Origin of Auer Bodies

By JAMES A. FREEMAN

ALTHOUGH the presence of Auer bodies in immature leukocytes is presumptive evidence of leukemia, the origin of these structures is not clear. Probably the earliest theory of the nature of Auer bodies is traceable to John Auer's\(^1\) original description of these structures in 1906. At that time he postulated that "the data are only strong enough to render a comparison justifiable" between human intracellular parasites and the "leukemia granules" which he described. He specifically referred to the Leishmann-Donovan body, but noted that the comparison was highly speculative. Roth\(^2\) in 1913, after finding Auer bodies in the myeloblast of a patient with tuberculosis, felt that these structures represented an unusual form of the tubercle bacillus. Unsuccessful attempts to transmit Auer bodies by inoculation experiments and by cultures on various media by Ottenberg\(^3\), Aibara,\(^4\) Ishikawa\(^5\) and McManus\(^6\) eliminated the possibility of these structures being an infectious microorganism. Nakashima's theory that Auer bodies were pathologic forms of azurophilic granules or immature neutrophilic granules\(^7\) received little attention, since it did not explain simultaneous occurrence of Auer bodies and azurophilic granules within the same cell. In 1947, Quattrin, recalling the structural similarity of Auer bodies, azurophilic granules and nuclear fragments, suggested a process of irregular germination or partial segmentation of nucleoplasm to form Auer bodies. Histologic and cytochemical evidence supporting this theory, however, was lacking. After extensive histochemical study of Auer bodies and cytoplasmic granules, Ackerman in 1950 proposed that an alteration of the acid pH occurred in immature azurophilic granules of cells with fusion of granules into Auer bodies instead of normal development into mature specific granules.\(^8\) He also suggested that differences in the size and shape of these structures could be explained by the degree of cytoplasmic alteration, although the stimulus to the alteration was unexplained. His theory, in essence, is an extension of Nakashima's proposal.\(^7\) Utilizing electron microscopy, Ito interpreted the Auer body to be a fused mass of granules which were formed within the endoplasmic reticular system.\(^9\) Akasaka\(^10\) also ascribed to Ito's theory; however, none of the Auer bodies in his published illustrations were within the endoplasmic reticulum. Low and Freeman, and later Freeman in 1960 failed to confirm Ito's findings.\(^11,12\) Instead, they found the granules in the ground

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substance of the cytoplasm, and noted a striking similarity of fine structure of Auer bodies from azurophilic granules; however, distinct stages of transition man's findings indirectly supported Ackerman's theory of development of Auer bodies from azurophilic granules; however, distinct stages of transition were not demonstrable.

The present study was undertaken in an effort to delineate the possible transition of azurophilic granules to Auer bodies. It was felt that in vitro tissue culture growth with a gradual loss of acidity of the culture media, possibly producing an alteration of the pH of the cellular cytoplasm and cytoplasmic granules, might promote a more ready visualization of the dynamic changes involved in the process of Auer body formation. Furthermore, it was suspected that in vitro tissue culture might promote persistence of Auer bodies in leukocytes for a longer period of time, thereby making the Auer bodies available for further study.

