Cytoplasmic Fibrils in Mixed Lymphocyte Cultures

By JOHN W. PARKER, HARUKI WAKASA AND ROBERT J. LUKES

An ultrastructural comparison of the cells which develop in cultures of mixtures of lymphocytes from allogenic donors and those which result from stimulation by phytohemagglutinin (PHA) has demonstrated morphologic differences. One of the most striking of these is the frequent presence of bundles of fibrils in transformed lymphocytes of the mixed lymphocyte cultures. There have been scattered reports, in recent years, listed by Biava,1 of cytoplasmic fibrils in a number of different cell types. Bessis2 described a crescent shaped fibrillar structure in the cells of acute granulocytic leukemia in 1957, and since then similar collections of fibrils have been reported in other hematologic cell types in both malignant and benign conditions.3-5 Reports of the presence of cytoplasmic fibrils in lymphocytes have apparently been limited to a description of bundles of fibrils in thoracic duct lymphocytes,6 occasional inconspicuous fibrils in peripheral lymphocytes,7 and rare individual fibrils in lymphocytes exposed to PHA.7 The latter is in agreement with our own experience. The frequent occurrence of bundles of cytoplasmic fibrils in mixed lymphocyte cultures, therefore, has been most impressive and, in fact, appears to provide an unusual opportunity for (1) the morphologic characterization and localization of the fibrils and (2) the determination of their chemical composition by cytochemical and autoradiographic technics.

The fibrils were observed not only in the blast cells of the mixed lymphocyte cultures, but also in a high percentage of lymphocytes with increased cytoplasm and organelles. These will be referred to as activated lymphocytes to distinguish them from the small or dormant peripheral blood lymphocytes. They appear to be similar to the blood lymphocytes commonly classified as medium or large, or in some instances atypical lymphocytes.

Whether the presence of the bundles of fibrils in the mixed lymphocyte cultures in contrast to their absence in phytohemagglutinin cultures is a morphologic expression of a fundamental difference in the stimuli—i.e., anti-
genie vs. nonspecific—cannot be determined from the data presently available. Work is in progress to clarify this point.

**Methods**

**Lymphocyte Cultures**

Lymphocyte suspensions were prepared from the blood of several healthy adult male and female donors by gelatin sedimentation, as previously described, with the exception that the final cell count was adjusted to one million lymphocytes per ml. This was accomplished by centrifuging the supernatant containing 90-95 per cent lymphocytes for 5 minutes at 800 rpm and resuspending the lymphocytes in appropriate volumes of TC 199, containing 20 per cent autologous serum and 100 units of penicillin and 100 mg of streptomycin per ml.

The mixed lymphocyte cultures were prepared by mixing 1 ml of the lymphocyte suspension from one donor with 1 ml from another donor. The mixtures were cultured in Leighton tubes and several were prepared for each pair of donors. Control cultures containing 2 ml of lymphocyte suspension from the individual donors were cultured separately with 0.2 ml of PHA-M (Difco) and still others without PHA.

The tubes were tightly stoppered and incubated at 37 C. for 6 or 7 days without change of medium or gas phase. The PHA cultures were usually allowed to incubate for only three days, since there was marked degeneration of cells with longer incubation. In two, however, the culture medium was changed in all tubes on the fourth day and the tubes were incubated for an additional three days, resulting in better cell survival. Additional PHA cultures were also examined at 24, 48, and 96 hours.

**Electron Microscopy**

Each of the groups of cultures was pooled and centrifuged at 800 rpm for 10 minutes. After removal of the supernatant, the cell buttons were fixed for 15 minutes in veronal buffered 1 per cent osmium tetroxide. The specimens were transferred, after fixation, to small plastic centrifuge tubes and centrifuged again for 4 minutes in a Beckman Microfuge to obtain compact pellets. The pellets were dehydrated through graded concentrations of alcohol and embedded in Epon or Araldite. Sections were cut on an LKB or Porter-Blum ultramicrotome with glass knives, stained for 30 minutes in saturated uranyl acetate in methanol, and examined with a Hitachi H8-7S electron microscope.

