Jpn. J. Cancer Res. 81, 376–382, April 1990

Light and Electron Microscopic Study of Vacuolated Cells in Immunoblastic Lymphadenopathy-like T-Cell Lymphoma

Yuko Hirose,[1] Shiro Shimizu, Ritsuko Yoshioka, Junko Tachibana, Susumu Sugai, Tomoo Takiguchi and Susumu Konda

Division of Hematology and Immunology, Department of Internal Medicine, Kanazawa Medical University, 1-1, Daigaku, Uchinada-machi, Kahoku-gun, Ishikawa-ken 920-02

Three cases of immunoblastic lymphadenopathy (IBL)-like T-cell lymphoma were analyzed immunologically, and the ultrastructure of mononuclear cells in the lymph nodes and peripheral blood was examined. In the peripheral blood, light microscopic examination revealed vacuolated lymphocytes. These vacuolated lymphocytes formed rosettes with sheep erythrocytes, and they were CD3- and CD4-positive using the avidin-biotin method in cases 1 and 2. Electron microscopic examination revealed two kinds of abnormal lymphocytes. One kind was of B-cell nature with rich lamellated rough-surfaced endoplasmic reticulum and mitochondria. The other kind had a large cytoplasm, in which Golgi apparatus, some endoplasmic reticulum, some mitochondria and a few vacuoles were seen. Some of these vacuoles had remnants of mitochondrial cristae or were enlarged endoplasmic reticulum. The vacuolated T lymphocytes and activated lymphocytes of B-cell nature disappeared with chemotherapy but reappeared with relapse of the disease. These observations suggest that vacuolated cells are related to pale cells in lymph node sections. In other words when these vacuolated cells are found in the peripheral blood of patients with lymphoid malignancies, IBL-like T-cell lymphoma can be suspected.

Key words: Immunoblastic lymphadenopathy-like T-cell lymphoma — Ultrastructure — Pale cells — Vacuolated cells

In 1975, Lukes and Tindle[1)] proposed a disease entity of immunoblastic lymphadenopathy (IBL) for the lymphoproliferative disorders of the B-cell system. IBL is characterized by a morphologic triad: proliferation of arborizing small vessels, prominent immunoblastic proliferation, and amorphous acidiphilic interstitial material. Shimoyama *et al.*[2)] and Watanabe *et al.*[3)] reported patients with histological profiles similar to those of IBL with a proliferation of immunoblasts as well as the so-called "pale cells" of T-cell nature and termed this disorder immunoblastic lymphadenopathy-like T-cell lymphoma (IBL-like T-cell lymphoma).

Recently, we encountered three cases of IBL-like T-cell lymphoma. May-Giemsa-stained peripheral blood smears revealed lymphocytes with vacuolation together with lymphocytes with basophilic cytoplasm (activated B lymphocytes) and pathologic lymphocytes with nuclear atypism. We speculated that these lymphocytes with vacuolation were characteristic of IBL-like T-cell lymphoma, and we studied them light and electron microscopically as well as immunologically. We also reviewed peripheral blood smears of 37 cases of T cell malignancy other than IBL-like T-cell lymphoma and 12 cases of activated T lymphocytosis in viral infection or drug allergy.

[1] To whom correspondence should be addressed.

MATERIALS AND METHODS

Immunologic analysis Cells obtained from sliced lymph nodes, heparinized peripheral blood, or ascites were examined for rosette-forming capacity with sheep erythrocytes (E), IgG-Fc (EA-γ) and complement (EAC) as described elsewhere.[4)] Ficoll-Hypaque-separated mononuclear cells were cytocentrifuged and stained with May-Giemsa, acid phosphatase and periodic acid-Schiff (PAS). Binding of antibodies to mononuclear cells of peripheral blood or lymph nodes was assessed by the indirect immunofluorescence technique or with a cytofluorograph system (50-F, Ortho Diagnostics). The monoclonal antibodies were purchased from Ortho Pharmaceutical Corp., NJ (OKT3(CD3), OKT4(CD4), OKT8(CD8), OKIa1(HLA-DR)), Becton Dickinson, Mountain View, CA (Leu1(CD5), Leu2a(CD8), Leu3a-(CD4)) and from Coulter, Hialeah, FL (B1(CD20)). Ficoll-Hypaque separated mononuclear cells were cytocentrifuged and stained with monoclonal antibodies by the avidin-biotin method, or incubated with gold colloidal particles coated with goat anti-mouse immunoglobulin following incubation with monoclonal antibodies.

