Hypereosinophilic syndrome with abundant Charcot-Leyden crystals in spleen and lymph nodes

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INTRODUCTION

Hypereosinophilic syndrome (HES) is characterized by eosinophilia in the peripheral blood, leading to various organ injuries and sometimes fatality [1]. Charcot-Leyden crystals (CLCs) were discovered in parasite-infected tissues and in the sputum of asthmatic patients in the 1850s, and their presence is known to be a classic hallmark of eosinophilic inflammation [2]. A recent study by our group indicated that tissue CLCs are associated with disease severity, and we revealed the mechanism of CLC formation, namely the involvement of eosinophil extracellular trap cell death (EETosis) – a nonapoptotic cell death [3]. EETosis is characterized by rapid intracellular calcium influx, programmed cell death (PCD) and extracellular protein release [3]. This study provides new insights into the pathophysiological roles of CLCs in severe eosinophilic inflammation.
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**CASE REPORT**

A 57-year-old man had previously consulted a physician for general malaise. He developed marked eosinophilia (white blood cells, 37,730/μL; eosinophils, 74%) and multiple lymphadenopathy and splenomegaly. He was transferred to our department of hematology for further investigation. Extensive screenings for reactive causes of eosinophilia including parasite infection were all negative. Serological studies revealed levels within the reference ranges of myeloperoxidase and antiproteinase 3 antineutrophil cytoplasmic antibodies, as well as markedly elevated serum IL-5 (994 pg/mL), GM-CSF (456 pg/mL), and IgE (7056 IU/mL). No gene abnormalities of *FIP1L1-PDGFRα*, *PDGFRβ*, or *FGFR1* were detected, and no blasts or atypical lymphocytes were observed in the bone marrow. A diagnosis with idiopathic HES was given, according to the World Health Organization diagnostic algorithm for eosinophilia [5]. He was treated with 30 mg/day (0.5 mg/kg/day) of prednisolone but was resistant to steroid treatment. Imatinib administration in combination with prednisolone had no satisfactory effect. Two years after the starting treatment, he was referred to an Emergency Department with movement difficulties and was hospitalized for acute renal failure and intestinal infection. Intensive care was provided, but he gradually deteriorated and died 2 months after admission.

Pathological dissection revealed the accumulation of numerous eosinophils in various organs, including enlarged lymph nodes throughout the body, spleen, bone marrow, and subcutaneous tissue. Hematoxylin-Eosin staining of the spleen revealed that the parenchyma was almost necrotic, and many spindle-shaped or hexagonal CLCs were observed (Fig. 1A, C). Abdominal lymph nodes contained eosinophilic abscess with a large number of CLCs (Fig. 1B, D). A small number of CLCs were also observed in the bone marrow, which showed hyperplasia of eosinophils without monoclonality (not shown). We further assessed ultrastructural morphologies using transmission electron microscope. The fixed sections were mounted on uncoated 200-mesh copper grids (Ted Pella, Redding, CA, USA) as previously described [3], and viewed with an electron microscope (H-7650, Hitachi, Tokyo, Japan). As shown in Fig. 2, there were amorphous spindle-shaped CLCs in the interstitial tissue adjacent to lytic eosinophils. The morphologies of the lytic eosinophils included intact electron-dense cell-FEGs, disintegrated plasma and nuclear membranes, and chromatolysis, indicating typical EETosis. Eosinophils with apoptotic morphologies, such as nuclear and cytoplasmic condensation, were not observed. These observations were in line with our previous ultrastructural observations of CLCs in different organs (skin, colon, and nasal tissue) from various eosinophilic diseases [3].

The CLC protein was initially found to exhibit lysophospholipase activity but was later assigned to the galectin superfamily, specifically, galectin-10 [6]. Galectin-10, exclusively expressed in human eosinophils, is a major constituent of the cells, comprising 7%–10% of total eosinophil proteins [6]. The dynamic change in the cytoplasmic localization and extracellular release of galectin-10 by the EETosis process contributes to CLC formation [3]. To confirm molecular localization in the tissue, immunofluorescent staining for galectin-10 and DNA was assessed. The staining specificity is shown in the Fig. 3A–D. As expected, various sizes of CLCs were stained with anti-galectin-10 Ab (Fig. 3E, arrows). Small punctate

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Fig. 1. Charcot-Leyden crystals in spleen and lymph nodes. Tissue samples were assessed by hematoxylin-eosin staining. Charcot-Leyden crystals (arrows) in spleen (A, C) and lymph node (B, D) autopsy tissue; acidic hexagonal crystals were observed. Using light microscopy (Nikon ECLIPSE 80i, Nikon, Tokyo, Japan), the low-power field image shows infiltrating abundant eosinophils. Scale bars are (A, B) 100 µm and (C, D) 10 µm. The arrows indicate Charcot-Leyden crystals (C, D).

Fig. 2. Electron micrograph for eosinophil in abdominal lymph nodes. Abdominal lymph node from hypereosinophilic syndrome patient was prepared for conventional transmission electron microscopy. Charcot-Leyden crystals (CLCs) with a bipyramidal structure and free eosinophil granules (FEGs) were evident. EET, eosinophil extracellular trap.
staining of galectin-10, probably indicating EETosis-mediated extracellular vesicles [3, 4], was also observed. The EET characteristics of loss of nuclear shape and mesh-like DNA were frequently observed close to the CLCs and vesicles. The DNA did not colocalize with galectin-10, further confirming our previous report on EETs [3].

DISCUSSION

Accumulating evidence suggests that EETosis accounts for the previously reported “cytolysis” or “lytic degranulation” of various diseases. Here, we report for the first time a fatal case of HES with numerous CLCs (i.e. crystalized galectin-10) in multiple organs associated with FEGs and EETs. These observations indicate that highly activated eosinophils underwent EETosis rather than prolonged cell survival or apoptosis. Apoptotic eosinophils are immediately cleared by phagocytes; however, EETotic cells, including FEGs and EETs, remain in the tissue because of the absence of a “find-me signal” [3, 4]. FEGs, containing toxic proteins, were shown to act as extracellular secretory components [7]. EETs are composed of DNA and histones that can activate a wide range of immune responses [4].

Recent evidence revealed the functional roles of CLCs. They activate the nucleotide-binding domain-like receptor protein 3 inflammasome in macrophages and are involved in the production of inflammatory cytokines [8]. CLCs enhances innate and adaptive immunity and act as type 2 adjuvants [9]. In addition, they induce inflammatory cytokine production by epithelial cells and activate neutrophils to cause neutrophil extracellular trap cell death (NETosis) [10]. The presence of CLCs in tissue from chronic sinusitis was associated with disease severity and the local IL-5 concentration [3, 9]. Considering the stability of CLCs in tissue, the deposition of abundant CLCs in multiple organs observed in current case may
have led to organ damage and the severity of the clinically uncontrolled disease. Interestingly, noncrystallized galectin-10 does not possess these effects, and a crystal-dissolving antibody reversed the CLC-mediated pathological condition in the mouse model [9]. Further studies are required to understand the pathophysiological roles of CLCs and may lead to the development of novel therapeutic modalities for severe eosinophilic inflammation.

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