Inflammatory Pseudotumor of the Spleen Associated With a Clonal Epstein-Barr Virus Genome

Case Report and Review of the Literature

Jason T. Lewis, MD,1 Robyn L. Gaffney, MD,1* Mary B. Casey, MD,1 Michael A. Farrell, MD,2 William G. Morice, MD, PhD,1 and William R. Macon, MD1

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Abstract

We report a case of an inflammatory pseudotumor (IPT) of the spleen occurring in an 81-year-old woman with a history of a monoclonal gammopathy of undetermined significance. Eighteen-month follow-up after splenectomy demonstrated no tumor recurrence or progression of underlying plasma cell disease. Histologic examination of the tumor demonstrated a polymorphic population of inflammatory and epithelioid and spindle cells. Immunophenotyping showed large numbers of T cells, B cells, and polyclonal plasma cells. The epithelioid and spindle cells were positive for vimentin and CD68 but lacked expression of follicular dendritic cell markers and actin. Epstein-Barr virus (EBV) genome was identified in the epithelioid and spindle cell population by in situ hybridization using probes specific for EBV-encoded RNAs (EBER1 and EBER2). Southern blot analysis of digested DNA extracted from the tumor using an EBV-specific probe (XhoI) demonstrated the presence of a single high-intensity band, indicative of EBV monoclonality. While there have been 2 previous reports of hepatic IPTs containing a monoclonal population of EBV-infected tumor cells, this is the first report of such an association occurring in the spleen. The presence of clonal EBV DNA suggests some splenic IPTs may be true neoplasms.

Inflammatory pseudotumor (IPT) is a mass lesion of unknown cause found in numerous sites, such as the orbit,1 respiratory tract,2 bladder,3 lymph nodes,1-8 liver,4,5,9-11 and spleen.4,5,7,12-14 The pathogenesis of IPTs is a topic of debate and includes infectious,4,9,15 autoimmune,14 and neoplastic processes.10 Cotelingam and Jaffe12 described the first splenic IPT and believed it was nonneoplastic with an inflammatory and reparative microscopic appearance. As in other locations, IPTs of the liver and spleen are composed of a polymorphic population of inflammatory cells, including T cells, B cells, and plasma cells accompanied by plump spindle cells. However, there are differences between splenic and hepatic IPTs and those arising in other sites. For example, Epstein-Barr virus (EBV) involves splenic and hepatic IPTs more commonly than IPTs of lymph nodes, and it is the spindle cell population that is infected in the former.4,5 The exact nature of these spindle cells has not been determined, but vimentin and actin are the most common markers expressed.4,11,16,17 This has led some investigators to postulate that the spindle cells are myofibroblastic.16,17 In addition, some IPTs express follicular dendritic cell (FDC) markers, such as CD21 and CD35.4,10,18 Interestingly, 2 hepatic cases with the FDC immunophenotype possessed a monoclonal EBV genome.10,19 Therefore, some IPTs that arise within the liver may be EBV-associated FDC neoplasms.18

We describe a patient with a splenic IPT that harbored a monoclonal EBV genome, apparently localized to the FDC marker– and actin-negative spindle cell population. This provides additional evidence that a subset of IPTs, and not just those that express FDC markers, is a clonal expansion of an EBV-infected cell.
Case Report

An 81-year-old woman, who had a 10-year history of a stable IgG, \(\lambda\) monoclonal gammopathy of undetermined significance, sought care because of epigastric pain of approximately 1 month’s duration. There were no associated fevers, chills, or weight loss. Her physical examination findings were within normal limits. However, a computed tomography scan of the chest and upper abdomen, performed in the workup for her abdominal discomfort, demonstrated a 5-cm, well-circumscribed, heterogeneous mass within the spleen that had a central area of low attenuation [Image II]. Further ultrasonic characterization of the mass yielded similar findings. A subsequent nuclear medicine technetium 99–labeled RBC study excluded a hemangioma, which is the most common primary benign splenic tumor.20 An ultrasound-guided biopsy then was performed. The biopsy cores demonstrated a reactive plasma cell proliferation based on polyclonal immunoglobulin light chain staining by paraffin immunohistochemical analysis. The patient decided to undergo follow-up for the mass with serial computed tomography scans, and these showed a slight increase in its size during a 7-month interval. Therefore, open splenectomy was performed without complications. She has had no recurrence or evidence of a lymphoproliferative disorder in the 18 months after the splenectomy.

Materials and Methods

Histologic Examination and Immunophenotyping

H&E-stained sections (4 µm) of the spleen were evaluated. Initial immunophenotyping of the fresh splenic mass was performed, as previously described,21 by multicolor flow cytometry using monoclonal antibodies directed against the following antigens: CD3, CD5, CD10, CD11c, CD14, CD16, CD19, CD20, CD22, CD23, CD38, CD45, CD56, and \(\kappa\) and \(\lambda\) immunoglobulin light chains (Becton Dickinson, San Jose, CA).

