

STUDY REPORTS

3. Title: Natural and Experimental Infection of Gibbons with Herpesvirus hominis.

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Objectives To establish the herpes virus etiology of naturally occurring cases of encephalitis in gibbons, to identify the viruses recovered from them specifically as Herpesvirus hominis and to test its pathogenic properties in experimentally infected gibbons.

Description Within a 6 month period, 4 gibbons of 131 in the Phra Buddhabat primate colony developed encephalitis. Histopathological and virological studies established a diagnosis in three of the cases as viral encephalitis of herpesvirus origin. Studies were carried out to specifically identify the viruses recovered from the gibbons. A serologic survey was carried out to determine the prevalence of herpesvirus infection in the colony. Gibbons were inoculated with the gibbon strain and others with a human strain of Herpesvirus hominis by a variety of routes to determine if gibbons could be experimentally infected, and if encephalitis invariably resulted.

Progress Four gibbons housed at the Phra Buddhabat primate colony developed encephalitis between 3 March and 28 August, 1967. Two of these animals exhibited descending, flaccid hemiplegia progressing to complete paralysis. Euthanasia was carried out when the animals became moribund. The animals were necropsied and gross and microscopic changes typical of herpesvirus encephalitis were seen in two of them. Agents producing cytopathogenic changes in human embryonic kidney and lung cells typical of herpesvirus were recovered from 3 of the 4 brains. Fluorescent antibody, Sellers staining and mouse inoculation tests for rabies were negative. The virus present in the brain of the first case was identified directly as Herpesvirus hominis at SMRL by neutralization with commercially prepared reference antisera in BS-C-1 cell cultures. More extensive testing of other aliquots of this brain suspension by Dr. Robert Hull, Eli Lilly Laboratories, corroborated this finding and ruled out Herpesvirus simiae. The other two strains were found to be identical with the first by plaque reduction neutralization tests (PRNT) in BS-C-1 cell cultures. Antiserum to Herpes T virus failed to neutralize the 3 gibbon virus strains.

A serological survey for herpesvirus antibodies in gibbons of the colony was conducted. Sera were sent to Dr. L.N. Binn, Division of Veterinary Medicine, WRAIR. Of sera from 127 animals tested, 25 (20%) neutralized 180 TCD₅₀ of Herpesvirus hominis. Although cross neutralization of the test virus by H. simiae antibodies is not ruled out, it appears that herpes virus infection had been fairly extensive in the colony sometime during the past.

For purposes of antigenic comparison, PRNT were done with the first gibbon strain and with a human strain recently isolated from the throat of a patient with encephalitis in korat. Antisera to the

gibbon and human strains each were prepared in 5 domesticated rabbits by a single exposure to the rabbits by the combined intradermal, intramuscular and intracorneal routes. Three weeks following inoculation, the rabbits were bled and the antisera pooled by strain, heat-inactivated and tested against the homologous and heterologous strains by PRNT. No differences between homologous and heterologous titers were observed (Table 2).

Table 2. Reciprocal Plaque Reduction Neutralization Tests of Gibbon and Human Origin Viruses with Rabbit Antiserum

Vaccination Virus Origin	No. Serum in Pool	Virus Neutralized	
		Human Strain	Gibbon Strain
Human	5	<u>200*</u>	160
Gibbon	5	700	<u>800</u>

* Reciprocal of titer neutralizing 50% of the indicated virus.

Four months after inoculation, 3 gibbon strain immune and 2 human strain immune rabbits were challenged on each flank with homologous and heterologous virus strains. Ten-fold dilutions (4×10^5 plaque-forming units) of both viruses were inoculated intradermally and concentrated virus suspensions were scarified into the cornea of each eye. The dermal erythema and edema which appeared and subsided within 18 hours was probably of an immune rather than an infectious nature. No skin lesions of the type seen on primary immunization occurred with either virus strain in any of the rabbits. Conjunctivitis occurred in two of the rabbits 5–8 days after inoculation, but this condition was present in both animals at the time of inoculation, casting doubt on its viral etiology. The failure of all the rabbits to develop herpes virus skin lesions indicated that solid immunity to both virus strains follows infection with either one of them, and supports the PRNT data which indicated antigenic similarity.

