The Ultrastructure of a “Fibrillar Formation” of Leukemic Human Blood

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Bessis and Breton-Gorius and later Bessis have recently described a newly discovered structure in the cytoplasm of cells of a case of acute granulocytic leukemia. Despite the fact that the cytoplasm of these cells is strongly hydrated, this structure was found to be fibrillar or filamentous in nature. When first observed, the fibrils formed a crescent-shaped zone. Hence, these investigators referred to this structure as the “crescentic zone.” The present investigation has demonstrated this crescentic zone to be a single architectural variant of a complex fibrillar formation, which, when fully developed, exhibits itself as a structure with a very high degree of organization. This paper will discuss the morphology of the “fibrillar formation.”

Materials and Methods

Blood samples were obtained from normal and leukemic patients by sterile venipuncture. The blood was withdrawn without stasis (a) in a 10 cc. silicon-coated syringe, fitted with an arquad-coated 20-gauge needle and transferred to a 10 cc. Lusteroid centrifuge tube, which had been precooled to 5–10 C., or (b) alternatively by needle drip directly into a tube coated with a suitable non-wetting agent. The cooled blood sample was centrifuged at 1500 r.p.m. for 15 minutes at 0 C. (R. C. F.-265: International model PR-2, refrigerated, angle head).

The buffy coat was aspirated with a silicon-coated pipette and transferred to a glass tube containing 5 cc. of 1/2 veronal buffered (pH 7.4) osmic acid, which had been precooled to 5–10 C. The specimen was fixed in the osmic acid for 1/2 hour. The material was then dehydrated by immersion in successive changes of 50%, 70%, 95% and absolute ethanol, remaining in each for 1/2 hour. The specimen was then placed in three changes of 5 parts n-butyl methacrylate plus one part methyl methacrylate catalyzed with 2%, by weight, of 2,4 dichlorobenzoyl peroxide, remaining in each for one hour. Between each successive step of the dehydrating and embedding procedure, the specimen was centrifuged at 1500 r.p.m. for 1 to 1/4 minutes at room temperature (R. C. F.—ca. 385: Clay Adams safeguard model). After each centrifugation, the supernatant fluid was decanted, the next fluid added and the tube manually agitated to produce a suspension. The last methacrylate suspension was permitted to settle by gravity in 00 gelatin capsules for 1/2 to 1 hour to avoid close packing.

The encapsulated specimens were then polymerized overnight in an oven with dry heat at 47–52 C. After polymerization the specimens were sectioned at ca. 1/20th to 1/40th...
micron thick using a Porter-Blum ultramicrotome. They were examined and photographed without removal of the methacrylate, with an RCA EML-1B and an RCA EMU-3 electron microscope. *

Smears of the buffy coat and direct smears of the peripheral blood were stained with Wright’s stain for light microscope analysis.

RESULTS

The general appearance of this fibrillar formation is illustrated in figures 1-6. During the course of investigation of a large number of these structures, we have observed it in various degrees of structural organization. By means of serial sections (figs. 2-4) one can demonstrate this fibrillar formation as a barrel-shaped structure or whorl open on both ends with the long axis of its fibrils oriented in a circular direction. These formations measure 1/2 to 21/2 μ in the smallest part of the oval diameter and 1/4 to 1 μ in depth. However, not all fibrillar formations exhibit such a high degree of structural organization. Cells may contain only a few closely packed fibrils, a crescent-shaped area or an oval formation of fibrils. *

The diameter of the individual fibrils is relatively constant, regardless of the degree of structural organization, averaging 75Å in diameter. Few fibrils are entirely straight, the majority being slightly wavy in appearance (fig. 5).

Contained within and associated with the complex fibrillar formation is a granular component. These granules are aggregated in a central position within the cytoplasm enclosed by the whorl of fibrils. They are found only by fortuitous sectioning through the central portion of the barrel-shaped fibrillar formation or by serial sections through the entire structure. The granules are highly osmiophilic and usually homogeneous, but a few have a clear core with a dense periphery. They are smaller than the specific granulation of the immature granulocytes (ca. 0.15-0.30 μ) and larger than the ribonucleoprotein granules of Palade (ca. 120-150Å). The diameter of these granules ranges from 175-200Å. In occasional instances, one can find moderately dense, very small granules (ca. 150-185 Å) in addition to the highly osmiophilic granular component (fig. 6).

The ground substance of the granular area is denser than the ground substance of the surrounding cytoplasm. The small rim of cytoplasm which is sometimes seen between the granular component and the internal aspect of the whorl of fibrils is comparable to the general cytoplasm of the cell (figs. 3, 6). Figure 1 is a schematic representation of the highly developed fibrillar formation, its relationship within the cell and its ultrastructure.

DISCUSSION

Because of the shape of this fibrillar structure when first observed, the term “crescentic zone” was applied and subsequently used by Bessis and Breton-Gorius.1 This term is an unfortunate one, since, in thin sections, any structure with a nonspherical form will appear in variously different shapes depending on the angle at which it happens to be sectioned. Nevertheless,

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*The RCA EML-1B electron microscope was from the Department of Anatomy; the RCA EMU-3 was made available through the courtesy of the Department of Pathology.
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Fig. 1.—Diagrammatic representation of a fibrillar formation, its relationship in the cell and its ultrastructure.

