Splenic Epstein-Barr Virus–Associated Inflammatory Pseudotumor

Chris Van Baeten, MD; Jo Van Dorpe, MD, PhD

- Splenic inflammatory pseudotumor (IPT) is an uncommon lesion with an inflammatory morphologic aspect that often poses a diagnostic challenge. The etiology of IPT can be infectious, autoimmune, reactive, or neoplastic. Splenic Epstein-Barr virus (EBV)–associated IPTs form a subset of splenic IPTs in which there is a spindle cell component infected by EBV. The best characterized and most frequent subgroup of splenic EBV-associated IPT is IPT-like follicular dendritic cell tumor. This review also focusses on EBV-associated splenic IPTs without follicular dendritic cell marker expression. These lesions are less well characterized, making the differential diagnosis with other splenic lesions even more difficult. Recently, increased numbers of immunoglobulin G4–positive plasma cells and the presence of numerous granulomas have been reported in EBV-associated IPTs, and this can add to the difficulties in recognizing the neoplastic nature of these lesions. Herein, we also review the epidemiology, clinical features, histologic morphology, immunohistochemistry, electron microscopy, and pathogenesis of EBV-associated IPTs.

Inflammatory pseudotumor (IPT) is a mass lesion of uncertain etiology that occurs in multiple anatomic sites. Inflammatory pseudotumor of the spleen is an uncommon lesion.1,2 Regardless of location, IPTs share certain histologic features. They are composed, in varying proportions, of spindle cells and a polymorphic inflammatory infiltrate that includes lymphocytes, polyclonal plasma cells, and histiocytes.2,3

The histologic definition of IPT encompasses a wide spectrum of lesions, including cases with an infectious, autoimmune, reactive, or neoplastic etiology. One pathogen known to be associated with IPT is the Epstein–Barr virus (EBV).1,2 Epstein–Barr virus–associated IPTs are distinct from conventional IPTs in that they occur predominantly in the spleen, liver, and lymph nodes. Not only does the frequency of EBV infection vary depending on site, but also there are differences in the infected cell types.1 Among EBV-associated splenic and hepatic IPTs it is the spindle cell component that is infected, whereas the virus preferentially affects the lymphocytes of EBV-associated nodal cases. The percentage of splenic and hepatic IPTs that are EBV positive is not well known. Some studies have reported the percentage of splenic EBV-positive IPTs to be as high as 60% to 67% of splenic IPTs.1,2

The best characterized and most frequent subgroup of splenic and hepatic EBV–associated IPTs is IPT-like follicular dendritic cell (FDC) tumor. To date, 32 cases of splenic IPT-like FDC tumor have been reported in the English-language literature.1,3–16 Very rare cases of IPT-like FDC tumor have been reported outside the spleen or liver.4,17 Inflammatory pseudotumor–like FDC tumor differs from conventional FDC sarcoma by almost exclusive localization in spleen and liver, frequent presence of systemic symptoms, indolent clinical behavior, an IPT-like appearance, and consistent association with EBV.4,9,16

A less frequent subgroup of splenic EBV–associated IPTs includes IPT-like lesions that are EBV associated, but in which the spindle cell component does not express FDC markers. This subgroup is less well defined than IPT-like FDC tumor. Fifteen well-documented cases have been reported in the spleen.1,5–7,18–23 The phenotype of the spindle cells in splenic EBV–associated IPT not expressing FDC markers is heterogeneous and controversial. Lesions with a presumed fibroblastic,1 myofibroblastic (“splenic inflammatory myofibroblastic tumor”),1,2,20,21 fibroblastic reticular cell (FBRC),17 or “fibrohistiocytic” phenotype have been reported.2,3,18,19,23 However, given a close relationship between fibroblasts, myofibroblasts, FBRCs, and FDCs with regard to their origin, differentiation, and transformation,3,9,17,24 it may well be possible that IPT-like FDC tumors and FDC marker–negative EBV–associated IPTs represent phenotypic subgroups of the same entity.