The occurrence of three confirmed and one suspected case of herpesvirus encephalitis in this small population suggested that Herpesvirus hominis infections of gibbons is highly virulent and may often be neurotropic. To test this hypothesis, two juvenile gibbons per virus strain were inoculated intradermally along the back and submucosally in the labial surfaces of the lips with 10-fold virus dilutions containing $0.4-4 \times 10^4$ plaque-forming units (PFU). The most concentrated suspension was also lightly scarified into one eye. One gibbon inoculated only with virus diluent by the same routes, served as a control. Definite lesions appeared on the backs of the gibbons inoculated with the human virus strain on postinoculation (PI) day 2. These lesions were red and raised, developed ulcerated centers by PI day 4 and then began to regress. These lesions again became inflamed and larger (up to 35 mm) on PI days 6 and 7. Secondary vesicles appeared around the primary lesion two days later. The skin lesions had completely healed by three weeks PI. Skin biopsies taken on PI day 11 contained typical type-A inclusion bodies in epithelial cells. In these same animals, oral lesions appeared as vesicles 3–4 days PI at all injection sites. The lesions increased in size and spread to other areas of the mouth including the tongue. Eight days PI,

large areas of sloughing had occurred and the lesions had become necrotic. Conjunctivitis was first noticed in these animals at 4-6 days. The infection progressed and within two days an extensive exudative keratoconjunctivitis with palpebral edema was evident. The corneas were ulcerated by PI days 9-10. By three weeks PI both of these gibbons had responded with high titered serum antibodies which neutralized both strains of virus equally well (Table 3), again confirming the antigenic identity of the strains.

Table 3. Serum Neutralization Antibody Titers In Young Gibbons 3 Weeks After a Single Exposure to Herpesvirus hominis of Gibbon and Human Origin.

Gibbon Number	Herpes Strain	Antibody Titer To	
		Gibbon Strain	Human Strain
5	Virus-free	<u>< 10</u>	< 10
6	Gibbon	<u>< 10</u>	< 10
7	Gibbon	< 10	< 10
8	Human	1080	<u>950</u>
9	Human	5120	<u>5120</u>

Although antigenically identical, a biological difference between the two strains became apparent. In contrast to the human strain, the gibbon strain produced only very slight erythema which persisted for 24 hours. No eye or oral lesions were seen, nor was detectable neutralizing antibody to either homologous or heterologous strains in evidence either three weeks or 5 months after inoculation. The control gibbon was similarly negative. Interestingly, the gibbon strain virus produced less severe lesions in the rabbits inoculated for antiserum production.

Approximately 5 months after the initial exposure, attempts were made to reactivate possible latent virus in all the gibbons by injecting large doses of epinephrine. Each animal received 2.0 ml. of a 1:1000 dilution of epinephrine hydrochloride intramuscularly. The animals were observed daily for evidence of reactivation. Ten days after treatment one animal (VM-9), previously infected with the human strain virus, developed redness, swelling and small vesicles on the lower lip. Herpesvirus hominis was recovered from vesicle fluid. There were no definitive findings in the other gibbons.

Six months after the initial exposure, all of the gibbons, including the control, were inoculated intradermally in titration and into the cornea as were the rabbits. Concentrated suspensions of both viruses were inoculated into opposite sides of the buccal mucosa. Gibbons previously infected with the human strain did not develop skin, mouth or eye lesions with either strain. The gibbons previously inoculated with the gibbon strain virus and the control animal developed skin, eye and mouth lesions with both strains. The course of the infections were similar to that of the human strain virus in the first experiments. Production of extensive lesions by the gibbon strain virus only in the challenge experiments is difficult to explain. Both primary exposure and challenge infection were done with the same lot of virus administered by the same routes, pointing to some unexplained changes in host susceptibility as the underlying factor.

Summary Four cases of encephalitis occurred in a gibbon colony of 131 individuals. Three of these were virologically diagnosed as Herpesvirus hominis encephalitis. Serological evidence indicated that 20% of the colony had been infected with herpes virus at some time in the past. Neutralization tests with the prototype gibbon virus and a local human H. hominis strain revealed no antigenic differences; immune rabbits had solid homologous and heterologous strain challenge immunity. Inoculation of the human strain virus into gibbons produced localized lesions and high-titered serum antibody following exposure by the intradermal, oral mucosal and corneal routes. The gibbon strain, on the other hand, produced no definite lesions, nor was antibody detectable after exposure. However, the gibbon-strain inoculated animals, and one previously uninoculated control animal, proved fully susceptible to infection with both strains 6 months later. The acquisition of susceptibility to the gibbon strain is not understood.