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25 August 1982; revised 5 October 1982

Virus-Induced Alterations in Homeostasis: Alterations in Differentiated Functions of Infected Cells in vivo

Abstract. *The noncytopathic lymphocytic choriomeningitis virus displays a tropism for the anterior lobe of the murine pituitary gland. Virus replicates in cells that make growth hormone. This results in a diminished synthesis of growth hormone with a concomitant clinical picture of retarded growth and hypoglycemia. However, there is no morphologic evidence of either cell necrosis or inflammation in the anterior lobe of the pituitary. Hence, during infection in vivo, a noncytopathic virus may turn off the "differentiation" or "luxury" function of a cell while not killing that cell (loss of vital function). This in turn can disrupt homeostasis and cause disease. This model illustrates a novel way whereby viruses may cause disease.*

During the course of infection, viruses injure cells by two distinct mechanisms. The first is a lethal attack by toxic products of the viral genome or disruption of regulatory (vital) functions needed for the cells' survival. Thus, by virtue of its cytopathic properties, the virus itself directly destroys the cell (*1*). The second mechanism is indirect and occurs with noncytopathic as well as cytopathic viruses. Here, the virus produces antigens foreign to the host or alters host antigens on cell surfaces. Such cells, when recognized by the host's immune system, are killed (*2*). The end product of both mechanisms is similar and provides a well-recognized morphologic picture of cell destruction in vivo, usually accompanied by inflammatory cell infiltrate characteristic of virus infections (*2, 3*). In contrast, some viruses or variants that arise in vivo can cause a noncytopathic persistent infection (*4*). Studies in vitro indicate that such virus-infected cells can have normal morphologic appearance, growth, and cloning rates, but demonstrate dysfunctions in their differentiated (luxury) cell function (*5*).

The immediate goal of the study described here was to determine whether a virus could persist in vivo in cells with specialized or differentiated functions and by the process of infection alter these functions but not kill the cell. As we show here, a relatively noncytopathic virus, lymphocytic choriomeningitis virus (LCMV), can infect cells of the anterior lobe of the pituitary gland that normally make growth hormone (GH). Morphologically these infected cells appear normal. Further, the pituitary tissue offers no evidence of either cell lysis or an inflammatory infiltrate. Most important, the production of GH by these cells diminishes significantly, and the result is a marked impairment in growth of the infected mouse.

Newborn C3H/St mice were inoculated with 60 plaque-forming units (PFU) of LCMV Armstrong strain 1371 (*2, 5*) cloned three times (clone 3B). Control, matched litters were either inoculated with medium (virus diluent) or not inoculated. The mice were weighed when they were 3, 6, 16, and 20 to 21 days of age. [Most C3H/St mice (> 99 percent) die by 30 days of age (*6*). The cause of death is uncertain, but is believed to be metabolic; a histopathologic survey of cells from organs performing vital functions is essentially normal (*6*).] Randomly selected representatives of LCMV infected and control groups were killed when they were 3, 6, or 16 days old, and their pituitary glands were removed for immunohistochemical, virological, biochemical, and morphological studies.

To locate viral antigens and GH we used pituitary glands that had been quick-frozen in liquid nitrogen, cut into 4-μm sections, and fixed. We examined these sections by immunofluorescence techniques (*2, 5*). The viral antigens we sought were the three known structural polypeptides of LCMV: two glycoproteins that insert into the cell's surface and one nucleoprotein found only in the cytoplasm of infected cells (*7*). Reagents to identify these components were murine monoclonal antibodies to LCMV, its two glycoproteins, and the nucleoprotein (*8*); others were monospecific monkey antibody to murine GH (*9*), goat antibody to murine immunoglobulin G (IgG) conjugated to either fluorescein isothiocyanate or rhodamine, and rabbit antibody to human IgG conjugated to fluorescein isothiocyanate (*10*).

