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Distribution of Guinea Pig Herpeslike Virus in Brain and Other Tissues of Naturally and Experimentally Infected Animals[1] (39412)

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In experimental infections with different herpesviruses it has been found that the viruses may persist in a latent state for prolonged periods of time without causing apparent disease (1). Latent herpes simplex virus infection of the trigeminal ganglia of mice and rabbits has been studied (2-5) but the distribution of this virus in the central nervous system has not been as extensively explored. Furthermore, even less is known about the neural distribution of other herpesviruses during long-term infection.

A herpeslike virus was isolated from guinea pigs (6), and latent infection of the brain with this virus in experimentally infected animals was studied in our laboratory (7). However, the distribution of this virus in different parts of the brain was not investigated. Because of the importance of latent and persistent viral infections of the nervous system, it was of interest to study the quantitative distribution of virus in the brain and the trigeminal ganglia of guinea pigs naturally and experimentally infected with the guinea pig herpeslike virus (GPHLV). In addition, the distribution of GPHLV in blood, spleen, salivary gland, and other tissues was determined and compared to that of virus in the nervous system.

Materials and methods. Virus stock and cell culture. GPHLV strain Y407, isolated from a naturally infected strain 2 guinea pig was used (6). The virus was passaged in Hartley guinea pig kidney (GPK) cell monolayer cultures four times before use. Virus infectivity titers of the stock virus varied between 10^6 and $10^7 TCID_{50}$/ml. Primary GPK monolayer cultures were prepared from GPHLV-free Hartley guinea pigs, by trypsin dispersion of kidney cells as previously described (8).

Animal source and inoculation. Adult strain 2 and strain 13 guinea pigs were obtained from Dr. C. H. Evans and Dr. D. Rosenstreich, respectively, of the National Institutes of Health. Hartley guinea pigs were purchased from CAMM Research Institute, Wayne, N.J. Hybrid guinea pigs were obtained through cross-breeding of strain 2 and Hartley guinea pigs. GPHLV negative Hartley or F_1 hybrid guinea pigs were observed for any evidence of disease. suspension by the intraperitoneal (ip) or 0.1-0.2 ml by the intracerebral (ic) route. Animals were kept in separate cages and were observed for any evidence of diseases.

Detection of virus. In order to determine onset of natural GPHLV infection, 0.5 ml of heparinized whole blood, obtained from anesthetized animals by heart puncture at monthly intervals, was inoculated onto GPK monolayer cultures. Animals were considered GPHLV positive when virus-induced cytopathic effect (CPE) was observed. Naturally and experimentally infected animals were sacrificed by exsanguination under ether anesthesia. The brain, trigeminal ganglia, spleen, and salivary gland were then removed aseptically. In order to obtain satisfactory samples of the cerebral cortex, diencephalon, brainstem, cerebellum, and trigeminal ganglia, the following procedure was employed. The brainstem was cut at the level of the superior colliculus, and the forebrain and diencephalon were gently raised and lifted out of the cranium. This brain tissue was then sagittally cut and the dience-

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phalic area including the basal ganglia, thalamus, and hypothalamus was separated from the cerebral cortex. The cerebellum and the brainstem were separated by cutting the cerebellar peduncles and the middle of the medulla oblongata. Care was taken to cut the trigeminal roots before lifting the brainstem out of the cranium. Each trigeminal ganglion was dissected free of the basal meninges and separated from the trigeminal root. Tissues were handled separately and were trypinisized as described previously (8). Serial 10-fold dilutions of packed cells prepared from tissues of GPHLV-infected animals were inoculated into tube cultures of GPK cell monolayers, 3–4 tubes per dilution. Virus isolation from heparinized whole blood was performed in a similar manner. Infectivity titers of the 10-fold diluted cell suspensions were determined by the 50% endpoint method (9). In some instances trypsin-dispersed infected cells were planted in Leighton tubes as primary cultures. All cultures were examined for the presence of CPE and/or virus induced intranuclear inclusions.

