ULTRASTRUCTURAL, HISTOPATHOLOGIC AND IMMUNOHISTOCHEMICAL STUDIES IN ORAL HISTIOCYTOSIS X

YEONG-LEI HUANG, YING-TAI JIN*, YAT-HANG YAN, CHENG-CHUNG LIN and LI-MIN LIN

The origin and pathogenesis of histiocytosis X disease still remain unclear. Neoplastic proliferation of histiocytes must be distinguished from the accumulation of histiocytic cells in response to an "appropriate" stimulus. The cellular nature of proliferating histiocytes has been thought to be derived from Langerhans cells, because they share similar morphologic features and immunological expressions. Two cases of oral histiocytosis X (eosinophilic granuloma, Letterer-Siwe disease) were studied with light, electron microscopic and immunohistochemical techniques. Sections showed tumor cells were positively stained for S-100 protein. The ultrastructural studies of the tumor cells showed trilaminar or tennis racket shaped cytoplasmic organelles which could not be distinguished from those in the epidermal Langerhans cells. These results suggest that the origin of cells in histiocytosis X is probably the Langerhans cell or its precursor. Our findings would also support, at least partially, that histiocytosis X may be fundamentally an abnormal proliferation of Langerhans cells.

Key words: oral histiocytosis X, Langerhans cell, birbeck granule

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The term "histiocytosis X" was suggested by Lichtenstein[1] in 1953 as the general designation for a disease complex including eosinophilic granuloma (EG), Hand-Schüller-Christian disease (HSC), and Letterer-Siwe disease (LS), indicating they were different forms of one basic pathologic entity. The disorders primarily affect children and young adults and occur in three forms involving varying degrees of proliferation of nonreactive histiocytes. Cline and Golde[2], in a review of histiocytic disorders, have categorized the nonreactive histiocytes according to the degree of cellular differentiation. The three forms range in degree of involvement and severity: (1) monostotic and polyostotic eosinophilic granuloma with one or more bony lesions; (2) chronic disseminated histiocytosis X which is a less serious type and has skeletal and extraskeletal lesions (HSC); (3) acute or subacute disseminated histiocytosis X which is the most fatal type and has widespread skeletal and multiple organ systems involvement (LS).

The origin and pathogenesis of this disease process remain unclear[3-7]. It has been suggested that the proliferative cells of histiocytosis X derive from abnormal Langerhans cells or its precursor[8-10]. Besides the similar unique ultrastructural features of organellar-cell marker (Birbeck granules)[11], the identification of Langerhans cells using immunohistochemical method has relied on the demonstration of a nervous system specific protein, S-100 protein[12].

The purpose of the present investigation was to study two cases of oral histiocytosis X by electron and light microscopic, and immunohistochemical methods in an attempt to clarify and explain the origin of proliferative

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histiocytes in histiocytosis X disease.

MATERIALS AND METHODS

Patient one

A 36-year-old Chinese male complained of severe tooth mobility, painful swelling and loss of masticatory function at the right posterior tooth area. A periapical radiography was taken and showed slightly osteolytic alveolar destruction of the right first molar area. A diagnosis of simple periodontitis was made by the local practitioner and the patient received only periodontal curretage in the local area. No significant improvement was noted for four months and the mobility of the above mentioned area became more severe. He was then referred to the dental clinic of Kaohsiung Medical College. After careful examination, panoramic (Fig. 1) and periapical radiographs demonstrated severe osteolytic alveolar bone destruction (floating-in-air appearance) in the right mandibular premolars to the first molar area. The right mandibular premolars and first molar were noted to have 3^+ mobility and the response of the pulp vitalities was within normal range. Further physical examination failed to demonstrate systemic spread and no other systemic disease was observed. A tentative diagnosis of EG was made and a incisional biopsy was taken from the osteolytic lesion.