To identify GH-producing cells by light or electron microscopy, we used the pituitaries of mice that had been exsanguinated by cardiac puncture and perfused with 3 percent glutaraldehyde (*11*). In several instances, thin sections of the pituitaries on gold grids were reacted with monkey antibody to murine GH, and then with goat antibody to monkey IgG conjugated to 8 to 10 nm gold particles (*12*).

Figure 1 shows that LCMV antigens were localized in the cells of the pituitary gland's anterior lobe. It also shows the corresponding clinical picture of an undersized virus-infected mouse. All of the 25 virus-infected mice were undersized at 16 days. The mean weight of the infected mice (± standard deviation) was

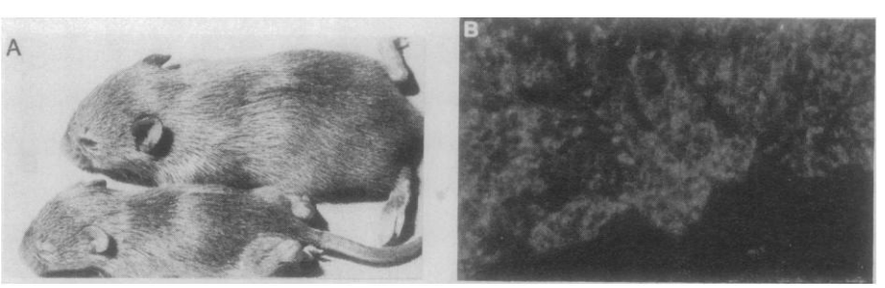

Fig. 1. (A) A 16-day-old C3H/St mouse persistently infected at birth with LCMV (bottom) and an uninfected littermate control (top). (B) Demonstration of viral antigen in the cells of the anterior pituitary of 16-day-old C3H/St mouse persistently infected with LCMV. The frozen section (4 μm) was reacted with monoclonal murine antibody to LCMV nucleoprotein and then with rhodamine-conjugated goat antibody to murine IgG. Cells of the middle and posterior lobes of the pituitary did not express viral antigens.

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Table 1. The growth of 6- and 16-day-old control mice and mice infected with LCMV at birth, and concentrations of GH in the pituitary gland. The data are expressed as means ± 1 standard deviation. *P* values are for the associated *t*-test ($N = 6$).

Age (days)	Infected LCMV	Growth		Concentration of GH	
		Weight (g)	*P* value	In μg/mg pituitary	*P* value
6	No	3.0 ± 0.5		4.8 ± 2.1	
6	Yes	2.7 ± 0.3	.08	5.8 ± 2.3	.4
16	No	7.4 ± 1.5		32.5 ± 6.4	
16	Yes	4.0 ± 1.0	<.001	19.9 ± 5.1	<.01

4.0 ± 1.0 g; that of the matched controls ($N = 30$) was 7.4 ± 1.5 [$t(43) = 9.1$, $P < .001$]. The viral antigens in the infected mice were restricted primarily to cells of the anterior lobe of the pituitary gland; these antigens were found, infrequently, in cells of the middle or posterior pituitary lobes of only one mouse of 25 mice studied. By means of the monoclonal antibodies to the LCMV polypeptides we found that at 16 days the anterior pituitary cells from only 4 of 25 infected mice expressed LCMV glycoprotein; in contrast, pituitary cells from all 25 mice expressed the viral nucleoprotein. For these studies we used either adjacent sections of the same cell or double staining by two fluorochrome dyes, a technique that enables one to study two different polypeptides in the same cell (*13*). In contrast, at days 3 or 6, cells from the anterior pituitaries of all five mice examined expressed all viral polypeptides. Hence, as the time of infection increased, there was an apparent restriction in synthesis of viral glycoprotein without impairment of nucleoprotein synthesis, a pattern noted in cells persistently infected with LCMV in vitro (*2*, *14*).