Epidemiology

Splenic IPT-like FDC tumor is a very rare tumor, estimated to account for less than 1% of all benign or malignant primary splenic tumors.2 It occurs exclusively in adult patients, at a mean patient age of 59.5 years and a median age of 57.5 years, ranging from 39 to 79 years. There is a slight female predominance (male to female ratio of 15:17). The female predominance is less marked than for IPT-like FDC in the liver.12 Although precise information regarding the race of the patients in the reported cases is not always available, there is a possibility that the tumor has a...
racial or geographic predominance, because more than 80% (26 of 32) of cases are reported from Eastern Asia (China, Japan, and Korea).

Splenic EBV-associated IPTs that are FDC marker negative are even more uncommon. The epidemiologic data are comparable with those from splenic IPT-like FDC tumor. Patient age ranges from 24 to 87 years (mean age: 61 years; median age: 66 years). In this phenotypic subgroup, 33% (5 of 15) of cases are reported from Eastern Asia.

**CLINICAL FEATURES**

As many as 50% of splenic EBV-associated IPTs are found incidentally on imaging obtained for other indications. The most common complaint is left upper quadrant or epigastric pain. Epstein-Barr virus–associated IPTs are sometimes accompanied by systemic symptoms such as weight loss, malaise, fatigue, and fever. There are no specific markers to identify EBV-associated IPT, but anemia, elevated C-reactive protein levels, polyclonal hypergammaglobulinemia, and elevated serum levels of soluble interleukin-2 receptor have been reported in several studies.15,18

On imaging, splenic EBV-associated IPTs are often circumscribed, nodular lesions. Epstein-Barr virus–associated splenic IPTs have been reported to be hypoechogenic or anechoic sonographically.6,15,16 They are without distinguishing magnetic resonance imaging or computed tomography (CT) imaging features.7,14,16,22 Epstein-Barr virus–associated splenic IPTs appear hypermetabolic on fluoro-deoxyglucose F 18 positron emission tomography integrated with CT.10,13,14,22 Lymphoma and metastasis, the most important preoperative malignant differential diagnoses, are indistinguishable from EBV-associated IPT by imaging. The diagnosis of splenic EBV-associated IPT is typically only made after splenectomy.

**GROSS PATHOLOGY**

Grossly, splenic EBV-associated IPTs usually present as solitary, well-circumscribed, firm and tan or yellow-white masses (Figure 1, A). Tumors range in size from 1.5 to 22.3 cm, with a mean of 7.3 cm and a median of 5.8 cm in greatest diameter. Areas with necrosis, hemorrhage, or sclerosis may be macroscopically visible. Prominent calcification is not a feature of splenic EBV-associated IPT, but small calcifications have been reported.14,16 No remarkable abnormalities are found in the surrounding areas of the spleen.

**HISTOPATHOLOGY**

Histologically, EBV-associated IPTs are well demarcated from the surrounding splenic parenchyma, with a complete or incomplete fibrous capsule (Figure 1, B). They have the morphologic characteristics of splenic IPTs in general. Loosely aggregated or dispersed ovoid to spindle-shaped cells are admixed intimately with abundant lymphocytes and plasma cells, but they are sometimes difficult to identify among the chronic inflammatory cells (Figure 1, C). They have a moderate amount of pale to faintly eosinophilic cytoplasm with indistinct cell borders. The cell nuclei are elongated to oval, with small or more distinct nucleoli, vesicular chromatin, and thin smooth nuclear membranes (Figure 1, D). Binucleate or multinucleate spindle cells are occasionally present.4,15 Mitotic figures are usually very rare. Some EBV-associated IPTs show a population of spindle cells with larger irregular nuclei, coarsely condensed chromatin, and multiple, large eosinophilic nucleoli.4,9,16 In rare cases, Reed-Sternberg–like tumor cells are present.4 In some cases, focal, vague fascicles or storiform arrays formed by spindle cells are present. Very rare cases of IPT-like FDC tumor contain a component that is morphologically indistinguishable from conventional FDC tumor, with compact syncytial tumor cells exhibiting a storiform growth pattern and lightly sprinkled with small lymphocytes.4

