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Report

Mast Cell Degranulation in Erosive Eosinophilic Cholecystitis with Charcot-Leyden Crystals: Evaluation by Mast Cell Tryptase/CD117 Ratio: Report of a Case and a Comparative Study with Chronic Cholecystitis

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A patient with cedar pollen allergy and a previous history of gallstones presented with episodes of abdominal pain in the right upper quadrant, thus, laparoscopic cholecystectomy was performed. Macroscopically, the gallbladder contained several erosive lesions, and microscopic examination revealed erosions and a dense eosinophilic infiltration admixed with degradation products of eosinophils and Charcot-Leyden crystals suggestive of erosive eosinophilic cholecystitis. Immunohistochemical staining revealed infiltration of the lesions with membranous CD117 (c-kit)-positive mast cells with negativity for metachromatic granules and mast cell tryptase (MCT), suggestive of mast cell degranulation. Assessment and comparison of mast cells counts in the mucosa of 11 cases with chronic cholecystitis and the present case revealed that MCT/CD117 ratio was extremely low in erosive eosinophilic cholecystitis areas (0.53–1.14 vs 0.25, respectively). These findings are consistent with the view that evaluation of the MCT/CD117 ratio with CD117 count is a valuable tool for assessing mast cell activity because determination of mast cell count by MCT or CD117 staining alone may fail to detect degranulated mast cells or the degranulation phenomenon, respectively.

Key words: erosive eosinophilic cholecystitis, Charcot-Leyden crystals, mast cell degranulation, mast cell tryptase/CD117 ratio, toluidine blue metachromasia

Introduction

Eosinophils are specialized inflammatory cells that are mainly involved in type I hypersensitivity reactions (anaphylaxis) and immune reactions against parasite infestations. They contain several proteins including major basic protein in crystal form in specific granules, eosinophil cationic protein, and neurotoxins, all of which are toxic to the cells, as well as to parasites[1)2)]. Eosinophils also contain histaminases, arylsulfatase, and phospholipase, suggesting that they may also play a role in attenuating inflammation.

Eosinophilic cholecystitis (EC) is a rare type of acute cholecystitis, first reported in 1949[3)]. It is sometimes acalculous and not necessarily associated with peripheral eosinophilia. The signs and symptoms of this type of cholecystitis are not usually different from those of other types of acute cholecystitis. The diagnosis is thus usually confirmed by histological examination[4)]. The etiology appears to be heterogeneous, including allergic reaction[5)], parasitic infestations[6)], hypereosinophilic syndromes[7)], association with eosinophilic gastroenteritis[8,9)], and idiopathic causes[10)].

Charcot-Leyden crystals are hexagonal bipyramidal crystals that are made up of Charcot-Leyden crystal protein (galectin 10) found in eosinophils and basophils[11)]. They have been identified as a hallmark of eosinophil-associated allergic inflammation. Charcot-Leyden crystals have been observed in

many kinds of disorders including: bronchial asthma; allergic fungal/parasitic inflammation of the upper and lower respiratory tract[12][13], hepatobiliary system[14] and intestine[15][16]; hematological malignancies involving the eosinophilic series[17]; cancers characterized by eosinophilic infiltration[18]; skin diseases[19]; and eosinophilic myocarditis[20]. Further the eosinophilic lesions containing such crystals have been associated with parenchymal tissue destruction[19][20]. To the best of our knowledge, there have been no reports on the detection of Charcot-Leyden crystals and mast cell degranulation in EC. We report a case of idiopathic erosive EC with presence of Charcot-Leyden crystals, and emphasize the significance of mast cell degranulation in comparison with normal and chronic cholecystitic mucosa.

Case Report

A 43-year-old male patient with a history of cedar pollen allergy presented with episodic right hypochondriac pain. He was under follow-up for gallstones detected during a routine group medical examination two years previously, in the absence of any signs and symptoms. There was no peripheral eosinophilia (WBC 6,000–6,700/μl; eosinophils 2.2–2.7%). Laparoscopic cholecystectomy was performed as treatment, and the postoperative course was uneventful.