Patient two

A 5-year-old, underdeveloped and poorly nourished boy was admitted to the pediatric ward of Kaohsiung Medical college Hospital for generalized yellowish discoloration of the skin, weakness, abdomen distention and icteric conjunctiva in the previous half month. At the time of admission, a tentative diagnosis of hepatomegaly was made and symptomatic therapy was begun. The pediatrician noted severe loosening of left mandibular primary molar and mandibular left first permanent molar. The patient was referred to our dental department for further examination. The panoramic radiograph (Fig. 2) showed the presence of a poorly outlined circumscribed radiolucent lesion with discontinued border at the lower margin, extending from the ramus to the primary canine area of the left mandible. In addition, multiple osteolytic bony involvements (including the left temporal area, left femoral and left pelvic bones) were observed by roentgenographic examination. An incisional biopsy specimen was taken from the osteolytic lesion of the distal area of the left mandibular primary molar area.

Electron microscopy

Tissue samples were obtained from the lesions and processed for electron microscopic examination. Specimens were fixed in 2% paraformaldehyde and 2.5% glutaraldehyde in 0.1 M cacodylate buffer solution, pH 7.4–7.6 for 1.5 hrs. After a thorough washing in 0.1 M cacodylate buffer containing 7% sucrose, blocks were post-fixed in 1% osmium

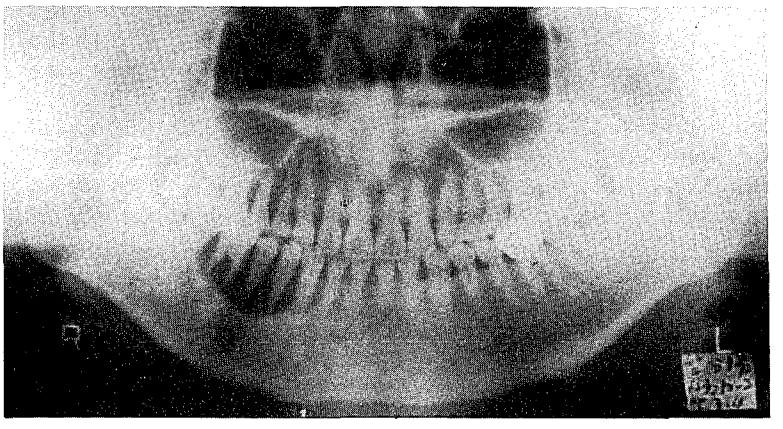

Fig. 1. Panoramic radiograph showing the osteolytic lesion at the right mandibular posterior teeth. The appearance is suggestive of the customary description of "teeth suspended in space; hanging in air".

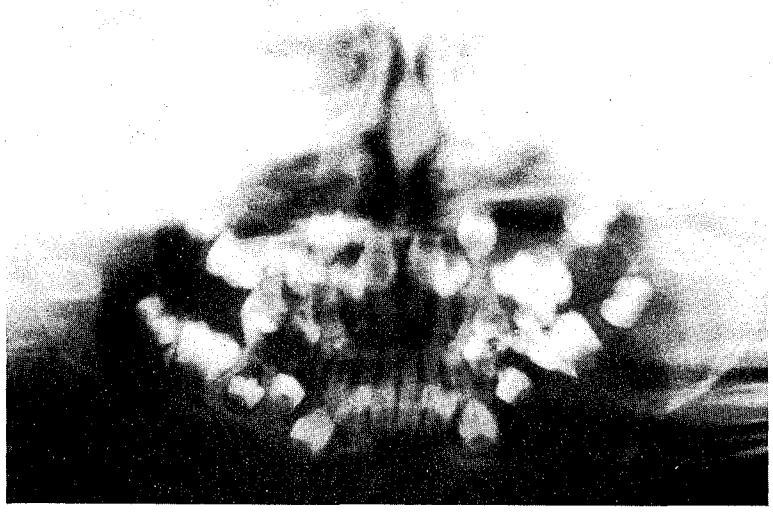

Fig. 2. Panoramic radiograph showing the presence of poorly circumscribed radiolucent osteolytic lesion with discontinued border at lower margin, extending from the ramus to the primary canine area of the left mandible.

tetroxide in 0.1 M cacodylate buffer for 1.5 hrs. And stained in 0.5% aqueous uranyl acetate for 1 hr. The blocks were dehydrated through a graded series of ethanol and embedded in epon. Thin sections were cut on a porter-Blum MT-2B ultramicrotome, stained with uranyl acetate and lead citrate, and examined under a Hitachi H-500 transmission electron microscope.

