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A Nonhuman Primate Model for the Study of the Cervical Oncogenic Potential of Herpes Simplex Virus Type 2[1]

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Summary

A primary genital infection with herpes simplex virus type 2 can be established in Cebus monkeys with the development of genital lesions in approximately one-half of the infected animals. The lesions occur on the vulva and on the cervix and occasionally are observed at other sites. As in humans, spontaneous genital recurrences can occur in infected monkeys. Veneral transmission from genitally infected female monkeys to males have occurred. The presence of herpes simplex virus antibodies prior to herpes simplex virus type 2 genital inoculation does not appear to influence the rate of genital infection as compared to animals with undetectable antibodies in their acute serum.

In view of the available evidence suggesting an association between HSV-2[3] and cervical cancer, experimental animal models have been sought to study this possible association. Although investigations of the oncogenic potential of HSV-2 in mice and hamsters have been conducted (4, 7, 9), similar studies in nonhuman primates would be particularly valuable. The ideal nonhuman primate model would be one that had the following characteristics: (*a*) the infection in the animal would mimic the human infection in its clinicopathological, virological, and serological manifestations; (*b*) the animal would survive infection, so that it could be followed over the years for the possible development of cancer; (*c*) the animal could be reinfected subsequent to the primary infection; (*d*) the rate of spontaneous cervical cancer would be minimal in the animal; however, the animal should be susceptible to oncogenesis by other herpesviruses; (*e*) the animal, for practical consideration, would be readily available and not untowardly expensive.

We present in this report information showing that the Cebus monkey fulfills these criteria and also review current studies with this model.

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[2] Presented by.

[3] The abbreviation used is: HSV-2, herpes simplex virus type 2.

Nonhuman Primates Tested

Several genera of nonhuman primates were tested by our group and others (1, 2, 6). A gradation in susceptibility and responsiveness of the various simian species to genital inoculation with HSV-2 was noted. Rhesus monkeys (*Macacca mulatta*) and squirrel monkeys (*Saimiri sciureus*) could not be infected. Baboons (*Papio cynocephalus*) could be infected but did not demonstrate clinical lesions. Marmosets (*Saguinus oedipus*) could be readily infected genitally with HSV-2 but succumbed soon after being inoculated. On the other hand, Cebus monkeys, either *Cebus albifrons* or *Cebus apella*, were found to be susceptible to infection and demonstrated clinicopathological, serological, and virological responses similar to those noted in human females infected with HSV-2. These findings together with the fact that infected Cebus monkeys would survive infection made us choose this animal for further study.

In order to ascertain that spontaneous cervical anaplasia (dysplasia, *in situ* cancer, or invasive cancer) was not prevalent in female Cebus monkeys, a preliminary cervical cytological study was done in 83 *C. apella* monkeys from the breeding colony at the Harvard School of Nutrition. Of the 83 four- to seven-year-old monkeys, only 1 showed mild dysplasia, and this was not considered to be significant. In the conditioning phase of our current studies, there has been no evidence of cervical anaplasia in an additional 130 *C. apella* or *C. albifrons* monkeys, age 3 to 5 years.

It has been shown that Cebus monkeys are susceptible to oncogenesis by other simian herpesviruses (3). It is also important to note that no natural herpesvirus has as yet been isolated for these 2 species.

Strain of HSV-2 to Use for Genital Infection

The question has recently been raised of possible differences among HSV-2 strains in their antigenicity or oncogenic potential [see earlier discussion by Nahmias *et al.* (8) in this Symposium]. It thus became important to ascertain possible differences among various HSV-2 strains used for genital infection of Cebus monkeys. Twelve strains were selected that had 1 or more of the following characteristics: (*a*) isolation from women with cervical cancer; (*b*) ability to transform baby hamster cells *in vitro* (11); (*c*) association of

the strain with sarcomas when inoculated into newborn hamsters (9).

