Ultramorphological and Ultracytochemical Studies on Tubuloreticular Structures in Lymphoid Cells

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SUMMARY

Tubuloreticular structures that are frequently present in the cytoplasm of lymphoid cells grown in vitro were studied with cytochemical methods at high resolution. The structures are easily digested by Pronase and pepsin; they are not sensitive to trypsin or RNase. From the results obtained, we conclude that the tubuloreticular structures are of proteinaceous nature and that they are mainly composed of proteins of the acidic type. Their significance in the cell is not yet known and, therefore, further investigation is required.

INTRODUCTION

Cytoplasmic inclusions of tubuloreticular structure have been observed repeatedly in cells of different origin, and their significance has been discussed frequently. It was suggested that they may represent normal constituents of the cells (2, 12, 26, 47) or that they may be an expression of cellular changes due to some pathological disorder (5, 14-16, 24, 38-40, 46). On the basis of their morphology, i.e., their structural similarity to viral nucleoprotein of the paramyxovirus group, it was proposed that the inclusions may represent viral nucleocapsids (9, 17-20, 27, 35, 41). However, to date, the origin and significance of these structures are still a matter of speculation. Therefore, a knowledge of their chemical composition would be valuable, providing a basis for a better understanding of the nature of these cellular components.

In recent years, cytochemical techniques have been developed that permit the study and electron microscopic evaluation of cellular constituents on ultrathin sections. We have applied these methods to lymphoid blast cells that contain relatively large numbers of the structures under discussion in relatively high frequency, and the results of these investigations are the basis for this report.

MATERIALS AND METHODS

Cell Cultures

The majority of the cells studied were isolated from a biopsy of a recurrent parangangioma. The cells grow as lymphoid blast cells in suspension, as described previously (22), and they have now been 2.5 years in culture (Tissue Culture Isolate T-13). Other cells used were obtained from a lymphocytic lymphosarcoma (23). These cells also grew in suspension as an established line of lymphoid blast cells and were kept under similar conditions (Tissue Culture Isolate T-24).

Fixation and Embedding

For routine examination, the cells were fixed by dilution of the cultures with equal volumes of 3% glutaraldehyde. The fixation was carried out at 2°C for 1 to 2 hr. After fixation, the cells were sedimented into a pellet by centrifugation at 900 X g, washed with Sorensen's buffer, postfixed with chromo-osmium solution (10), and flat embedded in Epon-Araldite (32). Sections of silver interference were cut with an LKB Ultrotome, double stained, and examined in a Siemens-Elmiskop 1A or 101 with a 50-μm objective aperture and an accelerating voltage of 80 kV.

Other cells were fixed with 3% glutaraldehyde in a similar manner (but for only 15 min) and were embedded in GMA3 (Polysciences, Inc., Warrington, Pa.) according to a method described by Leduc et al. (30).

Enzyme Studies

Proteases. Different proteolytic enzymes were used to characterize the tubuloreticular structures. They were applied to sections of silver-to-gold interference of routinely embedded cells which were pretreated according to a technique described by Monneron and Bernhard (33). The enzymes were Pronase (Grade B; Calbiochem, Los Angeles, Calif.), 0.5% in distilled water, pH 7.4; pepsin (Worthington Biochemical Corporation, Freehold, N. J.), 0.25% in 0.1 N HCl; and trypsin (Worthington), 0.5% in Tris buffer, pH 8.0. Control specimens were treated in a similar manner, with solutions that lacked the enzyme.

RNase. The distribution of RNA was investigated by means of cell digestion with RNase (Worthington). The method used was described by Swift (44). The enzyme was used as a 0.1% solution in distilled water, pH 6.8, adjusted with 0.01 N NaOH. The digestion took place after the cells were fixed with glutaraldehyde by suspending the cells in the enzyme solution.

3The abbreviation used is: GMA, glycolmethacrylate.
RESULTS

Morphology of the Cells. The lymphoid blast cells grow in small clumps in suspension. They are characterized by a large nucleus, which contains evenly distributed chromatin, and by a relatively narrow rim of cytoplasm. As seen in Fig. 1, cytoplasmic organelles are rare and consist mainly of round to oblong mitochondria, rough endoplasmic reticulum, and a few lysosomes. Infrequently, myelin figures have been observed in the cytoplasm. Ribosomes are numerous and are grouped into clusters of polysomes. As shown previously, the cells of line T-13 contain a cytomegalovirus (22), whereas those of line T-24 are infected with the Epstein-Barr virus (23).

