Tubular Complexes of Endoplasmic Reticulum in Lymphoblastic Lymphoma:

Case Report

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Distinctive intracytoplasmic tubular complexes have been identified occasionally by electron microscopy in a wide variety of hematologic and nonhematologic disorders. The mechanism of induction and significance of these tubular complexes are unknown. Tubular complexes were identified in the majority of bone marrow lymphoma-leukemia cells in a patient with documented lymphoblastic lymphoma in lymph node. These complexes varied in size but in general ranged from 800-1500 nm, and consisted of masses of nonparallel, twisted, smooth, 40-nm tubules. Continuity with adjacent endoplasmic reticulum was evident in some of the complexes. Cytochemical characteristics of the malignant cells included strong, focal, paranuclear acid phosphatase reactivity and strong, stippled nuclear terminal deoxynucleotidyl transferase positivity. Flow cytometric analysis showed a DNA-RNA content pattern consistent with acute lymphoblastic leukemia and typical of T-cell lymphoma. This represents the first report of such tubular complexes in a presumed T-cell malignancy.

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Electron microscopy has demonstrated intracytoplasmic tubular complexes only rarely in hematologic malignancies. Similar structures have been identified more frequently in endothelial and lymphoreticular cells from patients with viral and autoimmune disorders. Various names have been applied to these tubular complexes including tubuloreticular inclusions or structures, tubular complexes of endoplasmic reticulum, and virus-like particles. The significance of tubular complexes is uncertain, although several investigators have suggested a viral etiology for these structures.

This report presents a case of lymphoblastic lymphoma in which the leukemic phase of the disease was studied by electron microscopy, cytochemistry, and flow cytometry. Ultrastructurally, 80% of the leukemic cells contained distinctive tubular complexes of endoplasmic reticulum. Leukemic cells were positive by PAS, acid phosphatase, and terminal deoxynucleotidyl transferase (TdT) immunofluorescent staining. Flow cytometric analysis of cellular DNA and RNA content distribution revealed a pattern typical of T-cell lymphoma. The clinical, cytologic, and enzymatic features of this malignancy are strongly suggestive of a T-cell lymphoma. Tubular complexes have not been described previously in a T-cell malignancy.

Case Report

This 35-year-old man had been in good health until August 1979, when right cervical lymphadenopathy that was unresponsive to antibiotic therapy developed. Excisional biopsy of a 4 x 3 x 3 cm cervical lymph node showed lymphoblastic lymphoma. Physical examination revealed generalized lymphadenopathy involving bilateral posterior cervical, bilateral axillary, and left epitrochlear regions. There was no hepatomegaly or splenomegaly; the liver and spleen scans showed no abnormalities. A chest radiograph also showed no abnormalities. Bilateral pedal lymphangiograms showed extensive bilateral intra-abdominal and pelvic lymphadenopathy, which radiographically was consistent with malignant lymphoma. The initial peripheral leukocyte count was 4700 with 20% immature convoluted lymphoid forms. The hemoglobin and platelet count were normal. The bone marrow was normocellular with 10% convoluted and nonconvoluted lymphoblasts.

The patient received five courses of MOPP and one course of bleomycin and vinblastine with no evidence of response; lymphadenopathy and circulating immature lymphoid cells persisted. Four months after presentation, the bone marrow was markedly hypercellular with 95% primitive convoluted and nonconvoluted lymphoblasts. Following additional che-
Chemotherapy (Adriamycin, vincristine, and prednisone), much of the lymphadenopathy resolved. However, extensive bone marrow infiltration persisted. One year after presentation the leukocyte count was 5000 with occasional immature forms, and the bone marrow was hypercellular with 70% lymphoblasts.

**Materials and Methods**

The cervical lymph node was fixed in buffered formalin, sectioned at 4 μm, and stained with hematoxylin and eosin (H & E), periodic acid-Schiff (PAS), and methyl green-pyronine (MGP). Sequential bone marrow aspirate and biopsy specimens were prepared according to previously described techniques. Bone marrow aspirate smears were stained with Wright-Giemsa, PAS, myeloperoxidase, Sudan black B, and acid phosphatase. Unfixed aspirate smears were stained for TdT using an immunofluorescent procedure and examined under fluorescent light at 450 nm. Flow cytometry was performed on a bone marrow aspirate specimen.
FIG. 3. Electron micrograph of lymphoma cell showing nuclear clefting with some peripheral condensation of chromatin. A nuclear bleb is present. A distinct tubular aggregate is present in the cytoplasm (arrow) (×30,000).

imen according to previously described methods following the patient's initial failure to respond to therapy. Distributions of DNA and RNA content were evaluated using the model of Johnston et al. Heparinized bone marrow aspirates were obtained for electron microscopy. The specimens were processed according to standard techniques. Sections were cut at 600 Å, stained with uranyl acetate and lead citrate, and examined in a Siemens 102 transmission electron microscope.

