A Preliminary Report on Investigation of Oncogenic Potential of Herpes Simplex Virus Type 2 in Cebus Monkeys


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Summary

A total of 301 female and 133 male Cebus monkeys have been placed under study during a 3-year period. Females are inoculated intradermally into the cervix every 6 months; 225 receive virus and 76 receive control material. More than 50% of the animals were infected on primary inoculation, and a similar percentage was found on the 1st reinoculation.

Males are housed with females at 1:1 to 1:3 ratios. Eighteen % of the males exposed to virus-inoculated females have become infected. To date, a total of 55 pregnancies have produced 14 live births and 8 abortions. The remaining 33 animals are still pregnant. No neonatal herpes simplex virus type 2 infections have been identified.

Cytological changes of mild (atypia) to moderate (dysplasia) anaplasia have persisted for 12 to 32 months in 13 virus type 2 infections have been identified.

Introduction

The association between infection with HSV-2 and cervical carcinoma in humans is well documented (1-3, 5, 6, 7, 14-16, 18, 19, 20-24). Studies of the virulence of HSV-2 in laboratory nonhuman primates and its oncogenic potential in rodents have been reported (4, 7-11, 13, 17). One nonhuman primate model, the Cebus monkey, responds to infection with HSV-2 in a manner similar to that of humans (8, 9, 13). This study, utilizing the Cebus monkey model, was undertaken to determine the pathogenesis of HSV-2 in persistently reinfected female Cebus monkeys and its oncogenic potential in this genus.

Materials and Methods

The study utilizes 434 Cebus monkeys: 301 females and 133 males. For the females, 225 receive inoculations of virus and 76 receive control material at 6-month intervals. Because pregnancy is believed to play a role in the pathogenesis of cervical cancer in humans, males are housed with the females at ratios of 1:1 to 1:3, depending on the compatibility of each group. The development of the study is shown graphically at 3-month intervals (Chart 1). Those animals used in early studies (prior to March 1973) to determine the ability to infect, reinfect, and detect infection in female Cebus monkeys with HSV-2 have been retained; therefore, no control animals are available for that group. A lack of available animals during 1973 severely curtailed the development of the study. However, the supply became adequate in 1974 and the colony was rapidly increased to the required number.

Initially, inoculations were accomplished by inserting cotton pledges saturated with approximately 1.0 ml inoculum into the vagina and leaving them in place for 24 hr. The intradermal injection of 0.2 ml of virus into the cervix was found to produce a higher rate of infection and the same type of lesions seen with topical inoculation. Therefore, the intradermal inoculation procedure was adapted and is now used for all inoculations. Vaginal swabs, cervical smears, and blood sera are collected at appropriate intervals after each inoculation.

Pregnant females are inoculated without regard to the stage of their pregnancy. Neonates and aborted fetuses are examined for lesions, virus shedding, and serum antibody at birth. Tissues from aborted fetuses are examined for histological evidence of infection.

Vaginal and penile swabs are collected with 0.9% buffered NaCl solution (pH 7.2)-moistened cotton swabs which are rinsed in vials containing 2.0 ml of Hanks' balanced salt solution containing gentamycin. Samples are immediately frozen and held at -70°C until virus isolation attempts are made on primary rabbit kidney cell cultures.

Cervical smears are collected by firmly brushing the external cervix with a 0.9% phosphate-buffered NaCl solution (pH 7.2)-moistened cotton swab. The swab is then rolled gently on a clear glass slide and fixed while wet with an aerosol fixative (Spraycyte, Clay-Adams, Parsippany, N. J.). Smears are stained by the Papanicolaou technique and examined for cytological abnormalities.

Blood serum is separated into 2 samples and frozen at -20°C until use. One sample is utilized for immediate testing and the 2nd is stored for reference. The microneutralization
technique is used to detect HSV-2 neutralizing antibodies (11). A small group of control females receive inoculations of 10% salicylic acid in mineral oil every 6 months. This produces severe lesions with subsequent healing in the absence of HSV-2. The material is smeared onto the cervical os with a cotton swab. Samples are collected from these animals on the same schedule as from the virus-inoculated animals.

