The Plasmablasts in Castleman Disease

To the Editor

In their article, Hsi et al1 described the broad spectrum of histopathologic features of Castleman disease (CD). We would like to report one additional peculiar case, rich in human herpesvirus-8 (HHV-8)–infected plasmablasts (PBs), which we recently observed in our institution. The normal PB is a short-lived, very mobile B cell that can secrete antibodies but retains characteristics of an activated and proliferating cell.2 The PB could be considered the precursor of the more mature noncycling plasma cell (PC). Both naive and memory B cells can differentiate into PBs when activated with or without T-cell help, with or without antigen.3 The maturation of PBs in PCs is characterized by the cessation of proliferation and loss of mobility. B-lymphocyte–induced maturation protein 1 (Blimp-1)/PR domain zinc finger protein (PRdm-1) is essential for PC maturation, repressing the expression of genes that maintain B-cell identity (Pax5) and others that promote B-cell proliferation, such as Bcl6 and MYC.3 The PB is characterized by the following phenotype: CD19+, CD27++, CD38–/+ , CD20–/+, cIgM+.4 The PBs are rare in normal lymph nodes; they probably live around the mantle zone of the germinal centers, and then they exit into peripheral blood and may survive for a short period only unless they are recruited into mucosa or bone marrow niches by chemokine receptor expression.5,6 In pathology, PBs characterize only rare human disorders, most notably the microlymphomas10,11, polyclonal for the k/λ immunoglobulin (Ig) light chain. Neoformed germinal centers were colonized by a second, exceptionally abundant, HHV-8–infected PB population (CD138–, IgA–, IgM+) (Image 1D) Image 1F. Double staining for HHV-8/CD20 showed only very rare double-stained cells Image 2A1. IHC revealed complete restriction of the HHV-8+, IgM+ PBs for the λ immunoglobulin light chain Image 2B1. Epstein-Barr virus–encoded small RNA (EBER) was negative, excluding a germinotropic lymphoproliferative disorder.11 The Ki-67 proliferation index was very high in PBs (>80%); cyclin D1 and CD34 were negative. The cells were Blimp-1/PRdm-1 weakly positive.12 Double IHC staining for IgM and Myc revealed a variable and focal nuclear reactivity Image 2C1. Minimal apoptosis rate was demonstrated by phosphorylated histone H2AX (γ-H2AX) staining. Fluorescence in situ hybridization (FISH) was negative for the detection of t(8;14)(q24.1;q32). Moreover, PBs had a diploid copy of chromosomes 8 and 14 Image 2D1. The HHV-8–infected B cells in CD are usually a small fraction of the cellularity. In our case, their large number was exceptional. Some authors have proposed the term microlymphomas to describe similar cases with possible transition to overt plasmablastic lymphoma.4,13 Myc expression, which has been shown to be upregulated by HHV-8 latent nuclear antigen (LANA),14 has not been reported previously in this disease. The diploid status and the absence of Myc translocation by FISH examination were more consistent with a reactive or preneoplastic nature of the PBs, as reported by others with molecular data, such as Ig gene clonality.15 It is thought that Myc is ubiquitously expressed in proliferating cells, where it controls cellular growth pathways via transcriptional and nontranscriptional mechanisms, including ribosome biogenesis, metabolism, the cell cycle, DNA replication, and telomere maintenance.16 The upregulation of Myc, possibly via posttranscriptional stabilization by LANA,14 may be the first of multiple steps toward the development of a frank malignancy (eg, plasmablastic lymphoma or primary effusion lymphoma). As many authors have shown, transcription factors associated with B cells (Pax5) or, more specifically, germinal center markers (Bcl6) are downregulated in normal and pathologic PBs, whereas the...
A, Computed tomography showed involvement of the mesentery by pseudonodular lesions compatible with a lymphoproliferative process. B, At the histological examination, a lymphoid proliferation, partially organized in neoformed germinal centers, appeared (H&E, ×4). C, Lollipop features compatible with Castleman disease (H&E, ×20). Note the plasmablast (PB) (large cells) and plasma cell (PC) component of the lesion. D, Significant CD138+ (red)/human herpesvirus-8 (HHV-8)– mature PC population was involved in the process. HHV-8/latent nuclear antigen (LANA)+ PBs (brown) were completely CD138– (immunohistochemistry, double staining, ×10). E, High levels of IgA were detected with immunofluorescence (IF) in PCs (IF, ×20). F, Strong IF reactivity in PBs for IgM (IF, ×20).
as an expression of bone marrow involvement by CD,\textsuperscript{18} vanished after R-CHOP therapy. In conclusion, HHV-8+ PBs are highly proliferating cells with a minimal apoptosis rate and a momentary impossibility to differentiate into PCs. In our “micromodel” IHC, double stainings confirmed a sort of compartmentalization between PBs and PCs.\textsuperscript{4} The mature CD138+ PCs were attracted to the site of the CD lesions by chemotactic factors (interleukin-6), but they did not derive locally from a direct differentiation of PBs. Finally, our pattern of expression of Myc and the good clinical course of the patient should recommend caution in considering the presence of sheets of IgM-restricted plasmablasts outside of terminal differentiation program can be demonstrated with expression of Blimp-1, Xbp1, and Irf4/Mum1. However, PBs usually are completely CD138−, consistent with their transitional stage toward PCs. This is an important diagnostic key comparing other plasmablastic “proliferations,” such as CD138+ plasmablastic lymphoma. Downregulated CD20 expression among the PBs was also interesting if we consider the role of successful rituximab therapy.\textsuperscript{17} In our patient, 6 R-CHOP (rituximab-cyclophosphamide, doxorubicin, vincristine, and prednisone) cycles of chemotherapy resulted in a regression of all abdominal lesions. Moreover, a synchronous polyclonal plasmocytosis, as an expression of bone marrow involvement by CD,\textsuperscript{18} vanished after R-CHOP therapy. In conclusion, HHV-8+ PBs are highly proliferating cells with a minimal apoptosis rate and a momentary impossibility to differentiate into PCs. In our “micromodel” IHC, double stainings confirmed a sort of compartmentalization between PBs and PCs.\textsuperscript{4} The mature CD138+ PCs were attracted to the site of the CD lesions by chemotactic factors (interleukin-6), but they did not derive locally from a direct differentiation of PBs. Finally, our pattern of expression of Myc and the good clinical course of the patient should recommend caution in considering the presence of sheets of IgM-restricted plasmablasts outside of
follicles as a sufficient reason for a diagnosis of HHV-8+ large B-cell lymphoma.1

