Case of hypereosinophilic syndrome with gastrointestinal involvement showing tissue eosinophil cytolysis

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ABSTRACT

Hypereosinophilic syndrome (HES), which is characterized by eosinophilia in the peripheral blood, often causes various organ disorders, including those of the gastrointestinal (GI) tract. The eosinophils play a key role in inflammation in eosinophilic GI disorders (EGIDs), including HES with GI involvement. Here, we report a case of HES with GI involvement that showed major basic proteins (MBPs) deposition in the absence of marked eosinophil infiltration in the mucosa of the GI tract. An 11-year-old boy presented with nausea and epigastric pain for one week. He had a history of idiopathic HES with eosinophilic cystitis, diagnosed at the age of 2 years. He had been taking a low dose of corticosteroids for 9 years. The peripheral blood eosinophil count was 2,254/μL. Endoscopy revealed a swelling of the duodenal bulb mucosa. Histological findings of the duodenal mucosa revealed chronic inflammation, but no evidence of significant eosinophil infiltration and we could not diagnose him with HES with GI involvement or EGID. Immunofluorescent staining for MBP and galectin-10 was performed to detect intact and cytolytic eosinophils (eosinophil extracellular trap cell death: EETosis). Marked MBP deposition was evident in a small number of intact eosinophils in tissues from the duodenum, gastric antrum, and terminal ileum. The current case illustrates the utility of immunostaining for the detection of persistent eosinophilic inflammation, especially when cytolytic eosinophils are dominant.

Keywords: Eosinophilic enteropathy; Eosinophils; EETosis; Hypereosinophilic syndrome

INTRODUCTION

Hypereosinophilic syndrome (HES) is a heterogeneous group of disorders characterized by persistent eosinophilia (eosinophil count ≥ 1,500/mm³ for longer than 6 months) and organ involvement/dysfunction [1, 2]. Gastrointestinal (GI) involvement is the third-most commonly reported clinical manifestation in HES [3] and HES with GI involvement is classified as a primary eosinophilic disorder [4]. No diagnostic criteria have been established for patients with HES with GI involvement; however, diagnostic criteria have been proposed for eosinophilic gastrointestinal disorders (EGIDs) [5].
The pathogenesis of HES with GI involvement or EGIDs has yet to be completely elucidated. However, a chronic Th2-type reaction is thought to be involved in EGIDs [6]. The eosinophils contain four major cationic proteins, including the major basic protein (MBP), which play important roles in eosinophil-mediated inflammation [7, 8]. The eosinophils release these granule proteins via piecemeal degranulation, exocytosis, and cytolysis. Recent evidence has shown the in vivo occurrence of active cytolysis, that is, eosinophil extracellular trap cell death (EETosis) [9, 10]. Here, we report a case of HES with GI involvement in which MBPs, in the absence of marked eosinophilic infiltration, were significantly deposited in the GI mucosa, suggesting EETosis-mediated tissue damage.

CASE REPORT

An 11-year-old boy presented with persistent nausea and epigastric pain for one week. His medical history showed that he developed nausea with eosinophilia at the age of two years. He was diagnosed with FIP1L1-PDGFRA fusion-negative HES with eosinophilic cystitis and had been taking a small dose of corticosteroids for 9 years. The patient had no family history of eosinophilic disorder. Physical examination on admission revealed epigastric tenderness. Laboratory tests revealed eosinophilia (2,254 eosinophils/µL), and other blood test results were within normal limits, including IgE. Ultrasonography revealed duodenal wall thickening with no evidence of ascites. Upper GI endoscopy revealed mucosal swelling of the duodenal bulb with white mucus and partial erosion (Fig. 1). The mucosa appeared normal, except for the duodenal bulb. Colonoscopy revealed normal mucosa in the terminal ileum and entire colon.