(a) The fibrillar formation is a cytoplasmic component which, on occasions, may be large enough to invaginate the nucleus.
(b) Small fibrils compose the whorl that makes up a structurally complete fibrillar formation. The central area is cut away to show the location and relationship of the granular component.
(c) This figure is a cross-section through the center of a fibrillar formation. The dense homogeneous granules lie within the center. Sometimes a thin rim of cytoplasm lies between the granular component and the fibrils.

A purely morphologic descriptive term is desirable since nothing is known about the etiology or function of these structures. Since the structures are basically fibrillar in nature, the term "fibrillar formation" is morphologically acceptable.
Figs. 2-4.—These electron micrographs are 3 of 10 serial sections through the entire barrel-shaped fibrillar formation (ff) sectioned somewhat obliquely. Figure 2 is sectioned obliquely at one of the open ends of the formation, figure 4, at the other, and figure 3 somewhere near the center. The nucleus (n) is invaginated by the structure.

Fig. 5.—This electron micrograph is a lateral view of the fibrillar formation. The fibrils are oriented in a circular direction. A single fibril measures ca. 75Å in diameter (at arrows).
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Fig. 6.—The dense, homogeneous granular component (g) associated with the fibrillar formation (ff) is well demonstrated in this electron micrograph. However, the fibrillar formation in this cell is not a complete whorl. The small rim of undifferentiated cytoplasm (uc), sometimes found between the granular component and the fibrillar component, is visible.

Although the over-all dimensions of the fibrillar formation are such that it might be visible with the light microscope, it is not visible with Giemsa or Wright stains and cannot be identified in fresh, unstained preparations. This may be due to the fact that its individual structural components are too small to be resolved by the light microscope and the area thus appears to be undifferentiated cytoplasm.

Fibrillar formations were observed in 3 cases, two of acute monocytic leukemia, and one of acute granulocytic leukemia, in the course of examining the peripheral blood of 23 cases of various types of leukemias (1 acute granulocytic, 6 chronic granulocytic, 3 acute lymphocytic, 6 chronic lymphocytic, 3 acute stem cell, 1 plasma cell, 2 acute monocytic, 1 subacute lymphocytic) and 9 cases with normal peripheral blood pictures. In one case of acute monocytic leukemia approximately 75% of the blast cells contained fibrillar formation with a high degree of structural organization; in the second, only 10% of the cells showed a complete whorl formation. In the case of acute granulocytic leukemia, the fibrillar formation was found only occasionally in promyelocytes in the form of a few fibrils or a semi-oval without the granular component. With the rare exception of a few isolated fibrils, we have not observed the fibrillar formation in leukocytes of normal or leukemic (granulocytic) human bone marrow. Occasional small groups of fibrils, similar in dimensions and appearance have been observed in leukocytes of normal bone marrow of experimental animals. However, these groups did not exhibit
any degree of structural organization comparable to the fibrillar formations and no granular component was associated with them.

DiMayorca observed a thick peripheral membrane enclosing an area which contained a highly osmiophilic granular component in a cell from a chronic granulocytic leukemia. He implied that this organoid was associated with the fibrillar formation. However, the fibrillar formation in his specimen was not unequivocally demonstrable. We have never observed a limiting membrane surrounding the dense granular area associated with the fibrillar formation.

It would be conjectural to theorize as to the function or the etiology of these structures, but it is notable that they have been found predominately in blast forms or in promyelocytes. Although small aggregates of fibrils have been rarely found in normal human marrow cells, none have exhibited as intricate a structure as the fibrillar formation observed in this study. This could suggest a high degree of development, in a specific pathologic state, of a very simple form of organoid only occasionally seen in normal immature cells. Since repeated observations have revealed small groups of fibrils, crescentic forms and, in serial sections, a highly developed fibrillar whorl, a sequence of development from small aggregates of few fibrils, such as have been observed in cells of normal bone marrow, to the highly organized whorl of pathologic cells, is suggested.

**Summary**

A previously unsuspected cytoplasmic organoid, fibrillar in nature, was discovered by Bessis and Breton-Gorius. This structure exhibits a very high degree of structural organization, and appears as a barrel-shaped fibrillar formation, opened at both ends and enclosing an associated granular component within. Comparable fibrillar formations are rarely found in normal cells, even in the simplest form of a few closely packed fibrils. In its highest degree of structural organization, the whorl, the fibrillar formation has been found only in the blast or promyelocytes stages. Although the overall dimensions of this structure are well within the resolving limits of the light microscope, it is not visible in fresh, Wright stained or Giemsa stained preparations.

**Summario in Interlingua**

Un previemente non suspicite organoide cytoplasmic, de natura fibrillar, esseva discoperite per Bessis e Breton-Gorius. Illo exhibi un grado multo alte de organisation structural e se presenta como un complexo fibrillar barriliforme que es aperte a ambe extremitates e include un associate componente granular al interior. Comparabile formationes fibrillar se incontra rarmente in cellulas normal. In lor plus complexe organisation structural illos es verticilliforme. In iste conformation illos has essite trovate solmente in cellulas del stadios blastic e promyelocytic. Ben que le dimensiones total de iste structura non es infra le limites del resolution de microscopios a lumine, illo non es visible in preparatos fres colorate secundo Wright e Giemsa.
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