Electron microscopy The cells in suspension, rosette-forming cells, and cells coated with gold colloidal parti-

cles were fixed in 1.0% glutaraldehyde for 1 h, then fixed in 1.0% OsO_4 for 1 h. The specimens were dehydrated in graded acetone series and embedded in Epon 812. Ultrathin sections were made and stained with lead citrate. The specimens were examined under an H-500 electron microscope.

CASES

Case 1 A 62-year-old male developed generalized lymphadenopathy, hepatosplenomegaly and right pleural effusion in March, 1987. Laboratory data revealed an erythrocyte sedimentation rate (ESR) of 117 mm/h, RBC of $3.44\times10^6/\mu l$, hemoglobin of 10.3 g/dl, and hematocrit of 31.3%. The white blood cell count was 7900/μl with 2.5% bands, 80% segmented forms, 6.0% lymphocytes, 9.0% monocytes, 0.5% eosinophils, 0.5% basophils and 1.5% atypical lymphocytes. Vacuolated lymphocytes were also seen, but they did not appear in the differential count. His serum protein was 8.4 g/dl, of which 41.2% was gamma globulin. Serum IgG level was 4120 mg/dl, IgA was 430 mg/dl and IgM was 140 mg/dl. The level of LDH was 382 U/liter. The bone marrow was normocellular, and pathologic cells were not found. Lymph node biopsy revealed proliferation of pale cells, immunoblasts with dark cytoplasm and arborizing blood vessels. A diagnosis of IBL-like T-cell lymphoma was made (Fig. 1). Data from surface marker analyses revealed a predominance of CD3(+) and CD4(+) cells (Table I). Atypical lymphocytes and vacuolated lymphocytes disappeared in June, 1987 after chemotherapy with vincristine, cyclophosphamide, adriamycin and prednisolone but reappeared with exacerbation of the disease in November, 1987. He died from interstitial pneumonitis due to cytomegalovirus infection in April, 1988.[5]

Case 2 A 68-year-old male developed low grade fever, generalized lymphadenopathy and lower abdominal pain in March, 1988. On admission, he had generalized lymphadenopathy in the cervical, axillary and inguinal areas. His liver was palpable 5 cm below the right costal margin, but his spleen was not palpable. Shifting dullness and fluid wave were present in the abdomen.

Laboratory data revealed an ESR of 109 mm/h, RBC of $3.56\times10^6/\mu l$, hemoglobin of 11.2 g/dl, and hematocrit of 34.0%. The white blood cell count was 15,500/μl, 22% bands, 50.5% segmented forms, 18% lymphocytes,