Histological analysis of the duodenal bulb and proximal descending duodenum revealed prominent lymphocytic and plasma cell infiltration, but eosinophil infiltration was less than 20/high power field with eosinophil degranulation (Fig. 2A, B). Eosinophilic involvement could not be identified, because significant eosinophilic infiltration was not observed in the esophagus, stomach, or lower GI tract, including the terminal ileum. However, HES with GI involvement or EGID was strongly suspected on the basis of the underlying disease and endoscopic findings. To assess for eosinophilic involvement, we performed immunostaining for eosinophil-specific proteins, MBP, and galectin-10 in GI biopsies [11]. Intact eosinophils were double positive (gal-10+/MBP+), whereas lytic EETosis cells and free granules were only
positive for MBP (gal-10−/MBP+) [12, 13]. Comparison of hematoxylin and eosin staining and immunostaining revealed diffuse MBP deposition without marked intact eosinophil infiltration in the mucosal lamina propria of the duodenal bulb, gastric antrum, and terminal ileum (Figs. 2, 3). Small punctate galectin-10 staining, indicating extracellular vesicles, was also recognized [12]. The presence of cell-free eosinophilic granules and chromatolytic cells [12] indicates the presence of EETosis in GI tissue. Based on the clinical course and histological findings, the patient was diagnosed with HES with GI involvement. After increasing the corticosteroid dose, the GI symptoms improved, and the blood eosinophil counts decreased to within normal limits. However, tapering the dose of corticosteroids caused the flare-up of GI symptoms and an increase in the eosinophil counts. The patient’s condition was determined to be steroid-dependent, and oral tacrolimus was initiated, with target serum trough levels of 5–10 ng/mL. The administration of tacrolimus permitted the reduction of the corticosteroid doses (3 mg/day), and the patient’s symptoms remained in remission for over 1 year.

Fig. 2. Histological analysis of the duodenal bulb.
Histological analysis of the duodenal bulb reveals inflammatory cell infiltration in the mucosa. The boxed areas in A and C (x200) are magnified in B and D (x1,000), respectively. Identical sections are stained with hematoxylin and eosin (A, B) or anti-major basic protein (MBP) antibody (red), anti-galectin-10 antibody (green), and Hoechst 33342 (blue) (C, D). Intact free eosinophil granules are noted in close proximity to the chromatolytic eosinophils (arrow). Immunohistochemical staining reveals extracellular MBP deposition. Several intact eosinophils are stained with both galectin-10 and MBP (arrowheads). Immunostaining has been performed as previously described [12].
The patient and his parents provided permission to publish the features of his case and we obtained the written informed consent for publication from them.

**DISCUSSION**

We have reported a case of HES with GI involvement and diffuse deposition of eosinophil granule protein MBP in the absence of tissue eosinophilia. The patient with a history of HES presented with GI symptoms (pericardial pain and nausea), and endoscopic findings revealed swelling of the duodenal bulb. Our routine histological examination showed a chronic inflammatory pattern with no evident eosinophilic infiltration of the mucosa, which did not lead to a diagnosis. Since the underlying disease was HES, eosinophil involvement was strongly suspected, and immunostaining for eosinophil granule protein revealed cell-free intact granules, extracellular MBP deposition, and nuclear chromatolytic changes in the absence of significant eosinophilic infiltration in the duodenal mucosa, gastric antrum, and terminal ileum.

Tissue deposition of granule proteins in the absence of eosinophil accumulation has been reported in various conditions such as Hodgkin disease, parasitic infection, chronic urticaria, atopic dermatitis, and endomyocardial disease [8]. The mechanism of extracellular MBP deposition in the absence of eosinophils may involve cytolytic EETosis. EETosis is characterized by the release of eosinophil extracellular traps and intact, free extracellular granules, which can cause damage to various cells and tissues [8, 9, 14]. The deposition of histotoxic granule proteins and other cellular components that act as alarmins, induced by
EETosis, might have caused chronic inflammation. Further studies are required to elucidate the pathophysiological role of EETosis in GI disorders related to eosinophils.

Although distinguishing between HES with GI involvement and EGIDs can be difficult [15], eosinophils are the major effector cells in both conditions. The presence of eosinophilic inflammation may be underestimated by conventional pathological techniques in cases of EETosis-occuring tissues. Peterson et al. [16] demonstrated greater MBP deposition in biopsies of symptomatic patients than in biopsies of asymptomatic patients with eosinophilic esophagitis, but peak eosinophil counts in tissues did not differ between symptomatic and asymptomatic patients. Upon cytolysis, cytoplasmic galectin-10 can rapidly spread throughout the tissue and is undetectable by immunostaining [13], whereas MBP deposition in the tissues lasts for longer periods of up to 6 weeks [17]. The current case illustrates the utility of immunostaining for the detection of persistent eosinophilic inflammation in the GI tract, especially when cytolytic eosinophils are dominant.

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