HUMAN LEUKEMIC T AND B LYMPHOID CELLS : COMPARISON OF THEIR ULTRASTRUCTURE

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ABSTRACT

Human leukemic lymphoid cells were examined by transmission electron microscopy with special reference to the comparative morphology between T and B cells. Among 16 lymphoid leukemias studied, 2 out of 9 acute lymphoblastic leukemias and 1 out of 7 chronic lymphocytic leukemias were of the T cell type. The remaining 6 chronic lymphocytic leukemias were of the B cell type and 7 acute lymphoblastic leukemias had no detectable surface markers. One case of hairy cell leukemia was also studied.

Ultrastructural differences between the two cell types were found in the amount of rough endoplasmic reticulum, in the presence of clustered dense granules, and in the surface morphology. T cells contained only a few short strands of rough endoplasmic reticulum, while B cells had much, and often long strands. Dense granules were gathered in small clusters in T cells but not in B cells. The cell surface was almost smooth in all T leukemic cells, while it was villous in many B leukemic cells. In some B leukemic cells, however, the surface was as smooth as the T leukemic cells.

KEY WORDS B cells / T cells / lymphoid leukemia / rough endoplasmic reticulum

Human lymphoid cells are grouped into two types, thymus-dependent (T) and thymus-independent (B) cells, which are identified by their surface markers (1). Numerous studies have been carried out to identify the T or B cell origin of leukemias by using these surface marker expressions on leukemic cells. In contrast to the relative ease of identifying most chronic lymphocytic leukemias (CLL) as being a B cell malignancy (B-CLL) by the presence of B cell surface markers, acute lymphoblastic leukemias (ALL) appear to be heterogeneous, having a T cell type (T-ALL) as a minor subgroup and a "null-cell" type (N-ALL) as a major subgroup.

Attempts to find morphological differences between T and B cells in leukemias have had little success (for review, see 11), except for differences in enzyme activity (3) and in surface morphology (for review, see 9). We have recently found a difference in the amount of rough endoplasmic reticulum (RER) between human T and B cells in normal peripheral blood, in mitogen-stimulated cultures as well as in permanent lymphoid cell lines (5). The present study was carried out to determine whether this difference could also be found in leukemic T and B cells.

MATERIALS AND METHODS

Nine cases of ALL, 7 cases of CLL and 1 case of hairy cell leukemia (HCL) were examined in this study. As shown in Table 1, they were

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selected on the basis that (a) the patients were not undergoing chemotherapy, (b) the leukocyte count was high enough to permit surface marker studies and electron microscopic studies to be made using the same sample, and (c) more than 80 % of the mononuclear cells of the sample were determined to be leukemic by leukocyte classification of Giemsa-stained smears and by determining the presence of surface markers.

Mononuclear cells were separated by Ficoll-Hypaque density gradient centrifugation from peripheral blood of each ALL and CLL patient or from spleen cell suspension of the HCL patient. Surface markers examined were (a) non-immune rosette-formation with sheep erythrocytes (E rosettes), (b) rosette-formation with bovine erythrocytes coated with antibody (EA rosettes) or with antibody and complement (EAC rosettes), and (c) the presence of surface immunoglobulins detected by membrane fluorescence using anti-human immunoglobulins (sIg) (1). T cells were identified by the ability of E rosette-formation and the absence of sIg. B cells were identified by the ability of EAC rosette-formation and the presence of sIg.

For electron microscopy, mononuclear cell

pellets were fixed with phosphate-buffered 2 % glutaraldehyde followed by 1 % osmium tetroxide, dehydrated by ethanol and embedded in Epon epoxy resin. Thin sections were doubly stained with uranyl acetate and lead citrate.

For quantitative examination of the amount of RER, low magnification pictures ($\times 3,000$) were taken randomly. They were later enlarged to $\times 15,000$ photographically. Pictures of more than 100 lymphoid cells showing nucleus were examined for each specimen. Rectangular sheets (75×37.5 mm) as well as the depicted cytoplasm were cut out from prints. Each rectangular sheet represented $12.5 \mu\text{m}^2$ of the original thin section. The total length of RER on all pieces of the cut out cytoplasm (L, mm), total weight of the cut out cytoplasm (Wc, mg), total weight of the rectangular sheets (Ws, mg) and total number of the rectangular sheets (N) were measured.