All splenic EBV-associated IPTs contain a dense lymphoplasmacytic infiltrate, sometimes with the formation of small lymphoid follicles. They often contain areas where the lymphoid cells are so densely packed and the spindle cells so inconspicuous that the possibility of low-grade lymphoma must be considered. Foamy histiocytes are sometimes present. The presence of multinucleated giant cells and epithelioid granulomas is a common finding (Figure 1, C). Rare cases of EBV-associated IPT show extensive coalescent epithelioid granulomas, raising the possibilities of an infective process or sarcoidosis.8,9 Scattered neutrophils or eosinophils are focally present in some tumors. Very rare cases show focally aggregated eosinophils or eosinophilic abscesses.9,16

Within EBV-associated IPTs, there are often areas of lower cellularity. Most tumors show interspersed necrotic and/or hemorrhagic foci. Necrosis can be extensive in large tumors. Some cases show an important degree of hyalinized fibrosis. A feature that is often reported is the presence of blood vessels exhibiting ectasia and deposition of fibrinoid material in the wall. Sometimes, aggregations of these blood vessels can be reminiscent of cavernous hemangioma.4,9,16

**IMMUNOHISTOCHEMISTRY, IN SITU HYBRIDIZATION, AND DOUBLE-LABELING STUDIES**

On immunohistochemistry, a variable proportion of the spindle cells in IPT-like FDC are positive for at least 1 of the FDC antigens, including CD21, CD35, and CD23, although the staining can be patchy and focal. The staining for the FDC markers CD21, CD35, and CD23 is usually membranous and sometimes highlights the delicate interconnecting processes of the tumor cells. Other FDC markers that have been reported positive in this tumor are clusterin, CNA.42, γ-synuclein, and D2–40.1,5,8,15 In most studies CD35 appears to be the most sensitive and reliable marker for this tumor.8,12,16 The spindle cells are often also positive for smooth muscle actin (SMA), but negative for desmin, caldesmon, CD31, CD34, S100, anaplastic lymphoma kinase (ALK), and CD30.

Splenic EBV-associated IPTs that do not stain for FDC markers form a more heterogeneous phenotypic subgroup.1–3,7,18–23 The spindle cells in this subgroup show expression of vimentin, SMA (Figure 1, E), and/or CD68, suggesting fibroblastic, myofibroblastic, or histiocytic differentiation. In 1 reported case, there was positivity for SMA and keratin, which could suggest an FBRC origin.21

The background lymphocytes in EBV-associated IPT are predominantly CD3+ T cells, admixed with fewer CD20+ B cells.4,6,13,14,18,19,21,22,24,25,28–30 Choe et al40 reported a series of 6 cases of IPT-like FDC tumor with markedly increased immunoglobulin ( Ig) G4–positive plasma cells ranging from 27 to 128 cells per high-power field. The ratio of IgG4–positive cells to IgG–positive cells in these cases was 25% to 75%.

The nuclei of the spindle tumor cells in EBV-associated IPTs are positive for EBV-encoded small RNA (EBER) by in
Figure 1. Macroscopic, microscopic, and immunohistochemical features, and in situ hybridization (ISH) of a case of Epstein-Barr virus (EBV)–associated inflammatory pseudotumor. A, Gross image showing a well-circumscribed subcapsular lesion. B, The tumor (right) is sharply demarcated from the uninvolved splenic parenchyma (left) by a thin fibrous capsule (arrowheads). C, A dense lymphoplasmacytic infiltrate obscures the spindle cell component. The inflammatory infiltrate shows some multinucleated histiocytes (long arrows) and plasma cells containing Russell bodies (short arrows). D, High magnification demonstrating the spindle cell component. The spindle cells have indistinct cell borders and contain an oval to elongated nucleus with vesicular chromatin and a distinct nucleolus. The cell nuclei are slightly irregular. E, The spindle cell component stains strongly for smooth muscle actin (SMA). In this case, staining for CD21, CD23, and CD35 was negative (not shown). The SMA-positive cells have processes that form a reticular network between inflammatory cells. F, There is strong EBV positivity in the tumor (right), but not in the uninvolved splenic parenchyma (left) (hematoxylin-eosin, original magnifications ×50 [B], ×400 [C], and ×1000 [D]; original magnification ×400 [E]; EBV-encoded small RNA ISH, original magnification ×50 [F]).
situ hybridization (ISH), whereas the inflammatory cells in the background are negative (Figure 1, F). More than 70% of cases show focal or diffuse cell membrane staining for latent membrane protein (LMP) 1 in the tumor cells.4,8,9,16 Immunostaining for human herpes virus 8 is negative in splenic EBV-associated IPT.8

Double-labeling studies with ISH for EBER and an antibody against a marker for FDCs, SMA (Figure 2, A), or CD20 (Figure 2, B) can be of help to determine the specific cell type involved by EBV.

