

An Ultrastructural Study of Urticaria Pigmentosa

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To investigate ultrastructural characteristics of mast cells in urticaria pigmentosa in comparison to mast cells in other conditions and to search for the possible differences of ultrastructural features among different types of urticaria pigmentosa as well as those from normal mast cells, 4 cases of urticaria pigmentosa and 1 case of mastocytosis associated with capillary hemangioma were studied by both light and electron microscopic examinations. The cases of urticaria pigmentosa consisted of one case of blister type from a 3 month old male infant, 2 cases of maculo-papular type both from 10 month old male infants, and a case of nodular type from a 14 month old boy. Ultrastructural features of mast cells in urticaria pigmentosa, in general; a) appeared relatively immature; b) mast cells in nodular type of urticaria pigmentosa were mostly round shaped while other types showed spindle or oblong shapes; c) mast cells in the maculo-papular type and those in hemangioma were similar and resembled normal mast cells; and d) marked degranulation of mast cells in the form of expulsion of granules, perigranular vacuole formation and intracellular dissolution of granules for the blister type of urticaria pigmentosa.

Urticaria pigmentosa is a unique disease characterized by an increase of mast cells in the skin and occurs clinically in three forms: 1) Urticaria pigmentosa arising in infancy or early childhood without a significant systemic lesion; 2) Urticaria pigmentosa arising in adolescence or adult life without a significant systemic lesion; and 3) Systemic mast cell disease (Klaus and Winkelmann, 1962). Cutaneous lesions of urticaria pigmentosa are divided into 5 types: a) the maculo-papular, b) multiple nodular or plaques, c)

solitary large nodule, d) diffuse erythrodermic, and e) telangiectatic macular (Klaus and Winkelmann, 1965).

Mast cells are identified by the presence of specific metachromatic granules in their cytoplasm, and are known to contain at least three distinctive chemical substances, namely, histamine, heparin and serotonin (Holmgren and Wilander, 1973; Riley, 1955; Hagen *et al.*, 1959). Various types of irritation cause degranulation of mast cells and release of histamine and other substances, which produce whealing or blister formation (Jorpes, 1939; Davis *et al.*, 1958; Cramer, 1964; Selye, 1965).

Ultrastructural studies of mammalian mast

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cells in normal and diseased conditions and the process of degranulation have been reported by several investigators (Smith and Lewis, 1957; Bloom *et al.*, 1958; Hibbs *et al.*, 1960; Bloom, 1963; Smith, 1963; Hashimoto *et al.*, 1966; Freeman, 1967; Kobayashi *et al.*, 1968; Kobayashi and Asboe-Hansen, 1969; Lagunoff, 1972; Asboe Hansen, 1973; Moriyasu and Yamamura, 1973; Naveh *et al.*, 1975). They reported with uniformity of mast cell morphology in various conditions, except that Hashimoto *et al.*, (1966) reported some differences of mast cell morphology between the nodular and macular types of urticaria pigmentosa.

Previously we have studied mast cell changes in various skin diseases other than urticaria pigmentosa and found that mast cells are increased in various types of dermatoses especially at the periphery of the lesion and was particularly associated with fibrogenesis (Kim and Lee, 1978). The present study is carried out to investigate ultrastructural characteristics of mast cells from 4 cases of urticaria pigmentosa and 1 case of mastocytosis associated with capillary hemangioma to see if there is any difference in ultrastructural features among different types of urticaria pigmentosa and compare to other conditions.

MATERIALS AND METHODS

Materials consist of skin biopsies from 1 case of mastocytosis associated with capillary hemangioma and 4 cases of urticaria pigmentosa. The major portion of the biopsy specimens were fixed in 10% neutral formalin and processed for routine paraffin embedding for light microscopic examinations. About 5 μ thick sections from paraffin block were stained with hematoxylin-eosin and used for

histologic examination. The dominici method was used for the demonstration of metachromatic granules of mast cells. A portion of the biopsy specimens were cut into multiple small pieces of about 1cu mm size and fixed in 1% osmic acid solution buffered to pH 7.4 with phosphate buffer.

