Electron Microscopic Study in a Case of Solitary Mastocytoma

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The authors report a solitary mastocytoma with a solitary red infiltrated plaque on the dorsum of the right foot for 2 months. Histologically there were numerous mast cells infiltrating the dermis. Electron microscopy revealed CLCs located in phagosomes of activated macrophages as well as in the stromal tissue, close association between CLCs formation and damaged eosinophils was documented. Charcot-Leyden crystals (CLCs) have been found in many conditions associated with eosinophilia, but their occurrence in skin diseases is very rare. These occurrences showed the evidence that the formation of CLCs in a mastocytoma correlated to the individual and related to the biology of mast cells, basophils, eosinophils and macrophages. Phagosomes probably acted as the localization of CLCs formation. The pathological role of CLCs in a mastocytoma needs further investigation.

Keywords : *Mastocytoma , Electron microscopy*

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Charcot-Leyden crystals (CLCs) are characteristic bipyramidal, circular to elliptical shaped structures observed in variety of skin conditions often in association with eosinohilic cellular inflammatory reactions. They are difficult to observe on light microscope, but can be better observed under the electron microscope. We studied one case of mastocytoma on the presence of CLCs in the skin lesions by ultrastructural study.

Case Report

A 10-month-old Thai male boy presented with a solitary, red infiltrated plaque on the dorsum of his right foot for 2 months. Examination revealed a 1.0 x 3.0 cm infiltrated lesion, which was brown red in color and rectangular in shape (Fig. 1); positive Darier's sign was observed. No systemic symptom was observed.

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Light and electron microscopy

The plaque of this case was excised and examined by light and electron microscopy. The 4 mm sections were stained with hematoxylin and eosin and with 1% toluidine blue, pH 7.0, to identify mast cells. For routine electron microscopic observation, the tissue was fixed in 2.5% glutaraldehyde buffered

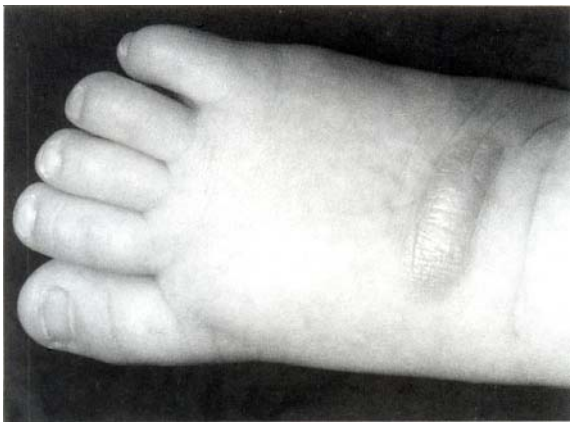

Fig. 1 Mastocytma: This solitary, red infiltrated plaque appeared on the dorsum of his right foot

with phosphate (PH 7.4) at 4 C and postfixed in 1% osmium tetroxide in the same butter for 1 h. After dehydration in a series of graded concentrations of alcohol, they were embedded in Epon. The 1 mm semithin sections were stained with toluidine blue to select the appropriate areas for study. Ultrathin sections were cut by an LKB Ultratome N with a diamond knife and double stained with uranyl acetate and lead citrate, and examined and photographed under an Hitachi H-600 electron microscope at an accelerating voltage of 75 kv.

Result

The histopathological findings showed papillamatosis and a heavy infiltrate of mast cells extending through the entire dermis (Fig. 2). Mast cells were characterized by the presence of metachromatic granules in their cytoplasm with toluidine blue staining. An increased number of eosinophils was observed around the mast cells aggregates, especially in the mid and lower dermis and subcutaneous tissue.

Electron microscopic evaluation showed the inflammatory infiltrate consisted mainly of mast cells in mid dermis but a relative abundance of eosinophils and macrophages were also seen. Mast cells which appeared to be normal, had numerous long villous projections and characteristic granules (Fig. 3). Degranulating mast cells had granules at various stages of degeneration. Large numbers of eosinophils with degranulation may result in tissue injury through direct toxicity to host cells and may modulate hypersensitivity reaction by their effect on inflammatory mediators. Macrophages were filled with several light dense, smooth vesicles in peripheral cytoplasmic areas and large membrane-bound vacuoles with variable contents, including the damaged eosinophils and their released granules.