Results

Light Microscopy and Cytochemistry

Sections of lymph node showed an extensive diffuse paracortical infiltrate of small to medium sized lymphoid cells. These cells had barely visible cytoplasm, moderately sized nuclei with very finely dispersed nuclear chromatin, inconspicuous nucleoli, and frequent irregular nuclear contours. Mitotic figures were numerous. Occasional spared germinal centers were evident (Fig. 1).

The lymphoma-leukemia cells in the bone marrow ranged from 10–18 μm in diameter and had minimal to moderate amounts of pale blue cytoplasm and large, irregular, immature-appearing nuclei with uniformly dispersed chromatin. One to two nucleoli were visible in each nucleus, but they were not prominent. Occasional round to oval pale areas of cytoplasmic clearing were seen, which were located predominantly in paranuclear regions. These areas of cytoplasmic clearing were present in the malignant cells prior to therapy, and persisted throughout the disease course. Using PAS stain, large blocks and granules of cytoplasmic reactivity could be seen within the majority of the leukemic cells (Fig. 2). The acid phosphatase stain showed fairly large, focal, granular areas of cytoplasmic reactivity that generally were paranuclear in distribution (Fig. 2).
In addition, a minority of the leukemic cells exhibited diffuse granular cytoplasmic reactivity. The immunofluorescent stain for TdT showed prominent stippled nuclear fluorescence in the majority of the tumor cells. Faint reactivity by MGP stain was evident on smear preparations, but tissue sections were negative. Special stains for myeloperoxidase and Sudan black B were negative.

**Electron Microscopy**

The leukemic cells varied in size and had minimal to moderate amounts of cytoplasm containing few organelles and numerous free ribosomes. More than 80% of the leukemic cells contained distinctive intracytoplasmic tubular complexes. Approximately one-third of the cells had multiple tubular complexes. Some complexes were located randomly throughout the cytoplasm and others were paranuclear with associated nuclear indentations and clefts (Fig. 3). The size of the complex varied from 200–3600 nm; most complexes ranged from 800–1500 nm. Individual tubules within the complexes were arranged in a nonparallel, twisted, and compact manner and measured from 30–50 nm in diameter (Fig. 4). The tubular membranes were smooth, although there were free ribosomes inside the tubular complexes. Tubular centers contained small amounts of flocculent material. At the periphery of many complexes direct continuity between the smooth tubular membranes and adjacent rough endoplasmic reticulum (RER) was evident. Other complexes appeared to be related to Golgi, although ultrastructural distinction between the Golgi and tubular complexes was usually obvious.

Additional ultrastructural features included monoparticulate glycogen granules and nuclei with frequent nuclear irregularities including indentations, blebs, pockets, and clefts. The nuclear clefting was often extreme, and nuclear lobes were frequently connected by only a strand of nuclear material. The nuclear chromatin was finely stippled with some condensation at the nuclear membrane. The nucleoli were relatively small; 1–2 were present in each nucleus.
Flow Cytometry

Biparametric analysis of acridine orange-stained bone marrow cells showed a low to intermediate RNA content of G1/2 cells consistent with acute lymphoblastic leukemia (Fig. 5).\(^{14,16}\) Compared with normal marrow, the RNA distribution of S-phase cells (20%) was less dispersed and skewed toward a higher RNA content, a phenomenon that we have observed in all T-cell lymphomas studied to date.\(^*\)

Discussion

Immunologic evidence suggests a T-lymphocyte origin for lymphoblastic lymphoma,\(^{17,18}\) although cases lacking surface markers (i.e., null cells) have also been reported.\(^{19,20}\) Our patient had widespread lymphoma that was clinically and cytologically consistent with lymphoblastic lymphoma (convoluted lymphocytic lymphoma).\(^{21,22}\) Although surface marker studies were not performed in our study, cytochemical evidence to support a T-cell origin for this malignancy is the strong, focal acid phosphatase reactivity.\(^{23,24}\) The TdT positivity, nuclear features by electron microscopy, and flow cytometric characteristics of the lymphoma-leukemia cells were all consistent with, but not diagnostic of, a T-cell malignancy.\(^{14,16,23,25,26}\)

The unique feature of this case was the ultrastructural identification of intracytoplasmic tubular complexes in the majority of the leukemia-lymphoma cells. These complexes consisted of anastomosing smooth, membranous, 40-nm tubules that usually ranged from 800-1500 nm, and were in continuity with RER. These complexes frequently were located within nuclear clefts, although they were also identified randomly throughout the cytoplasm.