In about 6 to 8% of the cells, tubuloreticular structures of moderate to high electron density have been observed (Fig. 1, arrow). Their size ranges from 1 to 2 μm, and their form is irregular. They are situated in dilations of the rough endoplasmic reticulum, and they consist of an array of small tubules, which may be packed rather tightly (Fig. 2) or which may be arranged in a loosely organized pattern (Fig. 3). In a few instances, both forms of inclusions (the loosely and tightly packed ones) were observed inside the same structure (Fig. 4). The tubules are hollow (Fig. 5), their diameter is about 25 to 35 nm, and their relatively uniform lumen measures approximately 20 nm in diameter. As can be seen in Fig. 5, they appear to be connected with the rough endoplasmic reticulum. By use of the tilting stage of the electron microscope, the close relationship of the tubules to the membranes of the endoplasmic reticulum could be demonstrated more clearly. In Fig. 6b, the section is not tilted; the membrane of the rough endoplasmic reticulum is cut obliquely (long arrow) and is in close and continuous contact with the tubuloreticular array (short arrow). Likewise, close association of both structures is pronounced when the section is tilted +25°, as can be seen in Fig. 6a. After the section is tilted to an angle that renders the endoplasmic membrane in sharp contrast (Fig. 6c), the close relationship of both structures is still present. The endoplasmic membrane appears now as a distinct line, whereas the connecting tubule of the inclusion retains its fuzzy character.

Digestion with Proteinases. Sections of control cells were incubated for different lengths of time in the appropriate buffer solution which lacked the enzyme under study. As can be seen in Fig. 7, these cells exhibit the tubuloreticular structures very distinctly, and the loss of electron density is negligible.

Treatment with Trypsin. Exposure to trypsin for different lengths of time (up to 4 hr) has no visible effect on the cells. Nuclear components and cytoplasmic organelles, including the tubuloreticular arrays, do not exhibit any changes in their fine structure. As seen in Fig. 8, the appearance of the inclusion is similar to that of the control shown in Fig. 7.

Treatment with Pronase. Incubation with Pronase provokes a considerable change in the appearance of the tubuloreticular structures. As illustrated in Fig. 9, an incubation for 15 to 30 min removes a large amount of the usually deeply stainable material from the inclusion, but the structure is still faintly recognizable, due to a net-like background. The membranes of the rough endoplasmic reticulum situated in different areas throughout the structure are now outstanding, due to the electron-lucent appearance of the remaining inclusion.

Treatment with Pepsin. The exposure of cells to pepsin resulted in changes similar to those observed after Pronase treatment. Also, as seen in Fig. 10, most of the tubuloreticular array is digested, whereas the surrounding cellular components do not show any aberrations from the normal.

Digestion with RNase. The digestion was carried out on glutaraldehyde-fixed cells by their suspension in the appropriate enzyme solution. Control cells that have been subjected to a similar treatment but that are lacking the enzyme in the incubation medium exhibit the tubuloreticular structures in nearly normal fashion, and RNA-containing structures, e.g., ribosomes, are well preserved (Fig. 11). In contrast, cells exposed to RNase are devoid of many of their RNA-containing components after completion of the digestion. For example, ribosomes are no longer visible (Fig. 12), and in other cells the granular part of the nucleolus is not identifiable. The tubuloreticular structures appear to be relatively heavily stained. As in the control (Fig. 11), their fine structure is not as well preserved as in routinely fixed and embedded material. The structures appear to be rather confluent, a fact that presumably may be due to the preparation method used. As a result, distinct cross-sections of the tubules have been seen only occasionally in these preparations.

Preferential Staining. As shown by Bernhard (4), preferential staining can be especially useful to reveal the presence of DNA- and RNA-containing structures in the cells. When the procedure is executed properly, deoxyribonucleoproteins lose all their contrast in ultrathin sections, but RNA-containing cellular components remain intensely stained. Our results show that the tubuloreticular structures do not change their electron density after being subjected to preferential staining. A comparison of Fig. 13 (control) with Fig. 14 reveals the similarity of the staining in both control and experimental sections, and the intensity of the staining corresponds well with that of the mitochondrial matrix which is known to be composed mainly of protein.