The gallbladder was 8 × 4 cm in size and contained small fragile stones or bile sands. Macroscopically, the surface mucosa was disrupted by several small erosive lesions (Fig. 1).

Histologically two pathological lesions were observed, erosive EC and chronic cholecystitis. There were several erosive lesions at the site where a dense eosinophilic infiltration was admixed with eosinophilic degradation material with or without Charcot-Leyden crystals (Fig. 2a, b) and was surrounded by a mixed population of inflammatory cells, including CD8-positive or CD4-positive small lymphocytes and eosinophils. CD68-positive macrophages were found to be either scattered or in aggregates in the lesions, but no CD138-positive plasma cells were found. Numerous CD117 (c-kit)-positive mast cells were also found to have infiltrated into these lesions (Fig. 2b, c) and the other areas of the gallbladder, albeit less densely. Immunostaining for mast cell tryptase, however, revealed that only a few mast cells were scattered in the EC areas with a decrease in granular staining (Fig. 2d). Toluidine blue and modified Giemsa staining also revealed absence of the metachromatic mast cells in these lesions despite the detection of mast cells in the smooth muscle layer and the subserosa (Fig. 2e, f).

Hematoxylin and eosin staining showed that the Charcot-Leyden crystals were eosinophilic and longitudinally bipyramidal or cross-sectionally hexagonal, and they appeared pale white in the immunostained slides (Fig. 2a, b); the crystals were not found to be refringent under polarized light.

The remaining regions of the gallbladder showed histological findings consistent with the usual chronic cholecystitis: areas of mucosal atrophy, mild infiltration by small lymphocytes and plasma cells with a few lymphoid follicles, and mild increase in fibrogenesis. Eosinophils were few in number.

1. Analysis of 11 Cases with Chronic Cholecystitis and Comparison with the Present Case

The number of mast cells/unit area (0.53 mm^2) in the mucosa of 11 cases with chronic cholecystitis ranged between 77 and 190 (mean 121.8 +/− 31.9) in CD117-stained sections and between 74 and 129 (mean 91.2 +/− 17.0) in MCT-stained sections (Table). The MCT/CD117 ratio ranged between 0.53 and 1.14 (mean 0.78 +/− 0.20). The value representing CD117 immunopositivity, MCT immunopositivity, and MCT/CD117 ratio of the present case with EC were 137, 125 and 0.91 in the mucosa, and 172, 43, and 0.25 in the erosive EC areas, respectively, showing that while there were more CD117-positive mast cells in the erosive EC areas than in the mucosa, there were less MCT-positive mast cells in the EC areas than in the mucosa. The box plot analysis of the MCT/CD117 ratio revealed that values of 0.95, 0.68, and 0.65 represented the 75th, 50th, and 25th percentiles, respectively, and that the values of the mucosa of all cases were within the upper and lower adjacent values (1.30 and 0.26), thus, the MCT/CD117 ratio in erosive EC areas alone was regarded as an outlier.