Immunoperoxidase studies then were performed on 4-µm sections of paraffin-embedded splenic tissue using the following antibodies, vendors, pretreatments (if any), and dilutions: CD20 (clone L26, DAKO, Carpinteria, CA; steam in citrate buffer; 1:60), CD21 (clone IF8, DAKO; protease; 1:5), CD35 (clone Ber-MAC-DRC, DAKO; protease; 1:5), vimentin (clone 3B4, DAKO; protease; 1:500), CD45RO (clone UCHL-1, DAKO; steam in citrate buffer; 1:60), CD23 (clone BU38, The Binding Site, San Diego, CA; steam in EDTA buffer; 1:20), and clusterin (clone 41D, Upstate Biotechnology, Lake Placid, NY; steam in EDTA buffer; 1:200). These studies were performed, as described previously,22 using the labeled streptavidin-biotin detection chemistry system on a Ventana ES autostainer (Ventana, Tucson, AZ) for all antibodies except CD45RO, which required manual staining. 3-amino, 9-ethyl-carbazole was the chromogen for all reactions.

In Situ Hybridization

In situ hybridization for the detection of EBV was performed on formalin-fixed, paraffin-embedded spleen tissue using the procedure described by Chang et al.23 A digoxigenin-labeled oligonucleotide probe cocktail directed against EBV-encoded nuclear RNAs (EBER1 and EBER2) was detected by sheep antidigoxigenin coupled to alkaline phosphatase (1:100 dilution, Roche Diagnostics, Indianapolis, IN). The hybridization products were visualized after developing in 5-bromo-4-chloro-3-indolyl phosphate/nitroblue tetrazolium plus levamisole solution (10 to 40 minutes), followed by counterstaining with 0.1% nuclear fast red (3 minutes), dehydration, and coverslipping. Double-labeling immunohistochemical and in situ hybridization studies also were performed on paraffin-embedded tissues using antibodies directed against CD20, CD21, CD23, CD23, CD45RO, CD68, clusterin, S-100, vimentin, SMA, and desmin in an attempt to further define the nature of the spleen.
EBV-infected cells. In situ hybridization studies were performed first, without counterstain, followed by immunoperoxidase studies (as described in the preceding section).

Molecular Analysis

Southern blot analysis was performed using a previously described technique.24 Briefly, following extraction and purification, lesional DNA was digested with the restriction endonuclease BamHI, resolved by agarose gel electrophoresis, and transferred to a nylon membrane that then was probed with a radiolabeled DNA probe specific for EBV terminal repeat sequences (XhoI). Hybridized bands were detected by autoradiography. Polymerase chain reaction and Southern blot studies to assess for clonal T-cell receptor and immunoglobulin heavy chain (IgH) gene rearrangements were performed as outlined by Lust.25

Results

The resected 200-gm spleen contained a solitary, well-circumscribed 6.5-cm mass. The cut surface of the tumor was tan and fleshy with areas of hemorrhage and central necrosis Image 2I. The remainder of the spleen was compressed but otherwise grossly unremarkable. Microscopically, incomplete fibrous bands separated portions of the lesion from the residual normal spleen Image 3A. Most of the tumor was hypercellular but punctuated in areas by zones of sclerosis and necrosis. The cellular regions were composed of a polymorphic population of inflammatory cells, including plasma cells, lymphocytes, polymorphonuclear leukocytes, and histiocytes, some forming non necrotizing granulomas. There were other cells present that varied from epithelioid to spindle shaped with large, ovoid nuclei Image 3B.

Flow cytometric immunophenotyping of the tumor revealed large numbers of T cells associated with a smaller population of polyclonal B cells. There also was a large population of polyclonal plasma cells, confirming the
previous paraffin immunohistochemical results on the splenic core biopsy specimen. Paraffin immunoperoxidase stains confirmed a predominance of CD45RO+ T cells in the splenic tumor accompanied by scattered CD20+ B cells. The epithelioid and spindle cells were positive for vimentin and demonstrated weak staining for CD68. There was equivocal lysozyme positivity in these cells and no staining with the remaining antibodies used.

In situ hybridization revealed EBV RNA (EBER1 and EBER2) within numerous epithelioid and spindle cells throughout the tumor Image 4I. The inflammatory cells were consistently EBER-negative. The EBV RNA-positive cells were focally positive for vimentin and CD68 but negative for CD20, CD21, CD23, CD35, CD45RO, clusterin, S-100, SMA, and desmin. A single EBV-specific band was detected by Southern blot analysis, indicating the presence of a monoclonal population of EBV-infected cells Image 5I. Additional molecular genetic studies (polymerase chain reaction and Southern blot analysis) failed to reveal evidence of clonal T-cell receptor or IgH gene rearrangements.