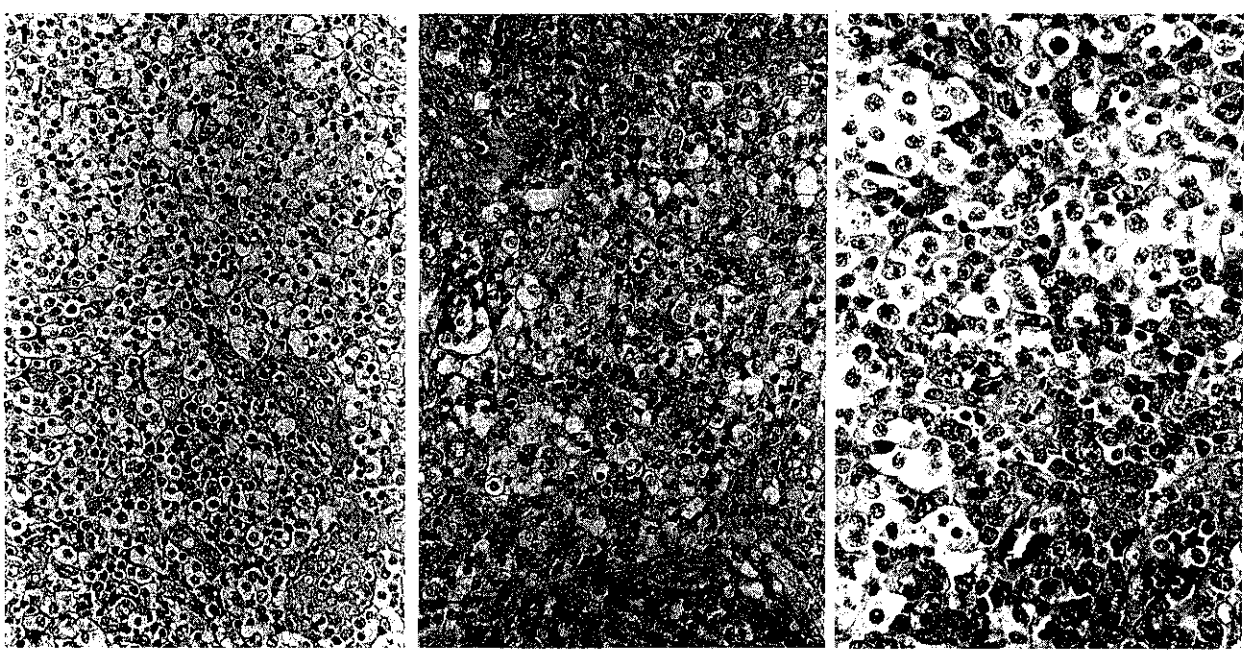

Fig. 1. Histology of lymph node biopsy from case 1. Obliteration of normal lymph node architecture, thick-walled small blood vessels, and sheet-like proliferation of pale cells are seen. H-E.
Fig. 2. Lymph node biopsy from case 2. Focus of pale cells with small round nuclei and a moderate amount of pale cytoplasm. H-E.
Fig. 3. Lymph node biopsy from case 3. Proliferation of pale cells with a moderate amount of pale cytoplasm, round nuclei and smaller plasmacytoid cells with dark cytoplasm. H-E.

Table I. Immunophenotypic Analysis of Mononuclear Cells from Patients with IBL-like T-cell Lymphoma (%)

		CD3 (OKT3)	CD4 (OKT4)	CD8 (OKT8)	OKIal	CD20 (B1)	S-Ig				
							IgM	IgG	IgA	κ	λ
Case 1											
PB	Mar 31 '86	70	67	39	45	25				7.6	5.5
LN	Mar 31 '86	85	67	32	50	23	0.5	6.0	1.2	1.0	3.9
PB	Nov 6 '86	70	56	40	72	33					
LN	Nov 10 '87	49	40	18	77	12	0.8	3.3	0	0.9	2.2
Case 2											
PB	Mar 22 '88	85	55	30	25	15					
Ascites	Mar 25 '88	85	65	20	30	15					
LN	Mar 22 '88	53	53	10	30	33	12.7	1.4	2.6	11.4	7.8
Case 3											
PB	May 29 '84	70	40	30	20	20				8.6	4.9
PB	Feb 1 '85	70	20	50	30	0					
PB	Apr 2 '85	80	40	55	40	40					
PB	May 10 '85	80	30	50	65	40					

Abbreviations: PB, peripheral blood; LN, lymph node; S-Ig, surface immunoglobulin.

4.5% monocytes, 0.5% eosinophils, 0.5% basophils, 1.5% atypical lymphocytes, 2.0% vacuolated cells and 0.5% pathologic cells. The total protein level was 5.9 g/dl, IgG was 1810 mg/dl, IgA was 421 mg/dl and IgM was 804 mg/dl. His level of LDH was 313 U/liter. Lymph node biopsy was done in the left inguinal area on March 22, 1988. A diagnosis of IBL-like T-cell lymphoma was made from the proliferation of pale cells and blood vessels (Fig. 2). Surface marker analyses of the peripheral blood, ascites and lymph nodes revealed that mononuclear cells were CD3(+) and CD4(+) (Table I). He frequently exhibited gastrointestinal bleeding and died in May, 1988 from respiratory failure.

