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Latent *Herpesvirus hominis* from Trigeminal and Sacral Dorsal Root Ganglia of *Cebus* Monkeys[1] (39523)

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Herpesvirus hominis type 2 (HVH-2) genital infection constitutes a uniquely important public health problem. This agent not only causes morbidity during primary infection but variable proportions of its victims also suffer recurrent disease. So far, experimental studies of latency and reactivation utilized rabbits and mice, and it remains uncertain whether the results can be extrapolated to primates. *Cebus* monkeys infected by intravaginal instillation of HVH-2 develop an infection similar to that seen in humans (1). To date, however, no systematic effort has been made to identify latent HVH-2 in experimentally infected simians. In this paper we present preliminary results indicating that HVH-2 induces a latent infection in sacral dorsal root ganglia of vaginally infected *Cebus* monkeys.

Materials and Methods. *Virus.* We used *Herpesvirus hominis* type 2, strain 333 (HVH-333), kindly provided by Dr. W. E. Rawls, Baylor College of Medicine, Houston, Tex. HVH-333 was originally isolated from a human penile lesion. After ultraviolet irradiation it transformed hamster embryonic fibroblasts and produced tumors in weanling hamsters (2). We grew the virus in FT cells (3). A single lot, titering 1×10^6 PFU/ml, provided the animal inocula.

Monkey inoculation and sampling. This study used sexually mature *Cebus albifrons* monkeys. Prior to the experiment all monkeys lacked evidence of HVH infection, as determined by multiple attempts to isolate virus from the oral cavity and genital tract and by the absence of serum neutralizing antibodies.

We infected monkeys intravaginally by depositing 5×10^5 PFU of virus into the vaginal vault and then inserting either a cotton pledget or a gelatin sponge. Three animals also received ocular inoculations, by dropping virus onto the eye and gently rubbing with a swab. Monkeys were examined and sampled three times weekly during the first month postinoculation, then twice weekly.

Virus isolation. Vagino-cervical, pharyngeal, and ocular swabs were inoculated onto FT cells for virus isolation. Finely minced tissue obtained at necropsy was homogenized and explanted or cocultivated with FT cells. FT cells inoculated with 10% tissue homogenates were checked for cytopathic effects weekly for 5 weeks.

Upon forming confluent monolayers, explanted cells were subcultured for further experimentation. Since thymidine analogs can induce nonpermissive cells to produce Epstein-Barr virus (4, 5) and cytomegalovirus (6) as well as depress interferon synthesis (7), we attempted a series of experiments using iododeoxyuridine (IUDR). For 4 consecutive weeks media from one set of subcultures were passed to FT cells and to FT cells grown 3 days in IUDR (20 mg/ml). Additional sets were grown with IUDR or cocultivated with FT cells which had been grown for 3 days in IUDR. We examined these cultures over 4 consecutive weeks, passing their media to FT cells. A fourth set of subcultures was maintained 3 to 4 weeks at 33° without medium change, then medium was passed to FT cells and IUDR-treated FT cells.

Presumptive HVH isolates were specifically identified as HVH-2 by tube neutralization and the immunoperoxidase antibody technique (8) using guinea pig hyperimmune serum to HVH-333 and an HVH type 1 strain.

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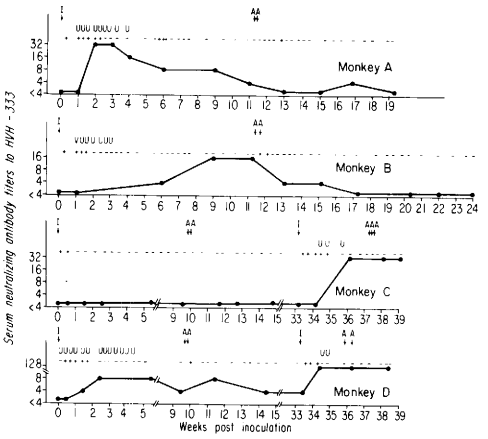

FIG. 1. Summary of clinical, serological, and virological patterns of monkeys A–D. ↓ I , HVH-333 inoculation; ↓ A , adrenalin administered; ●, neutralizing antibody titer; +, HVH-333 genital isolate; −, genitalia negative for HVH-333; U, genital ulcer; V, genital vesicle.

