EBV-positive Reactive Hyperplasia Progressed into EBV-positive Diffuse Large B-cell Lymphoma of the Elderly over a 6-year Period

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Abstract:
A 70-year-old woman with lymphadenopathy was admitted to hospital in 2008. Lymph node biopsy showed reactive lymphoid hyperplasia (RH) with monoclonal proliferation of EBV. Her lymphadenopathy regressed without treatment. In 2014, the patient presented with nasal obstruction because of a left nasal mass. She was diagnosed with EBV-positive diffuse large B-cell lymphoma (DLBCL) of the elderly based on the examination of a biopsy specimen of the mass. The IgH rearrangement in the specimens from the 2008 and the 2014 revealed that they were genetically identical. This is the first report of RH progressing to DLBCL, and suggests that EBV-positive B-cells in RH lymph nodes predict the evolution to DLBCL.

Key words: EBV-positive reactive hyperplasia, EBV-positive diffuse large B-cell lymphoma, EBV-positive lymphoproliferative disorders

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Introduction

Epstein-Barr virus (EBV) affects more than 90% of the adult population worldwide. Primary EBV infections in children are often asymptomatic, and EBV typically persists in an asymptomatic latent state in memory B-cells (1). Occasional reactivation from latency and virus production is triggered by environmental stimuli but tightly controlled by the immune system in healthy individuals. Suppression of the T-cell function by immunosuppressive agents or HIV infection, which usually plays a determinant role in controlling EBV-associated lymphoproliferative disorders (LPDs), increases the risk of EBV-positive B-cell LPDs (2-4). ‘EBV-positive diffuse large B-cell lymphoma (DLBCL) of the elderly’ is a provisional entity that was included in the 2008 World Health Organization classification of LPDs (5); the disease group is characterized by EBV-encoded small RNA-1 (EBER-1)-positive LPDs that occur in elderly individuals without predisposing immunodeficiency. It is also referred to by various other names, including “senile EBV-associated B cell LPD”, “age-related EBV-associated B-cell LPD”, and “EBV-associated B-cell LPD of the elderly” (5-8). Dojcinov et al. (9) categorized age-related EBV-positive B-cell LPDs in a Western population as follows: (i) reactive lymphoid hyperplasia (RH), (ii) polymorphic extranodal, or (iii) polymorphic nodal LPD, and (iv) DLBCL; and reported the clinical features, histology, immunophenotype, EBER, and clonality of the T-cell receptor and immunoglobulin genes. Disease progression is rarely reported (9), but it may occur in stages of multi-step lymphomagenesis. We herein describe a case in which EBV-positive RH progressed to EBV-positive DLBCL of the elderly over a 6-year period.

Case Report

A 70-year-old woman with cervical lymphadenopathy was admitted to our hospital in 2008. A physical examination revealed bilateral cervical and axillary lymphadenopathy. She had been healthy until the onset of disease. Laboratory find-
lymph nodes revealed follicular hyperplasia with plasma cell infiltration. EBV VCA IgM-negative. A biopsy of the right axillary antigen-positive (1:10), and anti-early antigen IgG- and IgG titer was elevated (1:2,560), and she was EBV nuclear antibody-negative. Her anti-EBV viral capsid antigen (VCA) within the normal range (160 IU/L). The patient was HIV-1,854 IU/L; her lactate dehydrogenase (LDH) level was increased levels of soluble interleukin-2 receptor (sIL-2R; 1,854 IU/L); her lactate dehydrogenase (LDH) level was within the normal range (160 IU/L). The patient was HIV-antibody-negative. Her anti-EBV viral capsid antigen (VCA) IgG titer was elevated (1:2,560), and she was EBV nuclear antigen-positive (1:10), and anti-early antigen IgG- and EBV VCA IgM-negative. A biopsy of the right axillary lymph node revealed follicular hyperplasia with plasma cell infiltration. Tingible body macrophages were observed in the follicles, which were positive for CD20 and CD21, and negative for bcl-2. In situ hybridization revealed EBER-1-positive cells outside the follicles that were large and positive for CD20 and bcl-2, indicating that they were B cells. The plasma cells were considered polyclonal because no clear monotypic light-chain restriction for kappa protein was observed (Fig. 1A). Both the monoclonal proliferation of EBV and IgH rearrangement were detected by Southern