GMA Embedding. As illustrated in Fig. 15, cells embedded in GMA and double stained exhibit clearly the nucleus, mitochondria, and lipid bodies. The ribosomes are intensely stained and outline the rough endoplasmic reticulum, being most commonly distributed on its surface in a chain-like manner. In contrast, and as is already known, membranous structures, i.e., the Golgi apparatus and the different cellular membranes, are not visible with this method of preservation. Likewise, the tubuloreticular structures are of low electron density, and their individual components are barely dis-
cernible, even when examined under higher magnification (Fig. 16a). By photographic procedure, a reversal (Fig. 16b) was prepared of Fig. 16a. Due to this photographic process, membranes appear as dark lines, but ribosomes are represented by an electron-lucent area. Cross-sections of the tubules of the tubuloreticular structures can be recognized (Fig. 16b, short arrow), and the tubules can be traced for some distance (long arrow) when arranged parallel to the surface of the sections. The width of the tubules, approximately 25 to 30 nm, is in good agreement with the results of measurements obtained on routinely embedded material.

DISCUSSION

Tubuloreticular structures have been observed repeatedly in different cells of normal tissues and in cells of persons with pathological disorders. A number of authors have given different names to these elements, and in Table 1 we present some of the more common synonyms used in different articles. They range from “undulating microtubules” to “reticular network” and “crystalline arrays.” In an effort to prevent further confusion, we would like to propose that these names be designated “tubuloreticular structures.” This term was mentioned first by one of the authors (A. J. Dalton) in 1971 at the Annual Meeting of the Electron Microscopy Society of America. The term describes precisely the appearance of the structures under study without limiting their different morphological aspects, which may range from loosely interwoven tubules to extremely orderly patterns of paracrystalline conformation. Since the structures have been found not only inside the cytoplasm in close proximity to the endoplasmic reticulum but also in perinuclear spaces (17, 20, 25) and inside the nucleus (25), no reference to their localization inside the cell is made in the proposed name.

The role of these structures in the cells is still obscure and mainly a matter of speculation. In Table 2, we have summarized the different conditions under which they have been found thus far. Under certain circumstances, they may represent a normal component of cells, being involved in highly specialized metabolic functions [as in the dendirctic organ of the catfish (47), in the photogenic grains of the luminous cells of the polynoid worm elytra (2), and in the cotton zygote (26)]. Better known, however, is the frequency with which the structures are found in various cells in certain autoimmune diseases. Most frequently, they have been observed in the endothelial cells of the glomeruli in systemic lupus erythematosus (5, 19, 24, 27, 37, 39, 41), and they have been associated with the endothelial cells of different organs in the following autoimmune diseases: chronic polymyelitis (9), dermatomyositis (14, 38), and scleroderma (14, 38), Sjögren’s syndrome (40) and Letterer-Siwe disease (46), the latter being a connective tissue disease of a different type. As Table 2 indicates, the structures have also been seen in a variety of tumors (15, 20, 25, 31, 43) and in virus-infected cells grown either in vivo (1, 7, 13, 16) or in vitro (3, 8, 11, 22, 28, 29).

From the preceding, it is apparent that we are confronted with a major problem in elucidating a common denominator for the presence of these structures. The most interesting factor thus far is their preference for cells belonging to the reticuloendothelial system. However, an evaluation of their meaning, even in these cells, seems to be difficult, since they have been observed at different cellular sites (i.e., the nucleus and the cytoplasm). Due to their frequent presence in cells of the reticuloendothelial system, it was proposed that the tubuloreticular structures may appear as a response of these cells to abnormal conditions, thus possibly representing a marker for cellular injury. Their possible involvement in the production of immunoglobulins has also been suggested (46).

