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Herpesvirus and Cervical Cancer Studies in Experimental Animals[1]

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Summary

Several studies with small laboratory animals in which low rates of malignant changes of the cervix have followed intravaginal inoculation with herpes simplex virus type 2 have been reported. Recently, we found that, following intravaginal inoculation, Cebus monkeys develop cervical infections and lesions similar to those found in humans. A large-scale investigation is now in progress to determine whether the repeated infection of Cebus monkeys with herpes simplex virus type 2 will produce malignant changes of the cervix.

HSV-2[2] infection is transmitted as a venereal disease in humans. In some populations, the frequency of infection is very low. In a recent study of approximately 500 pregnant women in Los Angeles (who were tested twice during pregnancy), we did not isolate HSV-2 from any of the 1000 vaginal-cervical swabs. Serological tests for the detection of antibody to HSV-2 in the serum of pregnant women throughout the United States are now in progress. To date, approximately 12% of the women we have tested have antibodies to HSV-2. For these studies, we are using the hemagglutination method of Fuccillo *et al.* (3). However, high rates of infection have been reported among sexually promiscuous women and those with cervical lesions (10).

Because of the possible association between genital herpetic infection and cervical cancer in humans, a number of virological-epidemiological studies have been conducted. While some of these investigations have shown higher rates of antibody to HSV-2 among women with cervical dysplasia, *in situ* carcinoma, and invasive cancer, the results have been variable and can only be taken to suggest a possible association (8, 12). However, the isolation of HSV-2 from cervical cancer tissue supports the association, as does the evidence for transformation of hamster kidney tissue cells *in vitro* by some strains of HSV-2 (1, 2).

Experimental animals have been used in attempts to produce HSV-2 vaginal-cervical infection and possible cancers. The major studies are summarized in Table 1. Initially, Nahmias *et al.* (11) reported the inoculation of 1581 newborn hamsters by various routes (intrathoracic, s.c., or i.p.) with 16 strains of HSV-2. Nine of 710 hamsters that survived more than 3 weeks developed mesenchymal sarcomas at the site of inoculation. No HSV-2 antibody could be detected in the sera of the tumor-bearing hamsters, and virus could not be recovered from the neoplasms. Subsequently, Nahmias *et al.* (10) and Muñoz (7) reported that intravaginal HSV-2 inoculation of mice was occasionally associated with cervical atypia or carcinoma. In recent collaborative investigations, Nahmias *et al.* (9) and London *et al.* (6) found that, when inoculated intravaginally, Cebus monkeys develop cervical infection and herpetic lesions similar to that of humans. Reinfection can be produced repeatedly. These observations have provided the basis for a large scale study with Cebus monkeys on the possible association of this infection and cancer of the cervix. The observations have been confirmed by Kalter *et al.* (4), who used related Cebus monkeys. Other studies by Larson *et al.* (5) showed the susceptibility of rabbits to intravaginal infection and death.

Our investigations of Cebus monkeys, repeatedly inoculated intravaginally with HSV-2, are summarized in Table 2. This study is supported under contract by the Viral Biology Branch of the Viral Oncology segment of the National Cancer Institute. It involves the collaboration of Dr. William London, in our laboratory, with contracts to Dr. Zenoble of Meloy Laboratories for the animal phase and to Dr. Nahmias of Emory University Medical School for the laboratory phase of the research. Statistical review and direction is provided by Dr. Jonas Ellenberg of the Office of Biometry of the National Institute of Neurological Diseases and Stroke. A pilot study was first conducted to compare 11 candidate HSV-2 strains for possible use in this study. The criteria for selection of a strain were: (*a*) it must be isolated from a woman who developed cervical cancer; (*b*) it must transform *in vitro* or cause production of sarcomas in hamsters; (*c*) it must cause genital infection in monkeys, preferably with lesions; and (*d*) it must cause infection (preferably with lesions) when the monkeys are reinoculated. The pilot study showed that most of the strains tested produced vaginal and cervical infection of the Cebus. This result was documented by virus isolation and antibody response. Only 5 strains produced lesions. The virus titer used did not seem to be a major factor. In a 2nd pilot study, we attempted to determine whether Cebus monkeys had a high natural rate of dysplasia or carcinoma of the cervix. A total of 83 adult female Cebus monkeys were tested by means of the Papanicolaou test. Only 1 had any evidence of abnormal cervical cells, and this was regarded as only possibly atypical. The full study (Table 2) was then initiated. It involves the repeated intravaginal inoculation of young adult Cebus monkeys with HSV-2 virus. The Benefield strain of virus, which was selected

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

Table 1
Experimental genital HSV-2 infection (summary of published reports)

Investigators	Animals	Techniques	Findings
Nahmias *et al.* (10)	Mice	Intravaginal HSV-2 pledget. Approximately 1000 mice were inoculated; 200 survived 1 month, 50 survived more than 1 yr	Two mice developed cervical atypia or carcinoma *in situ*, 1 after 2.5 yr
Muñoz (7)	Mice	Mice (140) were immunized first with UV-inactivated HSV-2, then with intravaginal HSV-2 pledget	Forty % developed cytological changes of herpes; 2 developed squamous cell sarcomas of the cervix (7 and 9 mo.)
Nahmias *et al.* (9) London *et al.* (6)	Cebus monkeys (*Cebus albifrons*)	Intravaginal HSV-2 pledget	Infection and ulcerative lesions; reinfection; clinical and laboratory findings similar to those of humans
Kalter *et al.* (4)	Baboons, Cebus monkeys (*Cebus apella*), and marmosets (*Cebus oedipus*) HSV-2	Intravaginal pledget	Baboons: subclinical infection only; Cebus: infection & ulcerative lesions; marmosets: fatal generalized infection
Larson *et al.* (5)	Rabbits	Vaginal douching, HSV-2	Perineal lesions; paralysis of hind limbs; loss of sphincter control; encephalitis and death

Table 2
Study design
Summary of attempts to induce cervical cancer in Cebus monkeys exposed to HSV-2.

Animals	No. tested	Mode and frequency of infection	Laboratory data	Duration of tests (yr)
Test	225 females, 75 males	Tissue culture fluid containing HSV-2 inoculated every 2 mo	Preinoculation, postinoculation; cultures taken at 4, 7, and 14 days; Pap smears	3-5
Controls	75 females, 25 males	Tissue culture fluid	Same as above.	3-5

from the pilot study because it produces lesions and meets the other selection criteria, is being used in the full study. The animals are being housed 3 females and 1 male to a cage. We expect many pregnancies. These conditions were selected to maximize the possible production of cervical cancers, on the basis of their reported association with cervical cancers in humans. Experiments will continue for a period of 3 to 5 years.

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