER-ISH positivity in Burkitt cells; (3) flow cytometric detection of a small abnormal monotypic lambda-restricted CD10-positive B-cell population; (4) HIV infection by serology and lymph node morphologic examination; (5) nonclonal IGH gene rearrangement by PCR; (6) nondiagnostic FISH results for MYC gene rearrangement; and (7) negative FISH results for MYC-IGH fusion; the case was diagnosed as HIV lymphadenitis associated with minimal involvement by an EBV-positive B-cell lymphoproliferative disorder with Burkitt-like features. Close clinical follow up, complete laboratory and radiologic work up, and a repeat biopsy if lymphadenopathy persisted or progressed were strongly recommended.

Four months later, a core needle biopsy of a persistently enlarged right axillary lymph node which increased from 1.3 cm to 2.9 cm in size was performed. Morphologic examination now showed a diffuse proliferation of predominantly medium sized cells with similar morphology as the nests of atypical lymphoid cells in the excisional biopsy consistent with Burkitt lymphoma with plasmacytoid differentiation (Figure 1I, inset). Frequent mitotic figures, apoptotic bodies, and occasional tingible-body macrophages focally imparting a

“starry sky” pattern were present. Flow cytometric analysis of the core needle biopsy showed the sample consisted almost entirely of abnormal B cells (97.3% of total WBC) with a near identical immunophenotypic profile as previously demonstrated (Figure 2E–H). Immunohistochemical studies also showed similar findings as the excisional biopsy (Figure 1I–P), notably a Ki67 proliferation index of 100% (Figure 1O). However, no CMV immunoreactivity by the neoplastic lymphoid cells was seen in the limited specimen. FISH study was positive for a t(8;14) MYC-IGH fusion in 45 of 60 (75%) interphase nuclei analyzed (Figure S1). IGH PCR gene rearrangement studies could not be performed due to complete exhaustion of specimen tissue following IHC and FISH studies.

Clinical Follow-up

Staging bone marrow biopsy and cerebral spinal fluid (CSF) sample did not show involvement by Burkitt lymphoma based on routine morphologic evaluation. Patient received 6 cycles of intensive chemotherapy with intrathecal prophylaxis, and a recent whole body CT scan demonstrated no evidence of disease. The most recent HIV viral load assay by quantitative PCR detected 24 copies/mL.

DISCUSSION

Based on the totality of the clinical, morphologic, immunophenotypic, radiologic, cytogenetic, and molecular findings of the initial lymph node excisional biopsy and subsequent core needle biopsy, the diagnoses of BmL arising in a background of HIV lymphadenitis and BL with plasmacytoid differentiation were confirmed, respectively.

Our case illustrated the early histopathogenesis of BL which occurred as very small nests of EBER-positive Burkitt cells scattered within prominent monocytoid B-cell areas in a lymph node of a person with newly diagnosed HIV infection. Although molecular studies for IGH gene rearrangement did not detect a clonal gene rearrangement in the first biopsy, this most likely was due to the paucity of Burkitt cells relative to numerous background non-neoplastic B cells (ratio of Burkitt cells to non-neoplastic B cells <5%) (Figures S2 and S3). This ratio was further supported by flow cytometric analysis (see above). Notwithstanding, the clonal relationship between the nests of Burkitt cells and BL can be strongly inferred based on (1) flow cytometric detection of the same monotypic lambda-restricted B-cell population in both biopsies in a similar proportion as seen on H&E stained slides with only a small subset of neoplastic B cells with dim CD45 expression in the second biopsy and (2) identical morphologic features, immunohistochemical profile, and positivity for EBER-ISH. It is particularly noteworthy that the nests of EBER-positive Burkitt cells were exclusively found within prominent areas of monocytoid B cells suggesting that these areas may represent a possible niche from which Burkitt cells originate and/or become infected by EBV in PLWHA.¹⁰ Circumstantial evidence supporting this possibility are well-known empirical findings that: (1) areas of monocytoid lymphoid hyperplasia in lymph nodes are frequently present in the setting of early HIV infection; (2) BL is more common in PLWHA than in other disorders involving the immune system where monocytoid lymphoid hyperplasia is typically not a salient lymph node morphologic finding; (3) BL usually occurs early in the course of HIV infection when CD4 T-cell counts are relatively high

(>200×10⁶/L) in contrast to diffuse large B-cell lymphoma (DLBCL); and (4) BL with plasmacytoid differentiation (as seen in our case) is particularly associated with EBV positivity and HIV infection.

