# Experimental *Herpesvirus hominis* type 2 Infection in Nonhuman Primates (36276)

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Although herpesviruses have been recovered from a number of different simian species, their pathogenic potential in the natural host and alternate species encountered subsequent to capture is not entirely known. On the other hand, increased virulence with infection of a foreign host has been repeatedly demonstrated (1, 2). The presence of the herpesviruses in nonhuman primates assumes a new significance when it is recognized that these viruses are: a) universally distributed among monkeys and apes, as well as other animals; b) associated with malignant lymphomas of simians (3, 4); and c) epidemiologically associated with human cervical cancer (5, 6).

The recent production of genital lesions in *Cebus* (*Cebus albifrons*) monkeys (7, 8) by human *Herpesvirus hominis* type 2 suggests that studies with nonhuman primates may be of value in the development of an experimental model system for the study of herpesvirus pathology, including cancer.

Nahmias *et al.* (7) in their study on type 2 herpesvirus infection of *Cebus* monkeys indicated that two other simian species—rhesus (*Macaca mulatta*) and squirrel (*Saimiri sciureus*) monkeys—were not susceptible to this virus as determined by the inability to demonstrate virus or antibody rise or the development of genital lesions. Other investigators have similarly demonstrated that the simian host response to herpesviruses was variable among the species examined (1). Thus, Melendez *et al.* (1, 3) have demonstrated that all New World monkeys do not react similarly to *H. saimiri* and other viruses. In our laboratory we have found that serologic surveys for antibodies to various herpesviruses in different species of nonhuman primates also reflect these species differences (9, unpublished data).

The purpose of this report is to extend the findings of Nahmias *et al.* (7) and London *et al.* (8) on *Cebus* (*C. albifrons*) monkey susceptibility to type 2 *H. hominis* as well as provide data on the response of the baboon (*Papio cynocephalus*) and marmoset (*Saguinus oedipus*) to this virus.

*Materials and Methods. Virus. Herpesvirus hominis* type 2 (HVH2) was the same strain (CUR) previously employed (7, 8). This virus was isolated from the genitalia of a female patient and passed three times in primary rabbit kidney (PRK) cell culture, followed by one passage in Vero cells. The titer was $10^{5.7}$ $TCID_{50}$/0.1 ml in these cells. Inoculation of monkeys was as described previously and consisted essentially of placing undiluted virus soaked cotton pellets in the vaginal vault as close to the cervix as possible (7, 8). These were maintained in place by a single suture through the labia for 24 hr at which time the sutures and pellets were removed and the animals sampled. After the third day, the animals were examined and sampled approximately every other day. A number of baboons (Table I) were also inoculated by the intramuscular, intravenous or intranasal routes with 0.5 ml undiluted virus

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TABLE I. Serum Neutralization Antibody Titers in Baboons Inoculated with *H. hominis* type 2.

| Animal | Route of inoculation | Weeks after inoculation | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | 0 | 1 | 2 | 3 | 5 | 6 | 7 | 8 | 10 |
| B724 F | Intravaginal | 4[a] | —[b] | 8 | 16 | 32 | — | — | — | — |
| B726 F | Intravaginal | <4 | <4 | <4 | 8 | 4 | 16 | 16 | 16 | 16 |
| B727 F | Intravaginal | <4 | <4 | 8 | <4 | 4 | 8 | <4 | <4 | 16 |
| B728 F | Intravaginal | <4 | <4 | <4 | 4 | <4 | <4 | <4 | <4 | 4 |
| B729 F | Intravaginal | <4 | — | 4 | 8 | 8 | — | — | — | — |
| B730 F | Intravaginal | <4 | <4 | — | <4 | 4 | 32 | 8 | 8 | 16 |
| B732 F | Intravaginal | <4 | 4 | 8 | 32 | 8 | 16 | 8 | 4 | — |
| B733 F | Intravaginal | <4 | — | 4 | 16 | 64 | — | — | — | — |
| B738 F | Intravaginal | 4 | — | 16 | 16 | 16 | — | — | — | — |
| B780 F | Intravaginal | <4 | — | <4 | <4 | 4 | — | — | — | — |
| B737 M | Intramuscular | <4 | — | 16 | <4 | <4 | — | — | — | — |
| B770 M | Intravenous | <4 | — | 8 | 8 | 16 | — | — | — | — |
| B802 M | Intravenous | 4 | — | 8 | <4 | 8 | — | — | — | — |
| B796 M | Intranasal | <4 | — | 4 | <4 | — | — | — | — | — |
| B804 M | Intranasal | 4 | — | 4 | 4 | 16 | — | — | — | — |

[a] Reciprocal of serum neutralization endpoint dilution.
[b] Not done.

suspension. One male marmoset was given 0.5 ml undiluted virus intranasally and two drops placed on the corneal surface.

