GUINEA PIG MODEL FOR CUTANEOUS HERPES SIMPLEX VIRUS INFECTION*

W. R. HUBLER, JR., M.D., TROY D. FELBER, M.D., DOUGLAS TROLL, M.S., AND MICHAEL JARRATT, M.D.

ABSTRACT

Previous animal models for cutaneous herpes simplex infections have been unsatisfactory because of atypical zosteriform progression of lesions and high animal mortality rates. By using a vaccination technique we have been able to produce lesions in guinea pigs which closely mimic human herpes simplex infection.

Herpesvirus hominis produces a spectrum of disease in man from vexing recurrent herpes labialis to life-threatening eczema herpeticum (Kaposi's varicelliform eruption) and herpes encephalitis. Myriads of therapeutic regimens have proved ineffective. Paramount to the study of disease therapy and pathogenesis is the development of an animal model in which the clinical disorder under study can be consistently and accurately reproduced. Thus far, animal models for cutaneous herpesvirus infections have failed to fulfill these criteria.

As early as 1923 Teague and Goodpasture induced herpesvirus lesions in guinea pigs by intradermal injection of herpes simplex vesicle fluid into scarified skin [1]. The lesions, however, could be induced only in skin which had been repeatedly pretreated with topical crude coal tar. Furthermore, the lesions progressed from the inoculated site to involve entire dermatomal areas in a zosteriform fashion. Zoster-like progression of lesions following focal cutaneous herpes simplex inoculation was observed also by Tanaka and Southam in newborn rats [2], by Sydiskis and Schultz in Swiss albino mice [3], and recently, by Constantine et al in hairless mice [4]. Although the phenomenon of herpesvirus spread along the distribution of cutaneous sensory nerves offers potential for the study of varicellazoster and possibly of reservoirs for recurrent herpes labialis, the zoster-like lesions are not analogous to focal cutaneous herpes simplex infection in man. Another disadvantage of the animal models described above is that 50–70 percent of the animals die from central nervous system infection or progressive systemic viremia [2, 3, 5, 6]. In 1964, Force, Stewart, and Haff produced localized herpetic lesions in rabbits by the simultaneous intradermal injection of hyaluronidase and viral suspension [7]. The lesions were clinically similar to focal human infection with herpes simplex, but the addition of a tissue degradative enzyme introduced a variable in the animal model which is unlike the human infection. In 1964 Platt reported local infections in guinea-pig flank and tongue but did not elaborate on his method of inoculation [8]. In 1972, Kalter, Felsberg, and Heberling induced vulvar and vaginal lesions in nonhuman primates by suturing virus-soaked cotton pads in the vagina [9]. Tomlinson and McCallum produced cutaneous herpes simplex infections in guinea pigs with Herpesvirus hominis and a vaccination needle [10]; however, over one-half of the animals developed paralysis of the hind limbs 7 to 8 days after inoculation unless hyperimmune serum was injected 4 days prior to inoculation.

The purpose of this communication is to describe a method of inducing focal herpes simplex infection in guinea pigs which simulates human disease in clinical appearance as well as in duration and course of disease.

MATERIALS AND METHODS

Animals. Adult male and female albino guinea pigs (300–400 gm) were isolated from other laboratory animals and maintained in the customary fashion with Purina Guinea Pig Chow and lettuce once weekly.

Virus preparation and strain. The Stohr strain of Herpesvirus hominis was obtained from an active herpetic lesion on a patient with recurrent herpes labialis and identified as type 1. Virus stocks were harvested after a single cycle of growth in immature rabbit kidney cells in Eagle's medium. At 16 and 24 hr when cytopathic effects were widespread, cultures were frozen and thawed once; the harvest was clarified by centrifugation at 2000 rpm for 10 min; and the supernatant fluid was then stored at −80°C. The viral suspension was assayed in rabbit kidney cell culture and standardized to contain 1 × 10^6 plaque-forming units/ml (PFU/ml).

The DeGuewin strain was obtained from an active lesion of herpes progenitalis and identified as type 2, Herpesvirus hominis. Viral suspensions were prepared as described above.