The results of our cytochemical study give substantial evidence of the proteinaceous nature of these structures. Our digestion studies show clearly that the structures contain a high proportion of proteins that are easily attacked by Pronase and pepsin. Pronase is an enzyme of broad spectrum containing different active fractions, which include endopeptidases resembling trypsin, as well as elastase, aminopeptidase, and carboxypeptidase. The low specificity of Pronase is due not only to the presence of a mixture of these different enzymes but also to considerable variation from one preparation to another (45). Pepsin, on the other hand, hydrolyzes only peptide linkages, especially bonds such as leucine-glutamine, leucine-valine, phenylalanine-tyrosine, and phenylalanine-phenylalanine. The enzyme is relatively specific in hydrolyzing large peptides, whereas the breakdown of medium-sized peptides to tri- and tetrapeptides seems not to be selective (6). Trypsin
Tubuloreticular structures observed under the following conditions.

An excellent summary is published in Ref. 45.

Table 2

Tubuloreticular structures observed under the following conditions.
An excellent summary is published in Ref. 45.

Normal conditions

Dendritic organ of catfish (47)
Cotton zygote (26)
Luminous cells of polynoid worm elytra (2)
Endothelial cells of renal pulmonary and hepatic capillaries of rhesus monkey (12)

Autoimmune diseases

Systemic lupus erythematosus (endothelial cells of glomeruli) (17–20, 24, 27, 35, 37, 39, 41)
Discoid lupus erythematosus (endothelial cells, histiocytes, and fibroblasts of skin lesion) (21)
Chronic polymyositis (endothelial cells of muscle biopsy) (9)
Dermatomyositis (endothelial cells of skin and muscle biopsies) (14, 38)
Scleroderma (endothelial cells of skin and muscle biopsies) (14, 38)
Sjögren syndrome (endothelial cells of skin and muscle biopsies) (14, 38)
Lupus erythematosus (endothelial cells of glomeruli) (40)

Other diseases

Infectious mononucleosis (34)
Letterer-Siwe disease (46)

Tumors

Sticker sarcoma (31)
Osteosarcoma (25)
Liposarcoma (20)
Rhabdomyosarcoma (20)
Fibrosarcoma (20)
Kaposi sarcoma (20)
Hepatoblastoma (15)

In vivo

Experimental herpes encephalitis (macrophages and endothelial cells of rabbit brain) (1)
Equine viral arteritis (endothelial cells) (13)
Tumors in dogs and monkeys induced by Rous sarcoma virus (neoplastic cells) (7, 36)
Virus infected midgut of coleopteron (16)

In vitro

Monkey kidney cells infected with rubella virus (28)
Lymphoblast cells isolated from a human paraganglioma (22)
Lymphoma cells infected with herpes simplex virus (3)
Lymphoblast cells originated from patients with Burkitt’s lymphoma (8, 23)
Mouse liver cells infected with mouse hepatitis virus (11)

ACKNOWLEDGMENTS

We are very grateful for the skilled technical assistance of Mrs. J. Kondratick, Mrs. T. Ben, Mr. D. Jones, and Mr. B. Elliott, Jr.
appreciate the generosity of Dr. C. Weichan, Siemens AG, Berlin, Germany, who permitted us to use the tilting stage of the Elmiskop 101.

REFERENCES

Ultracytochemistry of Tubuloreticular Structures


Figs. 1 to 6. Fixation, glutaraldehyde and chrome-osmium solution; embedding, Epon-Araldite; staining, uranyl acetate-lead citrate.

Fig. 1. Lymphoid blast cell from Tissue Culture T-13. Round to oval mitochondria (M) are numerous. Lipid bodies (L) and myelin figures (MF) are observed infrequently. An osmiophilic tubuloreticular structure is present in the cytoplasm (arrow). The size of this structure is similar to that of mitochondria. X 10,000.

Fig. 2. Tubuloreticular structure of tightly packed pattern. The tubules are composed of material of high electron density. It is difficult to resolve the fine structure of the inclusion, due to the tight arrangement of its components. X 70,000.

Fig. 3. Tubuloreticular structure of loosely organized pattern. In cross-sections, the tubules appear round and contain an electron-lucent center (arrow). Membranes of the rough endoplasmic reticulum are numerous throughout the structure. X 70,000.

Fig. 4. Tubuloreticular structure containing components of different appearance. Densely packed tubules are arranged at the right side of the structure. Loosely interwoven, relatively long tubules are seen at the left side of the inclusion. X 60,000.

Fig. 5. Tubuloreticular structure of loose conformation at higher magnification. The hollow centers of the tubules are distinct (short arrow). The close association of these structures with the endoplasmic reticulum is demonstrated in 2 areas (long arrows); here, the connection between both the endoplasmic reticulum and the tubules appears to be confluent. X 110,000.