Several authors have described similar tubular complexes in a variety of hematologic and nonhematologic disorders including acute nonlymphocytic leukemia, melanoma, carcinoma, sarcoma, collagen vascular diseases, immune deficiency states, and viral infections.\(^{1,4,7,27,28}\) Table 1 shows the ultrastructural description of these complexes from several more recent reports. The complexes identified in our patient were ultrastructurally very similar to those designated as tubular complexes of endoplasmic reticulum by Parkin and Brunning in acute myelogenous leukemia.\(^{7}\) In contrast to their cases, the complexes in the lymphoblastic lymphoma cells were frequently multiple and located throughout the cytoplasm, although they did appear to be concentrated in paranuclear locations and within

\(^*\) Maddox A, Barlogie B. Unpublished observations.
deep nuclear clefts. The complexes in the lymphoma cells of our patient were also significantly larger than those described by others.4,7,27

Many authors have emphasized the similarity of intracytoplasmic tubular complexes to virus particles. Cells infected by viruses initially show a proliferation of endoplasmic reticulum and of Golgi with subsequent appearance of virus particles and of membranous or tubular masses in cytoplasm.29,30 Structures designated as membrane complexes,30 convoluted membranous masses,29 tubular structures,31 and reticular inclusions with tubular bodies32 have been identified in the cytoplasm of virus-infected cells. Intracytoplasmic inclusions sharing many ultrastructural features with true virus particles have been described in acute and chronic leukemias, Hodgkin's disease, and lymphosarcoma and have been termed virus-like particles.1-3

Both the pathogenesis and the proposed name for this distinctive intracytoplasmic complex are controversial. The term tubular complexes of endoplasmic reticulum, as designated by Parkin and Brunning, seems most appropriate because it accurately describes the ultrastructural morphology without implying an etiology.7 A variety of pathogenetic mechanisms for the tubular complex formation have been proposed. Some relate to cell injury and immunologic mechanisms, others suggest an underlying viral infection.3-5 Similar membranous complexes have been noted in renal tubular epithelial cells in animals receiving vinblastine.33 The effect in humans of prior chemotherapy on the development of these complexes is unknown. However, the complexes were present in the lymphoblastic lymphoma cells in our patient one month after chemotherapy was given, and cytoplasmic cleared areas possibly corresponding to these tubular complexes were present in pretreatment marrow specimens. The persistence of tubular complexes in successive bone marrow specimens over a 12-month period suggests that they represent a permanent cytoplasmic structure whatever their mechanism of induction. This case fulfills the morphologic and enzymatic criteria for a T-lymphocyte malignancy, and as such, is the first such case in which tubular complexes have been demonstrated ultrastructurally.

REFERENCES

1. Foa C, Foa J, Carcassonne Y. Morphologic study of virus-like particles in a case of acute leukemia. Cancer 1976; 37:1718-1724.
2. Cawley J, Karpas A. The ultrastructural demonstration of virus-like particles in human leukaemic cells. Eur J Cancer 1974; 10:559-562.
3. Seman G, Seman C. Electron-microscopic search for virus particles in patients with leukemia and lymphoma. Cancer 1968; 22:1033-1045.
4. Grimley PM, Schaff Z. Significance of tubuloreticular inclusions in the pathobiology of human diseases. Pathobiol Annu 1976; 11:221-257.
5. Gyorkey F, Sinkovics JG, Min KW, Gyorkey P. Morphologic study on the occurrence and distribution of structures resembling viral nucleocapsids in collagen diseases. Am J Med 1972; 53:158-159.
6. Vernon M, Price P. Virus-like particles in geometric tubuloreticular structures. J Natl Cancer Inst 1976; 55:855-857.
7. Parkin JL, Brunning RD. Tubular complexes of endoplasmic reticulum in myeloblasts of acute myelogenous leukemia. Ultrastructural Pathol 1980; 1:55-65.
8. Futch HN. Histopathology Laboratory Manual. Houston: M.D. Anderson Hospital and Tumor Institute, 1979; 1-2, 116-118.
9. Brynes RK, McKenna RW, Sundberg RD. Bone marrow aspiration and trephine biopsy. An approach to a thorough study. Am J Clin Pathol 1978; 70:753-759.
10. Kaplow LS. Simplified myeloperoxidase stain using benzidine dihydrochloride. Blood 1965; 26:215-219.
11. Shaw MT. The cytochemistry of acute leukemia: A diagnostic and prognostic evaluation. Semin Oncol 1976; 3:219-228.