The most striking feature was a large amount of uniformly electron dense bipyramidal crystalloid structures measuring 2.0-6.0 mm in length and 0.3-1.8 mm in diameter, the hexagonal ones which contained an amorphous to finely granular matrix were seen in the cross-section. These crystals were membrane-free and easily seen in phagosome-like structures and in stromal tissue (Fig. 4). They were usually surrounded by or in close relationship to damaged eosinophil nuclear chromatin as well as broken eosinophil plasma membrane. These granules should not be confused with cytoplasmic lipid bodies, which occur in many mammalian cell types. Within the cytoplasm of macrophages, some CLCs were present with a smooth

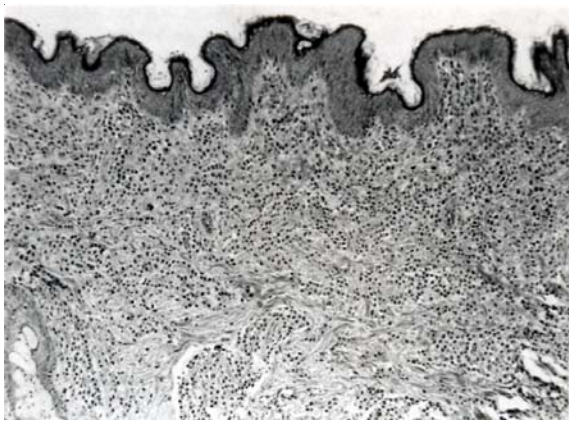

Fig. 2 Mastocytoma: The intense mast cell infiltration has resulted in papillomatosis of the epidermis (x 35)

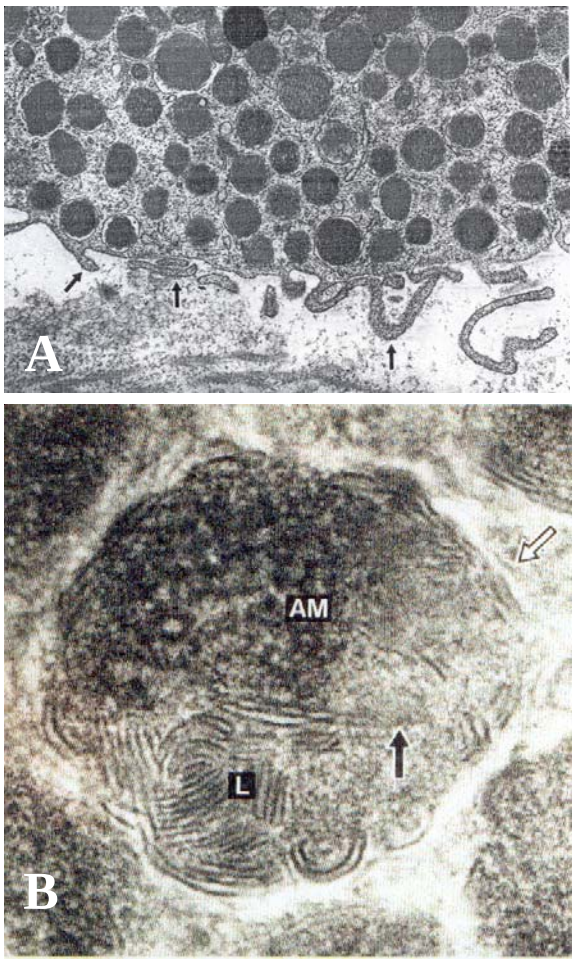

Fig. 3 The cellular infiltrate consisted mainly of mast cells in the mid dermis in the mastocytoma, the mast cell had numerous long villous projections (3A x 22,000), mast cell granules contain lamellar structure (L) and electron dense material (AM) (3B x 63,000)

membrane-lined or membrane-free appearance (Fig. 5 and 6). Relatively fewer numbers of CLCs, with varying electron density and forms, were contained in the phagosomes, it was not difficult to distinguish the various stages of CLC development (Fig.7), from the flocculous-like deposits to the mature appearance. There were rare macrophages showing cell damage and necrosis in the stroma. Two processes in which macrophages discharged CLCs into extracellular space could be observed (Fig. 8), one was of lysosomal exocytosis of CLCs, the other was when the pointed end of the CLCs directly pierced through the cell membrane. No CLCs were identified in any mast cell.