Serology. We used a plaque reduction assay to measure neutralizing antibody to HVH-333 (9). Sera were diluted 1:4, inactivated 30 min in a 56° water bath and serially diluted. Diluted serum, 60–120 PFU of HVH-333 and 15 hemolytic units of guinea pig complement were incubated for 1 hr at room temperature prior to plating on FT cells. Serum titers were defined as the serum dilution producing at least 80% plaque reduction.

Results. We infected and killed five *Cebus* monkeys. Monkeys A and B were sacrificed at 23 and 19 weeks following genital infection. Figure 1 summarizes their clinical courses, viral excretion, and antibody patterns. Although both developed herpetic vulvovaginitis, seroconverted, and demonstrated spontaneous or epinephrine-induced shedding of HVH-2, we failed to isolate virus from their tissues (Table I).

TABLE I. RECOVERY OF HVH-2 FROM *Cebus* MONKEY TISSUES.

	A 135			B 163			C 36			D 43			E 7		
Monkey Day killed Specimen	Homogenate	Culture	Explant	Homogenate	Culture	Explant	Homogenate	Culture	Explant	Homogenate	Culture	Explant	Homogenate	Culture	Explant
Pharyngeal swab	−			−			−			−			+		
Conjunctival swab							−			−			+		
Vagino-cervical swab	−			−			−			−			+		
Urine	−			−			−			−					
Vagina	−	−	−	−	−[a]	−[d]	−	−	−[b]	−	−[a]	−	+	+	+
Cervix	−	−	−[b]	−	−	−[d]	−	−	−[a]	−	−	−[d]	+	+	+[a]
Uterus	−	−[a]	−[b]	−	−	−[d]	−	−	−[c]	−	−	−	−	+	−
Ovaries	−	−[a]		−	−	−[d]	−	−	−[b]	−	−	−[e]	−	−	−
Bladder	−			−	−	−[d]	−	−	−[b]	−	−	−[g]	+	+	−
Kidneys							−	−	−[b]	−	−	−[g]	−	−	−
Inguinal nodes	−	−	−	−	−[a]	−[b]	−	−	−[b]	−	−	−[g]	−	−	−
Pelvic nodes	−	−	−	−	−[a]	−[b]	−	−	−[b]	−	−	−[g]	−	−	−
Paracervical fascia (ant)				−	−	−[b]	−	−	−[c]	−	−	−[g]	−	−	−
Paracervical fascia (post)	−	−	−[c]	−	−	−	−	−[a]	−	−	−	−	−	−	−
Liver	−			−	−	−	−	−	−[a]	−	−[a]	−[a]	−	−	−[a]
Spleen	−			−	−	−[d]	−	−	−[b]	−	−[a]	−[g]	−	−[a]	−
Spinal cord	−	−	−	−	−	−									
DRG L1, 2							−	−	−	−	−	−[g]	−	−	−
DRG L3, 4, 5, S1							−	−	−	−	−	−[g]	−	−	−
DRG S2, 3							−	−	−	−	+	−[g]	−	+	−
DRG C1, 2							−	−	−	−	−	−	−	−	−
Tonsils	−	−		−	−	−[d]	−	−	−	−	−	−[g]	+	+	−
Parotid gland	−	−[a]		−	−[a]	−[b]	−	−	−	−	−	−[f]	−	−[a]	−
Left ophthalmic nerve							−	−	−	−	−[a]	−	−	+	−
Right ophthalmic nerve							−	−	−	−	−	−[g]	−	+	−
Left trigeminal ganglion							−	−	−	−	−	−[g]	−	−	+[a]
Right trigeminal ganglion							−	−	−	−	−	−[g]	+	+	−

[a] No tissue growth; however, media were passed weekly.
[b] Media from explant passed to and explant cocultivated with IUDR-treated FT cells.
[c] Same as *b*, in addition explant incubated at 33° and media passed to untreated and IUDR-treated FT cells.
[d] Same as *c* in addition explant grown in IUDR.
[e] Explant grown in IUDR.
[f] Explant co-cultivated with IUDR-treated FT cells.
[g] Same as *e* and *f*.