**MATERIALS AND METHODS**

Iliac crest bone marrow aspirations were done with the Turkell or Illinois needles from 2 patients with acute myelomonocytic leukemia. First drop aspiration specimens were placed on a cover glass. Using two needles, marrow spicules were separated rapidly from the blood before clotting occurred. For electron microscopy the spicules were transferred directly into 1 per cent (w/v) osmium tetroxide in a 4.5 per cent (w/v) sucrose solution (pH 7.4). For tissue culture, the spicules were transferred into a sterile, covered petri dish containing 10 ml. medium 199 with 25 per cent (w/v) Hyland agamma human serum and 0.1 mg. phenol red indicator dye. Cultures were propagated for 48 to 72 hours in milk dilution bottles. Rapidity of growth and division were followed by transmission light microscopy. The pH of the medium was judged by color change from yellow (pH ca. 6.6) to orange-red (pH ca. 8.2). After 48 to 72 hours maintenance in tissue culture, 1 per cent (v/v) trypsin was added to separate the cells from the glass. The separated cells and medium were poured into a 15 ml. conical centrifuge tube and centrifuged gently (RCF = 656) to form a pellet. The medium was decanted. 1 per cent (w/v) osmium tetroxide was added, and the cells were resuspended by gentle agitation. After 1½ hours fixation at 4 to 10 C, the 1 per cent (w/v) buffered osmium tetroxide (pH 7.4) was decanted and phosphate buffered 10 per cent (w/v) formalin (pH 7.0) was added for 1 hour. At the end of this time the specimen was gently centrifuged (RCF = 377) to form a pellet and the formalin was decanted. A 2 per cent (w/v) solution of warm aqueous agar was added to the centrifuge tube with the pellet, was permitted to cool, and the pellet was separated gently from the sides of the glass, thus enabling the agar to encase and jell the specimen. The agar-jelled specimens were removed from the centrifuge tube, cut into small blocks, and dehydrated in graded ethyl alcohols according to the following schedule: 70 per cent (v/v), 95 per cent (v/v), absolute alcohol, 2 changes of the latter, 15 minutes each; propylene oxide: epoxy resin—1:1 (v/v) mixture, 30 minutes. The tissue was infiltrated with Maraglas epoxy resin (Freeman and Spurlock, Spurlock, Kattine and Freeman) overnight in a refrigerator at about 10 C. The tissue was then embedded in fresh epoxy resin in predried 00 gelatin capsules or in BEEM plastic capsules and cured in an oven with dry heat at 60 C. for 72 hours. Sections were cut on a manually operated Servall MT-1 (Porter-Blum) microtome. Specimens were examined and photographed in a RCA EMU-3F electron microscope at original magnifications of 6400 to 33,000 X and were photographically enlarged.

**RESULTS**

The structural integrity of the cells was determined by the intactness of plasma and nuclear membranes. Cells that showed defects in these membrane
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systems were felt to be either damaged in the preparatory electron microscopy technics or undergoing pathologic cellular alteration; hence, they were not studied. Observations were limited to those cells in which these membrane systems were intact.

Auer bodies were found in all forms and sizes from single round granules to large rod-shaped and diamond-shaped inclusions (Figs. 1, 2, 5-8). The earliest recognizable form of Auer body always was found intimately associated with granules within the cytoplasm (Figs. 1, 5, 6), although it was sometimes necessary to do serial sectioning to demonstrate this association. The granules were electron dense and structurally heterogeneous with no constancy of internal structure. Granules were surrounded by external limiting membranes. Some granules contained dense whorls of membranes, while others were finely granular. Disorganized or degenerated portions of organelles, a characteristic feature of cytolysomes, were not found in the dense granules associated with Auer bodies. Large vacuoles, up to 2 microns in diameter, were found near many of the granules (Figs. 5, 6). A few of the granules were continuous with, or were globular extensions of, the vacuolar content (Fig. 5). Within the vacuoles was found moderately electron dense, irregular, loosely arranged material having a filamentous appearance (Figs. 4-6). A granular electron dense material was interspersed between the filamentous material. Dense rod-shaped structures, consistent with classic Auer body size, shape, and fine structure, were found associated with the filamentous and granular material within the vacuoles (Figs. 4-7). Whenever the Auer body was seen in the rod-form, the vacuole was smaller and the content of the vacuole was granular only (Fig. 6). With the early formation of the elongate inclusion, a lamination of membranes in parallel array gave an appearance of myelin figures (Fig. 3). Each membrane measured about 75 Å and was separated by a clear space about 100 Å across (Fig. 10). The most “mature-appearing” Auer rods did not exhibit, with the same ease of recognition, the paralleling membranous component; instead, the rods appeared as coacervates of larger plaques sometimes possessing a central lucid core (Fig. 8). The central core was demonstrable only in the larger and more “mature” Auer rods.