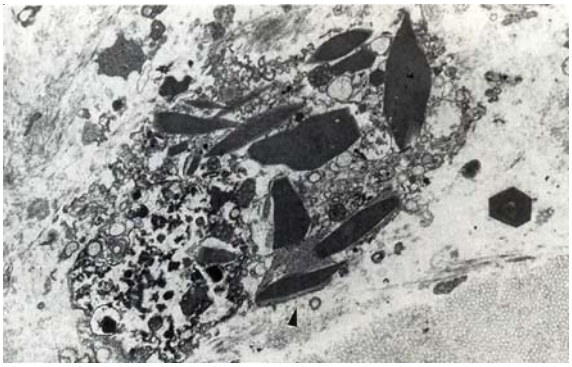

Fig. 4 Large numbers of bipyramidal and irregular CLCs were free in the stromal tissue, and admixed with damaged nuclear chromatin, cellular membrane debris, and numerous empty and partially empty granule chambers from the lysed eosinophils
Note 2 CLCs within the almost complete phagosome (arrowhead; x 6000)

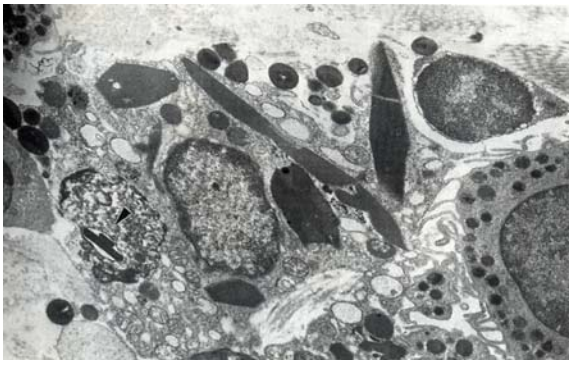

Fig. 5 An active macrophage cytoplasm was filled with numerous CLCs, damaged eosinophil, clear granules and empty vesicles. Two very dense, rod-like structures (arrowhead) might represent a pseudo-inclusion (x 8000)

Discussion

Solitary mastocytoma is an uncommon disease characterized by monotonous infiltrations of mast cells that extend from the papillary dermis to subcutaneous fat. They usually resolve by adulthood, and there is no satisfactory treatment. Electron microscope study in this present case showed that Charcot-Leyden crystals can occur in mastocytoma, but to our knowledge this is unusual[1-3]. Charcot-Leyden crystals were originally described in inflammatory eosinophil-rich skin disorders such as asthma and allergic or parasitic diseases, however, they have also been found in human basophils, macrophages[4],