The weight of a sheet representing $1 \mu\text{m}^2$ is expressed by $Ws / 12.5 N$, whereas the total cytoplasmic area by

$$Wc \div [Ws / 12.5 N] = 12.5 N Wc / Ws$$

Thus the length of the RER per unit area of

Table 1 *Diagnosis, Hematological Data and Surface Markers in Leukemic Patients*

Patient No.	Age (yr)	Sex	Diagnosis	Leukocytes ($\times 10^9/\text{cu mm}$)	Leukemic cells (%)	Surface markers			
						E	EA	EAC	sIg
1	0.5	M	ALL	394	95	5	0	3	1
2	1	F	ALL	100	94	5	3	5	8
3	5	F	ALL	65	90	6	1	2	2
4	8	F	ALL	128	95	8	0	8	NT
5	9	M	ALL	134	96	84	6	76	4
6	21	F	ALL	470	98	90	0	11	3
7	23	F	ALL	36	90	11	0	2	NT
8	24	F	ALL	6	98	17	0	10	NT
9	27	M	ALL	120	84	2	0	0	NT
10	50	M	CLL	48	90	1	0	66	NT
11	53	M	CLL	53	95	2	0	53	95
12	55	F	CLL	63	92	9	0	77	96
13	63	M	CLL	53	83	10	6	55	92
14	64	F	CLL	31	86	16	4	42	40
15	66	M	CLL	645	96	84	0	9	1
16	71	M	CLL	291	94	0	1	73	94
17	71	M	HCL	spleen	80	17	5	9	79*

Abbreviations: M, male; and F, female. NT, not tested. ALL, acute lymphoblastic leukemia; CLL, chronic lymphocytic leukemia; and HCL, hairy cell leukemia. For E, EA, EAC and sIg, see Materials and Methods. * Surface IgM and IgD were demonstrated even after incubation for 24 hr.

the cytoplasm ($\text{RER}/C, \mu\text{m}/\mu\text{m}^2$) is given by

$$\begin{aligned} & [1,000 \text{ L}/15,000] \div [12.5 \text{ N Wc}/\text{Ws}] \\ & = \text{L Ws}/187.5 \text{ N Wc} \end{aligned}$$

RESULTS

Two of 9 ALL cases were concluded to be of the T cell type by their surface markers (Nos. 5 and 6, Table 1). Leukemic cells in T-ALL are very homogeneous in their morphology either within one sample or between two samples (Fig. 2). The nucleus is round or indented and contains a fine condensation of chromatin and a prominent nucleolus. Nuclear pockets are occasionally seen (Fig. 1a). Numerous free ribo-

somes scattered throughout the cytoplasm are either monoribosomes or polyribosomes. Round mitochondria and a cluster of dense granules are gathered near the small Golgi complex which is located in the nuclear indentation. The cell surface is usually smooth.

Other ALL cases lacked detectable surface markers. The morphology of N-ALL is homogeneous within each case but exhibits considerable variety among cases (Fig. 3). The highly irregular nucleus contains poorly to very finely condensed chromatin, and one or a few prominent nucleoli. Nuclear pockets are seen more frequently than in T-ALL. Free polyribosomes are very numerous in the cytoplasm. RER is rare except for cells in one case (No. 2, Table

Fig. 1 Electron micrographs of nuclear pockets, *P* (a), annulate lamellae which connect with the rough endoplasmic reticulum and with the nuclear envelope (b; arrows), and accumulation of a fine filamentous substance in the rough endoplasmic reticulum (c). These structures are rarely found in normal lymphocytes, but are frequently seen in leukemic cells. a $\times 11,000$, b $\times 28,000$, c $\times 22,000$

1), which contain long strands of RER (Fig. 3b). Clusters of dense granules are found near the Golgi complex in 2 patients (Nos. 4 and 8, Table 1; Fig. 3a) where leukemic cells bear an ultrastructural resemblance to T-ALL cells. Glycogen particles are abundant in 3 cases. The cell surface is almost smooth or bears only a few microvilli in 4 cases. In 2 cases, cells project microvilli from a pole on their surface. A large process is sometimes seen from the same pole, so that the cell is hand mirror-shaped. In one case, the cell surface is uneven and bears some microvilli.

Six (Nos. 10-14 and 16, Table 1) out of 7 cases of CLL are concluded to be B cell malignancy. Leukemic cells in 5 B-CLL cases (Nos. 11-14 and 16, Table 1) resemble normal small lymphocytes in their size and nuclear morphology (Fig. 4a). The RER is moderately developed and its long strands are often seen. Dense and fine filamentous substance, which possibly represents immunoglobulin (2), is accumulated in the dilated cisternae of RER in one case (No.

13; Fig. 1c). The cell surface bears many microvilli in 3 cases. The surface is almost smooth in one case, while villous and smooth cells are mixed in another case.

In one B-CLL case (No. 10), leukemic cells are larger (Fig. 4b). The condensation of nuclear chromatin is less than other B-CLL and nucleoli are very conspicuous. The RER is moderately developed. The cell surface is smooth.

One case of CLL (No. 15) is determined to be a T cell type by its ability of E rosette-formation and the absence of sIg. Leukemic cells have a very irregular nucleus with moderately condensed chromatin and prominent nucleoli (Fig. 5). Strands of RER are few and short. A cluster of dense granules is often seen near the Golgi complex. Some areas of the cytoplasm appear felt-like due to presence of a very fine filamentous substance and by the absence of cytoplasmic organelles and free ribosomes. Only a few microvilli are projected from the cell surface.

