Multiple Intestinal Ulcers Associated with Primary Epstein-Barr Virus Infection in a Patient with Rheumatoid Arthritis Undergoing Methotrexate Therapy

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Abstract

A 47-year-old woman with a 2-year history of rheumatoid arthritis (RA) undergoing methotrexate treatment developed a perforated ulcer in the ileum for which she underwent emergency surgery. A histological analysis of the extirpated specimen presented a possible Epstein-Barr virus (EBV) infection in the ulcerative lesion without a feature of lymphoproliferative disorder. Interestingly, the patient’s serological tests with a paired serum diagnosed a primary EBV infection. The present case emphasizes the importance of being aware of severe enteritis as a possibility for patients with RA, for an accurate diagnosis.

Key words: Epstein-Barr virus, intestinal ulcer, methotrexate, rheumatoid arthritis

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Introduction

Methotrexate (MTX) is an effective immunosuppressive agent widely administered to patients with rheumatoid arthritis (RA) as a first line drug. Among the adverse effects of chronic treatment with low-dose MTX, such as bone marrow suppression, liver damage, and interstitial pneumonitis, lymphoproliferative disorders (LPDs) have recently been well-recognized. Epstein-Barr virus (EBV) infection is thought to play a role in development of MTX-LPDs. MTX can activate the release of infectious EBV from latently infected cell lines in vitro, and patients treated with MTX had significantly higher mean EBV loads in their blood than patients treated with immunosuppressing regimens that did not include MTX. On the other hand, patients with RA have been shown to have an impaired immune response to EBV and a higher systemic EBV load than healthy individuals. T cells from patients with RA seemed to be less competent in controlling the outgrowth of EBV-infected B cells. Moreover, patients with RA have more EBV-infected B cells than normal individuals. Despite the higher incidence of EBV infection in patients with RA either with or without MTX-treatment, manifestations related to EBV infection other than LPD have not been extensively documented. We herein describe a severe intestinal ulcer associated with a primary EBV infection that is histologically distinct from LPD in a patient with RA undergoing low-dose MTX treatment.

Case Report

A 47-year-old woman with a 2-year history of RA was referred to the emergency outpatient unit due to lower abdominal pain and a fever. She had been receiving MTX treatment for 2 years, starting at 6 mg/wk with increased...
Figure 1. Contrast enhanced computed tomography of abdomen showing an intestinal abscess (arrows).

Figure 2. The extirpated specimen showing an edematous ileum mucosa with longitudinal ulcers. There was a perforation at the bottom of the longitudinal ulcer surrounded by an abscess (***). Two longitudinal ulcers were found on the anal side of the abscessed fistula (*).

dose increments to 10 mg/wk, which successfully lowered disease activity [DAS28 C-reactive protein (CRP); 2.67] in a year. The same amount of MTX was continued for the maintenance of the patient’s controlled state of RA. Before the onset of her severe abdominal pain, she only had occasional epigastric distress, especially on the day of administration of MTX. She temporarily used non-steroidal anti-inflammatory drugs (NSAIDs) for pain control. The total amount of administrated MTX during the patient’s visit to the emergency unit was 1,018 mg. No other anti-rheumatic drugs or immunosuppressants, including steroids, were used.

The physical examination revealed mild tenderness on the patient’s left lower abdomen quadrant without abdominal guarding, however, a small tumor was palpable in the same region. She had a body temperature of 38.5°C. Laboratory studies showed an elevated white blood cell count (21,420/μL) with neutrophilia (19,320/μL) and monocytosis (1,390/μL), mild anemia (Hb 11.2 mg/dL), and increased CRP (11.7 mg/dL). Liver and kidney function and other major biochemical tests were within normal limits. Contrast-enhanced computed tomography (CT) scan showed slight peritoneal effusion, thickening of the intestinal wall, swelling of mesenteric lymph node, and an abscess in the ileum adherent to the omentum (Fig. 1). Despite her admission and subsequent initiation of systemic antibiotics, her symptoms did not improve. The patient’s CT scan demonstrated deterioration of inflammation around the intestinal abscess the next day and then she underwent emergency surgery. An abscess adhered to the greater omentum and sigmoid colon was found in the middle of the ileum. There were two thickened wall regions in the ileum on the anal side of the abscess. The affected region of the ileum, abscess, and omentum were resected all together. The extirpated specimen showed an edematous ileum mucosa with longitudinal ulcer (Fig. 2). There was a perforation at the bottom of the longitudinal ulcer that was surrounded by an abscess. Besides this, two longitudinal ulcers were found on the anal side of the abscessed fistula (Fig. 2).

Histological examination of the perforated region revealed hemorrhagic necrosis with diffuse lymphoid cell infiltration and accumulation of neutrophils and eosinophils that extended to the serosa (Fig. 3a, b). Most of the lymphoid cells were small or intermediate in size without nuclear atypia and no lymphoid follicles were formed (Fig. 3a, c). Mature-appearing plasma cells and histiocytes were also present (Fig. 3d). Similar histological appearances were found also in the two longitudinal ulcer regions at the anal sites. Elastic van Gieson staining demonstrated that the elastic fibrils in the arteries were intact (Fig. 4a). Any occlusion of the vessels by fibrin thrombi or the infiltration of inflammatory cells to the vessel walls were not observed, indicating that severe vasculitis was not likely present in the affected area (Fig. 4). Immunohistochemical analyses demonstrated that infiltrative lymphocytes consisted of mixed T and B cells expressing CD3, CD20, and CD79a. The lymphocytes were negative for CD5, CD10, CD30, and Bcl-2, thereby removing any impression of a LPD.