***ELECTRON MICROSCOPY***

Ultrastructural examination of the spindle cell component of IPT-like FDC tumors shows long, interweaving cytoplasmic processes, which are focally joined by desmosome-like junctions, consistent with FDC differentiation.12,25 Electron microscopic features of splenic EBV-associated IPTs without expression of FDC markers have not been reported.

***PATHOGENESIS***

Splenic EBV-associated IPTs show strong expression of EBER in the spindle cell component, but not in the inflammatory cells in the background or in the surrounding tissue, providing indirect evidence that EBV infection has an important role in its pathogenesis. Southern blot analysis showed monoclonal EBV genomes in 2 cases of IPT-like FDC tumor of the liver25,26 and in 1 case of FDC marker–negative EBV-associated IPT in the spleen,3 indicating that the EBV infection occurred before the monoclonal proliferation of the neoplastic cells and that EBV-associated IPT is a true neoplasm.

Epstein-Barr virus–encoded LMP1, which has been found to have an oncogenic role, is positive in more than 70% of cases of IPT-like FDC tumor by immunohistochemistry.4,8,9,16 Epstein-Barr virus genomes with a 30-base-pair deletion or point mutations in exon 3 of the LMP1 gene were found in 3 cases of IPT-like FDC tumor.5,25,27 These alterations are more prevalent in Asia.15 However, at the moment, it is not clear whether this is the reason why most EBV-associated IPT cases are reported from Asia. The alterations in exon 3 of the LMP1 gene do not seem to enhance the tumor-promoting activity of LMP1.5,25,27 CD21 expressed on FDCs is a well-known EBV receptor, and EBV can transform FDC cell lines in vitro.25 However, the role of CD21 in the pathogenesis of EBV-associated IPT is not completely clear, as in an important part of EBV-associated IPTs CD21 is not demonstrable immunohistochemically.

The origin of EBV-associated IPTs remains controversial. It has been hypothesized that splenic EBV-associated IPTs arise from a common mesenchymal cell and differentiate along different pathways.3,8,9,17,24 This process can occur via an FDC lineage expressing CD21, CD23, and CD35, or rarely via a myofibroblastic (or FBRC) lineage expressing vimentin and SMA. Rare cases have been reported that show another line of differentiation displaying only mesenchymal and histiocytic markers such as vimentin and CD68. Given a close relationship between fibroblasts, myofibroblasts, FBRCs, and FDCs with regard to their origin,24 it should not be surprising to see overlapping or hybrid phenotypic features of these cells in different lesions or within the same ones.

There is no clear association between splenic EBV-associated IPT and impaired host immunity. Concomitant malignancy, most commonly carcinoma (breast carcinoma [n = 2]27,28; gastric carcinoma [n = 2]7,8; EBV-positive gastric carcinoma with lymphoid stroma [n = 1]5; rectal adenocarcinoma [n = 1]9; and diffuse large B-cell lymphoma [n = 1]8) has been reported in 16.3% of splenic EBV-associated IPT cases. We have seen a case of splenic EBV-associated IPT complicating chronic lymphocytic leukemia.

Some studies report an important CD8+ lymphocytic infiltrate in splenic EBV-associated IPT,15,25,27 which may reflect an immune response against an LMP1 epitope, as LMP1 is one possible target to elicit a cytotoxic T-cell reaction. However, the exact role of EBV and of the EBV-infected neoplastic spindle cells in the IPT-like aspect of EBV-associated IPT is not known.
DIFFERENTIAL DIAGNOSIS

