EVIDENCE OF MURINE, VIRUS-INDUCED, PARAPROTEIN-PRODUCING LEUKÆMIA AND ITS RELATION TO OTHER VIRUS-INDUCED LEUKÆMIAS

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THE leukæmia-inducing viruses, described so far, have been recovered from tissues of leukæmic mice[1–4], from other tumours[5–8], or from hæmatopoietic tissue[9,10]. This article describes a recently developed leukæmia, induced by transplantation of hypertrophic, non-leukæmic mammary tissue. So far as I know, this is the first apparently virus-induced leukæmia, associated with paraproteinæmia, to be reported.

The mammary tissue (Fig. 1*a*) originated in a female virgin ($CBA \times DBA/2$) F_1 mouse, killed when moribund 29 months old. At the age of 2 months this mouse had been grafted—unsuccessfully—with leukæmic tissue from our transplantable plasma-cell leukæmia line 66 (ref. 11). Macro- and micro-scopic examination revealed hypertrophy of the mammary tissue, and heavy amyloidosis in most organs, but no leukæmia in any organ. Injection of minced mammary tissue subcutaneously into 5 mice induced a marked, generalized leukæmia, killing all 5 mice in 25–42 days. Inoculation of the leukæmic cells has resulted in the same leukæmia killing all mice grafted in 13–32 days. The leukæmia is an undifferentiated reticulum cell neoplasm (cells with large, irregularly shaped nuclei with rather low chromatin content and with homogeneous, slightly eosinophilic cytoplasm; estimated by Dr. H. E. Christensen, Laboratory for Rheumatic Research, University Institute of Pathological Anatomy, Copenhagen) (Fig. 1*b*). The karyotype of the cells in the first transfer passage showed 47 apparently normal chromosomes and no markers in 10 out of 10 cells examined by Dr. A. Frøland, University Institute of Human Genetics, Copenhagen. The serum protein pattern of all leukæmic mice shows a γ-paraprotein of very slow mobility (Fig. 2*c*). Numerous characteristic virus-like particles with a 'tail' (Fig. 3), similar to those described as typical for at least two leukæmogenic viruses[12,13], were observed by electronmicroscopy of negatively stained[14] preparations of plasma and spleen, concentrated by differential centrifugation[15]. (The electronmicrographs were prepared and photographed by Dr. F. Carlsen, University Institute of Biophysics, Copenhagen.) Four mice, injected intraperitoneally with viral extracts (prepared by differential centrifugation of leukæmic tissue) 32 and 12 days before subcutaneous inoculation of leukæmic cells, showed a survival-time of 30, 34, 38 and 38 days as compared with 21 and 22 days for the 4 controls, indicating the presence of a specific leukæmogenic virus in the 'immunizing' material. This conclusion is deduced from the earlier reports, that growth of leukæmic cells is inhibited by pretreatment with the corresponding leukæmogenic virus, but not with polyoma virus[16], which, on the other hand, inhibits the growth of polyoma tumours[17,18].