Using separate fluorochrome dyes to label either GH or viral nucleoprotein in the same cell, we noted that more than 85 percent of cells containing GH also contained viral antigen. This correlation was confirmed by electron microscopy and immunohistochemical techniques. The GH-producing cells contained replicating virus (Fig. 2). Despite abundant viral antigen expression in the anterior pituitary, neither anterior pituitary cell necrosis nor inflammation (indicated by the presence of lymphocytes, macrophages, host immunoglobulin, or the third component of complement) occurred. The lack of necrosis is probably related to the noncytopathic nature of the virus. The absence of inflammatory cells in the area of the anterior pituitary gland may be related to the decreased production of viral glycoprotein in anterior pituitary cells infected with LCMV, because viral glycoprotein must be expressed in sufficient quantity on the surfaces of infected cells for recognition and successful assault by the host's immune effector system (*2*).

By means of light and electron microscopy we found that cells of the anterior pituitary infected with LCMV still contained GH (Fig. 2C). We therefore measured the amount of GH in pituitary glands (*15*) from infected ($N = 6$) and control ($N = 6$) mice. At 16 days the pituitaries of LCMV-infected mice contained significantly less GH (19.9 ± 5.1 μg/mg) than the pituitaries of matched controls (32.5 ± 6.4 μg/mg) [$t(36) = 13.3$, $P < .01$]. At 6 days there was no significant difference between the infected and control mice in pituitary GH levels ($N = 6$) or the weights ($N = 20$) of the animals.

Growth hormone insufficiency may be accompanied by hypoglycemia (*16*). We therefore measured the concentrations of blood glucose, sodium, and potassium in 16 infected mice and 20 uninfected controls at 21 days of age. Both groups received a similar standard mouse diet. The infected mice ($N = 6$) had a mean blood glucose concentration (milligrams per 100 ml of blood) of only 73.6 ± 28.2, compared to a 170.8 ± 6.9 for controls ($N = 6$) (*t*-test, $P < .001$). Insulin-producing cells in the pancreas showed normal cytomorphology and there was no evidence of LCMV replication in such cells. Serum samples from virus-infected mice did not contain autoantibodies to either insulin-producing cells in the pancreas or GH-producing cells in the anterior lobe of the pituitary, suggesting that the hypoglycemia may be related, in part, to the dysfunction of the anterior lobe of the pituitary. Concentrations of sodium and potassium in the blood were similar in both groups.

These data show that the noncytopathic virus LCMV has a tropism for GH-producing cells of the anterior lobe within the murine pituitary. In vivo, the virus persisted in these cells without killing them and without attracting immune reactants. Thus, the host tissue for this virus appeared normal and provided no morphologic clues to the hidden but replicating virus within. However, these differentiated cells showed a functional disorder that was reflected in their inability to produce a normal amount of GH. The mechanism whereby a virus shuts down the differentiated function of a cell without turning off its vital (life) function is unknown, but there are at least three possibilities: (i) Synthesis of GH is but a small fraction of a cell's total synthetic output, and partial suppression of total cell synthetic capacity may impair differentiated function without affecting vital function. (ii) The virus may induce a specific alteration in an enzyme or transport system needed for the production and release of GH. (iii) A cell's capacity for membrane translocation and synthesis may be limited so that production of viral gene products competes with manufacture of GH, thus overloading the system.

Our finding that a noncytopathic virus can cause endocrine dysfunction adds to the recent evidence that viruses contribute to the etiology of endocrine diseases (*17*). However, the previous reports (*17*) contrast with our findings reported here in that the diseases were associated with