The method of genital inoculation of monkeys has been reported previously (2). Briefly, cotton pellets (approximately 2 cm long and 1 cm in diameter) were soaked in virus solution and inserted in the vaginal vault against the cervix where they remained for 24 hr before being removed. The animals were prebled and repeat bleedings were obtained 14 to 21 days after inoculation. The sera were tested for their HSV-neutralizing activity with a microneutralization test (5). Before viral inoculation, and 4, 7, and 10 days thereafter, cervical specimens are obtained for viral isolation attempts in primary rabbit kidney tissue culture and for cytological examinations for the detection of cervical anaplasia and for cellular changes associated with HSV infection (10). The animals were examined for lesions at those times, as well as at biweekly intervals for the following 2 weeks.

At least 2 female monkeys were inoculated with each of 12 strains of HSV-2; the titer of virus used varied from three to six 50% tissue culture doses per ml. Results of these studies demonstrated the following. (*a*) Eleven of the 12 strains caused genital infection. This was confirmed by serology or virological isolation. (*b*) The variability in the ability of the virus strain to produce a genital infection in the monkey could not be attributed to virus titer, since infection occurred with strains with titers as low as 10^3/ml. (*c*) Variability in the production of lesions occurred, so that the same strain could produce either clinically apparent or subclinical infection. Under these test conditions, 5 of the strains produced lesions.

Herpesvirus Induction of Cervical Cancer in Cebus Monkeys

It was decided to use the BEN strain of HSV-2 for long-term ongoing studies. This strain met all of the criteria noted above. The BEN strain of HSV-2 was isolated from an adolescent girl who developed cervical *in situ* cancer 3 years later. This strain was associated with sarcomas after its intrathoracic inoculation in newborn hamsters and was capable, in preliminary experiments by R. Duff and F. Rapp (personal communication), of transforming baby hamster cells *in vitro*. The virus had been isolated in primary rabbit kidney tissue culture cells and passaged 7 times in these cells. A large pool of virus, with a titer of $10^{5.5}$/ml, was prepared and stored at −70° in aliquots of 5 ml. Control material consisting of noninfected primary rabbit kidney cells from the same rabbits was also prepared and stored. The virus will be inoculated repeatedly into 225 young adult (3 to 4 years old) female monkeys, and noninfected tissue culture material will be used in 75 control female animals.

Since pregnancy might have an effect on promotion or acceleration of cervical carcinogenesis, inoculation and breeding of the female monkeys are being carried out concurrently. For this purpose, following a 60-day conditioning period, 2 or 3 female animals are placed in a cage with 1 male. Primary infection is attempted after the monkeys have been paired about 1 week.

Preliminary Observations

To date, attempts at producing genital infection of 89 female Cebus monkeys with HSV-2 have been made; 19 males have been exposed to these female animals. An additional 25 female monkeys have been inoculated with control (primary rabbit kidney) material and exposed to 10 males. One of the 89 female animals and 2 of 29 male animals were found to have HSV-neutralizing antibodies (1:10) in their serum on entry into the study. Genital HSV-2 infection was produced in the 1 female that had preexisting antibody. Lesions were noted overall in 50% of infected animals and were observed on the vulva, on the cervix, and occasionally on the mouth (Fig. 1). Cytological examination confirmed cervical involvement by the virus in 13 cases.

The duration of excretion of virus in animals with no HSV antibodies in their acute serum was approximately 1 week longer than if the animal had a prior HSV infection. Lesions in the 1st group were usually first evidenced at the 7-day examination and lasted for 7 to 14 days. Lesions were either absent, or of short duration, in animals with prior HSV-2 antibodies.

In order to determine the time at which genital reinfection would be successful, a group of monkeys that had been infected less than 3 months previously and another group infected 6 or more months earlier were reinoculated with HSV-2. It was found that reinfection could not be established in 10 of 12 animals infected within 3 months of reinoculation; on the other hand, reinfection could be established in 40% of 17 animals infected 6 or more months earlier.