Procedure. Guinea pigs were anesthetized with intraperitoneal nembutal sodium; the hair on the dorsum of the guinea pigs was plucked; and the area was shaved with electric clippers followed by the liberal application of a chemical depilatory (Surgex) for 10 min. The area was washed with water and dried with a soft cloth and warm air from a small hair dryer. A grid with six or eight squares was drawn on the epilated area with a marking pen.

0.02 ml of the undiluted viral suspension was applied to each square of the grid and inoculated with a spring-
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loaded vaccination instrument (Sterneedle, Pan Ray Division, Ormont Drug, Englewood, New Jersey) which produced a ring of six inoculation sites to a depth of 0.75 mm. A small vesicle formed at the puncture site of each of the six times, but in order to produce a more substantial clinical lesion the instrument was triggered 10 times in each square. The inoculation sites were allowed to air dry for 5 min before returning the animal to its holding cage. Each guinea pig was evaluated daily for the development of lesions.

RESULTS

Three days after inoculation the treated sites became erythematous and slightly edematous. At 4-5 days small vesicles appeared at penetration points of the vaccination tines. The vesicles gradually enlarged and, if in close juxtaposition, coalesced. The fully developed lesions consisted of discreet and coalescing vesicles on a 2-cm-diameter erythematous base (Figs. 1, 2). During the next 4-7 days, the vesicles dried, crusted, and progressed to complete healing by 10-12 days after inoculation. No animal developed zosteriform lesions or perished of neurologic involvement.

Using the viral concentration of $10^7$ PFU/ml, fully developed lesions appeared in every inoculated site and evolved as described above with only a matter of a few hours' variation from animal to animal. In a 2-year period over 500 guinea pigs were inoculated, with subsequent development of clinical lesions in virtually every inoculated site. However, inoculations with viral suspensions of less than $10^7$ PFU/ml failed to produce lesions with such consistency. Abortive, rapidly healing lesions were produced in animals previously inoculated. Consequently, new animals were used in each series of experiments.

To confirm the etiology of the vesicular eruption, the following series of examinations were performed:

1. Tzanck smears on a series of 10 guinea pigs revealed multinucleated syncytial cells characteristic of herpes-varicella viral infections (Fig. 3).

2. Histopathologic examination of biopsies of the vesicles revealed balloon cell degeneration, spongiosis, and intraepidermal vesicles containing acantholytic cells and multinucleated giant cells.

3. Fluid was taken from newly formed vesicles and assayed on rabbit kidney cell tissue culture. *Herpesvirus hominis* was recovered at a concentration of $3 \times 10^4$ PFU/ml.

COMMENT

Herpes simplex infections in man remain a therapeutic and pathogenetic enigma to investigators. The lack of an animal model for basic investigation has hampered the study of herpetic disease. The drawbacks in previously reported animal models include the following: frequent mortality or morbidity of the test animal after...
FIG. 2: Grouped, coalescing vesicles on an erythematous base simulated human herpetic infection.

FIG. 3: Multinucleated syncytial giant cells on Tzanck smears were characteristic of those from human herpetic lesions.
inoculation of virus, atypical lesions such as zosteriform progression after inoculation, inconsistent development of disease following inoculation procedures, difficulty of working with small newborn animals, and, in some models, requisite simultaneous injection of virus and degradative enzyme, or preparatory injection of hyperimmune serum to prevent neural dissemination.

The Stohr strain of Herpesvirus hominis was inoculated into hairless mice according to the technique described by Lieberman et al [5]. Five days after inoculation, lesions developed in a zosteriform pattern and there was 70 percent animal mortality. Thus the Stohr strain of Herpesvirus hominis was demonstrated to be as neurovirulent as the viral strain used by Lieberman et al.

We have presented a model which has the following advantages:

1. Guinea pigs are readily available, inexpensive, and large enough for easy handling and interpretation of experimental results.

2. The inoculation procedure is quick, 100% consistent in inducing lesions, and apparently innocuous to the experimental animal.

3. The lesions produced mimic human herpes simplex infections in clinical appearance, in evolution, and in duration of disease.

4. Percutaneous absorption of therapeutic agents in guinea-pig skin is analogous to that in human skin [11], thus allowing for extrapolation of therapeutic results in the animal model to the human disease.

We hope this reliable and practical animal model will stimulate further research in pathogenesis and therapy of Herpesvirus hominis.

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REFERENCES