The Benefield strain of HSV-2 was chosen for use in this study for reasons reported earlier (9). The virus is produced on primary rabbit kidney cultures in large pools and stored in 5.0-ml aliquots at −70°C until use. Virus pool titers have ranged from $10^{4.7}$ to $10^{5.2}$/ml. The control inoculum is uninfected primary rabbit kidney cultures from the same rabbits used for virus production. For inoculations, 2 vials of inoculum are thawed and pooled in a sterile beaker immersed in wet ice. Samples of inoculum are collected prior to the 1st and immediately after the last inoculation for virus titration.

Animals with persistent cytological anaplasia are evaluated monthly when each is examined for lesions, and vaginal swabs, cervical smears, and serum samples are collected. Cervical smears from these animals are collected in duplicate and are examined by 2 cytopathologists.

**Results**

Results from 129 primary inoculations and 87 1st reinoculations of female Cebus monkeys are summarized in Table 1. Animals that shed HSV-2, produced detectable HSV-2 antibodies or developed cytological changes of giant cells with intranuclear inclusions after either primary or reinoculation were considered to be infected. While lesions were detected in a large percentage of the virus-inoculated females, many occurred only on the cervix and were very subtle, indistinguishable grossly from traumatic lesions caused by the manipulation of the vaginal speculum. Therefore, they were not considered reliable for the diagnosis of infection. Infection rates using the above criteria were nearly identical after both primary and the 1st reinoculation. Slightly more than 50% of the females became infected.

Nearly 40% of the females retained HSV-2 antibody beyond the 6-month interval between inoculations. Virus shedding occurred at a much lower rate after reinoculation (16.1%) than after primary inoculation (47.2%). The rate among animals which had HSV-2 antibody at the time of reinoculation was even lower (11%). In animals that became infected, 88% shed virus after the primary inoculation and 30% shed virus after reinoculation.

Cytological changes of herpes infection were found infrequently (11%) after primary inoculation and were rare (2%) after reinoculation.

There were 77 males exposed to virus-inoculated females for which laboratory results were complete; 14 had become infected (18%). All infected males produced HSV-2 antibody; 10 developed penile lesions, and virus was isolated from 2.

To date, of 55 pregnancies in the colony, 39 were virus-inoculated and 16 were control females. There have been 22 deliveries, including 14 live births and 8 abortions. Three of the live births resulted in early neonatal deaths. The remaining 33 pregnant females are presently at various stages of gestation.

No virus isolations or histological evidence of HSV-2 infection in neonates and aborted fetuses have been found. Two of 3 animals from mothers with HSV-2 antibody had antibody levels identical to those of their mothers at the time of birth.

Persistent cytological changes of mild to moderate anaplasia have been found in cervical smears from 13 virus-inoculated animals. Five animals have had diagnoses of both anaplasia and dysplasia while 8 have had only anaplasia. The 5 animals with dysplasia were first infected 15 to 50 months ago, with a mean time since 1st infection of 38 months. The 6 females with persistent atypias were first infected 14 to 28 months ago, with a mean of 20 months. None of the control animals have shown persistent cytological changes. Results from salicylic acid-inoculated females are not available.

**Discussion**

The Cebus monkey has been shown to be an excellent nonhuman primate model for the study of HSV-2 infection (12). Epidemiological evidence linking HSV-2 to cervical carcinoma in humans has been suggestive but not conclu-
sive. However, studies in rodents and tissue cultures have shown HSV-2 to be oncogenic in these systems (10, 17). The development of cervical carcinoma in female Cebus monkeys inoculated with HSV-2, but not in control recipients, would provide additional evidence to strengthen the theory of HSV-2 etiology in cervical carcinoma of humans. This genus could then also provide a model for the study of carcinogenesis of cervical carcinoma and a valuable tool for the study of prevention of the disease through vaccines, diagnosis, and therapy.

The development of persistent changes of mild to moderate anaplasia in cervical smears from HSV-2-inoculated female Cebus monkeys is encouraging. These changes have been found only in animals that have been studied for 14 or more months. Only 66 virus recipients and 7 control females have been studied that long. Therefore, the incidence of anaplasia among these virus recipients is 19.7%. However, these findings must be viewed with caution, since none have progressed to carcinoma. If the long, latent period suggested for the development of cervical cancer in humans is similar for Cebus monkeys, several more years may be necessary to provide conclusive evidence of etiology if the theory is correct.

References

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