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.[1–3] Similar structures have been identified more frequently in endothelial and lymphoreticular cells from patients with viral and autoimmune disorders.[4,5] Various names have been applied to these tubular complexes including tubuloreticular inclusions or structures,[4,6] tubular complexes of endoplasmic reticulum,[7] and virus-like particles[1–3,6] The significance of tubular complexes is uncertain, although several investigators have suggested a viral etiology for these structures.[3,6]

This report presents a case of lymphoblastic lymphoma in which the leukemic phase of the disease was studied by electron microscopy, cytochemistries, 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 × 3 × 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 lymphoid cells. Following additional che-
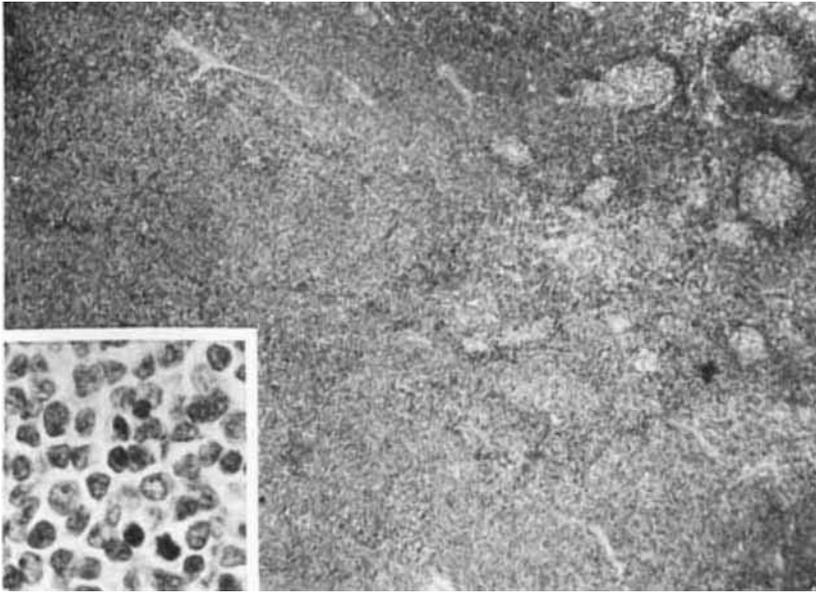
FIG. 1. Section of lymph node showing marked paracortical expansion with focal germinal center sparing. (Inset) High-power view of lymphoma cells with barely visible cytoplasm, irregular nuclei, and fine nuclear chromatin with inconspicuous nucleoli (H & E, X25; Inset X400).

