

THE ULTRASTRUCTURAL STUDY OF NON-HODGKIN'S LYMPHOMA CELL, CHRONIC LYMPHOCYTIC AND HAIRY CELL LEUKEMIA

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Non-Hodgkin's lymphoma cell leukemia (NHLCL), chronic lymphocytic leukemia (CLL) and hairy cell leukemia (HCL) are the diseases very similar to each other. The differential diagnosis is very difficult, especially when there are small lymphoid cells in peripheral blood and bone marrow under light microscope. We have observed 34 cases with electron microscope. The studies were correlated with clinical manifestation, cytology, pathology and immunologic histochemistry. Ultrastructural features strongly indicated the difference in three various diseases, although all the immunologic markers showed B-cell type. It is concluded that electron microscopic examination is of a definite significance in the diagnosis and successful treatment.

Key words: Ultrastructure; Non- Hodgkin's lymphoma cell leukemia; Chronic lymphocytic leukemia; hairy cell leukemia

NHLCL, CLL and HCL are the diseases very similar in clinic. Especially when there are small lymphoid cells in peripheral blood and bone marrow under light microscope, the differential diagnosis is very difficult. However, the differences among the three types of leukemia may exist in the ultrastructural features.

MATERIALS AND METHODS

Patients

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Between 1986 and 1995, a total of 34 patients were included in this study (NHLCL 16, CLL 13 and HCL 5). 28 were men and 6 women. The ages ranged from 9 to 76 (median 55 years). A diagnosis of leukemia of lymphocytic system was made according to the examination of peripheral blood and bone marrow. Biopsy of lymph node was performed in 22. Non- Hodgkin's lymphoma was confirmed in 12. Pathologists couldn't determine the classification between NHLCL and CLL in 10. Spleen aspiration revealed CLL in one patient. Immunologic histochemistry was analysed in 12. A panel of monoclonal antibodies composed of CD₄₅, CD₃, CD₇, CD₁₉, CD₂₀, CD₂₁, CD₄₅R and CD₄₅ RO was used in this study. B-cell markers included NHLCL, CLL and HCL in 5, 4 and 1 cases respectively. T-cell markers exhibited in 2 NHLCL cases. The course of the diseases ranged from 2 to 120 months (median 6 months). Incipient symptoms were enlarging superficial lymph node in 24, great spleen in 6. Other complaints were pale and proptosis of both eyes, tumor of skin, fever and dyspnea, and epistaxis etc. The laboratory data on admission were: hemoglobin 35 to 147 g/L (median 100 g/L); white blood cell count 4.5 to 150 $\times 10^9/L$ (median $22 \times 10^9/L$); lymphocytic absolute count 0.6 to $147 \times 10^9/L$ (median $19.8 \times 10^9/L$). Bone marrow aspiration showed hypercellularity. Small lymphocytes or Non- Hodgkin's lymphoma cells were 47% to 100%. The characteristic "hairy" appearance of cells was seen in 4 patients under light microscope.

Electron Microscopical Examination

Transmission electron microscope (TEM) was performed in all cases. Scanning electron microscope (SEM) was analysed in 3 patients. Pretreatment specimens were prepared in 27. Posttreatment specimens were obtained from 7 patients after intervals of several weeks. The specimens were collected from peripheral blood, bone marrow and lymph node in 16, 15 and 3 patients respectively.

RESULTS

Ultrastructural characterization of three diseases was described as follows:

NHLCL

The lymphoid cells measured 7-12 μm under TEM. The cell margins demonstrated pseudopodium and phalangeal processes. Therefore, the cells revealed an irregular contour. The nucleus was irregular outline, ranging from notches, distinct cleaves, deep invagination and long nuclear projection (Figure 1-3).

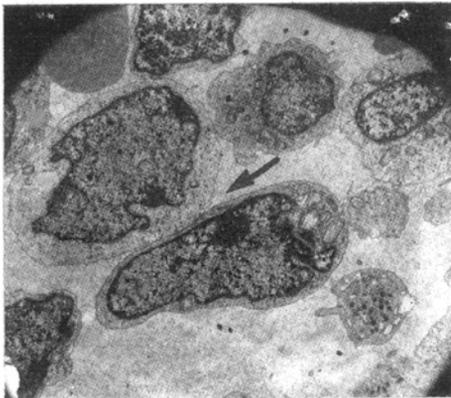


Fig 1. Bone marrow picture of NHLCL viewed under TEM. The nucleus shows Irregularity, the nucleoli locate eccentrically. $\times 4000$