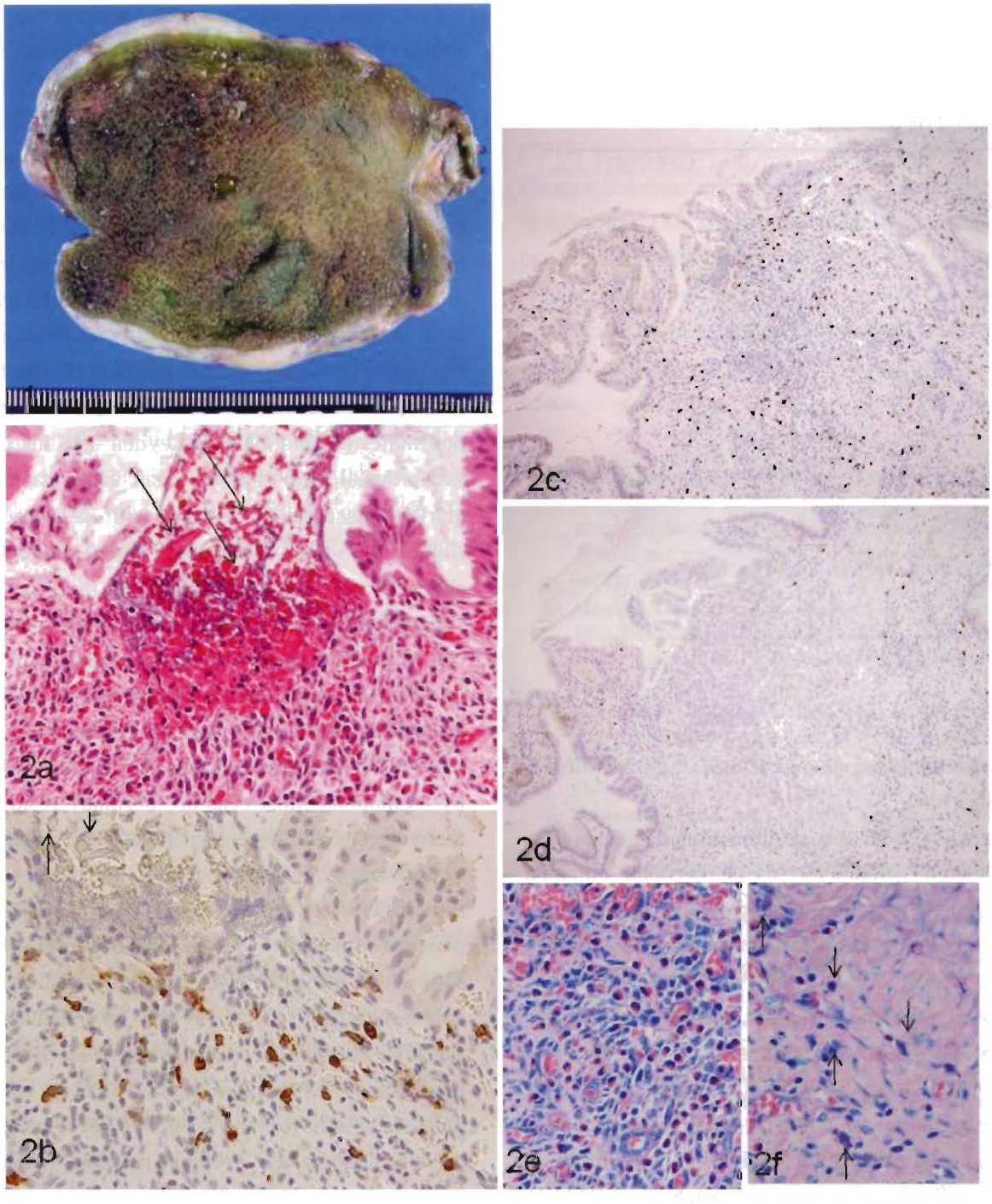

Fig. 1 A macroscopic view of the mucosal surface of the resected gallbladder. The velvety, mucosal pattern is disrupted by erosive lesions.

Fig. 2

a: Hematoxylin and eosin staining. The erosive lesion contains dense degradation material of eosinophils, as well as Charcot-Leyden crystals (arrows), surrounded by marked eosinophilic infiltration. Some crystals are needle-shaped in this section.

b: Many CD117-positive mast cells infiltrating the lesions of eosinophilic cholecystitis (EC) with pale white crystals, some of which are hexagonal in appearance (arrows).

c & d: Low power views of density of CD117-positive (c) and mast cell tryptase-positive (d) mast cells, the latter of which is low in number, suggesting mast cell degranulation in erosive EC areas.

e & f: Modified Giemsa stain. In the lesions characterized by dense eosinophilic infiltration, mast cells expressing membranous CD117 do not show granular basophilic cytoplasmic staining (e), although they do show such a staining pattern in areas of the muscular layer (f, arrows).