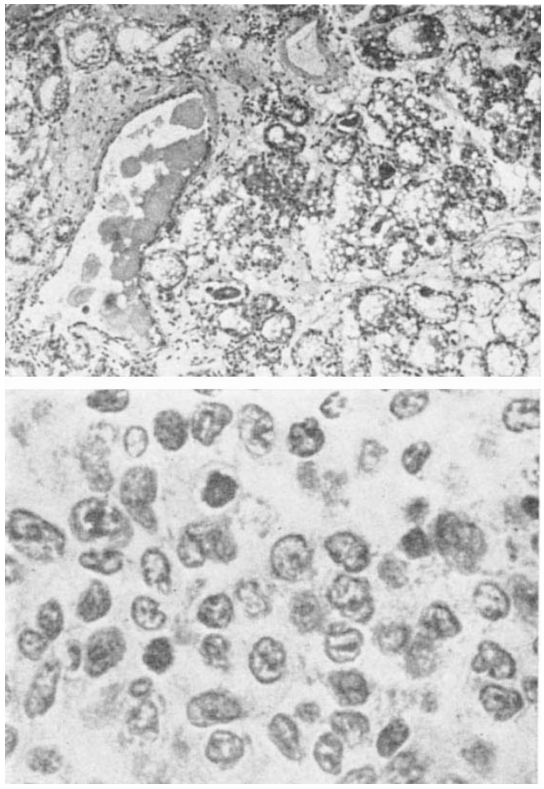
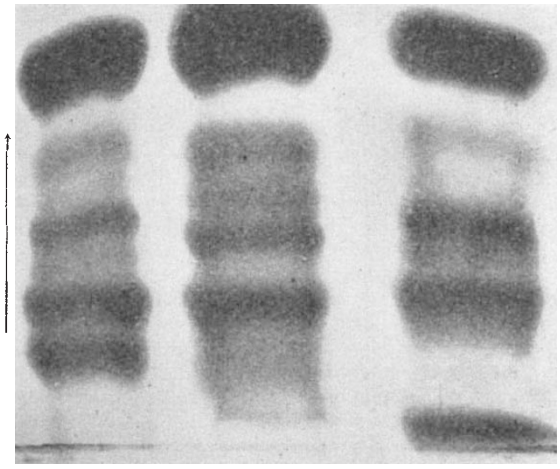


Fig. 1*a*, Mammary tissue used for transplantation. Hypertrophic glands and dilated ducts filled with secreted material are seen. (Hæmatoxylin and eosin, ×100); *b*, Cells of leukæmia induced by transplantation of mammary tissue seen in *a*, that is large polygoneal reticular cells with irregularly shaped nuclei. (Hæmatoxylin and eosin, × 900)



a *b* *c*

Fig. 2. Electrophoreses on cellulose-acetate strips. *a*, Serum from mouse of original line 66 with abnormal fraction in fast γ-region; *b*, serum from normal mouse; *c*, serum from mouse with leukæmia induced by transplantation of mammary tissue, shown in Fig. 1*a*. Abnormal fraction in very slow γ-region is seen

The fact that inoculation of non-leukæmic tissue resulted in a leukæmia, which furthermore is different in morphology, karyotype and serum protein pattern from line 66 (*vide infra*) which originally was grafted into the primary host, combined with the observation of characteristic virus-like particles and with the inhibition of growth by pretreatment with leukæmic tissue extract, points definitely to a viral ætiology of the leukæmia.

The origin of the leukæmia-inducing virus is unknown. Its introduction by the inoculated tissue from line 66 is

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suggested by the finding of typical virus-like particles in leukæmic mice of line 66 (*vide infra*) as well as from the following observation indicative of the presence of a leukæmogenic virus in line 66.

The injection of cell-free medium from tissue culture of leukæmic spleen from line 66 (ref. 4) (grown by Dr. A. Fjelde, University Institute of Genetics, Lund) after a latent period of 8 months gave rise to a generalized leukæmia in one out of 6 mice (spontaneous leukæmias were not observed in $(CBA \times DBA/2)$ F_1 hybrids until they were 18 months old or more[19]). This leukæmia was transplanted and has killed all mice inoculated during 22–59 days. Morphologically it is a slightly plasmoblastic or lymphoblastic reticulum cell neoplasm (polymorphous cells with the chromatin located beneath the strong nuclear membrane and with slightly basophilic cytoplasm) (Dr. H. E. Christensen). The karyotype of these cells in the first transfer passage was determined (Dr. A. Frøland) as 8 cells having 41, 2 cells having 42 chromosomes; no markers were present. The serum protein pattern shows no abnormal fractions (cf. Fig. 2*b*). Characteristic virus-like particles with a typical 'tail' have been found in negatively stained preparations of plasma and spleen (F. Carlsen).