Light microscopic and immunohistochemical study

Tissue samples were obtained from lesions and fixed in 10% neutral buffered formalin, processed and embedded in paraffin in the usual manner. Sections of 5 μm were attached to glass slides that had been previously coated with Elmer's glue (3% diluted with distilled water) for immunohistochemical study or with albumin for the regular histologic study. Scetions were deparaffinized in xylene, hydrated through a graded series of ethanol and rinsed with distilled water. The endogenous peroxidase activities of the tissue specimens were blocked by incubation in 3% hydrogen peroxide/methanol (1:4, 30 minutes), rinsed with PBS buffer and incubated in a moist chamber with 5% bovine serum (30 minutes) for blocking nonspecific immunoglobulin absorption. After the excess suppressor serum had been shaken off, sections were incubated with primary antibody (rabbit anti-S-100 protein, 1:300, Dako Corp., Santa Barbara, California) for 30 minutes at room temperature. After thorough washing with PBS, drops of linking antibody were added (goat IgG anti-rabbit IgG 1:100, Dakpatts) for 30 minutes, rinsed with PBS buffer (3 changes, 10 minutes each) and coupled to peroxidase antiperoxidase (30 minutes). After thorough washing with PBS, diaminobenzidine (DAB) (Sigma) (6 mg/10 ml PBS with 107 μl 35% hydrogen peroxide adding distilled water to 1.25 ml) was added as chromogen (50 minutes) and then rinsed with running water (5 minutes), then lightly counterstained with Mayer's hematoxyline, dehydrated, mounted with cover slide and examined under a regular light microscope.

RESULTS

Light microscopic findings

There were dense proliferations of histiocytes with characteristic indented nuclei, abundant collection of eosinophilic cytoplasm, and sheets of closely spaced histiocytes with foamy cytoplasm. The large mononuclear cells with oval or kidney-shaped nuclei and multinucleated giant cells observed in case one (Fig. 3) confirmed

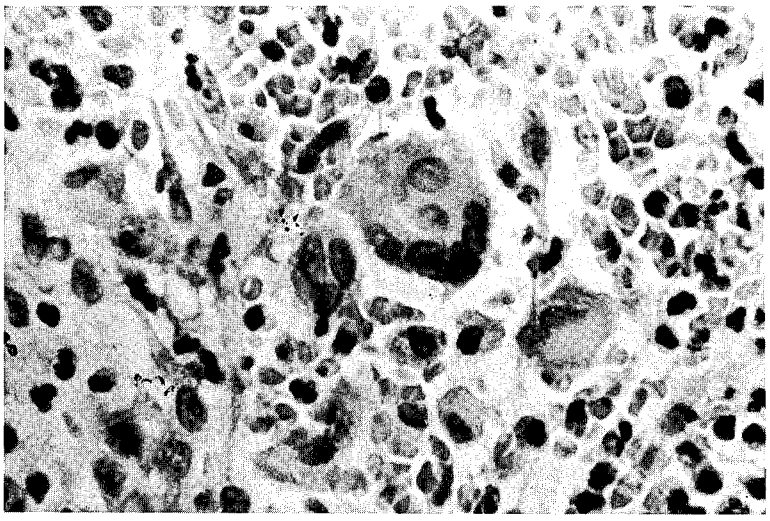

Fig. 3. Photomicrograph showing sheetlike mass of histiocytes with large ovoid nuclei, collections of eosinophilic leukocytes, foamy character of the compacted swollen histiocytes, and the multinucleated giant cells in the case of EG. (HE, x 400).