Microscopy of infected tissues. Portions of spleen, salivary gland, trigeminal ganglion, and brain tissue from each animal were fixed in 10% formalin or Bouin's solution followed by staining with H&E for light microscopy. Small portions of selected tissue samples were also fixed in 3% glutaraldehyde and examined by electron microscopy as described previously (10). For indirect immunofluorescence study, blood leukocytes, and trypsinized spleen cells were fixed with acetone and overlayed with anti-GPHLV serum produced in rabbits, before addition of fluorescein-conjugated anti-rabbit serum (11).

Results. Distribution of GPHLV in brain and trigeminal ganglion of naturally and experimentally infected guinea pigs. Guinea pigs naturally or experimentally infected with GPHLV were observed for periods of up to 15 months. Of the 25 animals studied, 13 naturally and 12 experimentally infected, virus was isolated from the trigeminal ganglia and several parts of the brain of all. Infectivity titers for dispersed cell suspensions of trigeminal ganglia and different parts of the brain are shown in Table I. The range of infectivity titers for the trigeminal and brain tissues was 1.7–2.7 log $TCID_{50}$/ml packed cells, with no significant difference between the naturally infected strain 2, strain 13, or hybrid guinea pigs and the experimentally infected guinea pigs.

Comparison of virus infection in brain, blood, spleen, and other tissues. Since the average infectivity titers of cell suspensions obtained from the different neural tissues did not differ significantly, the titers in the brain and trigeminal ganglia were compared with the titers in other tissues. As shown in Table II, the highest virus titer was found in the spleen regardless of mode of infection, including ic inoculation. The mean titer in spleen was 4.1 log $TCID_{50}$/ml packed cells with lesser titers in kidney, salivary gland, blood, and brain. The average titer in blood was slightly greater than in brain cell suspensions and slightly less than in cell suspen-

TABLE I. ISOLATION OF GPHLV FROM TRIGEMINAL GANGLIA AND DIFFERENT PARTS OF THE BRAIN OF NATURALLY AND EXPERIMENTALLY INFECTED GUINEA PIGS.

Guinea pig group	Route of inoculation	Strain of guinea pig	No. animals studied	Average infectivity titers of cell suspensions log $TCID_{50}$/ml packed cells				
				Tri. gang.[a]	C. Cortex	Dienceph.	Br.St.	Cer.
Naturally infected	None	2	3	1.8	2.2	1.8	1.7	1.7
		13	3	2.2	2.6	2.3	2.3	2.2
		F_1 hybrid[b]	7	1.8	2.2	2.1	2.1	1.9
Experimentally infected	ip	F_1 hybrid	4	2.1	2.2	2.0	2.0	2.1
	ip	Hartley	5	1.9	2.1	1.8	1.8	1.8
	ic	Hartley	3	1.5	1.8	1.8	2.0	1.7

[a] Tri.gang. = trigeminal ganglion; C. cortex = cerebral cortex; Dienceph. = diencephalon; Br. st. = brain stem; Cer. = cerebellum.

[b] Strain 2 crossed with Hartley.

TABLE II. COMPARISON OF INFECTIVITY TITERS OF CELL SUSPENSIONS OF BRAIN, SPLEEN, AND BLOOD FROM LONG-TERM GPHLV-INFECTED GUINEA PIGS.

Guinea pig group	Route of inoculation	Time postinfection (months)	No. animals studied	Average infectivity titers of cell suspensions log $TCID_{50}$/ml packed cells or whole blood[a]			
				Brain[b]	Blood	Spleen	Salivary gland, kidney, liver
Naturally infected[c]	None	1–5[d]	7	2.0	2.1	4.2	3.2
		7–15	9	1.8	2.3	4.0	3.0
Experimentally infected[e]	ip	4–5	5	1.9	2.7	4.0	Not done
	ip	7–10	5	1.7	2.1	4.2	3.1
	ic	4–5	3	1.7	2.1	4.4	3.0