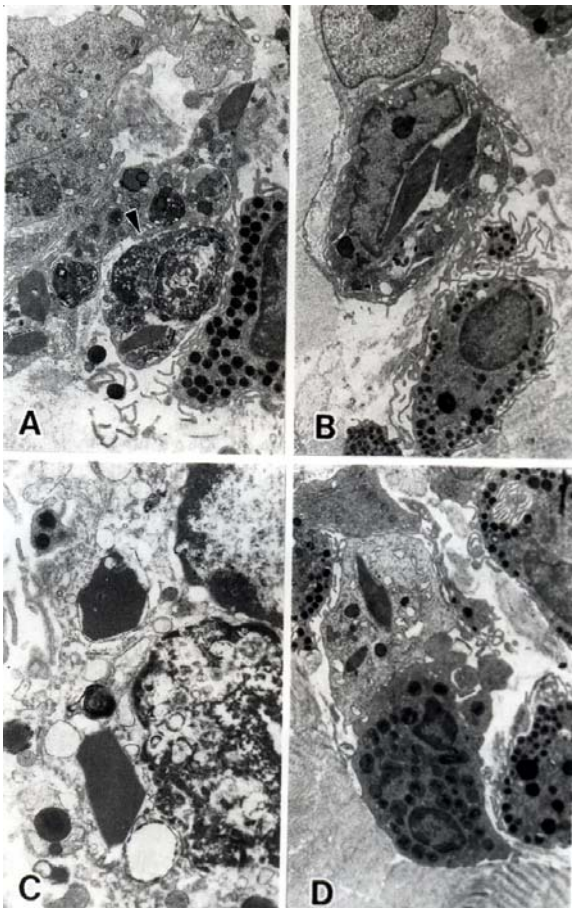

Fig. 6 Higher magnification of phagosomes in the cytoplasm of macrophages contained CLC as well as eosinophil cellular debris.
A: CLCs in close vicinity to the damaged eosinophil (arrowhead; x 6000), B: Two CLCs in a large macrophage vacuole (x 5000), C: Smooth membrane-enclosed CLCs adjacent to the damaged eosinophil nuclear and mpty large granules (x 10000), D: Macrophage and eosinophil were positioned close to each other (x 5000)

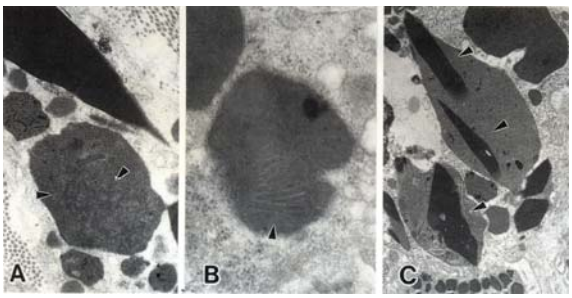

Fig. 7 The electron micrograph shows CLCs in various stages of development in the phagosomes of the macrophages
A: the flocculus-like deposits (arrowhead) with light electron density (x 20000), B: Sparse, needle-shaped crystals (arrowhead; x 35000), C: ClCs exhibited a mature appearance (arrowhead; x 10000)

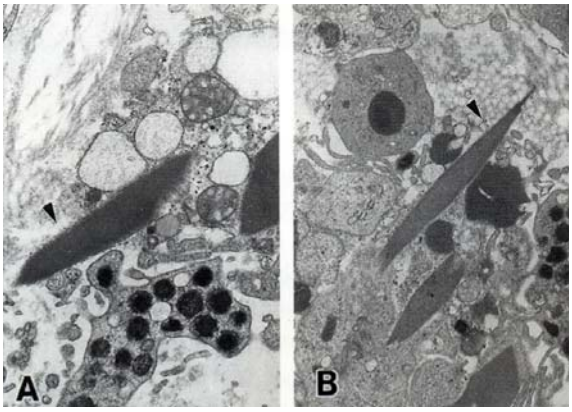

Fig.8 CLCs in stromal tissue adjacent to macrophages
A: Exocytosis of phagosome containing CLC (arrowhead; x 10000), B: The pointed end of CLC (arrowhead) pierced through the cytoplasm membrane (x 12000)

but neither cutaneous nor pulmonary mast cells contain them[5]. Eosinophil CLCs formed by a single markedly hydrophobic protein with a molecular weight of approximately 17400 Da that represents 10% and exhibits lysophospholipase activity[6]. 598-bp full length cDNA clone for this protein has recently been isolated and sequenced[7], they are typically seen extracellularly in the setting of abundant eosinophil degeneration but may also be seen intracellulary after phagocytosis by other leukocytes[8]. CLCs can be formed in vitro by the disruption of eosinophils in hypotonic or detergent solution[9]. In vivo, they were found in a variety of conditions characterized by tissue or peripheral blood eosinophilia, including asthma[10], pulmonary dirofilariasis[11], hepatic visceral larva migrans[8], Kimura's disease[12], myelodyspastic syndrome[13] as far as skin lesions are concerned, CLCs have only been observed in cases of mastocytoma[14], pemphigus vegetans[15,16] pemphigoid[17], herpes gestationis[17], hypereosinophilic syndrome[17] and the cutaneous lesion of histiocytosis X [18].