Case 3 A 75-year-old male was admitted with cervical, axillary and inguinal lymphadenopathy in May, 1984. His liver was not palpable but his spleen was palpable 1.5 cm below the left costal margin. His ESR was 144 mm/h, RBC $2.81\times10^6/\mu l$, hemoglobin 8.7 g/dl and hematocrit 26.9%. The white blood cell count was $4950/\mu l$, 21% bands, 63.5% segmented forms, 11.0% lymphocytes, 3.5% monocytes and 1.0% pathologic cells. A few vacuolated lymphocytes were seen on the smear, although they did not appear in the differential count. His total protein level was 8.0 g/dl, IgG was 2865 mg/dl, IgA was 180 mg/dl and IgM was 1871 mg/dl. His LDH level was 304 U/liter. Cervical lymph node biopsy was performed and a diagnosis of IBL-like T-cell lymphoma was made from the proliferation of pale cells, immunoblasts with dark cytoplasm and blood vessels (Fig. 3). He was treated with cyclophosphamide, vincristine and prednisolone and remission was obtained in November, 1984. He relapsed in January, 1985 and died in July, 1985 from interstitial pneumonitis. Surface marker analyses of his peripheral blood mononuclear cells revealed a predominance of CD3(+) and CD8(+) cells (Table I).[6]

RESULTS

Light microscopic study In case 1, a May-Giemsa-stained peripheral blood smear revealed lymphocytes with basophilic cytoplasm and lymphocytes with vacuolation. The vacuolated lymphocytes formed rosettes with sheep erythrocytes (Fig. 4) and they were stained positively with PAS and acid phosphatase. The vacuoles were not stained with PAS or acid phosphatase. Surface marker analysis with monoclonal antibodies revealed that these vacuolated cells were Leu 1(CD5)-positive, Leu 2a(CD8)-negative and Leu 3a(CD4)-positive. These vacuolated cells were, therefore thought to be helper/inducer-type T cells of peripheral origin. In case 2, some lymphoid cells with basophilic cytoplasm and lymphoid cells with vacuolated cytoplasm were seen. The vacuolated cells were E rosette-positive (Fig. 5A), Leu 3a(CD4)-positive (Fig. 5B) and Leu 4(CD3)-positive. In case 3, E rosette-forming vacuolated cells were seen (Fig. 6). In the review of the peripheral blood smears of 37 cases of T cell malignancy other than IBL-like T-cell lymphoma and 12 cases of activated T lymphocytosis, vacuolated lymphocytes were not detected in any of the cases.

Ultrastructure In the lymph nodes of case 1, two kinds of abnormal lymphocytes were seen. One kind was of B-cell nature with rich lamellated rough-surfaced endo-

