Recurrent Genital Herpes simplex Virus (HSV) Infection of Guinea Pigs

Marianne Scriba
Sandoz Forschungsinstitut Wien, Vienna, Austria

Received September 27, 1976

Abstract. Intravaginal infection of guinea pigs with HSV type 2 induced lesions resembling genital herpes in humans. Chronic latent infection with spontaneously recurring genital lesions developed in animals surviving the primary disease. Clinical observations and virological studies during the acute and chronic infection are reported.

Introduction

The pathogenesis of recurrent herpes simplex virus (HSV) infections is still far from being fully understood. Latent HSV has been shown to reside in sensory ganglia of men and experimentally infected animals (Baringer, 1975; Stevens, 1975) and recurrent lesions are thought to develop due to reactivation of this latent virus. The mechanisms responsible for the control of the latency or reactivation of this virus, however, are still an intriguing problem to virologists.

Several animal models for studying recurrent Herpes simplex infections have been described in recent years. Ocular infection of rabbits (Nesburn et al., 1967), footpad infection of guinea pigs (Scriba, 1975) and ear pinna infection of mice (Hill et al., 1975) can all result in chronic latent infections with spontaneous recrudescences at the site of the initial infection.

In view of the possible relation of genital herpes and cervix carcinoma, animal models for recurrent genital herpes should be particularly interesting. The present report describes a genital HSV type 2 infection of guinea pigs, giving rise to a chronic infection with frequent spontaneously recurring herpetic lesions.

Materials and Methods

Virus. HSV Type 2, strain 72, was grown in primary rabbit kidney (PRK) cells in Eagle minimal essential medium without serum.

Animal Experiments. Female white guinea pigs, weighing 200—400 g, were inoculated intravaginally by instilling $10^4$ plaque-forming units of HSV in 0.1 ml. The animals were examined for clinical symptoms daily through the first 3 weeks after infection and 3 times a week later on.

Direct Virus Isolation. Vaginal swabs were taken with small pieces of sterile cotton which were immediately placed into 1 ml of Hanks balanced salt solution containing $5\%$ inactivated fetal calf serum (Hanks-5). Recurrent vesicular lesions were ruptured and then swabbed in the same manner. Pertinent organs were taken from exsanguinated animals, ground in a mortar with sea sand, and made up to a $5-10\%$ suspension with Hanks-5. Blood was defibrinated and serum and cells separated by centrifugation. The cells were disrupted by 3 cycles of freezing and thawing, and the supernates obtained after low speed centrifugation
were used for virus assays. All specimens were assayed by inoculation into PRK cells, which were then observed for cytopathic effect for up to 2 weeks.

**Virus Isolation from Explanted Tissue.** All organs to be assayed by explantation as well as by homogenization were cut into 2 approximately equal parts. One of these was processed as described above. The second half was minced finely, washed once in hanks and treated with 0.5% trypsin for 20 min at 37°C. The trypsin was removed, the tissue fragments were washed once in medium and then placed on monolayers of PRK cells. The medium was changed twice weekly and the cultures were observed for cytopathic effect for up to 50 days.

**Identification of Isolates.** All isolates, after a second passage on PRK cells, were identified by neutralization with a rabbit anti-HSV hyperimmune serum.

**Results**

**Clinical Observations.** Two to 3 days after infection the animals showed signs of inflammation of the external genital tract. Vesicles appeared between day 3 and 5, developing into confluent pustulae between 5 and 7 days. Considerable urinary retention usually accompanied this stage. Between day 7 and 9 the pustulae were exfoliated; the entire perineum became purulent and sometimes necrotic. In addition, paresis of the hind legs developed and most animals eventually died between 9 and 20 days after infection. Approximately 20% of the animals were usually less severely affected and were able to overcome the infections; lesions healed gradually and complete recovery was established within 2–4 weeks. In these surviving animals recurrent herpetic exacerbations were observed on the vulva. In contrast to the primary infection, the recurrent infections never produced severe symptoms. One or 2 vesicles surrounded by a red halo appeared, which never persisted longer than 2–4 days. The recurrent infection was not restricted to the genital area. As early as 2 weeks after primary infection herpetic lesions appeared in the skin of the hind footpads and recurred there later. These plantar exacerbations resembled exactly those recurrent lesions observed after initial footpad inoculations (Scriba, 1975). Recurrent lesions would sometimes appear simultaneously in the external genital tract and in both hind footpads, but more often, the different sites were affected at different times (Fig. 1). The frequency of recrudescent infections decreased with time after primary infection in most animals. Eruptions in the genital area as well as in footpads were observed, however, up to 1 year after inoculation, the longest time which animals have been observed.

In order to investigate a possible influence of pregnancy on recurrent genital herpes, latently infected guinea pigs were mated. In the 6 guinea pigs observed through a considerable time no significant increase or decrease in the frequency and severity of recurrent herpes could be observed during gestation periods (Fig. 1).

**Virus Isolations.** During the first 7 days after inoculation virus was isolated from the vagina, but not from the uterus (Table 1). No virus spread into the blood or lymphatic system could be demonstrated, but HSV was found to migrate early into nervous tissues. Two days after inoculation, virus was already detectable in the nerves supplying the genital tract, and could later on also be recovered from lumbosacral dorsal root ganglia, the lumbosacral part of the spinal cord and the sciatic nerve trunks.