One patient (No. 17) is diagnosed as having

Fig. 2 An electron micrograph of cells from a patients with acute lymphoblastic leukemia of T cell type (Case No. 5, Table 1). Cells are round and have smooth surface contour. Cytoplasmic organelles are very scanty. D, a cluster of dense granules. $\times 6,300$

Fig. 3 Electron micrographs of cells from null-cell type subgroup of acute lymphoblastic leukemia. (a) Cells resemble those in T cell type of acute lymphoblastic leukemia, except for a more frequent occurrence of irregular-shaped nucleus and more abundant free polyribosomes. *D*, clustered dense granules. *F*, a bundle of fine fibrils. Case No. 4, Table 1. (b) Cells are seen to contain long strands of rough endoplasmic reticulum and glycogen (*G*). Case No. 2, Table 1. *a* $\times 5,400$, *b* $\times 10,000$

Fig. 4 Electron micrographs of cells from chronic lymphocytic leukemia, B cell malignancy. Cells are generally found to contain long strands of rough endoplasmic reticulum. (a) Leukemic cells resemble normal small lymphocytes. Case No. 11, Table I. (b) Leukemic cells are larger and have a nucleus with less condensed chromatin and a very prominent nucleolus. Case No. 10, Table I. a $\times 9,000$, b $\times 8,000$

Fig. 5 Cells from a patient with chronic lymphocytic leukemia, T cell type, showing the irregular-shaped nucleus with a conspicuous nucleolus. A nuclear pocket (*P*) and a cluster of dense granules (*D*) are seen. Felt-like area of the cytoplasm (*F*) is devoid of free ribosomes. Case No. 15, Table 15. $\times 7,500$

Fig. 6 Cells from a patient with hairy cell leukemia, showing numerous microvilli. Dense pericentriolar satellites (*S*) are conspicuous. *L*, lipid droplets. Case No. 17, Table 1. $\times 7,200$

hairy cell leukemia (HCL). Leukemic cells are considered to be B cell type by the presence of surface immunoglobulins (IgM and IgD) even after incubation for 24 hr. The cells extend long cell processes and microvilli (Fig. 6). Their nuclei contain moderately condensed chromatin and a nucleolus. Strands of RER are moderately developed, often long and in parallel arrays. Pericentriolar satellites are conspicuous. Dense granules and lipid droplets are often seen.

DISCUSSION

The findings in this study on the ultrastructure of leukemic cells (Table 2) roughly agree with those in previous papers (11, 15, 16, 17, 20). As a result of detailed examination, however, some differences are found between T and B leukemic cells in addition to those found in previous papers.

By examining thymocytes, peripheral blood lymphocytes and mitogen-stimulated cells from normal individuals and human lymphoid cell lines, we found that B cells had more developed RER than T cells, although this might not always be valid as the criterion of cell types (5). This is also true when leukemic T and B cells are compared as in the present study. The better development of the RER in B cells is attributed to the immunoglobulin synthesis in RER as discussed previously (5). The small amount of RER in most N-ALL cases seems reasonable, since the RER is generally scarce in rapidly proliferating and undifferentiated cells. One curious exception is leukemic cells in N-ALL patient No. 2, where the amount of the RER is similar to that in B cells. It is noteworthy that long strands of RER are often found in stem cell leukemias (17), although the role of the RER in these cells is unknown.

Watanabe *et al.* (19) and we (5) have reported that many T lymphocytes contain a cluster of dense granules near the Golgi complex. In the present study, such a structure is found in T type leukemic cells (T-ALL and T-CLL) but not in B type cells (B-CLL and HCL). It is interesting that clustered dense granules are found in 2 cases of N-ALL, which also have an ultrastructure more similar with T-ALL in many points than with other N-ALL. We are unable to correlate these 2 cases of N-ALL to T cell malignancy at the present. Further studies are required to clarify whether the presence of clustered dense granules has any diagnostic value for T cell malignancy even when the cells do not exhibit easily detectable surface markers.

On the surface structure of leukemic lymphoid cells, Polliack *et al.* (9, 10) summarized their extensive scanning electron microscope study by explaining that malignant T cells were predominantly the smooth type and B cells were most frequently the villous type, though a small number of B malignant cases were the smooth type or the mixed villous and smooth type. Our present study generally agrees with their results. Although the surface structure of lymphoid cells changes during their maturational stage and functional state as well as during processing for the fine structural study (12), the coincidence of the scanning electron microscope findings (9, 10) and the present transmission electron microscopic findings is notable. Both methods may produce similar artificial changes in the surface structure during processing.

We agree with the previous investigators (6, 7, 14) on the morphology of HCL. In addition, we must stress again that HCL cells often contain long strands of RER, which sometimes are in parallel arrays. The ribosome-lamella complex, which occurs in about half of the HCL cases (6, 7, 14), were not seen in the present case. As many investigators have suggested (4, 13, 18), we classified HCL as a B cell malignancy because of the presence of surface IgM, IgD and B cell antigen, and of a morphology similar to lymphoid cells rather than cells in the reticuloendothelial system. However, others disagree with this classification (8, 14).

This study demonstrates some quantitative morphological differences between leukemic T and B cells, although we cannot classify leukemia by the ultrastructure of the leukemic lymphoid cells alone.

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