Epstein–Barr virus expression in plasma cell neoplasms and its association with plasmablastic morphologic features

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Abstract Plasma cell neoplasms include plasma cell myeloma, plasmacytoma, primary amyloidosis, and light and heavy chain deposition diseases. Epstein–Barr virus (EBV) expression has rarely been reported to be associated with plasma cell neoplasms except for patients with a previous history of transplantation or severe immunodeficiency. In contrast, EBV expression has been reported in the majority of plasmablastic lymphoma, a subtype of diffuse large B cell lymphoma. We report three cases with a plasma cell neoplasm (two plasma cell myelomas and one case of plasmacytoma), where EBV infection was diffusely detected by in situ hybridization. All three patients were under the age of 40 years, and one of the three patients was an HIV-positive individual while the other two patients did not have any identifiable source for immunosuppression. All three cases showed multifocal plasmablastic features, which is concordant with the most recent reports about the possible association of EBV expression with plasmablastic morphologic features and increased proliferative activity.

Keywords Epstein–Barr virus · Plasma cell neoplasms · Plasmablastic features

Introduction

Plasma cell neoplasms encompass a spectrum of plasma cell disorders that include plasma cell myeloma, plasmacytoma, primary amyloidosis, and light and heavy chain deposition diseases [1, 2]. They are characterized by a monoclonal proliferation of immunoglobulin-producing plasma cells that represent post-germinal center, terminally differentiated B lymphocytes [1, 2]. Two types of plasmacytomas are recognized in the 2008 World Health Organization (WHO) classification: solitary plasmacytoma of bone (osseous plasmacytoma) and extraosseous plasmacytoma (extramedullary plasmacytoma) [1, 2]. Solitary plasmacytoma of bone usually presents as a lytic lesion involving the axial skeleton and frequently progresses to plasma cell myeloma in up to two-thirds of the patients, while the extramedullary type has a predilection for the upper aerodigestive tract and has a more indolent clinical behavior with only 15 % progressing to an overt plasma cell myeloma [1, 2].

True plasma cell neoplasms have been rarely reported to be associated with Epstein–Barr virus (EBV) expression. Plasmablastic lymphoma, a subtype of diffuse large B cell lymphoma with near-identical immunophenotype to plasma cell neoplasms, has been reported to show EBV expression in about two-thirds of the cases and near 100 % expression in the oral mucosa subtype [3, 4]. Post-transplant patients and severely immunocompromised patients, especially patients with established HIV infection, have been reported to have slightly increased incidence for EBV-positive plasma cell neoplasms although the cytomorphologic features of the neoplastic cells in such cases have been mainly described as plasmablastic or anaplastic [2, 5]. Plasmablastic cytomorphologic features are defined as plasma cells having larger concentrically placed nucleus with little or no cytoplasmic hof and exhibiting open or coarse chromatin, prominent nucleoli (usually central), and

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less abundant cytoplasm [6]. The anaplastic cytomorphologic features are defined as plasma cells with a hyperchromatic nuclei and more pleomorphic appearance with irregular nuclear and cytoplasmic contours [7]. Plasma cell myeloma patients with plasmablastic or anaplastic morphology tend to have a more aggressive clinical course and are generally considered to carry a more guarded prognosis [2]. We report three cases with a plasma cell neoplasm (two plasma cell myelomas and one case of plasmacytoma), where EBV expression was diffusely detected by in situ hybridization. All three patients were under the age of 40 years, and one of the three patients was an HIV-positive individual while the other two patients did not have any identifiable source for immunosuppression.

