High-Frequency Induction in vivo of Mouse Leukemia in AKR Strain by 5-Azacytidine and 5-Iodo-2′-deoxyuridine

It has been established for a long time that different carcinogenic stimuli or aging may results in the development of mouse leukemia[1]. More recently LOWY et al.[2] reported that exposure in vitro to 5-bromo- or 5-iodo-2′-deoxyuridine of AKR mouse embryonic cells induced the synthesis of virus particles in up to 0.5% of these cells, although ordinarily they contain little or no leukemia virus. Similarly, in the low susceptible strain BALB/c using mouse embryo cells in vitro, it has been shown that 5-bromo-2′-deoxyuridine induces different cell lines to form C-type virus[3]. The line of human lymphoblasts that did not show expression of Epstein-Barr herpesvirus, was made resistant to 5-bromo-2′-deoxyuridine. Following removal of the drug, virus particles were detected in the cells with the implication that 5-bromo-2′-deoxyuridine activated the repressed Epstein-Barr virus genome[4]. These and other similar experiments[5] are in accordance with the existence of the proposed oncogene, which is presumably in the DNA of all eukaryotic cells and is responsible for the conversion of a normal cell into a neoplastic one[6].

We now describe the effect of 5-iodo-2′-deoxyuridine[7] and 5-azacytidine in vivo, whereby an early induction of leukemia is demonstrated in the highly susceptible strain of inbred AKR female mice. Groups of animals received i.p. 5-iodo-2′-deoxyuridine[7] (LD_{50} 2.5 g/kg) or 5-azacytidine[8] (LD_{50} 115 mg/kg). Before and during the course of treatment, leukocytes were enumerated and differential counts registered. The dead mice were inspected for the macroscopic signs of leukemia.

Whereas the blood and differential counts in the controls did not change during the period of observation, the treated mice were leukopenic after 6 weeks of treatment. However, later on mild leukocytosis occurred (Figure 1). At that time occasional leukemic lymphoblasts and reticular cells were observed in the peripheral blood; segmented polymorphonuclears rose in number (50 to 60%). The time of observation was 120 days. During this period all control, untreated mice (40 animals) survived in good condition without any sign of the disease. The animals given 5-iodo-2′-deoxyuridine developed leukemia after 50 days of treatment, and during the next 10 days 67% of them succumbed with fully developed lymphomas (Figure 2). The administration of 5-azacytidine enhanced the incidence of leukemic deaths to 100% during the same time interval (Figure 2). The lymphoblastic infiltration in the livers of 5-iodo-2′-deoxyuridine- and 5-azacytidine-induced leukemias was 70 to 80%; the involvement of the thymus, the spleen and the lymph nodes was relatively inconspicuous. Both newly induced leukemic lines were transplantable, and 100% takes were observed in the mice of the same strain that received i.p. injections of leukemic liver cell suspensions.

The incidence of spontaneous AKR leukemia in female mice varies from 71 to 85%, begins approximately at 4 months of age, reaching its peak at 7 to 9 months, and then again declining[9]. Hematological changes set in only during the terminal stage of disease consisting in leukocytosis and in the appearance of an increased number of segmented neutrophils in the peripheral blood[10]. Thus the fact that 5-azacytidine accelerates the development of AKR leukemia in 4-month-old mice with the incidence of 100% is highly significant.

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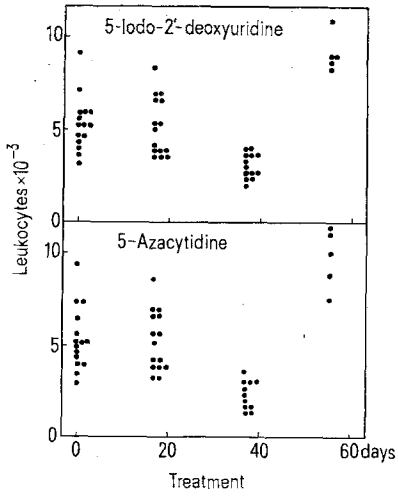

Fig. 1. White blood counts (WBC) in AKR mice treated with 5-iodo-2′-deoxyuridine and 5-azacytidine. For the schedule of the treatment and for the dosage see Figure 2. Each point represents WBC per mm³ from 1 mouse.

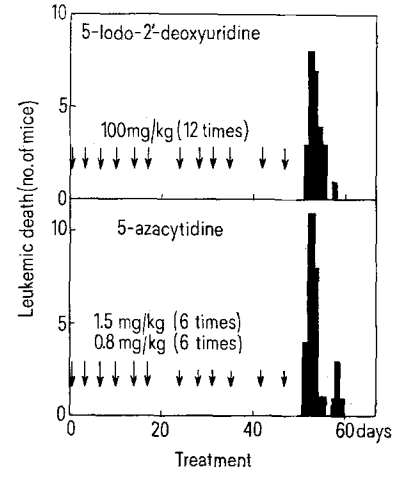

Fig. 2. Life-span of 2-month-old AKR female mice treated i.p. with 5-iodo-2′-deoxyuridine or 5-azacytidine. Each group included 40 female AKR mice (23–24 g). The dosage of 5-azacytidine was lowered after 16 days, since toxic manifestations occurred.

