Fibrillar Bundles in the Nucleus of Blood Lymphocytes from Leukemic and Nonleukemic Patients

By Stefano S. Stefani and Hiroshi Tonaki

During electron microscopic investigation on another project, the incidental observation was made of fibrillar bundles in the nucleus of blood lymphocytes obtained from a normal donor. Although several investigators have reported fibrillar formations in the cytoplasm of lymphocytes obtained from normal and leukemic patients, such intranuclear structures have apparently never been previously observed in human lymphocytes. The purpose of this investigation, therefore, was: (1) to study the morphologic aspect of these fibrils, (2) to determine the number of normal and leukemic individuals with these structures in their blood lymphocytes, and (3) to try to understand the significance of their presence in the nucleus.

Methods

Blood Lymphocyte Separation

Blood samples were obtained from 16 patients with chronic lymphocytic leukemia as defined by Dameshek and Guin5 and eight patients with Hodgkin's disease. In addition, the lymphocytes from 30 patients with no hematologic diseases (considered as controls) were also studied. Twenty milliliters of blood were obtained from each patient and sedimentation was carried out for 30-60 min. at 37°C. The leukocytes in the supernatant plasma were centrifuged, washed, and resuspended in the final medium which consisted of equal parts of TC199 (Difco) and compatible human serum.

To facilitate the search for these fibrils, lymphocytes were concentrated by means of the following procedure: The suspensions were introduced into a flat-bottomed T60 flask and incubated for 30 min. at 37°C. During this procedure, the granulocytes and monocytes adhere to the glass and 90-99 per cent of the nucleated cells in the supernatant are seen to be lymphocytes. This supernatant was collected for the electron microscopic study.

Electron Microscopy Preparations

The suspensions were then fixed from 16 hr to several days with 1.5 per cent glutaraldehyde in Millonig buffer (diluted 1:1 with the proper distilled water) with 0.13 per cent NaCl (wt./vol.) added to adjust the osmolality of the fixative to a value of 340 mOsM. The pH of the fixative was adjusted to 7.4. The suspensions were then postfixed with 1 per cent osmium tetroxide in Veronal buffer (pH 7.4 and 340 mOsM) for one hour. The cells were dehydrated through series of increasing concentrations of alcohol and

From the Electron Microscopy and Radiobiology Research Sections, Veterans Administration Hospital, Hines, Ill., and Department of Radiology, The Chicago Medical School, Chicago, Ill.

First submitted July 2, 1969; accepted for publication October 3, 1969.

This work was supported in part by the Leukemia Research Foundation, Inc., Chicago, Illinois.

Stefano S. Stefani, M.D.: Chief, Therapeutic Radiology Service, Veterans Administration Hospital, Hines, Ill., and Clinical Professor of Radiology, The Chicago Medical School, Chicago, Ill. Hiroshi Tonaki, B.S.: Electron Microscopist, General Medical and Surgical Research Service, Veterans Administration Hospital, Hines, Ill.
Fig. 1.—Normal lymphocyte. Pairs of arrows indicate borders of fibril bundles present in nucleus (N).

Fig. 2.—Normal lymphocyte. Intranuclear bundle of fibrils (arrows) shown in Y-shaped arrangement close to nuclear membrane (NM). Cytoplasm marked (CY), nucleus (N), mitochondrion (M).
FIBRILLAR BUNDLES IN THE NUCLEUS OF BLOOD LYMPHOCYTES

Fig. 3.—Normal lymphocyte. Several intranuclear bundles of fibrils sectioned at different angles and indicated by arrows. Nucleus denoted by (N), cytoplasm (CY), nuclear membrane (NM), mitochondria (M).

propylene oxide, and finally embedded in araldite mixture (Fluka). In each case more than 1000 sections of lymphocytes were studied at about 17,000 magnifications.

RESULTS

Bundles of fibrils were observed in the nuclei of blood lymphocytes of 15 of 30 nonleukemic patients studied. These fibrils were always found in the interchromatin space and were arranged in a parallel fashion forming a bundle of variable length and about 200 m. in diameter (Fig. 1). In one instance, bundles were seen to form a Y-shaped arrangement (Fig. 2). The fibrils appeared to have a beaded substructure and when seen in cross section had a
diameter of approximately 70 Å and did not seem to contain any lumen. In some instances (Fig. 3) several bundles cut at different angles were observed within a single section of the nucleus. Whether they represented separate bundles or one continuous bundle winding throughout the entire interchromatin material, could not be established.

In certain instances these bundles could be seen extending in the direction of the nuclear envelope (Fig. 2), although direct contact could not be firmly established. Further, no detectable connection between these structures and nuclear bodies or nucleoli could be demonstrated (Figs. 4 and 5).