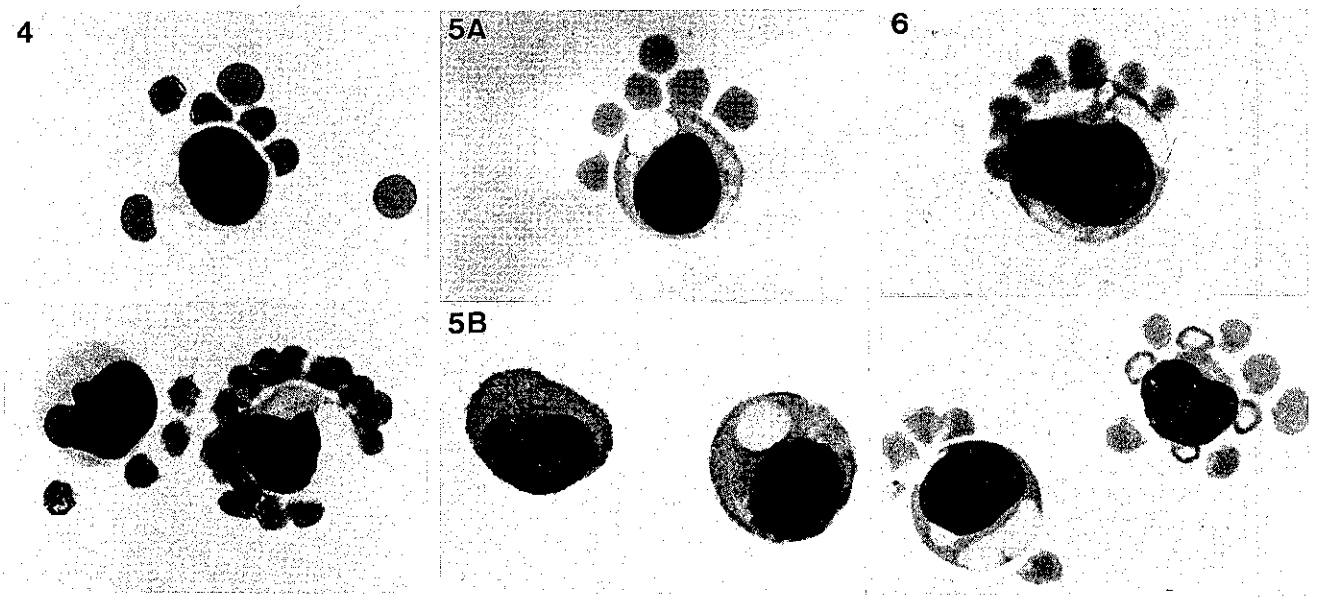

Fig. 4. E rosette-forming vacuolated lymphocytes in the peripheral blood of case 1. May-Giemsa stain.
Fig. 5. A. E rosette-forming vacuolated lymphocytes in the peripheral blood of case 2. May-Giemsa stain. B. The vacuolated cells stained positively with anti-Leu3(CD4) antibody. Avidin-biotin complex method.
Fig. 6. E rosette-forming vacuolated lymphocytes in the peripheral blood of case 3. Medium-sized and large vacuolated cells are seen. May-Giemsa stain.

plasmic reticulum and mitochondria. The nucleus has nucleoli and chromatin concentrated around its periphery (Fig. 7). The other kind was lymphocytes having a large cytoplasm with some endoplasmic reticulum, mitochondria and Golgi apparatus. Nuclei were indented with a moderate amount of heterochromatin and had nucleoli. These lymphocytes had gold particles that reacted with OKT4(CD4) on the surface (Fig. 8). In the peripheral blood, partially vacuolated lymphocytes and lymphocytes with large vacuoles were seen. E rosette-forming lymphocytes had a few small vacuoles (Fig. 9). The interior of the vacuoles were electron-translucent and some of these vacuoles had remnants of mitochondrial cristae (Fig. 10). In case 2, similar vacuolated cells were seen in the peripheral blood and ascites.

DISCUSSION

Watanabe *et al.*[3] reported five cases of adult T-cell lymphoma with hypergammaglobulinemia. Histologically, focal proliferation of monotonous pale cells and foci composed of polymorphic large, medium, and small cells were characteristic in all cases. Weiss *et al.*[7] and Watanabe *et al.*[8] thought that IBL and AILD are T-cell malignancies, which show a spectrum of histologic features from T-cell dysplasia to peripheral T-cell lymphoma (IBL-like T-cell lymphoma).

In further studies with monoclonal antibodies, neoplastic T-cells were found to be CD4+ or CD8+.[8-10] Our cases had histological features of IBL-like T-cell lymphoma with a proliferation of pale cells and basophilic cells resembling plasma cells in the lymph nodes and hypergammaglobulinemia. Surface marker analysis revealed that the pale cells were CD3+ and CD4+ helper/inducer-type T cells (cases 1 and 2) and CD3+, CD8+ cytotoxic/suppressor T cells (case 3). A difference between the number of CD3+ cells and the sum of CD4+ and CD8+ cells in case 1 of Table I might be explained in terms of a tendency of pathologic T lymphocytes to lose CD3 antigenicity. In the peripheral blood of both cases and in the ascites of case 2, vacuolated cells were also CD3+ and CD4+. The vacuoles were electron-translucent and most of them had remnants of mitochondrial or endoplasmic reticulum. The cytoplasm of vacuolated cells contained few organelles in spite of the large space. Nuclei had heterochromatin attached to the nuclear membrane and had small but distinct nucleoli. These features were similar to CD4+ lymphocytes in the lymph node. Watanabe *et al.*[3] reported that pale cells are rich in edematous cytoplasm