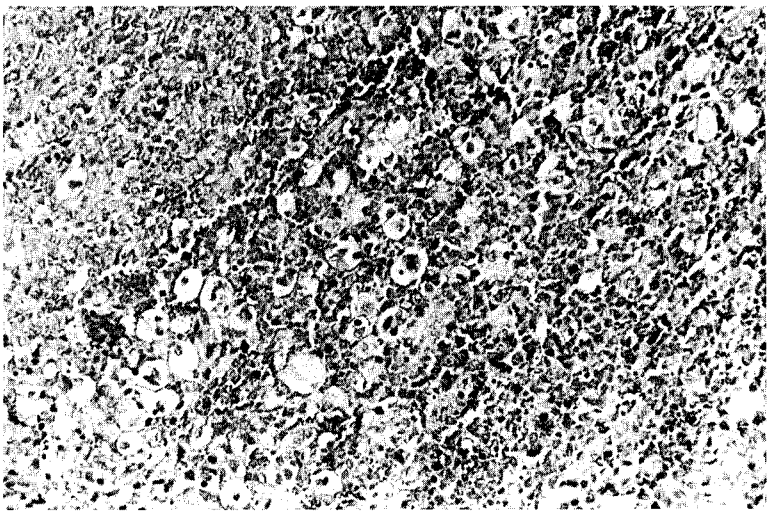

Fig. 4. Photomicrograph showing proliferative histiocytes with accumulation of eosinophils scattered throughout the sheets of histiocytes and many multinucleated giant cells in the case of Letterer-Siwe disease (HE, x 100).

the diagnosis of eosinophilic granuloma of the jaw bone. The histologic characteristics, small eosinophilic leukocytes, numerous multinucleated giant cells, and pleomorphism of individual histiocytes with abnormal mitotic figures observed in case two (Fig. 4) confirmed the diagnosis of Letterer-Siwe disease.

Electron microscopic findings

The eosinophlic leukocytes had numerous membrane-bound specific granules of varying sizes and shapes, and often had characteristic central crystalloid bodies in both patients. The active early splinter form of crystalloid bodies was also found in the

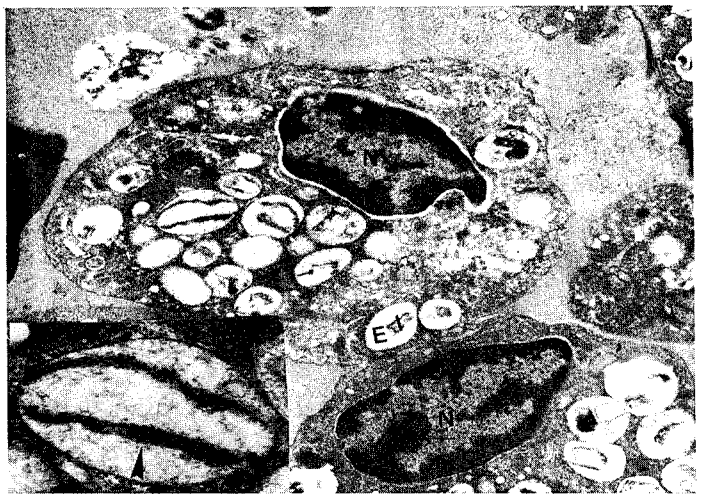

Fig. 5. Electron micrograph showing a typical eosinophilic granulocyte from an eosinophilic granuloma. The nucleus (N) is monolobed, and there are numerous membranelimited specific granules (G) which demonstrate a central crystalloid bar (I) and external less dense matrix (E). (origin magnification, x 6,000). Inset: High resolution electron micrograph showing various stages of development, including early splinter form (arrows). (origin magnification, x 20,000).