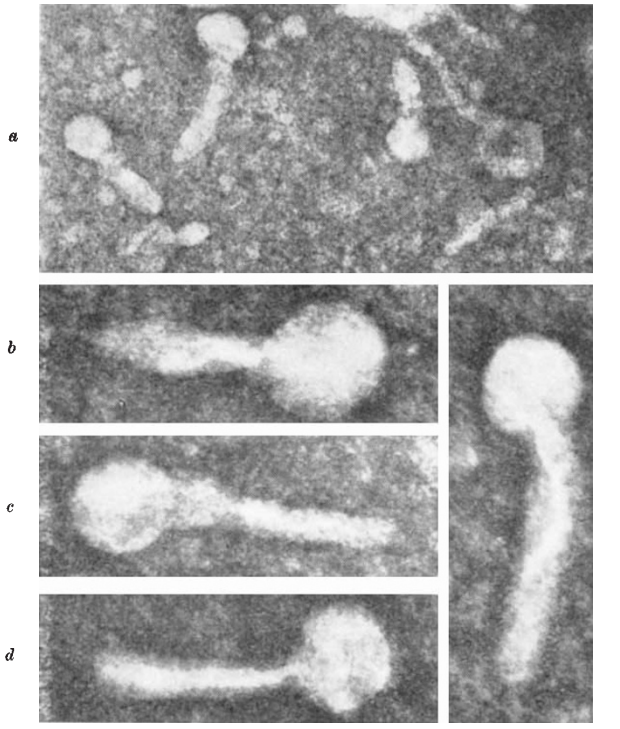

Fig. 3. *a*, Virus-like particles in electronmicrograph of negatively stained preparation of plasma from mice with leukæmia induced by transplantation of mammary tissue. (× *c.* 60,000.) *b–e*, Single virus particles in same preparation. (× *c.* 130,000)

This line of leukæmia differs from that induced by injection of the mammary tissue, and also from the original line 66 (ref. 11). The line 66 leukæmic tissue consists of small, round cells with chromatin-rich nuclei and with scanty basophilic cytoplasm intermingled with a varying number of large, polygonal cells with ample eosinophilic cytoplasm. These leukæmic cells were found[20] in 5 passages to exhibit a karyotype showing 42 chromosomes, of which two were distinct, big markers. The serum protein pattern shows an abnormal fraction in the fast gamma region (Fig. 2*a*). Virus-like particles have been seen in negatively stained preparations of plasma and spleen (F. Carlsen).

The findings reported here give supplementary information to previous observations on murine virus-induced leukæmias in showing the presence of a leukæmia-inducing agent in non-leukæmic, mammary tissue, resulting in the induction of a leukæmia associated with paraproteinæmia. The leukæmia produced by inoculation of tissue culture medium was obviously induced by an agent transferred from line 66. Most likely the leukæmogenic agent in the mammary tissue was transferred by the inoculated leukæmic tissue from line 66. If this assumption were accepted, the findings would indicate that different cell-free transmissions may result in leukæmias differing between each other in morphology and karyotype of the cells and in serum protein patterns, and differing also in these respects from the line of leukæmia, providing the ætiological agent. In conformity with these findings is the observed, though not conclusive, difference in cell morphology between individual leukæmias induced by the Moloney virus[21].

Experiments on cell-free transmission of the leukæmias mentioned are in progress.

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GIBBERELLIN-LIKE SUBSTANCES IN ALGAE

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GIBBERELLIN-LIKE substances have been reported to occur in several species of algae: mixed marine phytoplankton (unicellular algae)[1,2], two species of *Fucus*, *F. spiralis*[1,2] and *F. vesiculosus*[3], and a unialgal culture of a unicellular green alga, *Tetraselmis* (*Platymonas*[1,2,4]).

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Kato *et al.*[5] were unable to demonstrate any activity in extracts of *Chlamydomonas reinhardi*, which is closely related to *Tetraselmis*, and in *Macrocystis pyrifera*, which is a brown alga.

In my early work, paper chromatography was carried out using water as solvent; gibberellin-like biological