Occasional nuclei demonstrated cytoplasmic invagination. Intranuclear pseudoinclusion was present. Euchromatin was prominent. Heterochromatin was aligned along the nuclear membrane but it was less well clumped than in cases of CLL. It contained much more in more well differentiated cells. Nucleoli, one to four per nucleus, were conspicuous and eccentrically located in most of cells. The counting

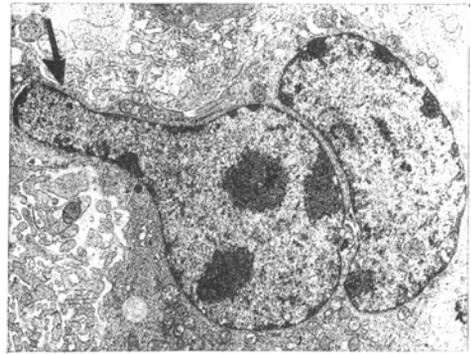


Fig 2. Bone marrow picture of NHLCL viewed under TEM. The nucleus shows long projection. $\times 4000$

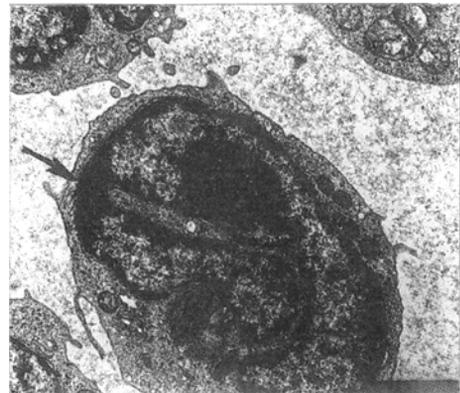


Fig 3. Bone marrow picture of NHLCL viewed under TEM. The nucleus shows deep cleave. $\times 8000$

rate of nucleoli was 80-90% (counted 200 cells). The cytoplasm was more abundant than that of CLL. Golgi apparatus was poorly developed, occasionally with centrioles and centrosomes. Swollen mitochondria (MI) was present, sometimes with vacuolar degeneration. Some rough endoplasmic reticulum (RER) dilated like cyst. In addition to the lymphoid cells, there were three more cell types: 1) The plasmoid cells contained abundant RER with linear arrangement (Figure 4). 2) The reticuloid cells showed bizarrely indented nucleus and large nucleolus placed against the nuclear membrane, reticular fibers may exist in the cytoplasm. 3) The monocytoid cells viewed kidney shaped nucleus. The cytoplasm was abundant with scattered granules. In lymph node section, occasional phagocytic cells were noted containing red blood cells and lymphocytes.

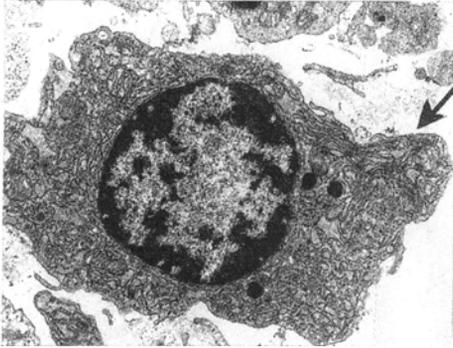


Fig 4. Lymph node picture of NHLCL viewed under TEM. The plasmoid cell presents abundant rough endoplasmic reticulum. $\times 4000$

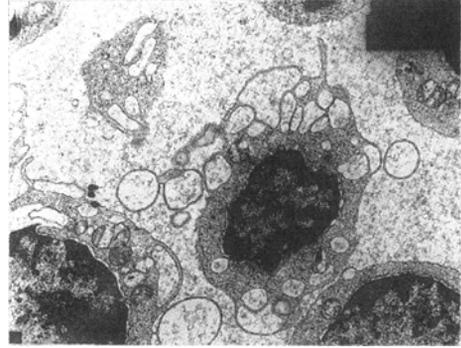


Fig 5. Blood picture of HCL viewed under TEM. the cell margin shows long processes, anastomosing to form circle. $\times 8000$

CLL

The cells showed small lymphoid cells (6-8 μm) with smooth surfaces under TEM. The nucleus was round or oval with regular outline. A small population of cells had slightly irregular or notched nuclei, and cells with cleaved were rare. Cytoplasmic invagination into the nucleus was not observed. Heterochromatin was densely clumped in a blocklike pattern. Nucleoli were small and often centrally located. The counting rate of nucleoli was 20-50%, occasionally may have 60%. The cytoplasm was scarce with a high nucleocytoplasmic ratio. MI was rare or clusters which often gathered on one side of cytoplasm, sometimes, a few large polymorphocytic cells were present. The nucleus revealed minor irregularity. Euchromatin was relatively much more. The nucleolus was conspicuous with eccentric location. The cytoplasm was relatively abundant.

