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Optional Column E Explanation Form

This form is intended as an aid to completing the Column E explanation. It is not an official form and its use is voluntary. Names, addresses, protocols, veterinary care programs, and the like, are not required as part of an explanation. A Column E explanation must be written so as to be understood by lay persons as well as scientists.

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1. Registration Number: 51-F-0016

2. Number _____30_____ of animals used in this study.

3. Species (common name) _____Guinea Pig_____of animals used in this study.

4. Explain the procedure producing pain and/or distress.

Herpes simplex virus 1 and 2 (HSV 1 and HSV 2) are pathogens of human oral or genital herpes. Infection of humans by HSV 1 and HSV 2 results in local virus replication at the inoculation site (eye, oral or genital); then it spreads through sensory nerves to the regional ganglia (trigeminal or dorsal root ganglia) where the virus establishes a latent infection. Those viruses in ganglia can reactivate to cause recurrent outbreaks of disease over the course of a person's life. Both viral and host factors are thought to play a role in the maintenance of HSV 1 or HSV 2 in the latent state, and these factors may also influence rates of reactivation.

The mechanism of HSV latent infection and reactivation are poorly understood. Specific viral genes appear to be involved in the establishment, maintenance and reactivation of latency.

The animal model is essential for the study of HSV pathogenesis and latency due to the poorly understood interaction between virus and host that allow latency to occur, and is necessary for the study of vaccines, antiviral drugs or passive protection of antibody before clinical testing. No computer simulation or cell culture model has successfully mimicked the above processes.

This study is designed to evaluate the effects of mutations in different regions of the virus genome on the course of latent infection. The guinea pig is the only model for HSV2 acute infection and spontaneous recurrent disease. Spontaneous recurrent disease must occur in order to assess the effects of the mutations made in the virus genome. Smaller animals such as the mouse can be latently infected with HSV2, but they do not experience spontaneous recurrent disease.

5. Provide scientific justification why pain and/or distress could not be relieved. State methods or means used to determine that pain and/or distress relief would interfere with test results.

There are six possible routes of infection used to study herpes simplex virus pathogenesis in animal models. Virus can be given via the skin, ear, vagina, eye, footpad or systemically. Herpes infection at any of these sites can spread to central nervous system tissue and lead to death of the

animal from HSV-induced neuritis. We use the vaginal route of infection because we are most interested in viral latency and the regional ganglia serving these tissues are most amenable to study.

HSV infection may cause no lesion, vesicle formation, or ulceration. Animals may experience pain during the acute phase of infection with HSV2 depending on the severity of infection. The acute infection lasts 15 days after which the virus is latent. During this latent phase, although animals have spontaneous recurrences, the lesions are milder-from a reddening of an area to a small vesicle--and are not believed to be painful.

Animals are carefully observed and scored daily for the development and location of lesions post-inoculation.

Scoring of Lesions

Score 0	No lesion
Score 1	Focal redness and few small vesicles
Score 2	Several vesicles
Score 3	Progressive vesicle formation
Score 4	Ulceration and maceration and/or hindlimb paralysis

Acute infections must be permitted to occur and resolve in order to study latency and spontaneous recurrence. Lesions of the severity of a score 3 or 4 are only seen in the acute phase of the infection. Scores of 3 and 4 are believed to be painful for approximately 5 to 7 days after they develop. Hindlimb paralysis and/or bacterial superinfection of the genital ulcers may also develop during this time. Both genital lesions and hindlimb paralysis usually resolve within 2 to 10 days after onset. The lesions may then recur with recurrences usually lasting 2-3 days. Between recurrences, animals are asymptomatic. With time, recurrences become less frequent.

Anesthetic or analgesic agents cannot be used, as these agents would attenuate the immune response, interfering with disease pathogenesis and/or reactivation of latent infection.

Up to 60% of infected animals develop lesions warranting a score of 3 or 4. Of these, approximately 10% develop persistent macerations in the genital area that do not resolve after 10 days. If the genital macerations have not resolved by the 11th day, or one hindlimb is paralyzed for 11 days, the animal is euthanized.

The investigator must inform the veterinary staff of the commencement of studies which include animals that will experience pain or distress. When animals are placed on studies in which morbidity is anticipated, the experimental endpoint must be clearly indicated on the cage card and contact information for investigators MUST be provided to the veterinary staff. This will allow the veterinary staff to ensure appropriate monitoring of animals which are expected to exhibit more than minimal or transient morbidity prior to the experimental endpoint. In animals that are showing signs of paresis or paralysis, the urinary bladder will be monitored for urinary retention. The urinary bladder will be expressed at least once a day in animals experiencing