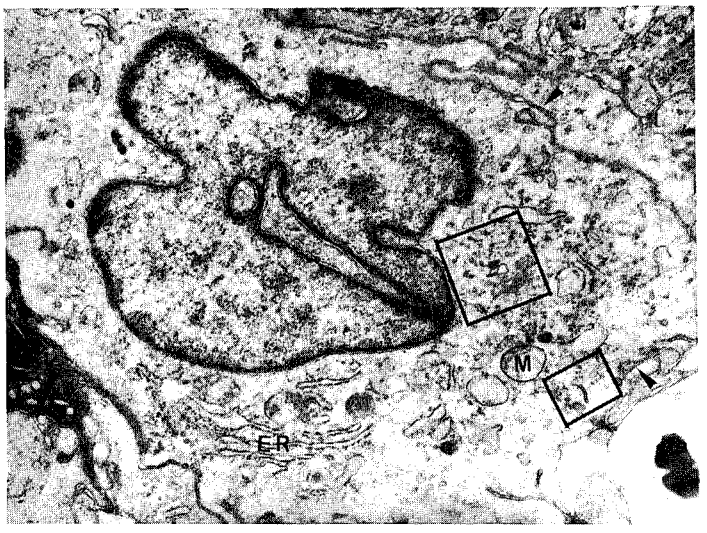

Fig. 6. Electron micrograph of a "histiocytosis X cell" showing a highly contorted and folded nucleus, numerous Langerhans cell granules, and ruffled plasma membranes (arrows). The cytoplasma contain scattered mitochondria(M) and strands of endoplasmic reticulum (ER) (original manification, x 8,000).

case of the eosinophilic granuloma (Fig. 5). The Langerhans cells with ruffled plasma membrane and enlarged folded nucleus were found in all biopsy specimens (Fig. 6). They can be recognized by the presence of pentalamillar Birbeck granules. The number of granule-bearing cells varied as did the number of granules per cell. Granules of histiocytes

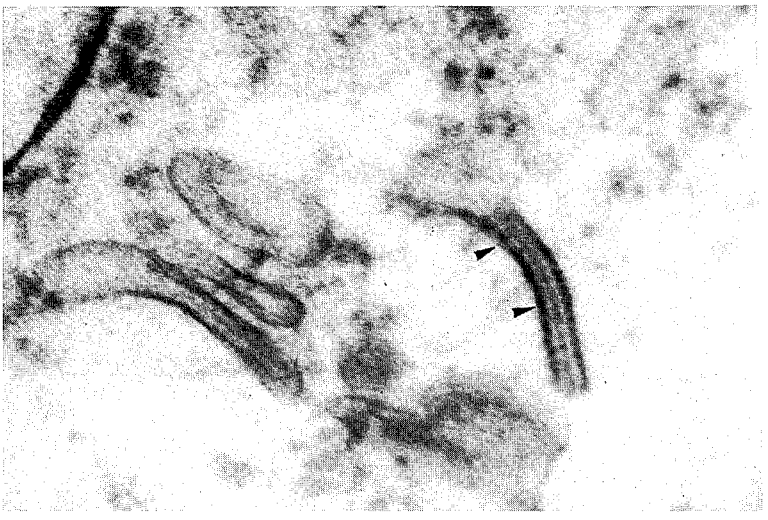

Fig. 7. High resolution of electron micrograph showing several small inclusion bodies (arrows) seen in the cytoplasma and in continuance with the cell membrane (original magnificaiton, x 104, 000).