HCL

The hairy cells measured 8-9 μm . The most striking feature was the protrusions from the cell surface. Under SEM there were numerous protrusions which was similar to villous, large fold and pseudopodium. Under TEM the cell margins showed long, thin, straight, curved and branched processes. The longest was 4 μm , anastomosing to form cyst and circle (Figure 5). The nucleus was slightly irregular. The nucleolus was one or inconspicuous. Only two patients viewed likely ribosome lamellar complexes (RLC). Sometimes cytoplasmic inclusion was noted.

The results showed that in four cases electromicroscopic findings were crucial to the diagnosis, and in 10 cases electron microscope served to confirm the diagnosis, unclassified between NHLCL and CLL. In the remaining cases, however, the ultrastructural diagnosis helped to confirm the light microscopic diagnosis. For example, in tow cases light microscopic examination of peripheral blood and bone marrow smears indicated fine microvillous processes of small lymphoid cells repeatedly, but electron microscope did not confirm the surface processes, leading to a final diagnosis of CLL. Similarly, light microscopic diagnosis of a patient's lymph node biopsy was NHLCL, CLL, or reactive hyperplasia, whereas electron microscope revealed features of NHLCL.

DISCUSSION

Diagnosis

According to the clinic features, NHLCL was divided into acute and chronic types by Schwarte in 1965. It was similar to acute lymphoblastic leukemia (ALL) and CLL respectively, but the clinical difference existed.¹ In recent years, the investigators have described two different opinions on their relations: 1) The origin of ALL and CLL was involved in bone marrow, but the disorder of NHLCL was involved in lymph node at first and then in bone marrow. The morphology revealed no distinct difference. Liang considered that when the leukemia of the small lymphocytic Non-Hodgkin's lymphoma was

complicated, it was equal to the CLL;² 2) Isaacs, et al. recognized that the morphology and clinical manifestation of these diseases were different.^{3,4} Our studies supported that the morphologic differences may exist between NHLCL and CLL, although both immunologic markers were B-cell type. According to the following ultrastructural characteristics of leukemic cells under TEM: the shape of cells, the regular degree of nucleus, the counting rate, size and location of nucleolus, cytoplasmic amount and nucleocytoplasmic ratio, distribution of heterochromatin and euchromatin, single or multiform cells (NHLCL had lymphoid, plasmoid, reticuloid and monocytoid cells), the degree of differentiation, the differential diagnosis could be established.

In this group, T-cell CLL couldn't be observed, but as previously described, ultrastructural features were: nuclear irregularities or convolutions, a few granules near the region of Golgi, numerous short microvillous processes.⁵ The difference exhibited between T- cell and B- cell CLL. Although T- Cell CLL occurred much less frequently, we must further study about the differential diagnosis between T- Cell CLL and NHLCL. In this study some B- cell CLL cases revealed high counting rate of nucleoli and a few prolymphocytes in the peripheral blood specimens, but it didn't belong to prolymphocytic transformation. In general, when the prolymphocytic cells increased over 15% in peripheral blood, the diagnosis could be established.⁶ Sometimes, the cells revealed microvillous under the light microscope, but it was smooth surface under TEM. The possibility may be association with preparation of smears. The fresh smears should be stained as quickly as possible for cytologic examination. Because typical RLC could not be showed, the subtypes in HCL and B- cell splenic lymphoma with villous lymphocytes should be further studied.⁷

Treatment

Accurate diagnosis was important in the defined therapy. Patients with NHLCL received CHOP-Bleo, COAP and BCNU+ VP16 regimens. For example, a case presented proptosis of both eyes and slowly enlarging superficial lymph nodes for 12 months. Tumor biopsy of upper orbit showed NHLCL, CLL or reactive hyperplasia. He was treated with prednisone for 6 months and had progressive disease. Based on the final diagnosis by using electron microscope, it

was B cell NHLCL chronic type and BCNU+ VP16 was used. He achieved complete remission for 36 months. Another case with B- cell monocytoid NHLCL received aforementioned regimen, high dose methotrexate and daunorubicin. He had no changes. At last, given single harringtonine, he achieved partial remission and maintained for 30 months. This type was previously reported very rare.⁸ In addition, two B-cell CLL cases were given chemotherapy according the NHLCL regimen at the local hospital. They were unresponsive to drugs. At the time of TEM diagnosis CLL, they were treated with leukeran and obtained partial remission for 6 and 20 months respectively.

Our observations further suggested that electron microscope was much more precise and specific than just using light microscope. It has a valuable adjunct for accurate diagnosis, important therapeutic and prognostic significance.

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