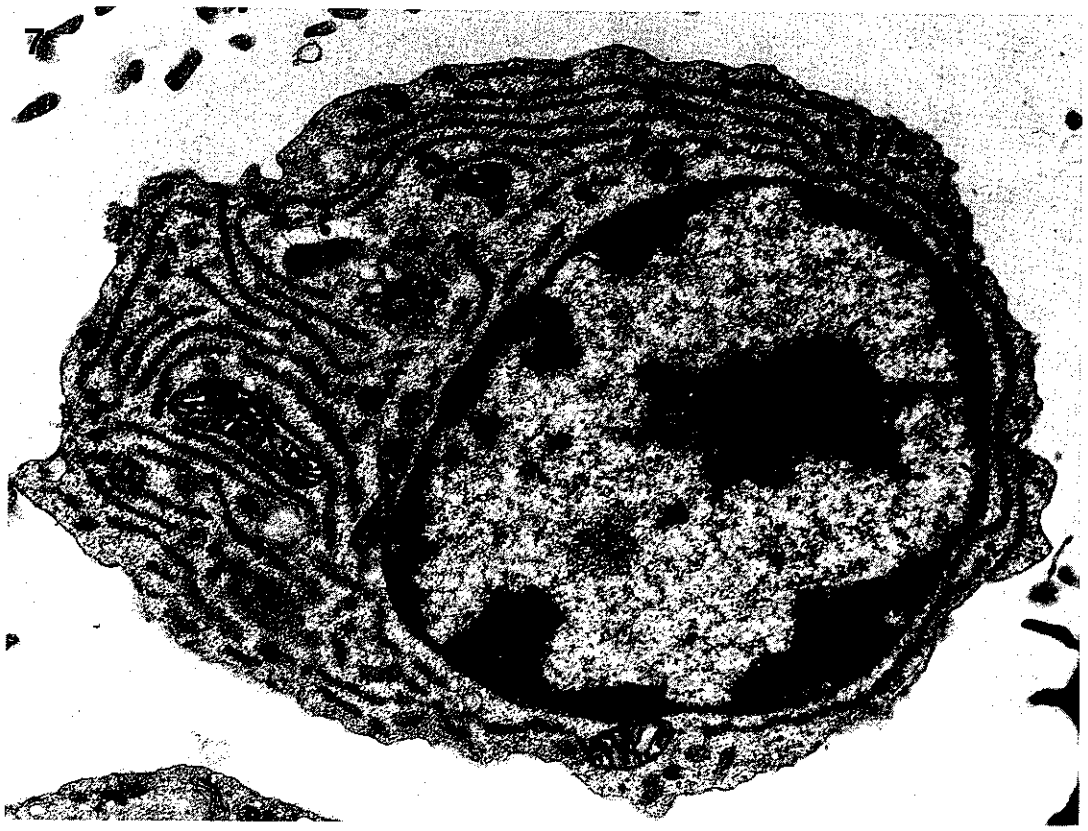

Fig. 7. Lymphocyte of B cell nature from a lymph node of case 1. This lymphocyte is rich in lamellated rough surfaced endoplasmic reticulum. The nucleus has a nucleolus and the chromatin is concentrated along the nuclear membrane. ×15000.

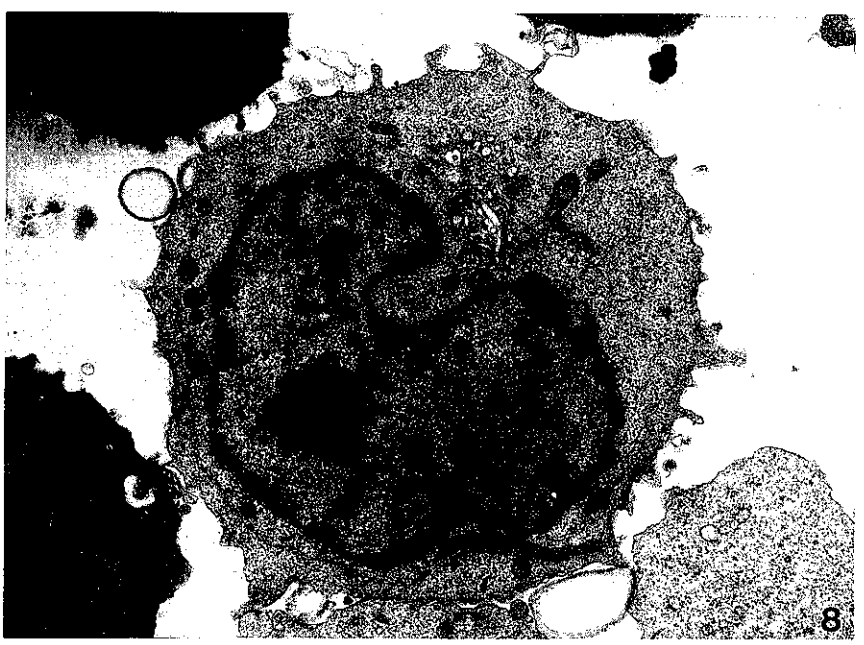

Fig. 8. Lymphocyte from a lymph node of case 1. This lymphocyte has a large cytoplasm with some mitochodria, some endoplasmic reticulum and Golgi apparatus. This lymphocyte has gold particles that reacted with monoclonal antibody (OKT4(CD4)) on the surface. ×12000.