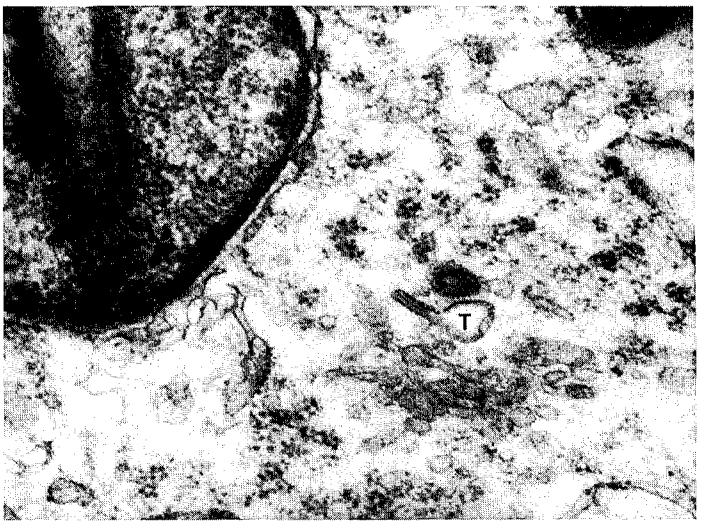

Fig. 8. High resolution of electron micrograph showing various forms of intracellular inclusion bodies. The typical granules are composed of parallel membrane and a central density (arrow). Some granules have dilated forming a "tennis racket" (T) appearance (original magnification, x 35,000).

could be found in continuance with the cell membrane. Cytoplasmic projection of the histiocyte folds back upon itself engulfing extracellular material or glycocalyx of opposed plasma membrane of the villus and the plasmalemma of the histiocytes to form a central line (Fig. 7). In addition to the normal cytoplasmic constituents, numerous small lamellar bodies were widely dilated at both ends, giving the lamellar bodies a "racket", "paddle" or "dumbbell" shape (Fig. 8). The lamellar bodies were located in the center of the cytoplasm and are often associated with the Golgi apparatus. Occasionally, the lamellar bodies were seen in association with the cell surface appearing to

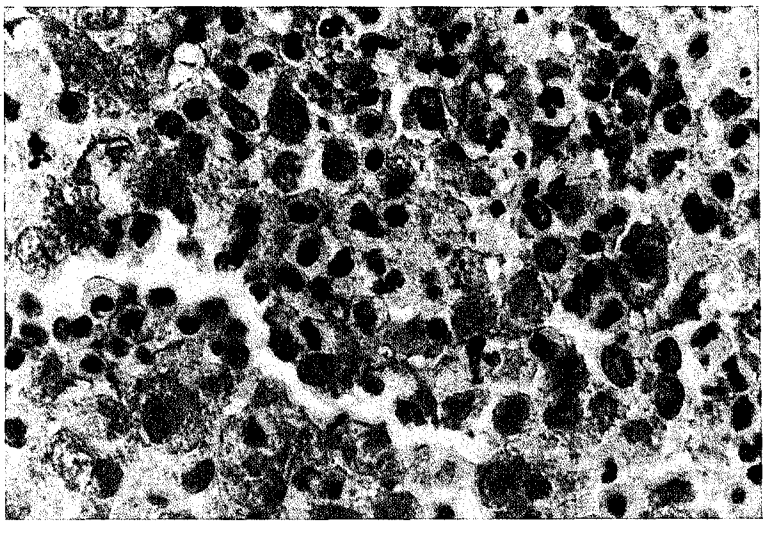

Fig. 9. Photomicrograph showing tumor cells exhibiting occassionally positive S-100 protein staining, the reaction of nucleus was more intense than cytoplasms in the EG (PAP with counterstain, x 400).

