Virus-like Particles in the Lymphocytes of a Patient with Chronic Lymphocytic Leukemia

By DOROTHEA ZUCKER-FRANKLIN

The viral etiology of avian leukemia has been recognized for more than 40 years while that of several types of murine leukemia has been established within the last decade. So far, little evidence has been forthcoming to suggest that similar agents may play a role in the human counterpart of the disease. Virus-like particles have been described in vacuoles within the cells of a few cases of acute human leukemia. However, such particles have never been seen directly in the cytoplasm of blood cells freshly removed from a human host afflicted with diseases of either neoplastic or infectious nature. For this reason, the following observations are reported.

Lymphocytes were isolated on three separate occasions from the peripheral blood of a patient with chronic lymphocytic leukemia. The specimens were obtained at monthly intervals during which the white cell count decreased from 240,000 to 8,000 without therapy. The diagnosis had been established about 20 years previously, and the patient received intermittent splenic radiation and Chlorambucil but was given no treatment during the 2 years preceding the collection of specimens. Within 20 minutes of their isolation, the lymphocytes were fixed in 1 per cent isotonic osmium tetroxide. They were dehydrated in increasing concentrations of alcohol and embedded in Epon 812. Thin sections were "stained" with uranyl acetate and/or lead hydroxide and examined in a Siemens Elmiskop 1 electron microscope. About half the cells exhibited some cytopathic changes consisting of clumped, unevenly distributed nuclear protein, an increase in nucleolar masses, and an increase in the number of small vacuoles and RNP particles in the cytoplasm. In 15–20 per cent of the cells, parallel rows of particles were observed in the cytoplasm (fig. 1a). Each row of particles was separated from the adjacent one by a membrane about 80 Å in diameter and several μ in length. The center rows of such particle aggregates were often indistinct or missing, suggesting a cylindrical or tube-like structure sectioned longitudinally. This impression was supported by the appearance of the aggregates in the cross-section illustrated in figure 1c. Here the particles are seen arranged in concentric circles. Each circle of particles was again separated from the next by a membrane. The central core consisted of a membrane-surrounded vacuole which, at times, also contained electron-dense particles of varying size. Filaments running at right angles to the long membranes separating individual...
Fig. 1—A. Longitudinal section of a cell aggregate. b. Cross-section of two particle aggregates. M—mitochondrion; P—particle aggregate; V—vacuole. Diagram of cell and reconstruction of particle aggregate. c.
particles were also resolved on occasion (see model fig. 1b). In addition to these organized arrays, particles have been observed lying singly or in groups dispersed throughout the cytoplasm. In this case they resembled RNP particles, though at high magnification “budding” from cytoplasmic filaments was suggested.

The exact structure of the particles has not yet been determined and awaits their isolation in purified form. They are most likely spherical with an irregular outline, and a diameter of 80 to 120 Å. It has not yet been possible to resolve the particle aggregates with the light microscope utilizing Wright’s and Giemsa, PAS, Sudan Black, or Feulgen stains. Phase microscopy of living or Epon-embedded sectioned cells also yielded negative results. Therefore, an estimate of the percentage of cells involved is difficult at this time. It is also too early to identify the particles with any known virus or to attribute to them an etiologic role in the patient’s lymphocytic leukemia. The longitudinal section of the particle aggregates resemble those published by Rifkind et al.\ of Echo 9 virus-infected tissue culture cells. The patient did not show clinical evidence of such an infection and viral agglutination studies, kindly performed by Dr. Millian at the New York City Board of Health, were negative. Tissue culture studies are still in progress.

The electron-microscopic appearance of the particle aggregates also bore no resemblance to the paracrystalline structures or “sticks” reported by Bernard et al.\ in the cells of a patient with long-standing lymphocytic leukemia. The authors suggested that the structures, which were fibrillar, represented an abnormal secretion of the cell. It is quite conceivable that the arrays of particles reported here could also be the product of abnormal cellular metabolism. However, intercurrent viral infections in patients with chronic lymphocytic leukemia are common, and the role of the lymphocyte in such infections is still obscure. It is noteworthy that the particles were not observed in any peripheral blood cells other than lymphocytes. Though the observation tempts to speculation, further interpretation is not warranted until the chemical and biological nature of the isolated particles has been unequivocally established.

SUMMARY

Lymphocytes from the peripheral blood of a patient with chronic lymphocytic leukemia were isolated and subjected to electron microscopy. Aggregates of virus-like particles measuring 80 to 120 Å in diameter were observed as organized structures in the cytoplasm of the cells. The true nature of the particles remains to be established.

SUMMARIO IN INTERLINGUA

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REFERENCES


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