In addition to the experimental route of genital HSV-2 infection, it became apparent that we should also examine

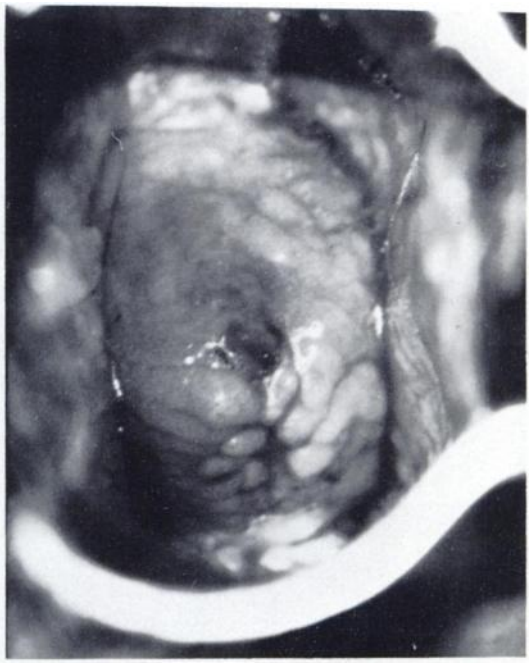

Fig. 1. Cervical herpetic lesion in a female Cebus monkey. Note the ulceration above the cervical os. × 2.

natural modes of transmission between animals and the possibility of spontaneous genital recurrences. Cross-infection by venereal transmission from infected females to males was noted in 2 instances. None of the males had HSV-2 antibodies prior to viral inoculation of their female contacts. Two male animals developed HSV-2 antibodies, and 1 was noted to have penile lesions (Fig. 2). As with genital HSV infection in female monkeys, the 2 males survived their infection. The lesions were similar to those observed in humans.

Direct cross-infection between infected and noninfected females or transmission by the male cage mate without evidence of infection in the male was also suspected in 5 instances. In these cases, even though the virus was inoculated into both females in the same cage, infection was demonstrated in only 1 animal within the 1st 2 weeks. The cage contact, negative for this period, began to show genital lesions thereafter (14 to 35 days), accompanied by a rise in antibody titer.

The possibility of spontaneous genital recurrences was also suggested in 2 animals in which the virus was isolated from the cervix 4 to 6 months after the primary infection; these monkeys were not in contact with other infected animals and were not reinoculated. Because of this finding, a more concerted study of 6 female monkeys previously infected with HSV-2 has been recently undertaken. Cervical swabs were obtained at weekly intervals and assayed virologically. Thus far, over a 12-week period, it has been possible to isolate HSV-2 from 1 animal.

Discussion

The HSV-2 cervical cancer hypothesis would be strengthened if it could be demonstrated that genital infection with HSV-2, but not control material, resulted in cervical anaplasia in a nonhuman primate model. The earlier reports showing that genital HSV-2 infection in female Cebus monkeys mimic infection in humans (2) have been extended in this study and indicate the following to date.

Fig. 2 Penile herpetic lesion on a male Cebus monkey at 18 days postinoculation of female cage contact.

A primary genital infection can be established in these animals with the development of genital lesions in approximately one-half of the infected monkeys. The lesions occur on the vulva and on the cervix, occasionally involving other sites, and the genital infection can often be detected by cytological techniques. The animals recover from the infection with no neurological or visceral complications, so that follow-up studies for the development of cancer are possible.

As in humans, spontaneous genital recurrences can occur in infected monkeys. The finding that exogenous reinfection is possible in these animals suggests that this mechanism might also be operative in humans. Reinfection was difficult to establish in recently infected animals. Since it is not clear at present whether primary or recurrent herpetic infection might be important in cervical carcinogenesis, attempts to reinfect at repeated intervals will be made.

Venereal transmission from genitally infected female monkeys to males, and occasional viral transmission between female cage mates, possibly by way of a male, has been observed.

The Cebus model of genital infection with HSV-2 offers several possibilities for the study of this virus in addition to its oncogenic potential. The model would be useful in vaccine studies and in immunological and serological studies, such as with nonviron antigens. Furthermore, with the increasing interest in the oncogenic potential of HSV-2 in male urogenital cancers, the preliminary observations that we have made with male genital herpetic infection in the Cebus monkey suggest further potential applicability of this model.

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