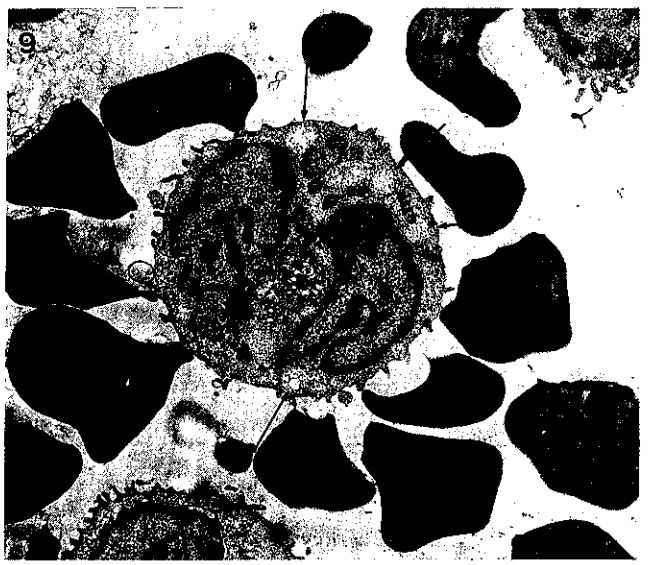

Fig. 9. E rosette-forming lymphocyte from the peripheral blood of case 1. This lymphocyte has mitochondria and Golgi apparatus but endoplasmic reticulum is not seen. A few small vacuoles are seen (arrows). ×7600.

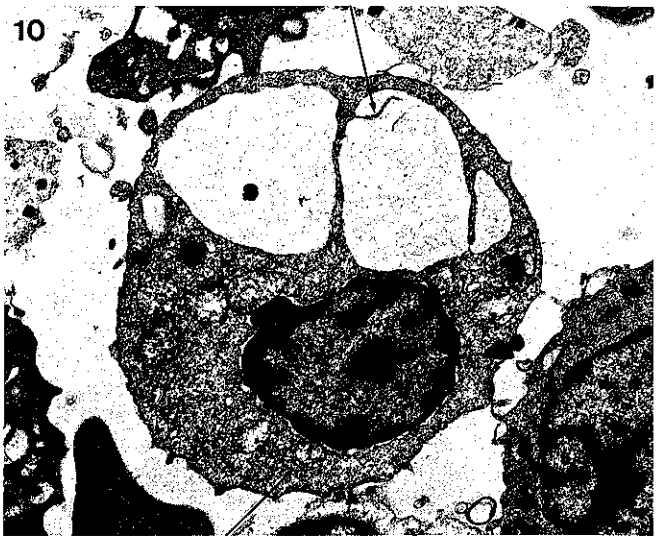

Fig. 10. Lymphocyte from the peripheral blood of case 1. This lymphocyte has large vacuoles. The inside of the vacuoles is electron-translucent and some of these vacuoles have remnants of mitochondrial cristae (arrows). ×9600.

containing a few organelles, so it seems probable that in cells with few cytoplasmic organelles, mitochondria or endoplasmic reticulum expand when the cells begin to degenerate. Ginn *et al.*[11] described marked expansion of mitochondria associated with lethal injury with ouabain. As the tiny vecuoles take up more and more water, the stands enlarge and flow together until they become one very large vacuole.[12] Therefore, we speculate that these vacuolated cells may be related to pale cells in IBL-like T-cell lymphoma. The presence of these vacuolated cells in the peripheral blood suggests IBL-like T-cell lymphoma. The other lymphoid cells with basophilic cytoplasm are thought to be of B-cell origin from the presence of lamellated endoplasmic reticulum in the cytoplasm.[13] In these B cells with lamellated endoplasmic reticulum, there is no space for the mitochondria or endoplasmic reticulum to swell, so vacuolation does not seem to occur. These cells are thought to produce gammaglobulins in IBL-like T-cell lymphoma. In other types of T cell malignancy, pathologic cells had lobulated and indented nuclei which occupied a large volume of cytoplasm, so there was not enough space for the cytoplasmic organelles to swell.[14] Both vacuolated T lymphocytes and basophilic activated B lymphocytes in peripheral blood disappeared in response to chemotherapy but they recurred with exacerbation of the disease. This further supports the relationship between vacuolated T lymphocytes and IBL-like T-cell lymphoma.