The alternative and reasonable possibility that the initial excisional biopsy findings did not represent the primary site of BmL is unlikely based on: (1) the excisional biopsy was of the largest lymph node; (2) the pattern of involvement in a nearby regional lymph node by a highly aggressive lymphoma as scattered predominantly small nests of neoplastic cells would be unusual; and (3) PET/CT SUV findings of 13.7 and 38.8 at the time of the initial and subsequent lymph node biopsy, respectively, support progression of BmL to BL. However, it is unclear in our case how nests of EBER-positive Burkitt cells with a germinal center immunophenotype preferentially resided within hyperplastic monocytoid B-cell areas, especially since EBER-ISH was completely negative in the hyperplastic follicles. Molecular genetic studies may help to determine if either the monocytoid or germinal center lymphoid population is related to the nests of Burkitt cells or BL.

An unusual and unexpected finding in our case was the reactivity of rare nests of Burkitt cells for CMV by immunohistochemistry in the original biopsy. Although co-expression of nests of Burkitt cells for CMV and EBER cannot be determined with certainty, there were some areas which showed positivity for both CMV and EBER (Figure S4 and S5). Rare cases of Burkitt lymphoma have demonstrated both CMV and EBV positivity.¹¹ Our findings further raise the possibility of a potential role of CMV in the pathogenesis of Burkitt lymphoma in PLWHA.¹²

As lymph node biopsies are seldom performed in the setting of HIV seroconversion, this case makes a strong argument that lymph node specimens should be obtained in PLWHA who have lymphadenopathy. Based on our findings, we believe that pathologists would be well-equipped to evaluate for occult BL which would allow for prompt and appropriate chemotherapeutic intervention.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

Histologic and immunophenotypic features of the first biopsy (top row) showed florid follicular and monocytoid lymphoid hyperplasia [$\times 100$] (A), small nests of Burkitt cells (A, arrows and inset [$\times 600$]) which coalesced to form ill-defined aggregates (A, arrowhead). Nests of Burkitt cells were positive for CD20 [$\times 400$] (B), BCL6 [$\times 400$] (C), CD10 [$\times 400$] (D), C-MYC [$\times 100$] (F), Ki67 [$\times 100$] (G), and Epstein-Barr Virus (EBV)-encoded RNA *in situ* hybridization [$\times 100$] (H), while negative for BCL2 [$\times 400$] (E). Rare nests of Burkitt cells were positive for CMV [$\times 600$] (H, inset). After 4 months of persistent and progressive localized lymphadenopathy, a second biopsy (bottom row) showed a diffuse proliferation of predominantly medium sized lymphoid cells with round to slightly irregular nuclei, finely granular/dispersed chromatin, multiple small peripheral nucleoli, and small to moderate amounts of eccentric amphophilic cytoplasm consistent with Burkitt lymphoma with plasmacytoid differentiation [$\times 200$] (I). Neoplastic cells were again positive for CD20 [$\times 400$] (J), BCL6 [$\times 400$] (K), CD10 [$\times 400$] (L), C-MYC [$\times 400$] (N), and Epstein-Barr Virus (EBV)-encoded RNA *in situ* hybridization [$\times 600$] (P); while negative for BCL2 [$\times 400$] (M); and showed a 100% Ki67 proliferation index [$\times 400$] (O).

Figure 2.

Flow cytometric analysis of initial and subsequent right axillary lymph node specimens gated on CD19-positive mononuclear cells (B cells) (A). Initial biopsy showed a very small abnormal B-cell population (highlighted in red) with bright monotypic surface immunoglobulin lambda light chain restriction and CD10 expression (B), bright CD38 expression (C), and normal expression of CD20 (C) and CD45 (D), in a prominent background of polytypic B cells (highlighted in blue) (A–D). Subsequent biopsy showed a predominant abnormal B-cell population (highlighted in red) with a nearly identical immunophenotype as seen in the initial biopsy without background polytypic B cells (E–H); a subset of abnormal B cells showed dim CD45 expression (H).