Discussion

IPT historically has been considered a benign, tumefactive process of unknown origin. It has been found in multiple sites, including the orbit,1 respiratory tract,2 bladder,3 lymph nodes,4-8 liver,4,5,9-11 and spleen.4,5,7-12,14 Regardless of location, virtually all IPTs share certain histologic features. They are composed, in varying proportions, of plump spindle cells and a polymorphic inflammatory cell infiltrate, which includes lymphocytes, plasma cells, and histiocytes. The majority of lymphocytes are T cells, with fewer numbers of B cells.4,10,14 In addition, the plasma cells have been shown to be polyclonal.3,4,6,10,11,13

Despite histologic similarities, studies have demonstrated site-associated differences among IPTs. For example, while EBV genomes have been identified in IPTs of the lymph nodes, spleen, and liver,4,5,10,16 there is a much greater incidence of EBV positivity in splenic and hepatic IPTs compared with those occurring in lymph nodes.4,5 Not only does the frequency of EBV infection vary depending on site, but differences in the infected cell types also are apparent. Among EBV-positive hepatic and splenic IPTs, such as our case, it is the spindle cell population that is infected, whereas the virus resides in the lymphocytes of EBV-positive nodal cases.4,5,10,18 Furthermore, as in our case, the concentration of EBV within IPTs is much higher than in surrounding tissue, providing indirect evidence that EBV infection has an important role in the pathogenesis of some IPTs.4,5,10,18

The nature of the spindle cell population in IPTs has been debated. Menke et al26 determined that many of the spindle-shaped fibroblast-like cells in nodal IPTs were probably monocyte derived because of their expression of CD68 or HAM-56. Neuhauser et al16 examined 12 splenic IPTs and found that SMA was the most commonly expressed marker (10/11 cases), followed by vimentin (7/10 cases). They also identified 2 of 10 cases that were positive for CD21, a
folicular dendritic cell marker.16 This supported the findings of Arber et al4 who, by using double-labeling studies, were the first to report a splenic IPT that contained EBER1-positive spindle cells that also expressed FDC markers. Selves et al10 subsequently reported a hepatic IPT that contained spindle cells positive for EBV and FDC markers.

The possible role of follicular dendritic cells in IPTs was further suggested by Cheuk et al.18 They described 11 FDC tumors that had the histologic appearance of an IPT, such as a spindle cell background and a predominant inflammatory component.18 All tumors showed positive staining for at least 1 FDC marker, and all showed labeling for EBV by situ hybridization. They postulated that some IPTs arising in the liver or spleen are actually EBV-associated follicular dendritic neoplasms and suggested that the term inflammatory pseudotumor-like follicular dendritic cell tumor might be a more appropriate designation for this group.27 However, they noted that some IPTs lacked EBV and FDC markers.27 Other investigators also have described splenic and hepatic IPTs that were negative for FDC markers, whether or not positive for EBV.4,5,16 In agreement with this apparent phenotypic variability, the EBV-infected epithelioid and spindle cells in our case were positive for vimentin and CD68 but negative for multiple other markers, including CD21, CD23, CD35, SMA, and desmin.

It has been theorized that follicular dendritic cells are of mesenchymal (stromal) cell origin,28 as are fibroblasts and myofibroblasts.29 Because of the variable immunophenotypic patterns seen in hepatic and splenic IPTs, it is possible that they arise from a common mesenchymal cell that is capable of differentiating along different pathways.

Figure 1. The majority would develop a myofibroblastic phenotype and be positive for SMA and vimentin. Others would acquire FDC characteristics and express CD21 and CD35. The final category, represented by our case, would remain less differentiated and express only mesenchymal and histiocytic markers, such as vimentin, CD68, and lysozyme.

Regardless of the exact nature of the spindle cells, we regard the presence of clonal EBV DNA as the most important finding in the IPT case reported herein. There have been 2 previous reports of clonal EBV infections in hepatic tumors with the histologic appearance of IPTs.10,19 However, both of those expressed the FDC markers CD21 and CD35. Our case, therefore, is the first splenic IPT with clonal EBV that lacked FDC markers. The presence of a clonal EBV genome suggests that the epithelioid and spindle cells within the tumor arose from a single infected cell, with concurrent expansion of that cell’s EBV genome.24 This phenomenon has been described in other neoplastic processes, such as Burkitt lymphoma30 and nasopharyngeal carcinoma.24 Thus, the finding of clonal EBV DNA in a splenic IPT adds further support for their being true neoplasms, possibly of mesenchymal origin, rather than reactive or reparative processes. A similar concept was proposed for the FDCs in a case of hyaline vascular Castleman disease by the recent demonstration of clonal high mobility group protein I-C (HMGIC) gene rearrangements within CD21+ cells.31

From the Departments of 1Pathology and 2Radiology, Mayo Clinic, Rochester, MN.

Address reprint requests to Dr Macon: Dept of Pathology, Mayo Clinic, 200 First St SW, Rochester, MN 55905.

* Dr Gaffney has relocated to the Department of Pathology, University of New Mexico Health Sciences Center, Albuquerque.

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