A portion of inflammatory cells was positive for EBV latent membrane protein 1 (LMP1). EBV early RNA (EBER) was strongly detected in the plasma cell-like population by in situ hybridization (Fig. 5a), indicating a presence of EBV infection. The EBER-positive cells were found from the lamina propria to the subserosa and surrounded some glands and vessels (Fig. 5b, c). In contrast, in the unaffected area of intestinal glands or in the area of serositis without ulceration, EBER-positive cells were barely detected (Fig. 5d, e). Immunostaining for the presence of cytomegalovirus was not evident.

Given the accumulation of EBV-positive cells in the ulcerative lesion in the ileum, serological examination for EBV infection was performed at 2 weeks post-surgery. The patient showed positivity for serum IgM antibody (10×) and IgG (160×) against EBV viral capsid antigen (EBV-VCA). Serum IgG antibody to EBV nuclear antigen (EBV-EBNA) and to EBV early antigen-diffuse and restricted (EBV-EA-DR) were negative. In the convalescent serum at 8 weeks post-surgery, EBV-VCA IgM antibody became negative.
while the EBV-VCA IgG antibody titer increased to 320x. EBV-EBNA IgG and EBV-EA-DR IgG were still negative, indicating that the patient had a primary infection of EBV.

**Discussion**

We herein describe EBV-associated multiple intestinal ulcers that developed in a patient with RA undergoing low-dose MTX treatment. The histological findings were different from EBV-associated LPD, which are often linked to MTX treatment.

EBV-positive mucocutaneous ulcer (EBVMCU) is a recently described clinicopathological entity secondary to either age-related immunosuppression or to the use of immunosuppressive agents including MTX (8). Patients with EBVMCU show circumscribed ulcerative lesion with indolent progression on the skin, oropharynx, or gastrointestinal tract. The pathognomonic histology of EBVMCU comprises of ulcers and polymorphous mixed lymphocytic infiltrates including atypical large B-cells and Reed-Sternberg-like cells uniformly expressing CD30 with EBER positivity, thus EBVMCU is considered as a clinical subtype of EBV-associated LPD (8). A recent study analyzing MTX-associated LPD (MTX-LPD) occurring in 20 patients with RA and 1 patient with polymyositis demonstrated that EBVMCU was found in 24% of MTX-LPD and in 42% of EBV-positive MTX-LPD, indicating that EBVMCU constitutes a certain fraction of MTX-LPD (9). However, in the present case, atypical large B cells or CD30-positive Reed-Sternberg-like cells and other types of immunoblastic cells were not found in ulcerative lesion, suggesting a pathogenesis distinct from that of EBVMCU underlies the perforated
ulcer formation in the ileum. Similar to the present case, although the number of the reports is small, cases with EBV-associated gastro-enteritis forming multiple ulcers without the features of LPD have been reported both in immunocompetent and immunoincompetent adults (10-15). It is not clear how EBV infection causes ulcer formation in the gastrointestinal tract. Tashiro et al. reported multiple ileum ulcers in a case with immunosuppression therapy given after allogenic stem cell transplantation (15). Histological analysis revealed EBER-positive cells surrounded some glands in the ileum mucosa and occasionally infiltrated into the glandular epithelium forming lymphoepithelial-like lesions, which are suspected to be the cause of erosions and ulcerations (15). In addition, in the area of severe inflammation, many vessels had narrow or occluded lumens, suggesting that an ischemic environment might accelerate ulcer formation (15). In accordance with this report, the present case showed an infiltration of EBER-positive cells around the glandular structure and vessels, indicating a similar mechanism might contribute to the ulcer formation. Even in the absence of the features of typical severe vasculitis, there might be an ischemic milieu in the affected ileum of the present case induced by, e.g., temporal usage of NSAIDs. Such ischemia may be the cause of circumstances that are prone to tissue damage by EBV infection. On the other hand, EBV-negative T cell infiltration into the gastric epithelium was seen in the other case of EBV-gastritis (14). In such conditions, it is speculated that EBV infection might elicit a strong cellular immune response mediated by cytotoxic T cells and inflammatory cytokines resulting in glandular damage and ulcer formation.

In patients with autoimmune diseases treated with immunosuppressive agents such as MTX, reactivation of EBV from the latent infectious state is not uncommon (4). However, the present case seemed to have a primary EBV infection. Primary EBV infection generally occurs subclinically in childhood and prolonged infection frequently causes infectious mononucleosis (IM) (16). Although typical symptoms of IM such as pharyngitis, lymphadenopathy, and liver dysfunction were absent in the present case, the patient’s serological tests with a paired serum diagnosed primary EBV infection. In the early phase of primary infection, the EBV-EA-DR IgG antibody test result is usually negative and gradually becomes positive. In the present case, EBV-EA-DR IgG antibody was negative even at 8 weeks and later after possible infection. We may not preclude the possibility that immunosupression by MTX treatment caused a delay in antibody production. Of interest, almost all of the reported cases of EBV-associated ulcerative gastritis occurred during primary EBV infection (10-14). An indication for understanding the mechanism for the correlation of IM and ulcerative gastritis is currently not available.

Figure 5. Detection of EBV early RNA (EBER) by an in situ hybridization analysis. (a) Low magnification image. EBER-positive cells surrounding the intestinal glands (b), and vessels (c). Absence of EBER-positive cells in unaffected glands (d), and in the area of serositis (e). Bar indicates 100 μm.
In conclusion, we encountered a rare case of multiple intestinal ulcers that possibly developed during a primary infection of EBV in an adult patient with RA undergoing low-dose MTX treatment. Although the EBV infection may not have solely caused the ulceration, the present case still highlighted a need for a high index of suspicion of ulcerative enteritis even in the absence of the signs of LPD or of EBV infection.

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

References