| Author(s) | Ultrastructural description | Disease |
|-----------|-----------------------------|---------|
| Parkin and Brunning, 1980 | Anastomosing network of smooth, 50-nm membranous tubules filled with amorphous electron-dense material; tubules are in continuity with dilated rough endoplasmic reticulum, are located opposite the Golgi, and indent the nucleus; occasional 86-nm particles present within complex | Acute myelogenous leukemia |
| Grimley and Schaff, 1976 | Irregular branched and anastomosing 25-nm tubules with a lucent core; tubules appear to arise from endoplasmic reticulum; total size of complex 100-300 nm | Malignant lymphoma |
| Tange and Chiba, 1979 | Bundles of 0.3-nm tubules in continuity with rough endoplasmic reticulum. Tubules located near Golgi; total size of complex 90 nm | Leukemia |
| Foa, Foa, and Carcassone, 1976 | Two separate inclusions identified; large round 100-nm particles composed of subunits; 30-50 nm clusters of twisted tubular structures located near endoplasmic reticulum; neither complex surrounded by membranes | Chediak-Higashi syndrome, Collagen vascular diseases, Immune deficiency states, Melanoma, sarcoma, carcinoma, Acute promyelocytic leukemia, Acute myelogenous leukemia (probable) |
| Posalaky and McGinley, 1979 | Anastomosing 35-nm tubular cytoplasmic inclusions | Malignant melanoma |
of tartrate resistant acid phosphatase. Technical considerations. Am J Clin Pathol 1978; 70:45-55.
13. Bollum FJ. Terminal deoxynucleotidyl transferase: Biological studies. In: Meister A, ed. Advances in Enzymology. New York: John Wiley and Sons, 1978; 347-374.
14. Barlogie B, Latreille J, Fu CT, Meistrich M, Andreeff M. Characterization of hematologic malignancies by flow cytometry. Blood Cells 1980; 6:719-744.
15. Johnston DA, White RA, Barlogie B. Automatic processing and interpretation of DNA distributions: Comparison of several techniques. Comput Biomed Res 1978; 11:393-404.
16. Andreeff M, Darzynkiewicz Z, Sharpless, T, Clarkson B, Malmened MR. Discrimination of human leukemia subtypes by flow cytometric analysis of cellular DNA and RNA. Blood 1980; 55:282-293.
17. Palutke M, Patt DJ, Weise R et al. T cell leukemia-lymphoma in young adults. Am J Clin Pathol 1977; 68:429-439.
18. Bouchetix C, Diebold J, Bernardou A et al. Lymphoblastic lymphoma/leukemia with convoluted nuclei. The question of its relation to the T-cell lineage studied in 13 patients. Cancer 1980; 45:1569-1577.
19. Bloomfield CD, Gajl-Peczalska KJ, Frizzera G, Kersey JH, Goldman AI. Clinical utility of lymphocyte surface markers combined with the Lukes-Collins histologic classification in adult lymphoma. N Engl J Med 1979; 301:512-518.
20. Koziner B, Filippa DA, Mertelsmann R et al. Characterization of malignant lymphomas in leukemic phase by multiple differentiation markers of mononuclear cells. Correlation with clinical features and conventional morphology. Am J Med 1977; 63:556-567.
21. Nathwani BN, Kim H, Rappaport H. Malignant lymphoma, lymphoblastic. Cancer 1976; 38:964-983.
22. Barcos MP, Lukes R. Malignant lymphoma of convoluted lymphocytes: A new entity of possible T-cell type. In: Sinks LF, Godden JO, eds. Conflicts in Childhood Cancer. An Evaluation of Current Management, vol. 4. New York: Alan R. Liss Inc., 1975; 147-178.
23. McKenna RW, Parkin J, Brunning RD. Morphologic and ultrastructural characteristics of T-cell acute lymphoblastic leukemia. Cancer 1979; 44:1290-1297.
24. Sondgaard-Petersen H, Boesen AM. Three different acid phosphatase patterns in leukemic lymphoid T-cells. Scand J Haematol 1979; 23:51-54.
25. Kung PC, Long JC, McCaffrey RP, Ratliff RL, Harrison TA, Baltimore D. Terminal deoxynucleotidyl transferase in the diagnosis of leukemia and malignant lymphoma. Am J Med 1978; 64:788-794.
26. Glick AD, Vestal BK, Flexner JM, Collins RD. Ultrastructural study of acute lymphocytic leukemia. Comparison with immunologic studies. Blood 1978; 52:311-322.
27. Tange T, Chiba S. Rough endoplasmic reticulum-associated tubular structures occurring in acute promyelocytic leukemia. Scand J Haematol 1979; 23:217-223.
28. Oshiro LS, Schieble JH, Lennett EH. Electron microscopic studies of coronavirus. J Gen Virol 1971; 12:161-168.
29. Tyson GE, Bulger RE. Vinblastine-induced aggregate of smooth endoplasmic reticulum in proximal tubular cells of rat kidney. Am J Anat 1974; 140:201-212.