Crystals of Protein Nature in the Cytoplasm of Lymphatic Cells in a Case of Lymphoreticular Malignancy

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C R Y S T A L S occurring in the cytoplasm of cells have been described by several authors.1-10 The most commonly known are protein crystals found occasionally in plasma cells and in their malignant counterparts, plasmocytoma cells.2,5,6,8,10 These crystals, which are found relatively rarely, seem to be composed of proteins with an admixture of some polysaccharide. They have been regarded as the crystalline form of material in the hyaline droplets or the so-called Russell bodies, and can occur coincidently in the same cells. Crystalline structures in plasma cells and plasmocytoma cells differ from Auer rods, since the latter are crystalloids and seem to be composed of nucleoprotein. On the strength of their staining properties, however, plasma cell crystals and Russell bodies seem to be related to the structures found by Goldberg in the so-called crystal cells.7,8

Electron microscopy of plasma cells crystals was done by Wellensieck4 and Thiéry.5 Both found a regular, fine linear arrangement with a spacing of about 100 A, suggestive of crystal formation. A similar arrangement was described by Bessis10 in crystals of plasmocytoma cells and in extracellularly occurring crystals in the plasma of a patient with cryoglobulinemia. Bessis also referred to a case of leukemia which was originally described by Bernard et al.9 The latter found crystal-like structures in the cytoplasm of lymphoid cells. The fine structure of these crystal-like structures was not suggestive of a crystalline arrangement, however. The staining methods used did not reveal the chemical nature of the structures.

It is the purpose of this paper to present some morphologic and chemical data of crystals similar to those described by Bernard and by Bessis. These structures were found to occur in the cytoplasm of lymphoid cells in bone marrow, lymph nodes, pleural exudate and occasionally in the peripheral blood of a patient with the tentative diagnosis of chronic lymphocytic leukemia.

C L I N I C A L D A T A

A white woman (Patient S.) was hospitalized at the age of 80 years in December 1961. Her chief complaints were: shortness of breath, anorexia, a sensation of heaviness in the epigastric region, belching and fatigue. She had had these complaints for about 2 years; more specifically, bone pains were absent.

Physical examination: An emaciated old woman with slightly elevated systolic blood pressure (170/80). There was no anemia. On percussion a bilateral pleural effusion was found. There was general enlargement of the lymph nodes. Liver and spleen were not palpable. No bone tenderness was demonstrated on percussion.

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Laboratory examination: Urinalysis and blood serology normal. Hematologic examination: Hgb. 13.2 per cent; RBC 4.46 million ml.; reticulocytes 1.7 per cent; platelets 232,000; WBC 73/00 ml.

Differential count: Eosinophils 1; stabs 1; segmented neutrophils 37; lymphocytes 58; monocytes 3; lymphocytes with crystal inclusions were found sporadically.

Bone marrow smear: Extensive proliferation of lymphoid cells with numerous intracytoplasmic crystalline inclusions (about one in every three cells, many cells containing more than one crystal). Crystals were also found in the cytoplasm of similar cells in the smears of the cervical and axillary lymph nodes. For a more detailed description of the crystals, see Morphology.

Serum chemistry: Sodium 144 mEq/L.; chloride 93 mEq/L.; potassium 5.2 mEq/L.; urea 42 mg. per 100 ml.; uric acid 0.19 mg. per 100 ml.; calcium 10.2 per 100 ml.; phosphorus 3.5 mg. per 100 ml.; alkaline phosphatase 2.2 mM. U. (Bessis); bilirubin total 0.8 mg. per 100 ml., 1 min. 0.1 mg. per 100 ml.; thymol turbidity 1 U. (McMaglan); cholesterol 151 mg. per 100 ml.

Paper electrophoresis: Albumin 3.36 Gm. per 100 ml.; α1 globulin 0.4 Gm. per 100 ml.; α2 globulin 0.9 Gm. per 100 ml.; β globulin 0.92 Gm. per 100 ml.; γ globulin 0.86 Gm. per 100 ml. Total 6.44 Gm. per 100 ml. (fig. 1A).

Pleura exudate: Culture sterile. Protein 3 Gm. per cent. Paper electrophoresis pattern essentially the same as that of serum (fig. 1B). Immuno electrophoresis of serum and of homogenized lymph node material in saline did not indicate the presence of paraprotein, macroglobulin or cryoglobulin.


Morphology

Tissue preparation procedures were:
1) Fresh material from lymph nodes was fixed in 4 per cent formalin. After dehydration in alcohol, the material was embedded in paraffin. Sections were stained with: a) hematoxylin-cosin; b) P.A.S. for the demonstration of polysaccharide; c) acrolein-Schiff for the demonstration of proteins, according to a method indicated by van Duyn;'1 d) Feulgen stain for the demonstration of DNA.