Clinical history, methods, and results

Case 1

A 39-year-old Hispanic male presented to the emergency room in early 2012 with a 2-month history of abdominal pain radiating to his back and with leg numbness for 2 days prior to presenting to the hospital. His past medical history was unremarkable except for hypothyroidism. A computed tomography (CT) and magnetic resonance imaging bone scans revealed lytic bony lesions at the cervical, thoracic, and lumbar spine as well as at the skull and left rib. A biopsy of the thoracic lesion revealed sheets of kappa-restricted plasma cells that showed strong expression of EBV by in situ hybridization (EBV-encoded RNA (EBER)) with a small subset expressing EBV-latent membrane protein 1 (LMP-1) by immunohistochemistry. The neoplastic plasma cells showed lack of expression for the B cell markers CD20 and PAX-5 as well as for cyclin D1, CD56, and human herpesvirus 8 (HHV-8). Focal plasmablastic features were noted on morphologic examination and an overall low Ki-67 proliferative activity was noted (10 %) although the plasmablastic areas showed slightly increased proliferative activity. The intensity of staining and percentage of EBER-positive cells were similar in both areas. Subsequent biopsies from the cervical spine and a right parietal mass showed an identical process with a similar immunophenotype. A bone marrow biopsy was performed and showed a slightly hypocellular marrow with active trilineage hematopoiesis and no evidence of involvement by a plasma cell neoplasm. EBER stain showed negative results when performed on the bone marrow clot biopsy sections. EBV serology testing was not performed. Cytogenetic studies performed on the bone marrow aspirate showed a normal male karyotype. Fluorescence in situ hybridization (FISH) with the dual-color MYC “split” probe was unsuccessful due to the decalcification method used to fix the cells but hinders DNA

probe hybridization and the visualization of the fluorescent signals. HIV testing by serology showed negative results. Serum protein electrophoresis showed a prominent beta-restricted band quantitated at 1.29 g/dl and was identified as an IgA kappa paraprotein by immunofixation. Urine electrophoresis and immunofixation showed free kappa light chains. Laboratory tests showed mild leukopenia, hypercalcemia, elevated serum free light chains, and elevated IgA levels. The patient also had mild anemia and mild thrombocytopenia. The patient’s mental status rapidly declined and a lumbar puncture was performed, which revealed involvement of the cerebrospinal fluid by the neoplastic plasma cells having the same morphological picture as the previous biopsy. The patient is currently receiving radiation therapy and has shown relative overall improvement despite the persistence of the monoclonal paraproteins in his serum.

Case 2

A 39-year-old Hispanic female who was diagnosed with HIV in 2000 presented to our institution in early 2008 with abdominal pain and distension for 5 days prior. Her CD4+ T cell count at presentation was 47 cells/ μ L. Multiple diffuse bony lesions were visualized on skeletal survey in addition to several ovarian and liver masses. An ultrasound-guided fine needle aspiration biopsy of the liver mass showed sheets of kappa-restricted plasma cells that express CD138 and CD56 by immunohistochemistry. The neoplastic plasma cells were negative for EBV-LMP-1, HHV-8, and cyclin D1 stains. By in situ hybridization, the neoplastic plasma cells were EBV (EBER) positive. EBV serology testing was not performed. Serum protein electrophoresis showed a band with restricted mobility in the fast gamma region that was quantitated at 3.25 g/dl. Immunofixation revealed an IgA kappa and IgG kappa biclonal gammopathy. Her urine protein electrophoresis showed free kappa light chains. A bone marrow biopsy from bilateral iliac crests showed 70–80 % marrow involvement by the neoplastic myeloma cells that exhibited anaplastic and plasmablastic features and demonstrated CD138 and kappa light chain expression by immunohistochemistry and flow cytometry. A subset of the neoplastic plasma cells within the bone marrow was positive for EBER stain. Cytogenetic studies performed on the bone marrow aspirate showed a normal female karyotype. FISH with dual-color MYC split probe did not yield unambiguous fluorescent signals. The patient succumbed to the disease 2 months later after complications from myeloma.

Case 3

A 38-year-old Caucasian male presented to our institute in late 2000 with altered mental status and epistaxis. A CT scan

of the head and neck revealed a lesion in the right nasal cavity extending into the right frontal lobe. A subsequent skeletal survey revealed multiple lytic bony lesions throughout the body. Laboratory testing showed elevated serum BUN and creatinine in addition to normocytic anemia. Testing for HIV-1 and HIV-2 by polymerase chain reaction (PCR) showed negative results. Serum protein electrophoresis showed a band in the fast gamma region that was quantitated at 1.25 g/dl. Immunofixation showed an IgG kappa monoclonal paraprotein. A free kappa light chain band was demonstrated in the urine by immunofixation as well. A biopsy of the rapidly growing nasal lesion revealed sheets of kappa-restricted plasma cells that exhibited plasmablastic morphology in many areas although areas of plasma cells with normal morphology were also seen. EBV studies by in situ hybridization (EBER) showed positivity in the vast majority of the neoplastic plasma cells including those with normal and plasmablastic morphologic features. The intensity of staining and percentage of EBER-positive cells were relatively similar in both areas (Fig. 1). The neoplastic cells were negative for EBV-LMP-1, HHV-8, cyclin D1, and CD56 by immunohistochemistry. A bone marrow biopsy of the iliac crest showed a normocellular marrow with active trilineage hematopoiesis and no evidence of involvement by clonal plasma cells. EBER stain showed negative results when performed on the bone marrow clot biopsy sections. Attempts to obtain MYC gene

