Herpeslike Virus Isolated from Spontaneously Degenerated Tissue Culture Derived from Leukemia-susceptible Guinea Pigs

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A herpeslike virus was consistently isolated from leukemia-susceptible strain 2 guinea pigs, but not from other strains known to be resistant to this leukemia.

Strain 2 guinea pigs (*Cavia cobaya*) have been of particular interest because of their unique susceptibility to a fulminant transmissible acute lymphoblastoid leukemia (L_2C) that arose spontaneously in strain 2 animals in the laboratories of Congdon and Lorenz (1). The leukemia (L_2C/NB) was continued as a stable line in strain 2 guinea pigs by Nadel at the National Cancer Institute and later in collaboration with Burstein at the Worcester Institute (5, 6). Strain 13 and Hartley strain guinea pigs were found to be resistant to the leukemia (3, 5). As part of a general study on latent virus infections in apparently healthy laboratory animals, kidney tissues from strain 2, strain 13, and Hartley strain guinea pigs were investigated. A herpeslike virus was obtained from tissue cultures derived from the strain 2 guinea pigs only, and the results of those studies are summarized in the present report.

Kidneys were removed from each guinea pig aseptically, and cell cultures were prepared. Islets of cell growth were observed 24 hr after planting (Fig. 1a-b), and complete cell sheets were obtained in 7 days (Fig. 1c-d). In a study of 17 strain 2 guinea pigs obtained from two separate sources, 15 showed cytopathic effects in their kidney cell cultures, manifested by focal areas of swollen rounded cells with intranuclear inclusions (Fig. 1e-f) characteristic of viral infection, 9 to 13 days after the cultures were prepared. On two occasions, virus was also isolated from cell cultures derived from spleen, liver, and lung tissue in addition to kidney, but the virus was not isolated at any time from homogenized tissue extracts. Virus could not be isolated from the kidney cells of the five strain 13 and the two Hartley strain guinea pigs which are known to be resistant to L_2C leukemia.

Electron microscopic examination of strain 2 guinea pig kidney tissue culture cells revealed

abundant virus particles with a morphology characteristic of the herpesvirus group (Fig. 2). Most of the virus particles were found in the nuclear matrix as free, single membrane-bound particles with or without electron-dense central cores. Closely packed double membrane-bound particles, with or without dense central cores, enclosed within vesicles were also seen within the nucleus. Occasionally, particles with double membranes were seen in the cytoplasm. The central core measured approximately 65 nm in diameter, the single membrane-bound particles averaged 115 nm in diameter, and the double membrane-bound particles were approximately 158 nm in diameter.

The virus also grew well in rabbit kidney cell cultures. However, monkey and hamster kidney cells as well as the chorioallantoic membrane of embryonated eggs appeared to be resistant to theavian herpesvirus infection. Serologically, the virus showed little, if any, relationship to the guinea pig cytomegalovirus described by Hartley et al. (2). The virus was not neutralized by antiserum to guinea pig cytomegalovirus as reported by Middlekamp et al. (4), although the guinea pig from which the herpesvirus was isolated did show low levels of neutralizing antibody to the virus isolated.

Since no virus was isolated from the kidney cultures of strain 13 and Hartley strain guinea pigs, but was consistently isolated from strain 2 guinea pigs, the relationship between the herpesvirus isolated and the sensitivity of strain 2 guinea pigs to L_2C/NB leukemia, as well as the virus-like particles observed in leukocytes of strain 2 leukemia guinea pigs (6, 7), is currently being investigated.

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FIG. 1. Growth of guinea pig kidney cell cultures and the appearance of cellular changes. Left panels, wet preparations, $\times 100$. Right panels, hematoxylin and eosin stained preparations, $\times 400$. (a, b) Freshly trypsinized kidney cells, 24 hr after planting. Note small islets of growth of kidney cells. (c, d) Confluent monolayers; 7 days after planting. (e) Focus of cellular degeneration; 10 days after planting. (f) Intranuclear inclusions; 10 days after planting.

FIG. 2. Virus particles in sections of primary kidney cell cultures of guinea pig LK33 10 days after planting. Note particles with dense and coreless centers enveloped by an inner membrane scattered in the nucleus. Particles enveloped by double membranes are seen in the vesicle. A single virus particle with dense central core and double membranes (arrow) is shown. N, nucleus. $\times 16,200$. Insert shows a single virus particle with dense central core and double membrane in the perinuclear region. $\times 97,200$.

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ADDENDUM IN PROOF

After this paper was submitted for publication, an additional study was made of 17 strain 2 and 8 Hartley guinea pigs. Herpesvirus was not isolated from any of the Hartley guinea pigs, but it was obtained from 16 of the strain 2 animals. Herpesvirus was also isolated from whole blood and buffy-coat prepa-

rations of 16 strain 2 animals, but not from the 12 serum samples tested.

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