*Animals.* The three simian species employed were derived from three separate sources: baboons (*P. cynocephalus*) were born in the SFRE breeding colony; the *Cebus* (*C. apella*) monkeys and marmosets (*S. oedipus*) were purchased through two commercial sources and used after a four week quarantine period and virologic examination. All animals were free of HVH2 infection prior to inoculation as determined by virus isolation attempts, although several animals had a low level (1:4) of neutralizing antibody (Tables I and III).

*Virus isolations.* Routine vaginal swabs were obtained at intervals of several days after the third day and inoculated into Vero cells for the isolation of virus. In addition, material from lesions and tissues obtained at necropsy was similarly examined.

*Serology.* Blood samples were obtained by venipuncture prior to virus inoculation and again at varying time intervals during the experiment. Antibody determinations were by serum neutralization (SN) test in microtiter plates with Vero cells using procedures standard for this laboratory (10). Between 30–50 $TCID_{50}$ virus were employed in all neutralization tests.

*Results.* Genital inoculation of the three simian species with type 2 herpesvirus resulted in considerably different gross clinical responses. The baboon was most resistant to development of clinical disease, the *Cebus* developed localized vulvar and vaginal lesions, and the marmoset responded with an overwhelming, generalized infection followed by death.

Of ten female baboons inoculated by the intravaginal route, six shed virus from 3–5 days, as determined by virus isolation from vaginal swabs (B724, B726, B727, B732, B738 and B780; Table I). Eight of the ten animals showed a significant rise in HVH2 antibody but no evidence of genital lesions was observed.

Virus was isolated from all eight *Cebus* monkeys for at least 10 days following inoculation (Table II). By the 17th day none of the animals were shedding virus. A purulent vaginal discharge was observed on day 3 post inoculation along with vaginal inflammation. Two animals also exhibited developing vesicular lesions on the dorsal commissure of the

TABLE II. *Herpesvirus hominis* type 2 Vaginal Shedding from *Cebus* Monkeys.

| Animal number | Days post inoculation | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 0 | 3 | 6 | 8 | 10 | 13 | 15 | 17 | 21 | 28 |
| CA12 | — | + | + | + | + | + | + | — | — | — |
| CA13 | — | + | + | + | + | + | — | — | — | — |
| CA14 | — | + | + | + | + | + | + | — | — | — |
| CA15 | — | + | + | + | + | — | — | — | — | — |
| CA16 | — | + | + | + | + | + | + | — | — | — |
| CA17 | — | + | + | + | + | + | + | — | — | — |
| CA18 | — | + | + | + | + | — | — | — | — | — |
| CA19 | — | + | + | + | + | + | + | — | — | — |

vulva. On day 6, five of the eight animals had lesions on the labia of the vulva. Eight days after inoculation, seven of the eight animals had developed ulcerated lesions on the labia of the vulva. Several animals also manifested vesicular lesions on the vaginal mucosa. The lesions started regressing by the fifteenth day. One female (CA15) never evidenced genital lesions, although she did exhibit a purulent vaginal discharge and vaginal inflammation. Lesions were not observed at a nongenital site on any of the animals.

Table III illustrates the seroconversion pattern of the female *Cebus* monkeys. All animals developed an increase in neutralizing antibody titer during the initial four weeks of examination. Evidence for a low level of herpesvirus antibody was detected on day 0 for CA14 and CA15.

TABLE III. Serum Neutralization Titers in *Cebus* Monkeys after *H. hominis* type 2 Inoculation.

| Animal | Weeks post inoculation | | | | |
|---|---|---|---|---|---|
| | 0 | 1 | 2 | 3 | 4 |
| CA12 | <4[a] | <4 | 16 | 16 | 32 |
| CA13 | <4 | 4 | 8 | 32 | 16 |
| CA14 | 4 | 4 | 16 | 16 | 16 |
| CA15 | 4 | 8 | 8 | 16 | 8 |
| CA16 | <4 | <4 | <4 | 16 | 16 |
| CA17 | <4 | 4 | 4 | 8 | 8 |
| CA18 | <4 | 4 | 8 | 8 | 8 |
| CA19 | <4 | 8 | 8 | 8 | 8 |

[a] Reciprocal of serum neutralization end point dilution.

Of the three species of monkeys tested, the marmoset appears to be the most susceptible to genital infection with HVH2. Of the four females involved, all manifested very severe genital lesions of the vulva, vagina, and cervix. The dose of virus used, however, also produced a fatal systemic herpes infection. The survival times of the females ranged from eight to nineteen days. All four animals were shedding virus vaginally up to the time of death. Post-mortem examination revealed severe, necrotic lesions of the urogenital tract, as well as lesions in various other organs.