The morphologic differential diagnosis of EBV-associated IPT is broad and includes inflammatory or reactive processes, and benign and malignant tumors. The easiest way to demonstrate the neoplastic cells in EBV-associated IPT is by ISH for EBER, which may be used as the first-line investigation.\(^4\) In a next step, a meticulous examination for an immunophenotype is needed for an interpretation of IPT-like tumors with EBV-positive spindle cells. To demonstrate an FDC lineage, a panel of markers (including CD21, CD35, clusterin, CAN.42, CXCL13, and D2-40) rather than sole markers should be included in the immunostaining, as IPT-like FDC tumors often show only focal or weak FDC marker immunopositivity.\(^9\) The differential diagnosis is even more challenging when the EBV-associated IPT lacks the typical immunophenotypic features of an IPT-like FDC tumor. Double-labeling studies with EBER ISH and an antibody against an FDC marker, SMA, CD3, or CD20 can help demonstrate the immunophenotype of the cells infected by EBV in difficult cases.

When an IPT is suspected, inflammatory and infective processes should be considered as a potential differential diagnosis, including mycobacterial spindle cell pseudotumor, IgG4-related disease; sarcoidosis; granulomas associated with altered immune function; and bacterial, fungal, protozoal, and parasitic infection.

Mycobacterial spindle cell pseudotumor is a rare localized lesion that can occur in the spleen in patients who are immunocompromised, particularly following solid organ transplantation and in those with AIDS.\(^3,32\) This lesion may be caused by tuberculous and nontuberculous Mycobacterium. Morphologically, it is composed of proliferating CD68\(^+\) spindle cells staining strongly for acid-fast bacilli.

Recently, a significant increase in IgG4-positive plasma cells has been described in a series of 6 cases of splenic IPT-like FDC tumor.\(^8\) Morphologic features suggestive of IgG4-related disease, such as a dense plasmacytic infiltrate and a storiform growth pattern, are also commonly seen in splenic EBV-associated IPT. Obliterative phlebitis can also be present in EBV-associated IPT.\(^8\) Granulomas and multinucleated giant cells, necrosis, and neutrophilic granulocytes are usually not features of IgG4-related disease and can be used in the differential diagnosis with EBV-associated IPT.\(^28\) The diagnosis of IgG4-related disease should probably only be rendered in the spleen after exclusion of EBV-associated IPT.

Epstein-Barr virus–associated IPT with extensive epithelioid granulomas can raise the possibilities of sarcoidosis, granulomas associated with altered immune function (chronic uremia, severe combined immunodeficiency, and selective IgA deficiency), and infections, including bacterial infection (chronic granulomatous disease, mycobacterial infection, tertiary syphilis, and brucellosis), fungal infection (histoplasmosis and blastomycosis), protozoal infection (leishmaniasis and toxoplasmosis), and schistosomiasis.\(^9\) Stains for microorganisms, including Ziehl-Neelsen, diastase–periodic acid-Schiff, and Grocott methenamine silver, should be performed to exclude these possibilities.

Splenic EBV-associated IPT should also be differentiated from inflammatory myofibroblastic tumor (IMT), Hodgkin lymphoma, interdigitating dendritic cell sarcoma, conventional FDC sarcoma, sclerosing angiomatoid nodular transformation of the spleen (SANT), lymphoplasmacytic lymphoma, EBV-positive lymphoproliferative disorder, and EBV-associated smooth muscle tumor.

In the series from Chen et al,\(^16\) 4 of 10 cases (of hepatic and splenic IPT-like FDC tumors) were initially misdiagnosed as IMT, owing to the remarkable inflammatory background obscuring the scanty tumor cells, and the tumor cells being positive for SMA. However, ALK is positive in about 50% of IMTs (of soft tissue), but negative in EBV-associated IPT. Most importantly, EBER expression is always negative in IMT.

Occasionally, the presence of Reed-Sternberg–like cells or of some CD30\(^+\) immunoblastic cells in a chronic inflammatory background can raise the differential diagnosis of Hodgkin lymphoma.\(^4,16\) Some cases of Hodgkin lymphoma may be accompanied by EBV infection. However, in Hodgkin lymphoma the tumor cells express CD30 and PAX5, and often CD15, and the number of EBV-infected cells is usually lower than in splenic EBV-associated IPT.