DISCUSSION

As far back as 1924 Nakashima theorized that Auer bodies might be related to azurophilic granules seen in both the myelocytic and monocytic cells, and proposed that Auer bodies were pathologic forms of azurophil granules. This theory was strengthened by demonstration of varying sizes and shapes of Auer bodies from small round granules to diamond-shaped and rod-shaped structures. However because of lack of an adequate explanation of the simultaneous occurrence of Auer bodies and azurophil granules within the same cell, the theory was ill-accepted. Bessis also demonstrated a series of Auer bodies from small granules to rods with central lucid core, indirectly suggesting a transition of granules to rods. It was Ackerman, however, and later Harada who demonstrated the histochemical similarity of Auer bodies and azurophil gran-
Fig. 1.—Single Auer rod in immature cell of monocytic series. The numerous cytoplasmic organelles, large nucleolus, and large number of ribosomes attest to the immaturity of the cell. Lipid accumulation, from incorporation of extracellular lipid by the cell, is present. Acute monomyelocytic leukemia after 48 hours in vitro tissue culture. The bars in the lower left hand corners represent 1.0 micron.
Fig. 2.—Multiple Auer bodies within the same cell. The Auer body at the right is round while the one at the upper left has an elongate diamond-shape. Numerous granules are visible in this cell. These granules correspond to the azurophilic granules seen in the light microscope after staining with Romanowsky dyes. Acute monomyelocytic leukemia after 48 hours in vitro tissue culture.
Fig. 3.—Monocytoid cell of acute leukemia after 48 hours in vitro tissue culture showing the massive development of hydrolytic enzyme granules (lysosomes) that occur with many leukemic cells in tissue culture. This case had no Auer bodies and was used as a comparison or “control” for the identification of Auer bodies. Compare with Figures 1 and 2. A myelin figure with its parallaling phospholipid membranes is seen. Compare this structure with the laminar membranes of the Auer bodies of Figures 7 and 10.
Fig. 4.—Early development of rod within globule associated with dense heterogeneous cytoplasmic granules. Acute monomyelocytic leukemia 48 hours after in vitro tissue culture.

ules. They found ribonucleic acid, acetyl-lipids, phospholipids, mucopolysaccharides, and oxidase and peroxidase enzymes in Auer bodies.

Electron microscopic studies of Auer bodies by Akasaka,† Freeman,‡ and Marchal, Bessis and Thiéry§ have revealed an internal laminar structure in the more mature rod-like structure consistent with known phospholipid mem-
Figs. 5–8.—Sequence of transition from cytoplasmic granules to rod-shaped Auer bodies. Large globules or vacuoles appear to develop from the granules (Fig. 5). Filamentous and granular densities develop within the vacuoles (Fig. 5). The content of the vacuole becomes more electron dense and paralleling filamentous membranes apparently form the rod-shaped structure (Figs. 6 and 7). As the rods become more mature, the laminar structure is not as evident (Fig. 8). Acute monomyelocytic leukemia 48 hours in vitro tissue culture.

brane structure in electron microscopy. Thus histochemical and electron microscopic analyses are compatible in this regard. The structures were found to be negative for lipase, glycogen, and deoxyribose nucleic acid. Recently, acid and alkaline phosphatase activities have been demonstrated in Auer bodies by Goldberg. Wetzel, Horn and Spieer, and Ackerman have pointed out the positive acid phosphatase activities of azurophil granules (in neutrophils) while obtaining negative results on specific neutrophilic granules. Thus, the latter 2 studies have shown similar enzymatic contents of Auer bodies and azurophil granules.
Fig. 9.—Auer body developing in intimate association with dense cytoplasmic granules. The boxed portion is shown at higher magnification in Figure 10. Acute myelomonocytic leukemia 48 hours in vitro tissue culture.

Fig. 10.—A laminar structure, morphologically consistent with phospholipid membrane structure, can be seen in developing Auer bodies. Compare the membranes with the myelin figure of Figure 3.

Locquin and Bessis have demonstrated the nucleoprotein nature of Auer bodies as well as Charcot crystals. Ackerman noted parallel development of the Auer bodies with change from the usual acid pH of the cell granules. He theorized that Auer bodies arose by fusion of azurophil granules when acid pH altered normal granule development. Although Freeman in 1960 described the Auer body as a coacervate of laminated homogeneous crystalline plaques with long axes of the plaques in the same plane as the long axes of the rods, Akasaka, whose findings were unknown to Freeman, had previously reported this observation in 1959. Demonstration of the marked similarity in fine structure of Auer bodies and cytoplasmic granules led Freeman to further support Ackerman’s suggestion that Auer bodies arose from fusion of granules. Furthermore, fused granular masses could be identified in close proximity to Auer bodies in Freeman’s electron micrographs. Although no certain morphologic transition from granules to Auer bodies was clearly demonstrable in Freeman’s earlier work, the consistent similarities in fine structural organization suggested such a circumstance.