Gross examination of the urogenital tract revealed severe necrotic lesions of the vulva. The vagina was filled with a viscous, purulent exudate, and the vaginal mucosa contained areas of petechial hemorrhage. The cervix exhibited areas of varying degrees of necrosis. Bladders of the marmosets were greatly distended and hemorrhagic. The neck of the urethra of these animals contained clotted blood and was overtly necrotic. Adrenals in all four animals were enlarged and their surfaces contained focal areas of necrosis. Similarly, the spleens of these animals also contained necrotic foci. Two of the marmosets showed lung consolidation.

Histopathologic examination of these tissues emphasized the gross findings. Coagulation necrosis with widely distributed hemorrhagic areas were seen in the vagina. The endometrium also evidenced some focal and diffuse necrosis. These necrotic lesions spread to the urinary bladder in all four animals producing focal to diffuse necrotic inflammation with or without hemorrhage in the mucosa and subserosa layers. Necrosis was also observed in the subserosal tissues of the small and large intestine and the uterus. Necrotic foci were observed in sections of liver, mesenteric lymph modes and adrenals. Herpesvirus intranuclear inclusions were distributed throughout and around these necrotic areas. Detailed pathologic manifestations of this study will be reported in a subsequent paper. The findings on the *Cebus* monkeys have been described previously (5). Table IV shows the results of viral isolation attempts from marmoset tissues obtained at necropsy.

TABLE IV. *H. hominis* type 2 Recovery from Marmoset Tissues Obtained at Necropsy.

| Tissue | Animal number | | | |
|---|---|---|---|---|
| | 45(M) | 48(F) | 52(F) | 54(F) |
| Liver | — | — | — | — |
| Adrenal | + | + | + | + |
| Spleen | + | + | + | + |
| Kidney | + | — | — | — |
| Vagina | | + | ND[a] | + |
| Lung | + | + | ND | ND |
| Bladder | + | + | ND | + |
| Testis | + | | | |

[a] ND = not done.

None of five male baboons inoculated with HVH2 intramuscularly, intravenously, or intranasally showed clinical signs of infection but converted to seropositive for neutralizing antibodies (Table I). A single male marmoset inoculated intranasally and by instillation into the eye became moribund and was sacrificed on the seventh day with widespread HVH2 infection (Table IV).

*Discussion.* Previous experimental genital infection of monkeys with HVH2 demonstrated that the *Cebus* monkey (*C. albifrons*) (7), in contrast to rhesus and squirrel monkeys, develops a clinical disease very similar to that seen in humans. The present experiments extend these findings by demonstrating that the baboon, like squirrel and rhesus monkeys, does not develop a clinical genital infection. A subclinical infection is suggested, however, by virus isolation for a short period and serological data. On the other hand, this virus induced 100% fatalities in the marmosets following vaginal inoculation. Although *H. hominis* (presumably type 1) has been reported previously as producing fatalities in marmosets as well as other simian species (1, 11), the overwhelming fatal infection observed following intravaginal inoculation in this experiment was unexpected.

The presence of pre-existing herpesvirus antibody in *Cebus* monkeys, CA14 and CA15, (Table III) cannot be attributed definitely to infection by HVH2 because of cross-reactivity observed among members of the herpesvirus group. It is possible that this antibody was responsible for the mild disease observed in CA15 but no such modification was noted in CA14. These results are similar to those obtained previously (7, 8) on reinfection of previously inoculated *Cebus* monkeys.

Successful demonstration of a susceptible host for genital herpesvirus infection offers an opportunity to explore certain experimental parameters heretofore limited to artificial systems or conjecture. Thus, this demonstration of genital infection permits further study into herpetic effects on the newborn, immunological responses of adult and offspring, neurological disorders due to herpesviruses, latent infections, and perhaps the relationship of herpesviruses to cervical cancer.

The *Cebus* monkeys used in this study were *C. apella* (R. Thorington, personal communication) not *C. albifrons* as used by Nahmias *et al.* (7) and London *et al.* (8). The similarity of response in these two species, however, suggests they are equally susceptible to infection by HVH2.

*Summary.* A graded response of the baboon (*P. cynocephalus*), *Cebus* (*C. apella*) and marmoset (*S. oedipus*) monkeys to infection via the female genital tract with *H. hominis* type 2 was demonstrated. A subclinical disease with limited virus production and antibody response was noted in the baboons. All *Cebus* monkeys showed clinical evidence of genital infection, extensive virus excretion and antibody response. Whereas baboons and *Cebus* monkeys survived inoculation with *H. hominis* type 2, a generalized fatal infection was observed in the marmosets.

This study was funded in part by U.S. Public Health Service grants RR05519, RR00278, RR00361, RR00451 and contract NIH 69-93 and WHO grant Z2/181/27. Dr. Nahmias is supported in part by grant T-521 from the American Cancer Society.

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Received Oct. 26, 1971. P.S.E.B.M., 1972, Vol. 139.