[a] In guinea pigs in previous experiments (6, 8, 12), GPHLV has also been isolated from lung, uterus, placenta, and thymus of long-term infected animals.
[b] Average of all parts of brain and trigeminal ganglia.
[c] Strain 2, 13, and hybrid F_1 guinea pigs.
[d] For naturally infected animals this was the time after virus was initially detected in blood.
[e] Hartley and hybrid F_1 guinea pigs.

sions from kidney or salivary gland. Occasionally brain tissues of some animals showed titers as high as those in whole blood. Other tissues, including lung and liver showed titers similar to those in kidney and salivary gland.

In naturally infected guinea pigs the presence of virus infection was initially determined by isolation of virus from whole blood. These animals were sacrificed 1–15 months after first evidence of virus infection, and virus was reisolated from the blood of all animals at time of sacrifice. None of the GPHLV-infected animals showed clinical evidence of nervous system or other disease during the period of observation.

Microscopy of infected tissues. Light microscopy of infected tissues from both naturally and experimentally infected animals did not show intranuclear inclusions or other pathology. Several attempts to visualize viral antigens on leukocytes and spleen cells from GPHLV-infected guinea pigs using indirect fluorescent antibody techniques were negative. Following electron microscopic examination, virions were not found in tissues from virus positive animals.

Discussion. The present study showed that in natural or experimental infection of guinea pigs with GPHLV, virus could be isolated in similar quantity from the trigeminal ganglion and several parts of the brain. Virus was present in greater quantity, however, in blood, spleen, kidney, and salivary gland than in the brain. In previous studies it was found that the GPHLV isolated from the blood of infected guinea pigs was associated with the leukocytes (10, 12). Latent infection of GPHLV in guinea pigs, therefore, may be somewhat similar to EB herpesvirus infection in humans. The association of virus with circulating leukocytes could explain the similarity between titers in the trigeminal ganglia and in the different parts of the brain sampled, without localization to any particular tissue. If virus isolated from the neural tissues was indeed due to contamination by leukocytes, it would indicate the importance of testing for the presence of viremia. Thus, determination of viremia, as well as study of the distribution of virus in the nervous system, would be necessary prior to demonstrating the localization of a virus to a particular part of the nervous system.

Infection with GPHLV is in keeping with some of the concepts of latent virus infection. Long-term infected guinea pigs are apparently normal, and examination of infected tissues by light and electron microscopy has not revealed evidence of histopathology or of viral inclusions or virions (8, 10). Similarly, infected leukocytes or spleen cells from guinea pigs have not revealed evidence of viral antigens following immunofluorescence testing. Thus, as in the present experiment, once infection has been initiated, the animals are chronically but subclinically infected. Demonstration of the

presence of virus in infected tissues has been accomplished only by primary cultivation, or cocultivation with susceptible cells of intact infected tissue cells, but not homogenates of infected tissues (6). In contrast, infection with guinea pig cytomegalovirus (GPCMV), another herpesvirus of guinea pigs, is quite different from infection of guinea pigs with GPHLV. In a separate study we found that intranuclear inclusions and virions were observed in tissues of guinea pigs infected with GPCMV, and infectious virus was readily isolated only from salivary gland (13).

Summary. Distribution of GPHLV in the trigeminal ganglion, brain, and other tissues of guinea pigs was studied. Virus was found in suspensions of all tissue cells from naturally infected guinea pigs, as well as animals inoculated ip or ic, up to 15 months after initiation of infection. Evidence of virus infection was detected by cultivation of cells or by cocultivation of infected cells with susceptible cell cultures, but not by other methods including immunofluorescence and electron microscopy. Quantitative determinations of virus in the trigeminal ganglia and different parts of the brain showed infectivity titers to be similar in these tissues. Since GPHLV was also found in blood and in high titer in spleen, and in other tissues tested, isolation of virus from neural tissues may have been due to contamination with infected blood leukocytes.

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