2) Formalin fixed frozen sections were stained with Sudan III.

3) Dried smear preparations from lymph node and bone marrow were stained in: a) May-Gruenwald-Gieinsa; b) P.A.S.; c) acrolein-Schiff (van Duyn11); d) Feulgen stain.

4) In addition, smear preparations were put in distilled water for one-half hour and then stained with acrolein-Schiff (van Duyn11).

4) For electron microscopy, tissue blocks of 1 mm.3 of bone marrow and lymph nodes were fixed in cold (4 C.), isotonic OsO4 solution buffered at pH 7.2 and sucrose was added to a final concentration of 4 per cent. After fixation for 1 hour, the blocks were
dehydrated in alcohol and embedded in methacrylate. Sections were collected on carboncoated grids. Some of the sections were stained in lead hydroxide. In addition, half thin sections of OsO₄ fixed, methacrylate-embedded material were stained with: a) P.A.S.; b) Feulgen; c) Trevan for the demonstration of DNA and RNA.

5) Fresh bone marrow material was sectioned in a cryostat at -20 C. Sections were incubated in several media in order to determine any activity of the following enzymes: ATP-ase, alkaline phosphatase, acid phosphatase, 5' nucleotidase, amino peptidase, esterase, and oxydase. The methods used for the demonstration of these enzymes were essentially the same as those described by Planteydt et al. For the demonstration of DPN H and TPN H the methods described by Nachlas and Seligman were used.

6) Smear preparations, paraffin sections and cryostat sections were studied in polarized light for possible birefringence.

RESULTS

1) Smear preparations: In both marrow and lymph node smears, one cell type predominated. This was a relatively large cell with a well-developed nucleus. The cytoplasm of these cells was clearly basophilic and slightly granular. No azurophilic structures were found in the cytoplasm. In a fairly high proportion of the cells, 5–10 μ long rods with a diameter of about 1–2 μ were found (fig. 3). Most of the rods had a more or less rectangular form, ending in a point on either one or both ends. The rods were not azurophilic. They sometimes seemed to lie across the nuclear mass. Some were found lying outside the cells, possibly as a result of rupture of the cell membrane with displacement of cytoplasmic material during the preparation of the smear. The rods took no stain except for the acrolein-Schiff (van Duyn) stain, which varied from pink to a brilliant magenta, indicating the presence
Fig. 3.—Micrograph of a May-Gruenwald-Giemsa stained smear preparation of a cervical lymph node. Several unstained intracellular crystals are visible (arrow). Magnification x 1200.

Fig. 4.—Micrograph of an acrolein-Schiff stained smear preparation of a cervical lymph node. The crystals are stained (arrow). Magnification x 1200.

Figs. 5 & 6.—Micrograph of intracytoplasmic crystals. In fig. 6 the membrane surrounding the crystal is continuous with the endoplasmic reticulum. Magnification x 56,000.
of proteins (fig. 4). When the smears were washed in distilled water for one-half hour prior to staining with acrolein-Schiff, no staining was found, however. This seems to indicate that the material in the structures is to some extent water-soluble.

2) **Paraffin embedded material of a cervical lymph node**: Microscopically the structure of the lymph node studied showed a marked deviation from the normal. Practically no follicles or germinal centers were found (fig. 2). In this material too, the cell picture was distinctly dominated by a relatively large cell with a more or less hyperchromatic nucleus. Although in some of the nuclei an indication of a disposition of chromatin clumps along the inner side of the nuclear membrane was seen, such as is generally found in plasma cell nuclei, the overall picture was that of more or less immature lymphatic cells. The cells showed a high nucleo-cytoplasmic ratio; most cells showed only a very narrow rim of cytoplasm. Mitotic figures were sporadically found. The dominant cell type was distributed diffusely throughout the lymph node, interspersed with elements of the reticuloendothelial system, a few typical plasma cells and neutrophils. Some infiltration of marginal and marrow sinuses was seen. None of the staining methods used revealed the presence of crystals. This finding contrasted sharply with the findings in the smear preparations.

3) **Frozen sections of formalin-fixed material** did not reveal the presence of intracellular sudanophilic material.

4) **Electron microscopy**: Electron microscopic examination of OsO₄-fixed material of both bone marrow and lymph node revealed that here, too, the overall picture was dominated by one cell type with lymphatic characteristics. The cells had a high nucleo-cytoplasmic ratio. Nucleoli were present. In many instances the nuclei showed an indentation. In the cytoplasm there occurred few mitochondria, a moderately developed endoplasmic reticulum, and other cytoplasmic constituents such as a relatively large number of multivesicular bodies. The endoplasmic reticulum development in these cells was much lower than that generally found in plasma cells.