**Results**

The per cent of blast cells in the mixed cell cultures was generally less than 10 per cent, in striking contrast to the degree of transformation in the PHA cultures. The partially or completely transformed lymphocytes in the PHA cultures showed the features previously described, i.e., cells with large irregular nuclei having fine loose chromatin and prominent dense, occasionally skein-like nucleoli, and abundant cytoplasm containing a variety of well-developed organelles. The transformed lymphocytes in the mixed lymphocyte cultures, although generally similar in appearance to the PHA cells, showed minor differences in numbers and types of cytoplasmic organelles. These differences will be described in more detail at a later date, in conjunction with cytochemical studies. The most striking difference was the presence of prominent bundles of cytoplasmic fibrils in the lymphocytes in the mixed cultures. Bundles of fibrils were not seen in any of the numerous PHA transformed lymphocytes examined, and only a very rare fine individual fibril was encountered in these cells. In contrast, the mixed lymphocyte cultures exhibited prominent bundles of fibrils in cells at all stages of transformation, even though
the percentage of blast cells was low. A small number of large macrophage-like cells were present and these cells occasionally contained similar cytoplasmic fibrils. However, the majority of the cells with fibrils were interpreted as large or activated lymphocytes, which appeared to correspond to cells of the types labelled B and C in Figure 1. These cells (Fig. 2) possessed nuclei which showed finely distributed chromatin and large nucleoli. In addition, the cytoplasm was more abundant and complex than that of the small lymphocyte. Although the percentage of blast cells encountered in the sections examined was low, the majority of these also contained fibrils. Twenty-five to 30 per cent of the cultured lymphocytes contained bundles of fibrils and these were all blast cells or activated lymphocytes. In the non-PHA control cultures, in which the proportion of activated cells was much smaller and blast cells were not encountered, the percentage of cells containing fibrils was approximately half that seen in the mixed cultures, and the bundles were generally not as well developed. However, essentially all of these cells showed an increase in cytoplasmic content and complexity.

The fibrils (Figs. 3, 4, and 5) were very similar to those that have been described in other hematologic cells.1-5 Their average diameter was 75Å and they were frequently arranged in compact wavy bundles. The heaviest concentration of fibrils was near the nucleus and in an occasional cell they appeared to encircle it (Fig. 2). Quite frequently they were present in nuclear invaginations and in many cells there appeared to be a loss in continuity of the nuclear membrane where the fibrils came in contact with or were in close proximity to the nucleus. In a few instances, fibrils, arranged perpendicular to the nuclear membrane, appeared to penetrate through a defect and merge with the nuclear chromatin (Fig. 4). The only other recurring association between the fibrils and other cell structures was their occasional apparent contact with mitochondria. This association was less convincing than that with the nucleus...
Fig. 2.—*Activated* lymphocyte from a mixed lymphocyte culture, illustrating prominent perinuclear bundles of fibrils. The area outlined is seen at a higher magnification in Figure 3. Osmium fixation, uranyl acetate stain; × 13,500.

Fig. 3 (left).—Inset from Figure 2 showing the close approximation of fibrils and nuclear envelopes. Osmium fixation, uranyl acetate stain; × 36,000.

Fig. 4 (right).—Cytoplasmic fibrils apparently in contact with nuclear chromatin and extending through a defect in the nuclear envelopes. Osmium fixation, uranyl acetate stain; × 35,700.
since it occurred much less frequently and the thickness of the sections in which it was seen allowed for superimposition.

When seen in cross section (Fig. 5) the fibrils contained no detectable lumens, and because of this and the size difference they were distinctly unlike the mitotic spindle fibers occasionally seen in these cultures or the microtubules reported in other cell types.

**Discussion**

Similar, if not identical, fibrils have been described in several different cell types, both epithelial and mesothelial in origin, including cells of the reticuloendothelial system. Although originally described in leukemic cells, they have also been reported in mononuclear macrophages and in reactive plasma cells, and it has been suggested that this type of fibril is a normal cellular component which is probably exaggerated in both reactive and neoplastic processes.

Apparently, the only report of bundles of fibrils in lymphocytes is one describing human lymphocytes from the thoracic duct, and the lymphocyte illustrated in that report shows evidence of cytoplasmic activation with an
increase in organelles. Inconspicuous, very fine fibrils associated with granules have been reported in the lymphocytes from the peripheral blood by Tanaka et al. and rare individual fibrils have been noted in lymphocytes exposed to PHA. The "strands" connecting mitochondria in the atypical lymphocytes of infectious mononucleosis, mentioned by Paegle, appear to be of a different nature.