The pathogenesis and mechanism of crystal formations concerning CLCs, remain entirely unknown. In the present study, the authors identified bipyramidal, hexagonal and amorphous CLCs in tissue macrophages and in the cutaneous lesion of mastocytoma. Although many authors had previously reported observations of mastocytoma, which also revealed CLCs[14], the findings in the present study documented for the first time a role for macrophages in the biology of CLCs in mastocytoma. Electron microscopic studies revealed that CLCs, as well as damaged eosinophils and their released granules, were present within macrophages. The number of CLCs, either intracytoplasmic crystals in macrophages or extracellular crystals in interstitial tissue, paralleled the degree of eosinophil infiltration and degranulation of eosinophils. The authors speculated that macrophage CLCs are derived from endocytosis of released eosinophil CLC protein, this mechanism primarily involves the uptake and transport of soluble CLC protein by endocytic smooth vesicles[17,19] CLC may also be produced by basophil and CLC protien has been localized to the plasma membrane as well as more recently to an additional cytoplasmic membrane bound crystalloid free granules compatible with persistent primary granules. Although most CLCs were membrane-free, some were also observed within phagosomes or surrounded by membrane-like structures which probably arose from large, swollen phagosomes. Phagosome membranes could not extend without limit with an increasing volume of CLCs, inevitably they became thin and remained close to the surface of the CLCs, so the appearance of these CLCs seemed to be membrane-free. When enlarged, CLCs could pierce through one end of the phagosome, separate itself from phagosome, becoming free in the cytoplasm and leaving the phagosome with the appearance of an empty vacuole in cross-section (Fig. 6C). Meanwhile, the authors observed both membrane-free and membrane-bound CLCs existing within macrophages, even in interstitial tissue.

Since there were rare macrophages with visible signs of cell injury, the authors believed that CLCs within the macrophages were released into the extracellular space mainly by either exocytosis of phagosomes containing CLCs or directly piercing

through the cell membrane (Fig.8). These findings provide an explanation why only the membrane-free and phagosomal CLCs were admixed with the remnants derived from the destroyed eosinophil, while no other components of macrophages could be identified in stromal tissue. The pathophysiological relationship between macrophages, eosinophils and mast cells in our cases is certainly complex. A reasonable explanation is that mast cells discharged a lot of mediators enclosed in their granules, such as eosinophil chemotactic factor anaphylaxis (ECF-A), into the surrounding tissue in response to a series of stimuli, which caused aggregation and degranulation of eosinophils, then, the soluble CLC protein from eosinophils was phagocytosed by macrophages, accumulated within the phagosome, then gradually became concentrated. Finally, the CLC protein might have crystallized in the phagosomes, in proportion to excessive amounts of CLC protein released from tissue eosinophils, to the insufficient capability of macrophages to degrade internalized soluble CLC protein, under certain optimal physiological conditions. Then the CLCs became larger, and some were discharged into the extracellular space.

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การศึกษาจุลทัศน์อิเล็คตรอนในผู้ป่วย Mastocytoma ชนิดก้อนเดี่ยว: รายงานผู้ป่วย 1 ราย

ปิติ พลังวชิรา, Hitoshi Yaguchi, **ปราณี พลังวชิรา**

ผู้ป่วยโรค solitary mastocytoma 1 ราย พบลักษณะผลึก charcot-leyden ซึ่งโดยปกติจะพบผลึกชนิดนี้ในโรคซึ่งมีอีโอสิโนฟิลส์สูงในกระแสเลือด แต่พบน้อยมากในโรคผิวหนัง ลักษณะทางจุลทรรศน์อิเล็คตรอนพบผนึกเหล่านี้ฝังอยู่ในฟาโกโซมของแมคโครฟาจ และบริเวณชั้นหนังแท้ ผลึกเหล่านี้เชื่อว่าเกิดจากอีโอสิโนฟิลส์ซึ่งถูกทำลายโดยอาจมีความสัมพันธ์เกี่ยวข้องกับแมสเซลล์ เบโซฟิลส์และแมคโครฟาจ พบว่าฟาโกโซมเป็นตำแหน่งสำคัญของการสร้างผลึกส่วนบทบาทสำคัญของผลึกเหล่านี้ใน mastocytoma ยังคงต้องทำการศึกษาค้นคว้าวิจัยต่อไป