They were dehydrated through graded alcohol, embedded in Epon 812, sectioned in 600 Å thickness, and stained with uranyl acetate and lead citrate. Ultrastructural observation was made with the Hitachi 11-E model electron microscope.

Case History:

Case 1: A 14 year old boy had a purplish subcutaneous lesion on the forehead which was present since birth, and an excision biopsy was performed.

Case 2: A 3 months old male infant had multiple blisters on the trunk and extremities since 2 months of age. He was born following a full term normal pregnancy and the skin was unremarkable at birth and remained clear until 2 months of age when blisters developed on the thigh and upper arms. The blisters became numerous and spread to the trunk without association of other symptoms.

Case 3 and 4: Both cases were 10 month old male infants with normal delivery. Skins were unremarkable at birth and remained clear until 8 months of age when brownish pigmented macular and papular urrticarial plaques developed on the entire body.

Case 5: A 14 month old boy complained of pruritic, erythematous brownish papular and nodular skin eruptions since 8 months of age, and the largest nodule measured 2×4 cm in size.

Light microscopic findings:

Case one showed features of capillary hemangioma associated with increased mast cells in the stroma within the hemangioma and in the periphery of the lesion. Mast cells were either spindle shaped or stellate (Fig. 1).

Cases 2, 3, and 4 showed band like infiltration of mast cells in the papillae and subpapillary regions of the dermis. Most of the mast cells were spindle shaped and were admixed with capillary endothelial cells, fibroblasts, and collagen fibers (Fig. 6, 11). In addition, subepidermal blister formation was noted in case 2 (Fig. 6). Histologically, case 2 was macular with blister formation, and cases 3 and 4 were maculopapular type. Case 5 showed nodular infiltration of pure mast cells in the papillary and subpapillary region, and the mast cells were mostly round with relatively wide intercellular space (Fig. 16, 17). Clinically and histologically, the case was a characteristic nodular type of urticaria pigmentosa. Special staining for mast cell granules demonstrated metachromatic granules mostly within the cytoplasm in cases 1, 3, 4, 5 and large numbers of granules in the extracellular spaces in case 2, blister form.

Ultrastructural findings:

Mast cells from case 1, mastocytosis in hemangioma, showed spindle or elongated shape containing large numbers of packed homogeneously dense granules and a round nucleus (Fig. 3). Granules frequently showed a partial crescent appearance due to the inclusion of a small clear vacuole (Fig. 2, 3). Higher magnifications of granules showed slight differences of electron density within an occasional granule but lamellar structure

was not observed (Fig. 4, 5). The nucleus was relatively round and chromatin particles showed marginal condensation. Microvilli were not prominent.

Mast cells from case 2, blister form, showed oblong or spindle shapes containing relatively scanty numbers of granules with marked perigranular vacuolation or halo formation and many electron-lucid vacuoles giving a honey-comb appearance. There were also many granules in the extracellular space (Fig. 7, 8.) The nucleus was elongated, irregular with marked indentation. Chromatin showed some peripheral condensation and prominent nucleoli were noted (Fig. 8). High magnification of granules showed irregular density and frequently lamellar shaped subgranular structure (Figs. 9, 10). Microvilli were present in moderate numbers and interdigitated with adjacent cells. Mast cells from cases 3 and 4, maculopapular type, were similar and are described together. They contained large numbers of rather homogenous granules packing the entire cytoplasm. No perigranular halo formation or electron-lucid vacuole was noted (Figs. 13, 14, 15). The nucleus showed a prominent nucleolus (Fig. 12). Higher magnification of granules showed some irregularity of electron density and an occasional ring form or scroll like subgranular structure (Fig. 14). Microvilli were prominent and interdigitated with adjacent cells (Fig. 13).