Besides the structures mentioned, crystals were seen in the cytoplasm of sections of about one in every ten cells. As a result of differences of sectioning angles, the crystal-sections showed considerable variety in their geometrical forms. It appeared that the geometrical forms of the crystals found in the sections fitted with the concept of a rectangular bar. However, occasionally crystal sections did not fit with this concept (figs. 5 and 7). In such cases, some of the crystals seemed to consist of a rectangular bar, having a square pyramid with its base apposed to either one or both ends of the bar (fig. 10).

When stained with lead hydroxide, fine structural details became visible inside the crystal. In some, a regular arrangement of thin lines running parallel to two opposed surfaces of the crystal was found (fig. 8). In many instances an orientation of lines in more than one direction was evident, as a result of which a mosaic pattern (fig. 9) had formed. This pattern, with a regular 100 Å arrangement in more than one direction, seemed to be in accordance with the concept of crystal formation. The latter pattern is illustrated in figure 9. Differences from crystal to crystal with respect to the
Figs. 7–9.—Electron micrographs of intracytoplasmic crystals. In the upper part of fig. 7 the crystal is ending in a point. Figs. 8 and 9 show a regular periodicity in one (fig. 8) and in more than one (fig. 9) directions. Magnifications: fig. 7, x 56,000; figs. 8 & 9, 112,000.
occurrence of one or more directions of the lines may be explained by differences in the angles at which the planes consisting of crystal components were hit by the electron beam.

In some of the cells, crystals were observed within infoldings of the nuclear membrane, but never inside the nucleus as might appear from the light microscopy of smear preparations. Electron microscopy revealed few crystals occurring outside the cytoplasm.

5) In the crystals, the reactions with the enzymes listed above (see tissue preparation procedures under Morphology) were all negative. This was noteworthy with respect to the acid phosphatase reaction, since if this reaction had been positive, it might have given an indication that the material in the crystals could possibly be regarded as stored material from outside the cells.16,17

6) Paraffin and cryostat sections as well as smear preparations were all studied in polarized light for possible birefringence of the crystals. No such birefringence was found, however.

**Discussion**

Clinically and morphologically, this case showed the characteristics of a lymphoreticular malignancy. We must therefore consider the following possibilities:

1) Lymphocytic leukemia, 2) lymphosarcoma, 3) plasmacytoma, 4) Waldenström's macroglobulinemia. Paper electrophoresis of blood serum and pleural exudate and immuno electrophoresis of serum and extract of homogenized lymph node material did not indicate the presence of paraprotein. Clinically, no Bence Jones protein was found in the urine, while light- and electron microscopy showed that the pathologic cells occurring in bone marrow and lymph node bore no resemblance to the typical cells of plasmacytoma or the cells occurring in Waldenström's macroglobulinemia. These findings make a diagnosis of plasmacytoma or Waldenström's disease rather improbable. This left us with the possibility that we were dealing either with lymphocytic leukemia or with lymphosarcoma. The finding of diffusely distributed pathologic cells in the bone marrow, lymph nodes, pleural exudate
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and sporadically in the peripheral blood, together with the morphologic characteristics of the lymphoid cells, make the diagnosis of lymphosarcoma less probable. We are therefore inclined to diagnose this case as chronic aleukemic leukemia.

Histochemical studies indicated that rods occurring in the cytoplasm of the pathologic cells consisted of a water-soluble protein. Electron microscopy showed that they had a crystalline structure. They were found inside cisternae of the endoplasmic reticulum. Study of the fine structure of the crystals revealed a regular 100 Å periodicity and because of this they resembled the crystals that are occasionally found in plasma cells and plasmocytoma cells. However, P.A.S. positivity, indicating the presence of mucopolysaccharides, was absent. This helped to differentiate further the crystalline material in our case from the crystalline or non-crystalline material in plasma cells, plasmocytoma cells and the cells in Waldenström's macroglobulinemia.

Since lymphatic cells in our case which contained the protein crystals did not show an increased activity of acid phosphatase in comparison with other cells of the same type, we have no indication that the protein in the crystals was taken up by the cells, with subsequent storage in the cisternae of the endoplasmic reticulum. Quite to the contrary, all evidence pointed to a protein being secreted by the endoplasmic reticulum with subsequent crystallization in the cisternae.

SUMMARY

The occurrence of crystals in the cytoplasm of lymphatic cells in a case of lymphoreticular malignancy—probably lymphocytic leukemia—is reported. Histochemistry revealed the protein nature of these crystals. It appeared that the protein was water-soluble, with no admixture of polysaccharides. Electron microscopy of the crystals showed a fine structure of regularly arranged thin lines with a spacing of 100 Å. The crystals were surrounded by membranes of the endoplasmic reticulum.

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