Technical Note

Buffered formalin-fixed, paraffin-embedded tissue was obtained from surgical specimens, and 3-µm sections were cut for H&E and special stains. IHC was performed after heat-mediated antigen retrieval on a DAKO autostainer (Glostrup, Denmark) using antibodies specific for CD20 (L26, DAKO), CD138 (B-B4, Serotec, Raleigh, NC), κ immunoglobulin light chain (KR-10-21-F3, DAKO), λ immunoglobulin light chain (N10/2, DAKO), IgM (polyclonal, DAKO), Pax5 (DAK-Pax5, DAKO), ki67 (MIB-1, DAKO), cyclin D1 (EP12, DAKO), CD34 (QBEnd 10, DAKO), and CD56 (C5.9, DAKO). Double staining for LANA-1 of HHV-8 (13B10, Ventana Medical Systems, Tucson, AZ) and CD20/CD138/κ immunoglobulin light chain (L26, DAKO), CD138 (B-B4, Serotec, Raleigh, NC), and CD56 (C5.9, DAKO). Double staining for LANA-1 of HHV-8 (13B10, Ventana Medical Systems, Tucson, AZ) and CD20/CD138/κ immunoglobulin light chain was performed using a Benchmark XT autostainer (Ventana Medical Systems). HHV-8 reactivity was tested with a 3,3′-diaminobenzidine (DAB) in chromogen solution, which yielded a brown color at the site of the target antigen, and counterstained with hematoxylin. CD20, CD138, and λ had a red 3-amino-9-ethylcarbazole peroxidase substrate solution as a chromogen (cytoplasmic or membrane staining). Indirect IF was performed on paraffin-embedded tissue using fluorescein isothiocyanate–conjugated IgM (polyclonal, DAKO) and IgA (polyclonal, DAKO) staining. Myc (1742-1, Y69, Epitomics, Burlingame, CA), Blimp-1 (6D3: sc-47732, Santa Cruz Biotechnology, Santa Cruz, CA), and γ-H2AX (JBW301, Millipore, Billerica, MA) staining was performed in alkaline phosphatase by nitro blue tetrazolium chloride and 5-bromo-4-chloro-3-indolylphosphate p-toluidine salt development. For the double Myc/IgM staining, DAB was used for IgM. Each antibody was tested with an appropriate positive control. EBER in situ hybridization was performed using a peptide nucleic acid probe (Histosonda, Cenbimo, Lugo, Spain). FISH was performed on formalin-fixed, paraffin-embedded sections using the MYC “break apart” DNA-FISH probe t(8;14)(q24,q32) (Thermo Scientific, Rockford, IL).