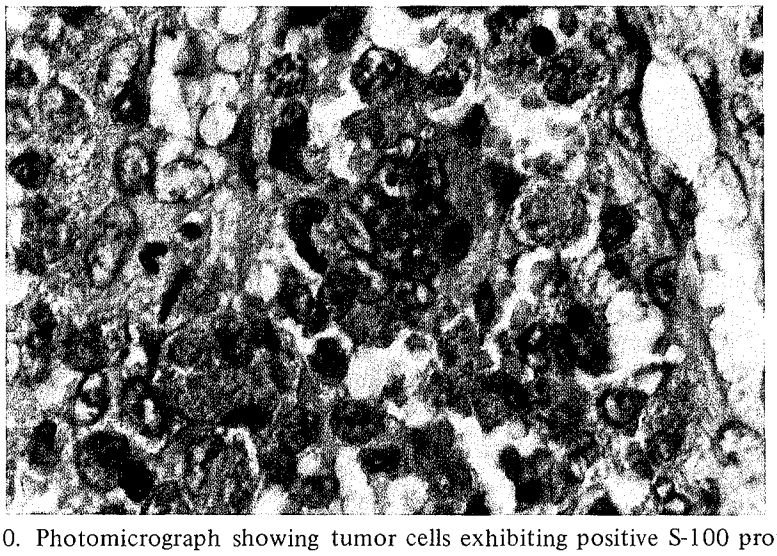

Fig. 10. Photomicrograph showing tumor cells exhibiting positive S-100 protein staining and negative staining for the large multinucleated giant cells, eosinophils and phagocytic histocyts in the LS (PAP with counterstain, x 400).

form by infolding the cell membrane. These lamellar bodies were not seen in regular phagosome containing macrophages.

Immunohistochemical findings

Affected histiocytes in the lesions of most histiocytosis X demonstrated positive immunostaining for S-100 protein. Both the cytoplasm and nucleus of these cells had positive results, but the reaction of the nucleus was more intense than that of the cytoplasm (Fig. 11 & 12). There was weak staining for the foam cells and negative staining for the large multinucleated giant cells and eosinophils.

DISCUSSION

Histiocytosis X is one of the neoplastic granulomatous lesions which mainly affect the reticuloendothelial system, characterized by eosinophilic and histiocytic cell proliferation that are considered to be related to the mononucleated-phagocytic system[13]. The cause of these disorders remains unknown. Accumulation of histiocytic elements may be a proper response to invasion by certain microrganisms or to the presence of abnormal or damaged erythocytes. But this proliferation of histiocytes is restrained and self-limited. In the proliferative histiocytes of histiocytosis X disease, no such constraints are evident. In the past few years, considerable progress has been made regarding the understanding of the origin and function of Langerhans cell[14]. Dense local accumulation of eosinophils seen in EG is thought to be a secondary inhibitant of the lesions due to the excessive production of eosinophilic chemotactic factor which is possibly produced by the lymphocytes[15]. However, on the cellular level, Cline and Golde[2] stated that the histiocytes in LS were more immature than those in HSC and EG. In their recent report, Groopman and Golde[13] stated that all stages of histiocytic maturation are common to all LS, HSC, and EG. The use of phagocytosis has been seen as an indicator of the character of the proliferating cells. However, the Langerhans cells rarely show phagocytic activity. Thus it seems that there is some confusion as to the significance of phagocytosis in histiocytosis X disease and its implication for judging maturity or differentiation of proliferating Langerhans cells. The frequent presence of multinucleated giant cells perhaps indicates that mononuclear histocytes have some capability for coalescence and transition to multinucleated form. The 20% of the oral lesions bearing the multinucleated giant cell have been noted[3]. In our histopathologic findings, the admixture of multinucleated giant cells was observed in both cases and individual histiocytes with abnormal mitosis were also noted in the case of LS. Therefore the influx of phagocytes became more prominent in these proliferating diseases.

In our electron microscopic study, the fine features of numerous specific granules demonstrated central crystalloid bodies in various stages of development; including early splinter form. The ultrastructure findings were similar to the hypereosinophilic syndrome which usually shows neoplastic potential. This may explain the frequent recurrence encountered in EG. Two separated histiocytic populations, the prominating histiocytosis X cells and phagocytic histiocytes, were also found in this study. The former cells were characterized by the presence of cytoplasmic inclusion bodies (Birbeck granules) and a lack of polymorphic phagosomes. The latter contain neither Birbeck granules nor lamellar bodies. These findings seem to be in favor of the existence of two histiocytic cells in histiocytosis X disease. Although there are some differences between lamellar bodies and Langerhans bodies[6] (mainly in size, presence of intranuclear X bodies, disappearance of Langerhans granules in culture), these differences are minor in comparsion with structure and spatial similarities. The argument of histogenecity for variants of histiocyosis X disease as a Langerhans cell origin had been described by Nezelof[16]: (1) the morphologic and histochemical similarities between Langerhans cells and granule-containing cells in histiocytosis X disease; (2) the frequent findings of skin, scalp or mucosal lesions in some forms of histiocytosis X, especially the LS variant; (3) the pattern in which the disease spreads, suggesting vascular routes of dissemination and (4) cells with lamellar bodies in the bloodstream.