motherapy (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).[8] Sequential bone marrow aspirate and biopsy specimens were prepared according to previously described techniques.[9] Bone marrow aspirate smears were stained with Wright-Giemsa, PAS, myeloperoxidase,[10] Sudan black B,[11] and acid phosphatase.[12] Unfixed aspirate smears were stained for TdT using an immunofluorescent procedure and examined under fluorescent light at 450 nm.[13] Flow cytometry was performed on a bone marrow aspirate spec-
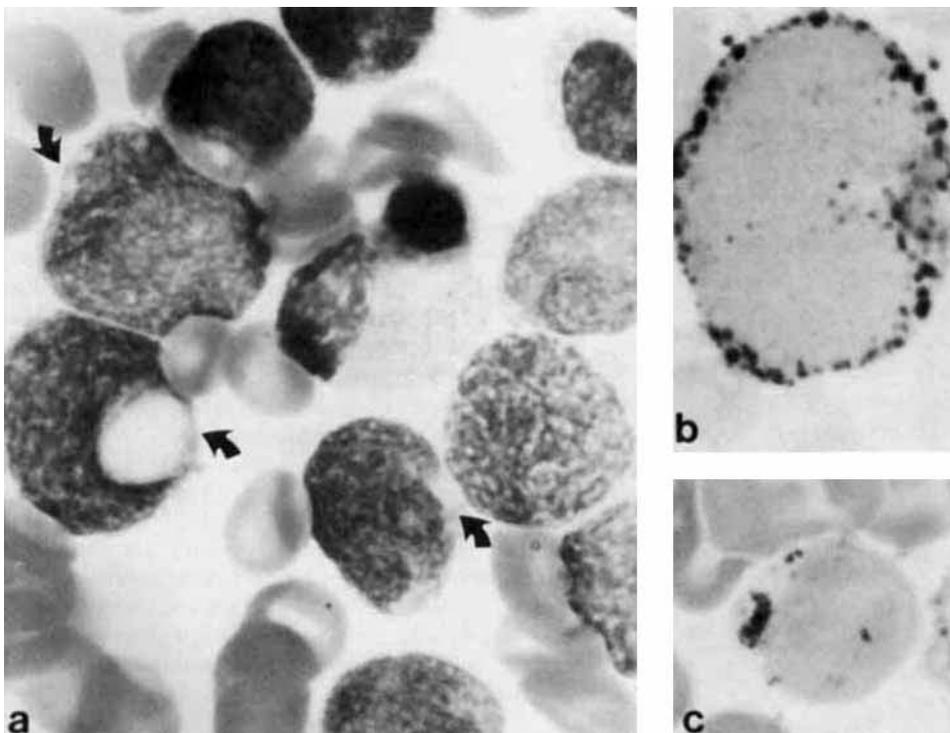
FIGS. 2A-2C. Bone marrow aspirate smear (A) showing pleomorphic population of lymphoma cells with variation in size and marked nuclear irregularity. Large pale oval intracytoplasmic inclusions are present in the lymphoma cells (arrows) (Wright-Giemsa, X1000). (B) PAS stain demonstrates numerous large intracytoplasmic aggregates (PAS, X1000). (C) A focal, strong paranuclear area of positivity is demonstrated by acid phosphatase stain (Acid phosphatase, X1000).
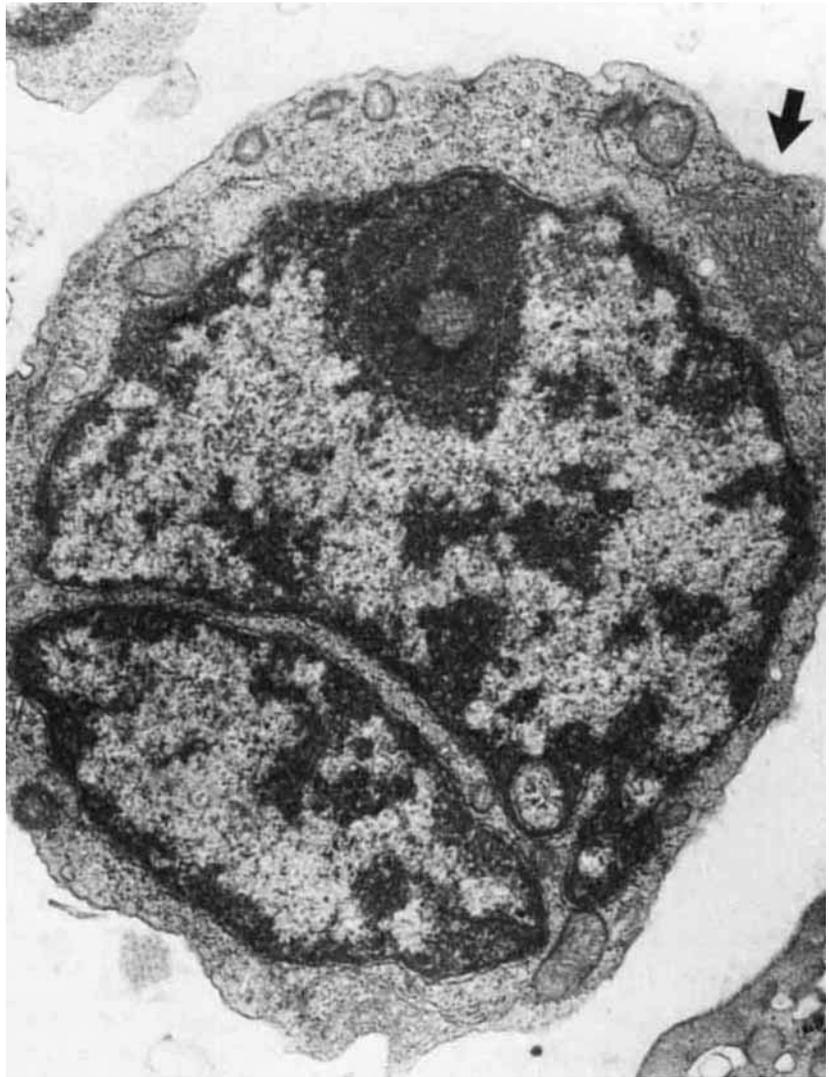
FIG. 3. Electron micrograph of lymphoma cell showing nuclear clefting with some peripheral condensation of chromatin. A nuclear bleb is present. A distinct tubular aggregate is present in the cytoplasm (arrow) (×30,000).