status by dual-color FISH were unsuccessful. The patient underwent treatment with vincristine, doxorubicin, and dexamethasone with pamidronate and radiation therapy but did not show initial response to treatment and was ultimately lost to follow-up and his living status is unknown.

Discussion

In contrast to plasmablastic lymphomas, true plasma cell neoplasms have been rarely associated with EBV infection although post-transplant patients and severely immunocompromised patients may have slightly increased incidence of having an EBV-positive plasma cell neoplasm [2, 5]. The distinction of plasmablastic lymphoma from plasma cell myeloma or plasmacytoma with plasmablastic features can be difficult at times and usually depends on clinical correlation with the presence of serum monoclonal paraproteins, bone marrow involvement, and radiographically evident lytic lesions, which are all factors that would favor plasma cell myeloma with plasmablastic features [7, 8]. Although the expression of EBV and a positive HIV status have been historically associated with a diagnosis of plasmablastic lymphoma, other more reliable factors have been postulated to support a diagnosis of plasmablastic lymphoma over myeloma such as markedly increased mitotic activity, presence of numerous apoptotic bodies, and paucity of maturing

Fig. 1 The composite picture represents two different fields from case 3. The upper half shows sheets of neoplastic plasma cells that show no atypical features. **a** Low-power magnification (×10) of the plasma cells and **b** high-power magnification (×40). **c** EBER expression (EBV by in situ hybridization) (×40) and **d** Ki-67 expression shows minimal proliferative activity (×40). The lower half of the composite picture shows

sheets of plasma cells with atypical and plasmablastic features and a higher proliferative activity. **e** Low-power magnification (×10) of the atypical plasma cells and **f** high-power magnification (×40). **g** EBER expression (×10) and **h** Ki-67 expression shows increased proliferative activity than the areas without the plasmablastic features (×40)

Table 1 Review of EBV-positive plasma cell neoplasm cases in correlation with plasmablastic morphology

Cases	Age	Sex	Diagnosis	Immune status	M component	Plasmablastic features	CNS lesions	Disease course	Comments
Chang et al. [5], first case	40	M	Extraosseous plasmacytoma	Competent	Absent	Yes	ND	F/U for 6 years shows remission	All 4 cases were EBER positive and LMP-1 negative except for one case
Chang et al. [5], second case	27	M	Plasma cell myeloma	Competent	IgG	No	ND	Died of disease in 6 years	that was focally positive for LMP-1
Chang et al. [5], third case	60	M	Plasma cell myeloma	Competent	IgG	Yes	ND	Died of disease in 8 years	
Chang et al. [5], fourth case	51	M	Plasma cell myeloma	Competent	IgG	Yes	ND	Died of disease in 1 year	
Qing et al. [12]	69	M	Plasmablastic lymphoma transforming from plasmacytoma	Competent	Absent	Yes, mainly plasmablastic with adjacent mature monoclonal plasma cells	No	Patient alive but with disease progression	EBER positive in plasmablastic cells, while the clonal mature plasma cells are negative
Sasaki et al. [10]	62	M	Plasma cell myeloma	Competent	IgA	Yes, in the maxillary sinus tumor while bone marrow showed non-plasmablastic morphology	No	Patient responsive to first cycle of intensive chemotherapy	EBER positive in maxillary lesion with plasmablastic features, while negative in the marrow with nonplasmablastic morphology
Wu et al. [11]	40	M	Extraosseous plasmacytoma	Suppressed HIV+	Absent	Yes, focal	Yes	Patient sent to hospice in less than a year	EBER positive, LMP-1 stain negative
Current report, first case	39	M	Plasma cell myeloma	Competent (mild leukopenia)	IgA	Yes, admixed with nonplasmablastic areas	Yes	Patient alive after 1 year but with deterioration of clinical status	EBER positive, LMP-1 stain positive
Current report, second case	39	F	Plasma cell myeloma	Suppressed HIV+	Biclonal IgA and IgG	Yes, admixed with nonplasmablastic areas	No	Died of disease in 2 months	EBER positive, LMP-1 stain negative
Current report, third case	38	M	Multiple plasmacytomas (osseous and extraosseous)	Competent	IgA	Yes, admixed with nonplasmablastic areas	Yes	No F/U available	EBER positive, LMP-1 stain negative