These structures when observed in a sample were found only in small numbers. We estimated that in these samples only one out of 70–100 cellular sections contained detectable intranuclear bundles of fibrils. On the other hand, cytoplasmic bundles were seen in all samples studied, and for any given sample occurred more often than intranuclear bundles; they were observed approximately 10–20 times more often than the intranuclear bundles, although both were not always found together in the same section.

No definite proof could be established concerning the significance of these nuclear bundles.

In spite of extensive searching, these fibrillar bundles were found in only three of 16 patients with chronic lymphocytic leukemia, and in one of eight patients with Hodgkin’s disease. The fibrils found in the lymphoma cases appeared similar to those found in normal patients.
Fig. 5.—Normal lymphocyte. Intranuclear bundle of fibrils (arrows) found in interchromatin material (ICM) near nucleolus (NC) surrounded by chromatin material.

DISCUSSION

The observation of these structures in blood lymphocytes of 15 of 30 patients without hematologic disease contrasts with their absence in the lymphocytes of 13 of 16 patients with chronic lymphocytic leukemia, and seven of eight patients with Hodgkin's disease investigated. Whether this difference in incidence is the expression of a different type of population or a different stage of maturation of the lymphocytes, or of a pathologic state, remains to be demonstrated.

In spite of the apparent low incidence of these bundles, it is our impression that they are not an unusual component of the nuclear structure of normal lymphocytes. They are rarely observed, however, because: (a) they appear usually as a small segment in the interchromatin region, and (b) they are difficult to identify unless the bundles are cut in their longitudinal section.

In our study, no proof of a direct connection between these structures and the nuclear membrane could be made. Several investigators have described similar structures in the cytoplasm of the human lymphocytes and have underlined the frequency of contact between these fibrils and the outer nuclear membrane. Similar findings have also been repeatedly observed by us during this investigation. Parker et al. reported also, that in a few instances fibrils arranged perpendicularly to the nuclear membrane appeared to penetrate through a defect and to merge with the nuclear chromatin. Although even some of our own sections were strongly suggestive of some connection
between the nuclear and cytoplasmic fibrils, none could show a definite connection. These bundles are located in the interchromatin space and do not appear to have any connection with nuclear bodies or nucleoli. Morphologically, these bundles do not resemble the fibrillar zone found in the nucleoli in human lymphocytes by Tokuyasu et al.\textsuperscript{7}

Similar intranuclear fibrillar bundles have been described in chick sympathetic neurons treated with deuterium by Masurovsky et al.\textsuperscript{8} These authors, too, noted some fibrils approaching close to the nuclear envelope and extending into the nuclear pore area.

The nature and the function of nuclear fibrils, as well as those at times observed in the cytoplasm of the same lymphocyte, are as yet unknown. Bundles of fibrils associated with virus particles have been described by Rowe and Capps,\textsuperscript{9} and by Bernhard and Granbolulan,\textsuperscript{10} in the nuclei of thymic cells of newborn mice infected with “mouse thymic virus.” Filaments have also been found by Patrizi et al.\textsuperscript{11} in the nuclei of human hepatocytes infected in vitro by herpes simplex virus. Similarly, Kalnins et al.\textsuperscript{12} detected bundles of fibrils in nuclei of human amnion cells, and of hamster cells, both infected in vitro with Adenovirus Type 12, and in the cytoplasm of Adenovirus Type-12-induced neoplastic cells from Syrian hamsters. In addition, they noted that these bundles of fibrils presented the same antigenic character as Adenovirus Type 12. The authors suggest that the bundles of fibrils could be considered as “viral footprints” and could serve as an indicator of a viral etiology in neoplastic cells. The bundles of fibrils described by us in normal and leukemic blood lymphocytes resemble morphologically those described by Kalnins et al. Whether the former could also be the expression of viral infection remains to be demonstrated.

Another possible explanation of these structures is that they represent a paracrystalline arrangement of nuclear material (possibly RNA) which may eventually move from the nucleus to the cytoplasm or vice versa. We could find no histochemical or autoradiographic study of these cytoplasmic bundles reported in the literature. However, autoradiographic studies using tritiated uridine are being carried out in our laboratory with the aim of testing the composition of this material.

**SUMMARY**

Fibrillar bundles were found in the nuclei of blood lymphocytes from 15 of 30 normal individuals. Similar fibrils were found in three of 16 cases of chronic lymphocytic leukemia, and in one of eight cases of Hodgkin’s disease. Nuclear bundles when observed in a sample were found only in small numbers. No points of continuity between the nuclear and the cytoplasmic fibrils could be found.

**ACKNOWLEDGMENT**

We are indebted to Mr. Carl Kirsh and Mr. Joseph Kompare for their skillful technical assistance.
REFERENCES

Fibrillar Bundles in the Nucleus of Blood Lymphocytes from Leukemic and Nonleukemic Patients

STEFANO S. STEFANI and HIROSHI TONAKI