5-Azacytidine is incorporated in vivo into RNA of mammalian cells[11], in distinction to 5-iodo-2′-deoxyuridine[7] which is taken up into DNA. It suppresses the growth of L1210 and of AKR leukemia[12,8] and inhibits the synthesis of antibodies[13]. Accordingly, the possibility must be considered that AKR leukemia could have been evoked on account of the immunosuppressive effects of 5-azacytidine by the impairment of the synthesis of corresponding antibodies. However, the immunosuppressive effect of 5-azacytidine is inferior to that of cyclophosphamide[13] which has failed to activate the virus synthesis in vitro[14]. Similar negative results in tissue culture were obtained also with cytosine arabinoside, 5-fluoro-2′-deoxyuridine, 6-azathymine, 6-azauridine, 6-mercaptopurine and 6-azaguanine[14]. It is clear that leukemia virus is present in tissues of AKR mice from the time of birth[15]. It is difficult to conclude at present whether the chemical treatment described is analogous to the induction of virus synthesis in vitro. However, our preliminary studies of virus titers in respective mouse tissues indicate that virus is being induced by 5-azacytidine also in vivo.

Zusammenfassung. Nachweis der leukämogenen Wirkung von 5-Azacytidin und 5-Jod-2′-Desoxuridin beim Stamm der AKR-Mäuse. Es wird angenommen, dass es sich hierbei um eine Virusinduktion handelt.

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A Study of the Cytology and Cell Culture of the Canine Transmissible Venereal Sarcoma

Canine transmissible venereal sarcoma (CTVS) is a neoplasm occurring naturally on the genitals of both male and female dogs. It is the oldest transplantable tumor known and is readily transplanted by sexual contact and by parenteral injection of viable tumor cells. The etiology, histogenesis, and the mechanism of universal 'take' and spontaneous regression of the tumor are poorly understood[1–3]. Although there is no serious objection to the belief that the CTVS is truly neoplastic, it has been considered by some competent pathologists to be infectious granuloma[4]. Ultrastructural examination of the tumor cells by previous workers has not revealed distinctive features and the reports of the presence of virus-like particles have been controversial[5]. We now report that the CTVS cells behave like tumor cells in culture and that a distinctive ultrastructural feature is indeed present.

Materials and methods. Canine transmissible venereal sarcoma. The original tumor (kindly provided by Dr. G. Brown of Farragut Animal Hospital, Knoxville, Tennessee) from the vagina of a poodle of mixed ancestry was removed surgically and portions were processed immediately for cell culture, chromosome analysis, histopathology, and electron microscopic studies. Other portions of the tumor were kept in Medium 199 overnight before being transplanted into dogs. Single cell suspensions and tumor pieces were prepared by mincing the tumor tissues with scissors without the use of trypsin. Non-preconditioned, apparently healthy, dogy of different ages were used for in vivo tumor passage. Either single cell suspensions or tumor pieces of (1–2 mm^3) suspended in serum-free Medium 199 were injected s.c. into the interscapular region. Chromosome, histopathology, and transplantation studies of the original and transplanted tumors confirmed that the tumor was indeed canine transmissible venereal sarcoma[1–7].

Electron microscopic studies. The original tumor was fixed in 3.5% glutaraldehyde and processed as previously described[8].

Cell culture. The tumor cells were cultured as monolayers in Medium 199 and Minimum Essential Medium (MEM) supplemented with 20% fetal calf serum or as suspensions in RPMI 1640 supplemented with 20% fetal calf serum. Final cell concentration of 1×10^6 and 2×10^6 viable cells per ml, as determined by trypan blue dye exclusion test, were used for initiation of the cultures. Trypsinization was done at the time of subculturing monolayer cell cultures.

Results and disscussion. Electron microscopic studies showed that large round or polygonal cells were most predominant in the original donor tumor. The nuclei were large, vesicular and with prominent nucleoli. Mitochondria, smooth endoplasmic reticulum and ribosome granules were sparse but the Golgi apparatus was relatively well developed and there were many small cytosomes in the cytoplasm. Mitotic figures were frequently found. In addition, cells resembling plasma cells, lymphocytes, and fibroblasts were mingled within the main tumor mass. Tumor cells with increased rough endoplasmic reticulum occasionally showed whorl like formation (Figure 1). To our knowledge, this structure had not been recorded by previous workers for this tumor. Similar, but not identical, concentric formations of rough endoplasmic reticulum have recently been observed in human osteosarcoma cells in culture[9], in the oocyte and folicular cells of the lizard *Lacerta sicula* during winter rest[10], and in the border cells of the adrenal glands of the Mongolian gerbil[11].

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