imen according to previously described methods following the patient's initial failure to respond to therapy.[14] Distributions of DNA and RNA content were evaluated using the model of Johnston et al.[15]

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).
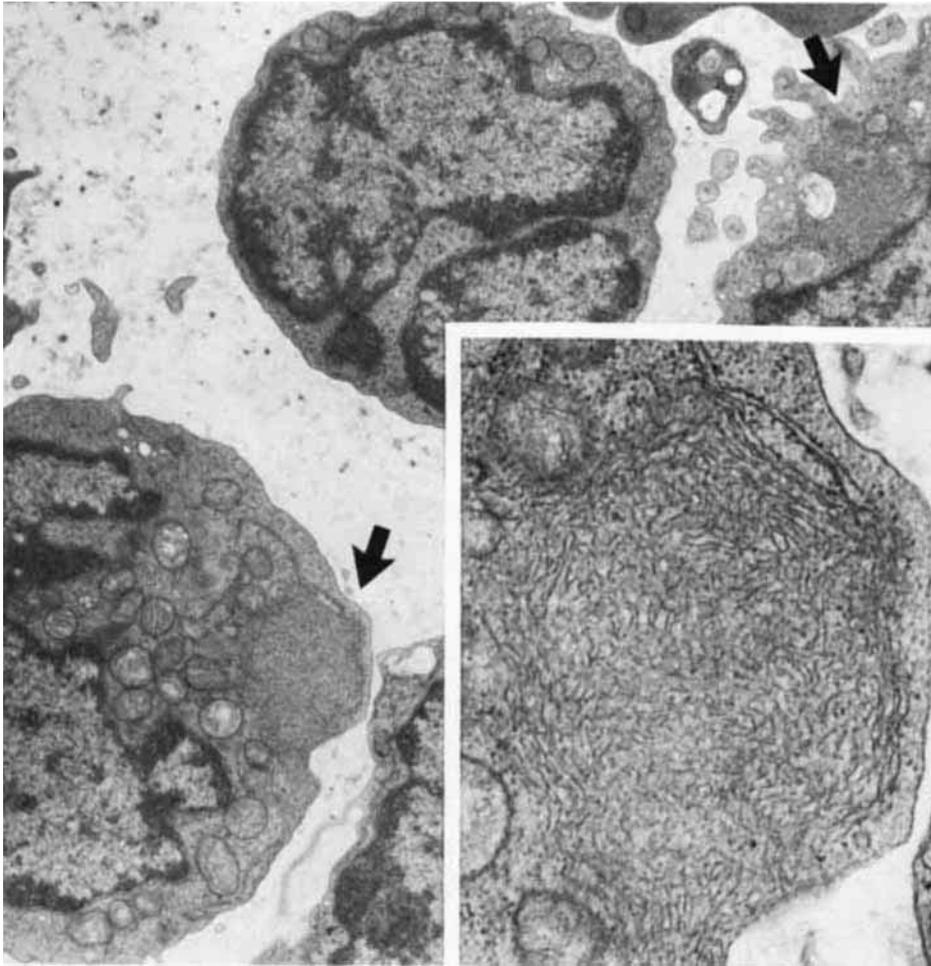
FIG. 4. Portions of three lymphoma cells are present, two of which contain large tubular aggregates of endoplasmic reticulum (arrows). One aggregate is paranuclear and the other is in the peripheral cytoplasm (×12,000). (Inset) At higher power the aggregate consists of large numbers of smooth, nonparallel 40-nm tubules. Continuity with adjacent RER is evident (×60,000).