ND not described

plasma cells [7]. Vega et al. have postulated in their study that EBV expression constitutes the differentiating factor between plasma cell neoplasms and plasmablastic lymphoma, where the latter show EBV expression in the majority of cases [3]; however, others have reported the detection of EBV in extramedullary plasmacytoma of the head and neck in immunocompetent individuals in up to 15 % of all cases [9]. Moreover, other investigators have reported the identification of EBV expression in immunocompetent patients with plasma cell myelomas and plasmacytomas, where the plasma cells in these cases exhibited plasmablastic morphology, and they concluded that the presence of EBV in plasma cell neoplasms is significantly associated with plasmablastic morphologic features suggesting that these tumors may have been driven by EBV to gain the plasmablastic morphologic features and a higher proliferation rate [5, 10–12]. Also, the identification of EBV expression in a subtype of patients with plasmablastic lymphoma that occurred as a result of plasma cell myeloma progression/transformation has been reported [13]. A more recent study has identified EBV expression by PCR in 33 % of bone marrows with plasma cell myeloma compared to only 10 % of normal bone marrow specimens [14], although the results may not be truly representative due to small sample size and the ethnicity of patients.

In healthy individuals who are seropositive for EBV, terminal differentiation of EBV-infected memory B cells into plasma cells has been reported to be associated with viral replication and initiation of the EBV productive (lytic) cycle [15, 16]. In contrast, individuals with plasmacytoid tumors frequently associated with EBV such as primary effusion lymphoma or plasmablastic lymphoma have been shown to establish a nonproductive (latent) cycle and exhibit a restrictive EBV latency pattern (latency I/II) [16, 17]. In addition, a strong reduction in plasma cell-associated markers such as CD38 and CD138 and a regression to a less mature B cell phenotype were observed in the EBV-infected plasmacytoid tumor cells as well as in few plasma cell myeloma cell lines examined in vitro [16]. As such, plasma cell myeloma or plasmacytoma cases that express EBV, whether as a result of an associated immunodeficiency or for unknown reasons, may follow the same path as plasmablastic lymphoma and exhibit a non-productive (latent) cycle, increase proliferation, and exhibit plasmablastic cytologic features starting in focal areas and probably eventually progressing to plasmablastic lymphoma.

Despite the fact that plasma cell neoplasms occur rarely in young individuals with the vast majority of cases occurring over the age of 50 and a median age at diagnosis of 70 years for plasma cell myeloma and 55 years for plasmacytoma (osseous and extraosseous) [2, 18], all three of our patients were under the age of 40 years at diagnosis and all three had presented with a very aggressive clinical

course including involvement of the central nervous system (CNS) in two cases. All three patients presented with morphologic evidence of a plasmablastic morphology whether within the majority of cells or within a subset of the neoplastic plasma cells. One patient was HIV positive, while the other two showed no definitive evidence of an associated immunosuppressive state. All three of our cases showed no reduction in CD138 expression and two out of the three cases were negative for EBV-LMP-1 expression by immunohistochemistry but positive for EBER by in situ hybridization, which is suggestive of a restricted EBV latency pattern, probably latency I. Based on our case series and other cases reported in the literature (Table 1), there may be indeed a notable correlation between EBV expression and the presence of plasmablastic morphologic features as well as with increased proliferative activity and a potential aggressive clinical course compared to the relatively more indolent and prolonged clinical course usually seen in EBV-negative plasma cell neoplasm cases, especially plasmacytomas. The EBV role may be through alternate pathway other than the LMP-1 protein-related neoplastic transformation. Further studies with a larger series of cases are needed to validate these findings.

Conflict of interest The authors declare that they have no conflict of interest.

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