Interdigitating dendritic cell sarcoma shares some morphologic features with IPT.\(^2,16\) However, interdigitating dendritic cell sarcoma usually has a less dense background inflammatory infiltrate, is SI00 positive, and is consistently EBV negative. The differentiation between IPT-like FDC tumor and conventional FDC sarcoma is also important, because the biological behavior of conventional FDC sarcoma appears to be more aggressive than that of IPT-like FDC tumor.\(^4,9,16\) Inflammatory pseudotumor–like FDC tumors have much more inflammatory infiltrate in the background. In contrast, most conventional FDC sarcomas are composed of numerous tumor cells admixed with a sprinkling of lymphocytes. Very rare cases of IPT-like FDC tumor contain areas that are morphologically indistinguishable from conventional FDC tumor.\(^4\) However, conventional FDC sarcomas are usually not associated with EBV infection.\(^4,22\)

SANT can closely mimic EBV-associated IPT.\(^8\) It is a benign, probably reactive lesion composed of angiomatoid (vascular) nodules surrounded by sclerotic stroma, fibroblasts, myofibroblasts, and inflammatory cells (plasma cells, lymphocytes, macrophages, and siderophages). Expression of FDC markers is not a feature of SANT, but very rare cases have been described in which there was positivity for EBER in spindle cells expressing SMA.\(^29\) It is possible that these very rare cases of SANT may represent transformation of an EBV-associated IPT.

Given the presence of a dense lymphoplasmacytic infiltrate and the frequent bland appearance of the neoplastic spindle cells in EBV-associated IPT, lymphoplasmacytic lymphoma should be considered in the differential diagnosis, but can be excluded by demonstrating light-chain restriction.

Age-related EBV-associated lymphoproliferative disorder can present as a polymorphous infiltrate containing numerous plasma cells and Reed-Sternberg–like cells.\(^8\) A rare case of splenic IPT has been described with an important granulomatous reaction and the presence of EBER in lymphohistiocytic cells.\(^30\) In these cases, the application of various FDC markers is essential. Care should be taken in the interpretation of these stains, because some of these lesions could harbor a residual FDC network. Double-labeling studies can be of help in these difficult cases.

Epstein-Barr virus–associated smooth muscle tumor occurring in immunocompromised hosts is another Epstein-Barr virus–positive lesion, but EBV-associated IPT
differences in the lack of spindle cells with eosinophilic cytoplasm and distinct cell borders (characteristic of smooth muscle cells), the presence of a heavy chronic inflammatory infiltrate, and the absence of staining for desmin.\textsuperscript{3,4,5}

TREATMENT AND PROGNOSIS

Epstein-Barr virus–associated IPTs are slow-growing lesions, and their prognosis is usually excellent.\textsuperscript{1,4-6} Total splenectomy is the mainstay of therapy without need of adjuvant therapy. Relapse from splenic EBV-associated IPT is exceptionally rare. Three cases with concurrent splenic and hepatic IPT-like FDC tumor have been reported.\textsuperscript{5,9,13} In 1 of these cases, the liver lesions were considered metastases from a large splenic IPT-like FDC tumor.\textsuperscript{10} This patient died at 2 months follow-up. Recurrences and metastases (including peritoneal dissemination and metastases in lymph nodes) from hepatic IPT-like FDC tumors have been reported more frequently (in about 25% of cases).\textsuperscript{9,12} Therefore, splenic IPT-like FDC tumors (and splenic EBV-associated ITPs in general) are considered low-grade malignant lesions and surveillance is currently suggested after resection. This is in contrast to splenic conventional EBV-negative IPT, which is considered a benign entity. However, IPT-like FDC tumors follow a more indolent course and are associated with longer survival than conventional FDC sarcomas.\textsuperscript{4,9,16}

CONCLUSIONS

Splenic IPT-like FDC tumors and EBV-positive IPTs without expression of FDC markers have in common that it is the (neoplastic) spindle cell component that is infected by EBV. They are probably immunophenotypic variants of the same entity and can be placed in the broader category of splenic EBV-associated ITPs. The pathogenesis of these lesions is not completely understood, but they are probably of mesenchymal origin. Epstein-Barr virus–associated ITPs, whether positive or negative for FDC markers, should be clinically evaluated, treated, and followed up in the same manner.

References