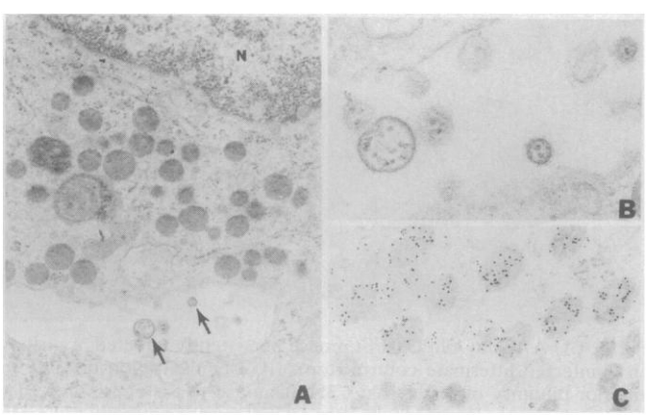

Fig. 2. (A) Electron micrograph of a GH-producing cell in the anterior pituitary lobe of a 16-day-old C3H/St mouse persistently infected with LCMV. Arrows point to LCMV; *N*, nucleus (×35,000). Panel (B) Enlargement of viral particle shown in (A) (×94,000). (C) Growth hormone in vesicles of cells in the anterior pituitary lobe of a 16-day-old C3H/St mouse persistently infected with LCMV. The section was reacted with monkey antibody to mouse GH and then in the goat antibody to monkey IgG conjugated to gold particles (×35,000).

lytic viruses uniquely tropic for endocrine-producing cells and may have also involved autoantibodies against hormone-producing cells.

If it is found that viruses can similarly impair other differentiated or specialized functions of cells that constitute the human immune, endocrine, nervous, and other systems, our findings could have important implications concerning several diseases of man currently of unknown origin.

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9 July 1982; revised 21 September 1982

Fast Axonal Transport in Squid Giant Axon

Abstract. *Video-enhanced contrast–differential interference contrast microscopy has revealed new features of axonal transport in the giant axon of the squid, where no movement had been detected previously by conventional microscopy. The newly discovered dominant feature is vast numbers of "submicroscopic" particles, probably 30- to 50-nanometer vesicles and other tubulovesicular elements, moving parallel to linear elements, primarily in the orthograde direction but also in a retrograde direction, at a range of steady velocities up to ±5 micrometers per second. Medium (0.2 to 0.6 micrometer) and large (0.8 micrometer) particles move more slowly and more intermittently with a tendency at times to exhibit elastic recoil. The behavior of the smallest particles and the larger particles during actual translocation suggests that the fundamental processes in the mechanisms of organelle movement in axonal transport are not saltatory but continuous.*

Processes of fundamental functional importance in the nervous system include the action potential, synaptic transmission, and axonal transport. The last process conveys substances, organelles, and tubulovesicular structures in both directions, toward the synapse (orthograde direction) and toward the cell body (retrograde direction).

Large membranous organelles in neurites have been reported to exhibit bidirectional saltatory motion (*1*). These structures have instantaneous velocities consistent with subsequent measurements of the bidirectional transport rates of radioactively labeled substances (*2, 3*). However, the 30- to 50-nm vesicles and other submicroscopic structures known or suspected to move along axons have only recently been directly observed in living cells (*4*). By means of video-enhanced contrast–differential interference contrast (AVEC-DIC) microscopy (*5, 6*) it has become possible to observe microscopic events corresponding to fast axonal transport. AVEC-DIC microscopy has considerably increased the detectability of structures as small as one-tenth the resolution limit of the microscope (for example, microtubules 25 nm and intermediate filaments 10 nm in diameter). With this method, direct observation of axonal transport in the squid giant axon is possible despite the large amount of light scatter present.

Carefully dissected, well-oxygenated squid axons with the ganglia attached to establish polarity of the preparation were viewed by the AVEC-DIC method at a magnification of 12,000 with a ×100 oil-immersion planopochromatic objective (numerical aperture, 1.3). Each microscopic field displayed an optical section (21.3 by 21.3 μm and about 0.2 μm thick) of an axon 0.5 mm in diameter. In a typical field, small particles (0.1 to 0.2 μm in apparent diameter) moved in one direction or the other (never reversing) at velocities up to ±5 μm/sec (±432 mm/day) and averaging 2.5 μm/sec (216 mm/day) at 21°C, while some of the larger organelles exhibited more intermittent movements (Fig. 1). Although the visual impression was that of almost uniform velocities of continuous movement, detailed analysis (*7*) revealed a considerable range of velocities (Fig. 2).

Individual particles were tracked over distances from 5 to 20 μm. In the cortex, movements were mostly orthograde, while in the endoplasm, particles could be seen moving in both directions. Or-

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