**Figure 1.** The histological findings of the biopsy specimens from 2008 (right axillary lymph node) and in 2014 (left nasal mass). A: Biopsy of the right axillary lymph node. The biopsy specimen of the right axillary lymph node showed reactive patterns that included follicular hyperplasia [i: Hematoxylin and Eosin (H&E) staining; magnification: ×20]. Plasma cell infiltration and epithelioid granulomas were present in the paracortical area (ii: H&E staining; ×200). The cortex and paracortical area were positive for CD20 (iii: CD20-immunostaining; ×200), in situ hybridization revealed that the paracortical area was positive for Epstein-Barr virus-encoded RNA (EBER) (iv: ×200) and CD138 (v: CD138-immunostaining; ×200). B: Biopsy of the left nasal mass. The histopathological examination of the biopsy specimen of the left nasal mass showed the monomorphic and dense proliferation of large lymphoid cells accompanied by necrosis (i, ii: H&E staining; ×10, ×200). The large cells were positive for CD20 (iii: CD20-immunostaining; ×200). The expression of EBER was identified in the large cell nuclei (iv: ×200).
blotting of lymph node cell-derived DNA (Fig. 2). We ruled out follicular lymphoma based on the pathological and immunohistological findings, and diagnosed the patient with EBV-positive RH. Her lymphadenopathy regressed without treatment. Thus, we intended to follow the patient with watchful waiting until the appearance of other symptoms. Her lymphadenopathy worsened in 2009, but again regressed without treatment.

In 2014, the patient presented with bilateral axillary lymphadenopathy, nasal obstruction, and nasal bleeding. Computed tomography revealed generalized lymphadenopathy and a left nasal mass. The laboratory findings revealed that her sIL-2R level (2,225 IU/L) was elevated; her LDH (225 IU/L) and hemoglobin (13.7 g/dL) levels were within the normal ranges. 18F-Fluorodeoxyglucose positron emission tomography/computed tomography revealed that these lesions were metabolically active. The maximum standardized uptake value was 23. Biopsy of the left nasal mass revealed effacement of the nodal architecture, accompanied by necrosis and diffuse large lymphocyte permeation. The monotonous cells had a basophilic cytoplasm and most had prominent nuclei; they were positive for CD20, bcl-2, MUM-1, and kappa chains, negative for CD10 and CD56, and the MIB-1 index was 70% (Fig. 1B). The patient was diagnosed with EBV-positive DLBCL of the elderly stage IVa, and underwent chemotherapy with R-CHOP (rituximab, cyclophosphamide, doxorubicin, vincristine, and prednisolone), which led to the improvement of her clinical symptoms and normalized her sIL-2R level; complete remission was achieved.

We analyzed the IgH rearrangement in both the axillary biopsy specimen from 2008 and the nasal mass biopsy specimen of 2014 by a PCR to detect immunoglobulin gene rearrangement. The PCR products of both samples had the same peak size (A: right axillary lymph node specimen, B: nasal mass specimen).

### Discussion

EBV-positive LPDs are categorized into four different histological subtypes, the relationship among which is unclear. A previous report described one case of RH that progressed to polymorphic nodal LPD, and two cases of RH that progressed to EBV-positive classic Hodgkin lymphoma (9). This is the first case report of a patient with RH that progressed to DLBCL. The progression was verified by a PCR to detect IgH rearrangement; the results indicated that these two LPDs were genetically identical. Our case report and other reports of these progressive states of EBV-positive LPDs support the hypothesis that expansion of EBV-positive B-cells in the RH lymph nodes predicts the evolution to a polymorphic subtype, and then to monomorphic DLBCL. EBV is involved in the development of various types of LPDs with diverse immune alterations. Ohshima et al. (11) proposed a clinicopathological categorization of EBV-associated T/natural killer (NK)-cell LPD in children and
young adults as follows: (i) category A1, polymorphic LPD without the clonal proliferation of EBV-infected cells; (ii) category A2, polymorphic LPD with clonality; (iii) category A3, monomorphic LPD (T-cell or NK cell lymphoma/leukemia) with clonality; and (iv) category B, monomorphic LPD (T-cell lymphoma) with clonality and a fulminant course. Categories A1, A2, and A3 possibly constitute a continuous spectrum. There might be multi-step processes subdivided into histological categories characterized by the tumor cell morphology in EBV-associated T/NK cells as well as B-cell lymphomagenesis. Our case provides evidence to support these hypothesized categories.

Age-related EBV-positive LPD is defined as an EBV-positive clonal lymphoproliferation that occurs in patients of >50 years of age with no known immunodeficiency (5, 12). Aging is thought to be a factor in immunosuppression. T-cell response dysregulation, the reduced output of new T-cells, the development of anergic memory cells, the loss of immunosurveillance, and deficient cytokine production, as well as limitations in the T-cell receptor repertoire are associated with immunosenescence (13). EBV-positive DLBCL was recently reported in young immunocompetent individuals (14, 15). Immune checkpoints of the programmed cell death 1/programmed cell death ligand-1 axis are dysregulated in young patients with EBV-positive DLBCL, similar to elderly patients (15, 16). These findings suggest that the downregulation of immune checkpoint receptors is one of the mechanisms of immunosenescence in these disorders. The findings of the present case suggest that further investigations might elucidate the mechanisms of multi-step lymphomagenesis in EBV-positive LPDs.

The authors state that they have no Conflict of Interest (COI).

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