Table Mast cell number in the mucosa of patients with chronic cholecystitis and in the mucosa and the lesions of erosive eosinophilic cholecystitis (EC) on the bases of CD117 and mast cell tryptase (MCT)-immunostaining

	CD117	MCT	MCT/CD117 ratio
Chronic cholecystitis			
Case 1	77	88	1.14
2	97	107	1.10
3	111	108	0.98
4	90	74	0.82
5	110	77	0.70
6	119	88	0.68
7	190	129	0.68
8	115	77	0.67
9	135	88	0.65
10	143	93	0.65
11	153	81	0.53
Mean	121.8	91.2	0.78
SD	31.9	17.0	0.20
The present case with erosive EC			
Mucosa	137	125	0.91
EC areas	172	43	0.2

SD: standard deviation.

2. Immunohistochemical Staining of Gallbladder Specimen

Formalin-fixed and paraffin-embedded, surgically-resected gallbladder tissue sections obtained from a present patient with erosive EC and 11 unselected patients with chronic cholecystitis without artifacts formed at the time of resection were immunostained using and anti-CD117 (IBL, Takasaki, Japan), anti-mast cell tryptase (MST; Novocastra, New Castle, UK), anti-CD68 and CD-138 (DAKO, Glostrup, Denmark), anti-CD4 (MBL, Nagoya, Japan) and anti-CD34 (Becton Dickinson, San Jose CA, USA) antibodies with the EnVision System (DAKO, Glostrup, Denmark). Modified Giemsa stain (MGS) and toluidine blue stain (TBS) were also performed. Mast cell numbers in the mucosa and erosive EC areas were counted in a unit area each (350×250 μm on the display of a digital camera) from six high power fields.

Discussion

In this case report, we provide evidence to show that the key patient had acute erosive EC, which explains the recent episodes of right hypochondriac pain. The cause of EC has been ascribed to a unique/hypersensitivity type of inflammatory response to altered bile[4]. Although the present patient had cedar pollen allergy, its relationship to the development of erosive EC is not too clear.

The histological features of EC are heterogeneous. In the previously reported cases, eosinophils were found in the intraepithelial as well as interstitial infiltrates[3], distributed predominantly in the mural/subserosal areas[4~10], or accompanied by mucosal destruction with fibroblastic proliferation[1]. In the present case, erosive EC manifested as a dense eosinophilic infiltration with eosinophilic degradation materials and Charcot-Leyden crystals in the subepithelial and erosive areas accompanied by marked mast cell infiltration with degranulation, suggesting a late phase type I hypersensitivity reaction[21].

Eosinophils have a special relationship with mast cells; the latter secrete several eosinophilic chemotactic factors, whereas the former secrete major basic protein that causes mast cells to release histamine which, in turn, is inactivated by histaminases by eosinophils. Mast cells are normally resident in many organs and tissues, including the respiratory system and gastrointestinal tract, but not in the adrenal fasciculata and reticularis zones and the neoplastic counterparts, which produce cortisol that suppresses mast cell proliferation[22]. Mast cells not only have a principal role in type I hypersensitivity reaction, but also have a major role in chronic inflammation. For example, mast cells have been hypothesized to have roles in hepatic fibrogenesis and sinus capillarization[23]. We have previously observed similar phenomena in experimentally-induced rat pulmonary fibrosis in which mast cell infiltration precedes fibrogenesis and induction of alkaline phosphatase activity in alveolar capillaries[24][25]. Thus, mast cells may also have a role in chronic cholecystitis[26].