Although the fibrils described in the present report and in other cell types are morphologically distinguishable from microtubules, as well as from tonofilaments, it has been suggested that they also are part of a cytoskeletal system. However, the chemical nature of the fibrils and their function in the cells containing them is as yet unknown.

If, as de Petris and Tanaka agree, fibrils are a normal component of certain cells of the reticuloendothelial system, but are exaggerated in pathologic process whether reactive or neoplastic, this might explain why lymphocytes have been reported by most observers as lacking them. Most of the lymphocytes examined by electron microscopy have probably been small, dormant, peripheral lymphocytes showing scanty cytoplasm with few cytoplasmic organelles. The cells containing the fibrils, in the present study, were large cytoplasmic lymphocytes or activated lymphocytes. Although similar fibrils might be expected in the atypical lymphocytes of infectious mononucleosis, the strands connecting mitochondria, illustrated by Paegle, do not resemble the fibrils observed in these mixed lymphocyte cultures. The fibrils in the thoracic duct lymphocytes described by Zücker-Franklin, however, are quite similar to those of the mixed lymphocyte cultures and the cells illustrated also have abundant cytoplasm with numerous organelles and appear to be activated rather than small dormant lymphocytes. The association of fibrils with activated and transformed lymphocytes may suggest that, for lymphocytes, fibril development is part of an increasing cytoplasmic complexity which is in response to an activating stimulus. The fact that they are not seen in the PHA cultures at 24, 48, 72, 96 or 168 hours may mean only that PHA in some way prevents their development. However, the possibility that the difference is due to a functional difference in the type of stimulus cannot be dismissed.

Another possibility that must be considered in explaining the presence of bundles of fibrils in lymphocytes in the mixed lymphocyte cultures is that the cells containing the fibrils were actually macrophages or transition forms between lymphocytes and macrophages. Macrophages were present in greater numbers in these cultures than in PHA cultures in which most of the neutrophils and monocytes disappear in the first 24 hours, apparently due to a toxic effect of the PHA. However, the cells that we have interpreted as activated lymphocytes and blasts were sufficiently different from macrophages to distinguish them by light and electron microscopy.

An interesting aspect of the presence of fibrils in these cells was the frequent contact noted between the fibrils and the nucleus. It appears unlikely that this was an artefact, since it has been observed in several instances in our cultures and has also been described by two other groups of workers. Both de Petris and Tanaka noted this association but reported only communica-
tion of fibrils with the outer nuclear membrane effect. The above workers also described an occasional apparent connection of fibrils with mitochondria, a finding that we also have noted. This connection is less convincing in our material, but again repeated observation by three independent groups studying different cell types suggest that the association may be meaningful. If this nuclear-mitochondrial connection by fibrils proves to be real, the implications of mechanical and/or chemical interaction are intriguing and the mixed lymphocyte cultures appear to offer an excellent model for studying such interactions.

Summary

Bundles of cytoplasmic fibrils are described in activated (increased cytoplasm and organelles) lymphocytes and blasts in human mixed lymphocyte cultures. Similar bundles were not observed in the transformed lymphocytes in PHA cultures. They were encountered in the activated lymphocytes in the control cultures, but with a lower incidence. The fibrils were usually perinuclear, were frequently in contact with the nuclear envelopes and occasionally penetrated through defects in the nuclear membrane into the nucleus. A less frequent and less convincing connection with mitochondria was also observed.

These morphologic observations seem to indicate that the transformed cells observed in mixed lymphocyte and PHA cultures are not identical.

Summary in Interlingua

Fasces de fibrillas cytoplasmatic es describite in activate lymphocytos e blastocytos (i.e., lymphocytos e blastocytos a augmento de cytoplasma e de organellas) in cultura mixte de lymphocytos human. Simile fasces non esseva observate in le transformate lymphocytos in cultura con phytohemagglutinin. Illos esseva incontrate in le activate lymphocytos in le culturas de controlo, sed illa lor incidentia esseva plus basse. Usualmente le fibrillas se trovava in sitos perinucleari, illos esseva frequentemente in contacto con le invelloppes nucleari, e illos penetrava occasionalmente a transverso defectos in le membrana nucleari ad in le nucleo. Un minus frequente e minus convincente connexion con mitochondrios esseva etiam observate.

Iste constatationes morphologic pare indicar que le transformate cellululas observate in cultura de lymphocytos mixte e in culturas a phytohemagglutinin non esseva identic.

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References

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