Mast cells from case 5, nodular type, showed a relatively round shape containing various numbers of dense homogeneous granules of relatively smaller size. Perigranular halo formation is not noted, but a few electron-lucid vacuole were noted (Fig. 18, 19, 20). The nucleus was slightly elongated and indented. The chromatin particles were distributed rather homogeneously and nucleolus was

obscure. Microvilli were well developed and interdigitated with adjacent cells. A large extracellular space was conspicuous (Fig. 18).

Beside specific granules, various amounts of other cytoplasmic organelles; mitochondria, RER, ribosomes, and Golgi apparatus were also noted. But when granules packed the cytoplasm, these organelles were often marked.

DISCUSSION

Our observations can be summarized as follows. Mast cells in hemangioma, non-urticaria pigmentosa, are spindle shaped, packed with homogeneously dense granules, a mature nucleus, and moderately well developed microvilli. These features are characteristics of normal mast cells reported by previous investigators. Mast cells from 4 cases of urticaria pigmentosa also showed more or less similar features except in the nature of the granules, and the character of the nucleus indicated a rather immature nature of the cells. Among histologic types of urticaria pigmentosa, the nodular type showed round shape with widened intercellular space, variation in amount of granules whereas in the mast cells of the maculo-papillary type spindle or oblong shapes were present. Evidence of marked degranulation was pronounced in the blister form.

Since the first description of mast cells by Ehrlich in 1877, tissue mast cells have been a source of much curiosity. A voluminous literature concerning chemistry, function and morphology of the mast cells has accumulated over the succeeding years. However, the morphologic studies of mast cells have been largely with the light microscope and usually in animals other than human (Hibbs *et al.*, 1960).

Electron microscopic studies of mast cells were only feasible after the development of suitable methods of fixation and embedding for electron microscopic preparations. Thus electron microscopic studies of mast cells were just begun about 20 years ago, from guinea pigs and hamsters (Smith and Lewis, 1957) and canine mastocytoma (Bloom *et al.*, 1958). Electron microscopic studies of human mast cells were made by Hibbs *et al.* (1960) and they reported at least two types of mast cells. The first type, most abundant in the dermis, is spindle shaped containing rather homogeneous granules, and the second type, distributed throughout the body, is round or oval shaped containing lamellar or scroll shaped subgranular structures. They also noticed an intermediate type.

Ultrastructural studies of mast cells in diseased conditions of the human have been reported by several authors (Orfanos and Stüttgen, 1962; Hashimoto *et al.*, 1966; Freeman, 1967; Kobayashi *et al.*, 1968; Kobayashi and Asboe-Hansen, 1969; Lagunoff, 1972; Moriyasu and Yamamura, 1973; and Naveh, *et al.* 1975). Most of these authors reported that there is no significant morphologic differences between mast cells in normal and diseased conditions or among different types of diseases, except that Hashimoto *et al.* (1966) reported that the mast cells in the nodular type of urticaria pigmentosa are round or oval while mast cells in macular urticaria pigmentosa or systemic mastocytosis are spindle shaped. The findings in our observations are rather in accordance with that of Hashimoto *et al.* (1966). However, the significance in the shape is not certain, as Asboe-Hansen (1973) stated that mast cells may assume various shapes depending on the surrounding tissue, and are either flat, spherical, spindle-

shaped, stellate or even filiform.

The substructure of mast cell granules have been studied by several investigators (Hibbs *et al.*, 1960; Combs, 1966; Freeman, 1967; Kobayashi *et al.*, 1968; Fujita *et al.*, 1969; Asboe-Hansen, 1973). They consist of three major components; a) moderately electron dense filaments in parallel arrangement often containing crystalloid structures; b) very dense finely granular material; and c) thick curved parallel lamellae suggesting finger print or scroll in their configuration. It is suggested that younger or immature granules show irregularity in density with several subgranules, while mature granules, exhibit compact homogeneous density (Combs, 1966; Kobayashi *et al.*, 1968; Fujita *et al.*, 1969).