Our immunohistochemical studies confirmed that some parts of the tumor cells of histiocyosis X disease have an immunohistochemical phenotype that is similar to that of Langerhans cells. The proliferating cells display strong S-100 protien reactivity. The presence of this protein in both nuclear and cytoplamic locations and the negative immunohistochemical staining of multinucleated giant cells and phagocytic histocytes were also observed in some areas of histiocytosis X disease. However, cells previously referred to as "histocytes" are actually of two different cell systems: the mononuclear-phagocytic system and the Langerhans (dendritic) cell system. Moreover, recent immunohistochemical investigations using monoclonal antibody also show a high

degree of specificity of OKT6 in the identification of Langerhans cells[17-19] and histocytes in histiocytosis X disease. T6 antigen may only be demonstrated by a fresh specimen in frozen sections. However, the morphology associated with frozen sections is inferior to that seen in paraffin material, making the topographic relationship of various cell types difficult to analyse. It is necessary to develop reliable markers for these cells on routine paraffin embedding block. The foam cells were relatively weak staining for S-100 protein. Our findings suggested that foam cells are probably not related to macrophage lineage.

Langerhans cells are thought to fulfill a crucial role in the T-cell mediated system[20-21]. Their proliferation may represent a disturbed interaction with T-cell, either due to the faulty function of Langerhans cells, or T-cells, or both. The defect of T-cells could be due to acquired immune deficiency perhaps initiated by viral infection, triggered by contact with environmental agents[22].

In summary, the presence of ultrastructural Birbeck granules (organellar markers) and immunohistochemical detection of S-100 protein in the proliferating cells of histiocytosis X disease support the concept that the histiocytosis X disease is a part of the spectrum of Langerhans cell proliferative disease[23,24]. A special population of lymphocytes may serve as important regulators of Langerhans cell proliferation. Loss of this regulatory function may be a significant factor in this proliferative phenomenon[25]. On the other hand, it may represent true neoplastic transformation. Further investigation of cellular constituents of cells in histiocytosis X disease may lead to the understanding of its cause, pathogenesis and prognosis.

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口腔X型組織細胞增多症之超微構造組織病理學及免疫組織生化學的探討

黃湧澧　靳應臺*　印憶恒　林正仲　林立民

X型組織細胞增多症(histiocytosis X)自1953年Dr. Lichtenstein，提出此爲一種組織細胞球的炎性及增殖性反應，並且認爲嗜伊紅性肉芽腫病（eosinophilic granuloma）、Hand-Schüller-Christen 病、Letterer-Siwe 病是一群臨床及組織相似之單一病型的不同臨床表現。其發生病因尚不清楚，但可能爲網內皮系（reticuloendothelial system）的一種障礙。增殖性的組織細胞球由於具有相似的超微構造及免疫組織化學的表現，以往學者認爲其可能與Langerhans cell 有密切關係，本篇研究報告是藉發生於顎骨中兩個不同類型的X型組織球增多症，分別爲36歲、男性症例，診斷爲嗜伊紅性肉芽腫；及5歲、男孩症例，診斷爲全身性Letterer-Siwe 病，進行組織病理學上、免疫組織化學上及超微構造的觀察，發現此腫瘤細胞對S-100 蛋白質在免疫組織化學染色產生陽性反應，並且細胞質內有三層板狀（trilaminar）及網球拍（tennis racket）形態之胞器（organelles），而無法與存在於表皮的Langerhans cell 區別，因此我們認爲爲X型組織球增多症可能源自Langerhans cell 或其先驅物。本研究的結果可以闡明X型組織球增多症（histiocytosis X）主要可能是一種不正常的Langerhans cells 的增殖。

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