There have been no reports describing the presence of mast cells in the gallbladder wall in EC cases, except for a brief description in a case with hypereosinophilic syndrome whose gallbladder contained scattered toluidine blue-positive mast cells in the mucosa[7]. In addition, there has been no report

describing mast cell degranulation in normal or diseased gallbladder, including chronic cholecystitis and EC.

CD117 has been regarded as a reliable marker of mast cells[27], such that mast cells have sometimes been regarded as an internal control for CD117 immunostaining of gastrointestinal stromal tumors[28]. Although CD117-positive Cajal-like cells have been observed in the gallbladder[29], the great majority of CD117-positive cells were regarded as mast cells in our study because Cajar cells are usually not resident in the gallbladder mucosa.

In the present study, the gallbladder showed a relative abundance of mast cells in the mucosa, muscular layer, and subserosa in the cases of EC and chronic cholecystitis (Table, Fig. 2). Moreover, mast cell infiltration and degranulation were characteristic features of the present erosive EC case. However, the detection of mast cell number and degranulation involve certain challenges. Detection of mast cell granules by mast cell tryptase, toluidine blue staining, or Giemsa staining may underestimate mast cell count. On the other hand, detection by immunostaining for CD117, which is localized in the cell membrane and not influenced by degranulation, may render a more accurate mast cell count, but may fail to detect mast cell degranulation. The evaluation by mast cell tryptase/CD117 ratio in addition to mast cell count by CD117 immunostaining might be a more powerful tool for investigating the role of mast cells in EC and other inflammatory lesions. An intensity score of mast cell granules (e.g., mast cell tryptase, toluidine blue metachromasia, and/or Giemsa staining, etc.) might also be a valuable parameter, although it was not done in this study.

In Hudson's study of mast cells identified by toluidine blue histochemistry in the gallbladder, active mucosal inflammation in chronic cholecystitis was associated with a decrease in mast cell numbers[26]. From the viewpoint of our present study, an apparent decrease in mast cell numbers in their study may in reality be due to failure to detect degranulated mast cells.

In conclusion, we report here a case of erosive EC characterized histologically by the presence of Charcot-Leyden crystals admixed with eosinophilic degradation products. The lesions were found to contain many mast cells, which were positive for CD117 but negative for mast cell tryptase, modified Giemsa staining, and toluidine blue metachromasia in EC areas. Our study may suggest that a significant role of mast cells in erosive EC via their active degranulation. MCT/CD117 ratio may be a more powerful tool as a parameter of degranulation for assessing mast cell activity in inflammatory processes.

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シャルコー–ライデン結晶を認めた好酸球性胆嚢炎の肥胖細胞の脱顆粒について—肥胖細胞の activity の指標としての mast cell tryptase/CD117 比の有用性—症例報告および慢性胆嚢炎との比較検討

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以前より胆石症を指摘されていた，スギ花粉症を有する43歳の男性が右季肋部痛で来院し，腹腔鏡下の胆嚢摘出術を受けた．摘出された胆嚢に数個のびらんを認め，その部に一致して，好酸球の変性産物とシャルコー–ライデン結晶をともなって強い好酸球浸潤が見られ，びらん性好酸球性胆嚢炎と診断された．

病変部（びらん）には免疫組織化学的にCD117（c-kit）が細胞膜に一致して陽性を示す肥胖細胞が多数存在したが，これらは異染性の顆粒およびmast cell tryptase（MCT）が陰性であった．このことは肥胖細胞が脱顆粒を起こしていることを示唆している．

本症例と，11例の慢性胆嚢炎の粘膜について肥胖細胞をカウントした結果，MCT/CD117比が本症例の病変部では著明に低いことがわかった(0.25 vs 1.14～0.53)．MCTの染色のみでは脱顆粒を起こした肥胖細胞が検出できず，またCD117による染色のみでは肥胖細胞が脱顆粒現象を起こしていることを認識できない．得られた結果はMCT/CD117比が炎症の過程における肥胖細胞のactivityを評価する有用な指標であることを示唆している．