The process of degranulation of mast cells is one of the high lights in mast cell studies. It has been extensively studied both in animals (Smith, 1963; Lagunoff, 1972) and from urticaria pigmentosa in humans (Hashimoto *et al.*, 1966; Freeman, 1967; Kobayashi and Asboe-Hansen, 1973; Moriyasu and Yamamura, 1973). Degranulation proceeds in two forms, either extrusion of entire granules or intracellular disintegration without extrusion. The extrusion of granules takes place through extensive membranes, resulting in extensive labyrinthine channels in the cell, through which the granules are released from their intracellular sacs (Lagunoff, 1972). Moriyasu and Yamamura (1973) further reported four types of degranulation; 1) Separation of protruded areas containing granules in the cell surface and the released granules are surrounded by plasma membrane, 2) perigranular vacuole or halo formation followed by expulsion of granules, in which released granules lack the plasma membrane. This type of degranulation is most frequently

found in whealing after stroking of urticaria pigmentosa. 3) Discharge of granules by disruption of intergranular cytoplasm without perigranular vacuole formation. 4) Loss of electron density without expulsion of granules, leaving a electron-lucid honey-combed appearance. In our cases, large numbers of type 2 and 4 degranulation as well as type 1 were observed in the blister form of urticaria pigmentosa and a mild degree of type 4 degranulation in the nodular type of urticaria pigmentosa. However, the maculopapular type of urticaria pigmentosa and mastocytosis in hemangioma revealed no notable evidence of degranulation.

Microvilli are constant feature of mast cells in both normal and diseased condition. Some variations in number, length, and interdigitation are noted, but their significance is not certain.

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LEGENDS OF FIGURES

- Fig. 1. Case 1 showing capillary hemangioma with several mast cells around blood vessels. H-E, $\times 430$.
- Fig. 2, 3 & 4. Electronmicrophotographs of mast cells in hemangioma showing spindle or elongate shapes, numerous packed dense cytoplasmic granules, some crescent forms by inclusion of a clear space, dense marginal condensation of chromatin particles, and moderately developed microvilli. $\times 16,000$; $16,000$; $22,500$.
- Fig. 5. Higher magnification of granules from Fig. 3, showing dense homogenous appearance of granular material and a small clear inclusion in one granule. $\times 30,000$.
- Fig. 6. Case 2, showing a large subepidermal vesicle and a dense band of mast cell infiltration in the floor of the vesicle. H-E, $\times 100$.
- Fig. 7. & 8. Electronmicrophotographs of mast cells from case 2, showing various amounts of granules with marked perigranular vacuole formation, some empty granules giving honey-combed appearance, marked irregularity of nuclear membrane, prominent nucleoli, and moderately well developed microvilli. A few extracellular granules are seen at left lower part. $\times 16,000$; $10,700$.
- Fig. 9. & 10. Higher magnification of granules from fig. 8, showing mottled density and somewhat lamellar substructures. $\times 22,500$; $30,000$.
- Fig. 11. Case 3 showing band-like infiltration of mast cells, admixed with endothelial cells and fibroblasts at papillary and subpapillary regions. $\times 100$.
- Fig. 12 & 13. Electronmicrophotographs of case 3, showing large amount of cytoplasmic granules and prominent microvilli with marked interdigititation without evidence of degranulation. $\times 10,700$; $16,000$.
- Fig. 14 & 15. Higher magnification of granules in fig. 13, showing some irregularity of granule density and scroll-like substructure. $\times 30,000$.
- Fig. 16 & 17. Case 5, showing massive tumorous infiltration of mast cells in papilla and subpapilla. Mast cells are mostly round and intercellular space is relatively wide. $\times 100$; 400 .
- Fig. 18. Electronmicrophotographs from case 5, showing irregular shapes with various amounts of granules, moderately indented nucleus with relatively homogenously distributed chromatin particles, and a prominent nucleolus. Microvilli are prominent, long and interdigitated. $\times 10,700$.
- Fig. 19 & 20. Higher magnification of granules from fig. 18, showing dense granular material and lamellar structure. $\times 30,000$