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 mono-particulate 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.

FIG. 5. Graph of flow cytometry results on bone marrow aspirate. DNA/RNA frequency distribution of bone marrow leukemia-lymphoma cells stained with acridine orange. Green fluorescence (abscissa) reflects double-stranded DNA and red fluorescence reflects single-stranded RNA (ordinate).
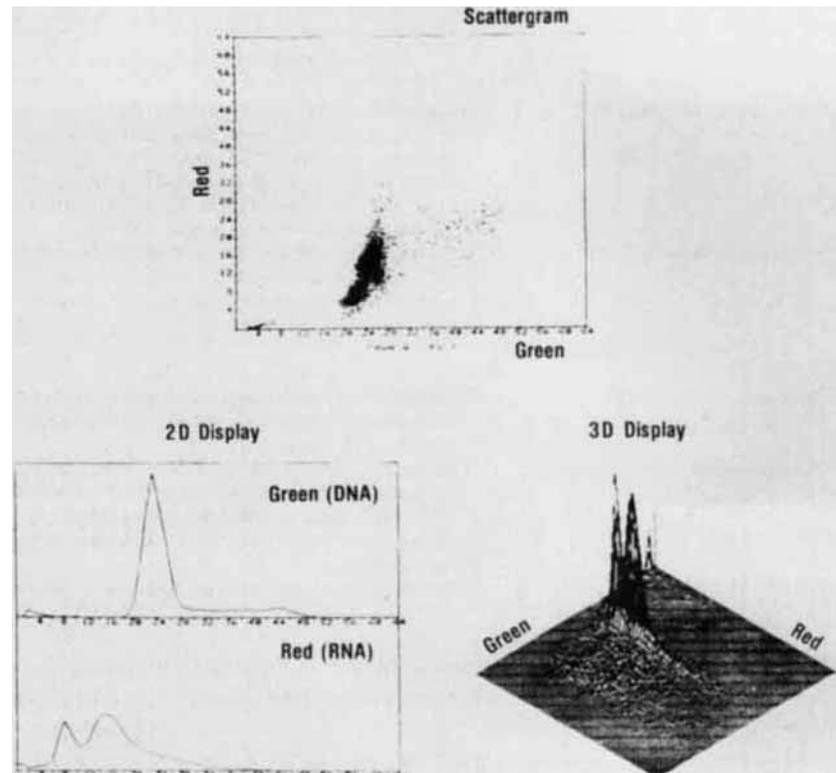
Flow Cytometry

Biparametric analysis of acridine orange-stained bone marrow cells showed a low to intermediate RNA content of G $1/_0$ 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

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* Maddox A, Barlogie B. Unpublished observations.

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

TABLE 1.　Diseases in which Tubular Complexes Have Been Identified

Author(s)	Ultrastructural description	Disease
Parkin and Brunning, 1980[7]	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 from the Golgi, and indent the nucleus; occasional 80-nm particles present within complex	Acute myelogenous leukemia
Grimley and Schaff, 1976[4]	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 Leukemia Chediak-Higashi syndrome Collagen vascular diseases Immune deficiency states Melanoma, sarcoma, carcinoma
Tange and Chiba, 1979[27]	Bundles of 0.3-nm tubules in continuity with rough endoplasmic reticulum. Tubules located near Golgi; total size of complex 90 nm	Acute promyelocytic leukemia
Foa, Foa, and Carcassone, 1976[1]	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	Acute myelogenous leukemia (probable)
Posalaky and McGinley, 1979[28]	Anastomosing 35-nm tubular cytoplasmic inclusions	Malignant melanoma

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 bodies[32] 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 corre-

sponding 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.

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