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References

The Authors’ Reply

The interesting case presented by Pagni and colleagues serves to further describe the spectrum of human herpesvirus-8 (HHV-8)–related plasmablastic proliferations in the setting of multicentric Castleman disease (MCCD). These were reviewed in the context of the 2009 Society of Hematopathology/European Association for Haematopathology Workshop on the Spectrum of Immunoproliferative Disorders and the Border Between B-Cell Lymphoma and Plasma Cell Neoplasms.1 The presented case of MCCD demonstrates an exuberant proliferation of HHV-8+ plasmablasts (IgM+, λ+, EBER–) within newly formed germinal centers in the mesenteric fat rather than within the lymph node microanatomy. Such newly formed germinal centers, which can be seen in nodal HHV-8+ MCCD in the perinodal adipose tissue but also during bone marrow involvement, do not necessarily indicate a higher risk of disease.2

As noted in our review, the biologic and pathologic features of progression from MCCD with collections of HHV-8+ plasmablasts and HHV-8+ large B-cell lymphoma have not been well characterized, and it was suggested that the presence of sheets of plasmablasts outside follicles that distort architecture might be sufficient for the diagnosis of bona fide large B-cell lymphoma.1 One objective criterion for large-cell transformation is the presence of a clonal immunoglobulin (Ig) rearrangement. The plasmablasts in MCCD are polyclonal for Ig rearrangement despite expressing monotypic Igλ light chains. So-called microlymphomas tend to be oligoclonal, whereas HHV-8+ large B-cell lymphoma is monoclonal.3 In the case of Pagni et al, the illustrations clearly show HHV-8+ plasmablasts present in collections that fulfill the description of a microlymphoma but not necessarily a large B-cell lymphoma. No IGH@-MYC or copy number abnormalities of chromosomes 8 and 14 were present, and so no positive evidence of genetic abnormalities was demonstrated. Unfortunately, no Ig rearrangement studies were performed. This information both for the whole process and for individual germinal centers would be important for assessing the clinical risk. Although a detailed clinical history is not provided, such as human immunodeficiency virus status or other prognostic features, such as performance status, the authors allude to a regression of unknown duration with immunochemotherapy.

In the context of the spectrum of HHV-8+ MCCD and HHV-8+ plasmablastic lymphomas, this case may represent the transition in which uncontrolled proliferation of these cells is beginning. However, it appears that standard treatment regimens for aggressive B-cell lymphomas were able to effectively eliminate this proliferation. Whether this is due to direct effects on the HHV-8+ plasmablasts alone or in combination with the immunomodulating effects on the background of MCCD is uncertain. Additional informative cases such as this are needed, with clinical and pathologic description and application of tools such as microdissection and gene rearrangement, or perhaps high-resolution single-nucleotide polymorphism array karyotyping or next-generation sequencing. These studies may help us define key genetic aberrations, elucidate the borders between neoplastic and nonneoplastic proliferations, and inform treatment choices.

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References