By propagation of immature leukemic cell in in vitro tissue culture in this study, it was possible to make static observations by electron microscopy of the dynamics of Auer body formation. It is not implied, however, that Auer body formation was stimulated by the tissue culture technics. There is no indication that the number of Auer bodies per cell or the absolute number of Auer bodies
was increased significantly by the in vitro tissue culture technic. It is more likely that this experimental approach promoted a longer intracellular existence, or slowed the dynamics of formation, of the Auer body to such a point that the structure persisted in the cell long enough to permit the observations made herein.

Auer bodies have been shown to contain oxidases and peroxidases, enzymes commonly found in mitochondria. Nevertheless, there is no morphologic evidence to suggest that the granules from which the Auer bodies develop originate as mitochondria. One cannot exclude totally the remote possibility that the granules originate as altered cellular structure combined with foreign material, such as foreign ribonucleoprotein. Clearly it was recognized that the Auer bodies, as theorized by Nakashima and later by Ackerman, formed from the granules within the cytoplasm of immature leukocytes. That the granules seen in electron microscopy are the same azurophilic granules as defined by Romanowsky dyes in light microscopy, is suggested by the size, position, shape and number of granules in the cells. Certainly the size is such that these granules should be visible with the light microscope. Since the fine structure of the known cytoplasmic organelles, such as mitochondria, centrioles and the Golgi complex, is characteristic, there is no question of confusion of these organelles with azurophilic granules. Therefore, the granules as described in this paper represent azurophilic granules of light microscopy.

Although it is possible that the large vacuole or globular extrusions found in continuity with forming Auer bodies could represent degenerative rather than formative phenomena, this is most improbable. Other cytoplasmic organelles and structures, even those nearest lysosomal granules, were unaltered morphologically, contrary to what would be expected if a degenerative process had occurred.

The transition of granules to Auer rods reported by Nakashima in 1924 is supported by this investigation, but the reason for, or the stimulus promoting, this transition is unclear. Ackerman suggested that change in acid pH of immature leukocytes promoted an alternate pathway of granule maturation, namely transition into Auer bodies instead of normal maturation to form mature azurophilic granules. Such a study as the present one cannot establish the validity of this hypothesis. However, it should be noted that with propagation of cells in vitro the pH of the medium gradually was made alkaline, as judged by the color change of the medium from yellow to orange-red with phenol red indicator. It is not inconceivable that the pH of the granules was altered, secondary to that of the cytoplasm, toward a more basic pH.

Since the introduction of the concept of the lysosomes by deDuve, it is now generally accepted that membrane-bound sacs of enzymes, called lysosomes, occur in many cell types. The specific granules of neutrophilic and eosinophilic granulocytes have been accepted as lysosomes, according to their enzymatic content. Since lysosomes are heterogeneous, usually electron-dense groups of structures with no constant internal structure, it is necessary to utilize histochemical techniques in electron microscopy for their definitive identification. However, presumptive identification can be made on the basis of
electron-dense, heterogeneous, membrane-limited groups of cytoplasmic granules in cells known to have lytic abilities. Inasmuch as the azurophilic granules described in this study correspond to this description, it is probable that they are lysosomes. Moreover, Goldberg has demonstrated recently alkaline and acid phosphatase activities, hydrolytic enzymes commonly associated with lysosomes, in Auer bodies.22 Further chemical and histochemical analyses, especially analyses for cathepsin, glucosidase, sulphatases, glucosamine and other hydrolytic enzymes, are necessary before it can be established with utmost certainty that the granules belong to the class of cytoplasmic particles currently identified as lysosomes. However, on the basis of currently available studies, it is most probable that Auer bodies develop from azurophilic granules which are lysosomal in origin; hence, Auer bodies are lysosomal in nature.

SUMMARY

Evidence accrued by electron microscopic study of in vitro tissue-cultured leukemic cells supports Ackerman’s histochemical evidence that Auer bodies are formed from azurophilic granules, a circumstance first postulated by Nakashima in 1924. The enzymatic content and structure of these granules conforms to that of lysosomes, according to deDuve’s concept. Therefore the Auer body actually represents an unusual form, or abnormal development, of lysosomes.

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