Monkeys C and D were reinoculated genitally and in the right eye 8 months after the primary infection. Sacrifice occurred 36 and 43 days later (Fig. 1). We isolated HVH-2 by cocultivation from pooled second and third sacral dorsal root ganglia (DRG) from monkey D, while all other tissues including lumbar, sacral, and coccygeal DRG were negative (Table I).

Monkey E was inoculated intravaginally and in both eyes with HVH-333. She developed herpetic conjunctivitis and exhibited vulvar herpetic lesions. When we killed her 7 days postinoculation the lesions remained HVH positive and she had yet to seroconvert. Several tissues yielded HVH-2 (Table I). The only positive tissues not directly contiguous with active infection sites were the ophthalmic nerves, trigeminal ganglia, and pooled second and third sacral DRG.

Discussion. Human HVH-2 genital infection has assumed increasing importance during recent years with respect to acute venereal disease, fetal and neonatal diseases, and cervical cancers. Our findings extend previous observations that *Cebus* HVH-2 infection closely resembles human genital disease clinically, virologically, and serologically (1). Thus *Cebus* should provide an excellent primate model to study the dynamics of HVH infection.

Our study also indicated that *Cebus* monkeys could serve as a model for examining viral latency in primates. Three of four animals manifested either spontaneous or epinephrine-induced recurrent viral shedding. Monkey D, killed 43 days postinfection, yielded HVH-2 isolates from pooled cocultivated second and third sacral DRG only. Other sacral, lumbar, and coccygeal DRG failed to yield HVH. HVH was not recovered from any tissues of monkeys A–C. In retrospect, however, we did not specifically dissect DRG from A or B. Although all tissues from monkey C remained HVH negative, she was the only animal not reshedding virus following epinephrine administration and may not have been latently infected. Monkey E was killed while still acutely infected. Virus was isolated from several tissues, all contiguous to actively infected areas. She also had HVH in two noncontiguous sites, the ophthalmic nerves, trigeminal ganglia and the second and third sacral DRG. These results are in accord with several mouse and rabbit studies. In these animals HVH appears to spread centripetally via intro-axonal transport and cause latent infection in neurons of sensory ganglia (10–13). Our results are also in accord with human cadaver studies reporting HVH isolations from cocultivated or explanted trigeminal ganglia (14–16) and sacral DRG (17). While human cadaver studies presume that virus in sacral DRG resulted from genital infections, only in mice has HVH been shown to spread from the vagina and cervix to lumbosacral ganglia and cause latent infection (13). Our study represents the first demonstration that genital HVH infection in primates results in viral latency limited to sensory ganglia specifically innervating the genitalia.

The findings from these five monkeys also indicated that latent HVH could only be recovered from sensory ganglia innervating the infected sites. We could not recover HVH from autonomic nervous tissue (paracervical fascia) nor from other tissues despite repeated attempts and a variety of techniques. Murine, rabbit, and human studies have not recovered latent HVH from other tissues (11, 15, 17–21). One previous study (22) examined tissue explants from *Cebus* monkeys following HVH-2 genital infection. They recovered HVH from explant cultures of vagina, uterus, bladder, ovary, adrenal, lung, kidney, and spleen from one to four of eight monkeys tested. This paper did not specify when postinoculation sacrifice occurred, nor did the authors state if neural tissue was examined. The five monkeys reported here represent our preliminary studies of *Cebus* genital HVH infection. Current experiments extend these preliminary observations and will examine the relationships of host age and immune response to genital HVH pathogenesis and latency.

Summary. Five *Cebus albifrons* monkeys were infected intravaginally with *Herpesvirus hominis* type 2. The resulting infection clinically, virologically, and serologically resembled that seen in humans. After the acute infection had ceased, one monkey shed virus spontaneously on two occasions

while two others shed following adrenalin administration. Herpesvirus was recovered from the second and third sacral dorsal root ganglia of two monkeys by cocultivation. Despite a variety of techniques, we could not recover virus from any other tissues. In addition, we recovered herpesvirus from cocultivated trigeminal ganglia of an ocularly infected monkey.

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