(Received December 8, 1989/Accepted February 22, 1990)

REFERENCES

1) Lukes, R. J. and Tindle, B. H. Immunoblastic lymphadenopathy. A hyperimmune entity resembling Hodgkin's disease. *N. Engl. J. Med.*, **292**, 1–8 (1975).
2) Shimoyama, M., Minato, K., Takenaka, T., Watanabe, S., Nagatani, T. and Naruto, M. Immunoblastic lymphadenopathy(IBL)-like T-cell lymphoma. *Jpn. J. Clin. Oncol.*, **9** (Suppl.), 347–356 (1979).
3) Watanabe, S., Shimosato, Y., Shimoyama, M., Minato, K., Suzuki, M., Abe, M. and Nagatani, T. Adult T-cell lymphoma with hypergammaglobulinemia. *Cancer*, **45**, 2472–2483 (1980).
4) Shimizu, S., Sugai, S., Konda, S., Yamanaka, Y. and Setoyama, M. A monoclonal surface immunoglobulin (IgM/D-L) with specificity for surface antigen of ox red blood cells in a patient with leukemic lymphoma. *J. Clin. Immunol.*, **5**, 397 (1986).
5) Yoshioka, R., Shimizu, S., Sugai, S., Tachibana, J., Hirose, Y., Takiguchi, T., Sawada, M. and Konda, S. Oligoclonal gammopathy and vacuolated lymphocytes in a patient with immunoblastic lymphadenopathy (IBL)-like T-cell

lymphoma. *Rinsho Ketsueki,* **29,** 1842 (1988) (in Japanese).
6) Takiguchi, T., Okada, H., Sawada, M., Tachibana, J., Imaoka, T., Shimizu, S., Hirose, Y., Sugai, S. and Konda, S. Two cases of IBL-like T-cell lymphoma with proliferation of reactive B-cells. *Rinsho Ketueki,* **26,** 318 (1985) (in Japanese).
7) Weiss, L. M., Strickler, J. G., Dorfman, R. F., Horning, S. J., Warnke, R. A. and Sklar, J. Clonal T-cell populations in angioimmunoblastic lymphadenopathy and angioimmunoblastic lymphadenopathy-like lymphoma. *Am. J. Pathol.,* **122,** 392–397 (1986).
8) Watanabe, S., Sato, Y., Shimoyama, M., Minato, K. and Shimosato, Y. Immunoblastic lymphadenopathy, angioimmunoblastic lymphadenopathy and IBL-like T-cell lymphoma-A spectrum of T-cell neoplasia. *Cancer,* **58,** 2224–2232 (1986).
9) Shimoyama, M., Tobinai, K., Minato, K. and Watanabe, S. Immunoblastic lymphadenopathy (IBL)-like T-cell lymphoma. *Gann Monogr. Cancer Res.,* **28,** 121–134 (1982).
10) Tobinai, K., Minato, K., Ohtsu, T., Mukai, K., Kagami, Y., Miwa, M., Watanabe, S. and Shimoyama, M. Clinicopathologic, immunophenotypic and immunogenotypic analyses of immunoblastic lymphadenopathy-like T-cell lymphoma. *Blood,* **72,** 1000–1006 (1988).
11) Ginn, F. L., Shelburne, J. D. and Trump, B. F. Disorders of cell volume regulation. I. Effects of inhibition of plasma membrane adenosine triphosphatase with ouabain. *Am. J. Pathol.,* **53,** 1041–1071 (1968).
12) Brown, W. V. and Bertke, E. M. Microtubules, vacuoles, and crystals. *In* "Textbook of Cytology," Chapter 16, pp. 297–302 (1969). C. V. Mosby Company, Saint Louis.
13) Konda, S., Takiguchi, T., Sugai, S., Hirose, Y. and Shimizu, S. Character of peripheral atypical lymphocytes. *In* "Cellular Pathology," ed. M. Kojima, pp. 3–22 (1984). Kindaishuppan, Tokyo (in Japanese).
14) Hirose, Y. Ultrastructure of adult T-cell leukemia cells and other malignant T-cells. *J. Kanazawa Med. Univ.,* **11,** 406–416 (1986).