Fig. 6. The close association of the tubules to the endoplasmic reticulum is clearly demonstrated by the use of the tilting stage of the electron microscope. In b, the section is in the un-tilted position. The relation of the rough endoplasmic reticulum to the adjacent tubule is obscure, due to the oblique sectioning of the membrane of the endoplasmic reticulum near the tubules of the inclusion (long and short arrows). In a, the same section is tilted +25°. The connection between tubule and endoplasmic reticulum is poorly resolved. In c, the section is tilted to an angle that renders the endoplasmic membrane in sharp contrast (long arrow). The adjacent tubule appears to be perpendicular to the membrane and is closely attached to it (short arrow). X 150,000.

Figs. 7 to 10. Digestion of the tubuloreticular structures with proteinases according to a method described by Monneron and Bernhard (33). Fixation, glutaraldehyde and chrome-osmium solution; embedding, Epon-Araldite; staining, uranyl acetate-lead citrate.

Fig. 7. Control section incubated in buffer solution. The structure is clearly recognizable, but it has lost some of its electron density (compare with Fig. 2). X 48,000.

Fig. 8. The exposure to 0.5% trypsin for 4 hr has no effect on the tubuloreticular structure. X 42,000.

Fig. 9. The section was exposed to 0.5% Pronase for 30 min. The tubuloreticular structure differs markedly from the control. It has very little contrast, and the outline of its tubules is only faintly visible. Other cellular structures, i.e., ribosomes present in the surrounding matrix and the membranes of the endoplasmic reticulum present in different areas of the structure (arrows), are well preserved. X 42,000.

Fig. 10. Treatment with 0.25% pepsin for 1 hr markedly affects the structure. Most of its components are digested, and the remaining residue is only poorly stained. Inside the inclusion, there is little evidence of formed elements. The surrounding cytoplasm and its organelles show no definite alteration from the normal. X 32,000.

Figs. 11 and 12. RNase treatment. Cells were fixed with 3% glutaraldehyde and exposed in suspension to 0.1% RNase in distilled water, pH 6.8. Postfixation, chrome-osmium; embedding, Epon-Araldite; staining, uranyl acetate-lead citrate.

Fig. 11. Control. The cells were suspended for 3 hr in distilled water. The tubuloreticular inclusion is recognizable, but its fine structure is not as well preserved as that in material embedded in Epon-Araldite by routinely used methods. The inclusion is of medium electron density. Ribosomes found free in the cytoplasm or attached to the membranes of the endoplasmic reticulum (arrow) are distinct, and mitochondria (M) are well preserved. X 51,000.

Fig. 12. RNase-digested specimen. The section contains a tubuloreticular structure which has been exposed to RNase for 3 hr. The inclusion exhibits an electron density comparable to that of the control (Fig. 11). In contrast to the control, the ribosomes are digested by the enzyme, leaving the endoplasmic reticulum without these characteristic particles (arrow). X 51,000.

Fig. 13. Control to Fig. 14. The latter represents a specimen that has been subjected to the preferential staining of Bernhard (4). The tubuloreticular array is only moderately stained, and its density appears similar to that of the adjacent mitochondria (M). The tubules of the inclusion are barely recognizable. In contrast, the surrounding cytoplasm is well preserved. X 60,000.

Fig. 14. The specimen was subjected to the preferential staining of Bernhard (4). The structure exhibits neither the appearance of RNA containing material nor that of DNA-containing structures. X 60,000.

Fig. 15. Lymphoid blast cell embedded in GMA and double stained. The nucleus (N), mitochondria (M), lipid bodies (L), and the Golgi apparatus (GA) are well preserved, and the ribosomes are outstanding. The tubuloreticular inclusion is of relatively low electron density (arrow). X 21,000.

Fig. 16. Tubuloreticular array at higher magnification. The cell is embedded in GMA and double stained. a, routine photograph; b, the reversal of a. In a, details of the structure are barely recognizable. In b, it is possible to trace some of the tubules (long arrow), due to the electron density of their enveloping membrane. Short arrow, cross-section of a tubule. A cluster of ribosomes is seen at R. X 70,000.
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