ELSEVIER

Antiviral Research 38 (1998) 43–53

Antiviral Research

# Herpes simplex virus zosteriform lesions with adoptive transfer of immune cells: a murine model which mimics human recurrent disease

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Received 13 August 1997; accepted 22 December 1997

**Abstract**

Existing murine models for cutaneous herpes simplex virus type 1 (HSV-1) infection have limited relevance to recurrent disease in humans, since the infection is usually primary rather than reactivated and infection occurs in the absence of an established immune response. To obtain a reproducible model to study the effects of topical antiviral therapy on recurrent disease we have adapted a mouse model which employs zosteriform spread of HSV-1 in the presence of adoptive transfer of immunity (ATI) which mimics human recrudescent lesions. Mice were infected with HSV-1 by scarification at the lateroventral line of the neck; 2 days later, the mice received adoptive transfer of immune cells from the cervical lymph nodes of syngeneic mice that had been infected in the ear pinna with the same strain of virus 7 days earlier. ATI resulted in a heightened inflammatory response in the target tissues for virus replication. Virus was cleared more quickly from the infected tissues in comparison with mice similarly inoculated without ATI, however, the intensity and duration of the inflammation was greater. The model was then used to test the effect of a topical formulation of foscarnet. The results presented demonstrate that the ATI model can provide useful data concerning the efficacy of topical antiviral chemotherapy in man. 

*Keywords:* Herpes; Immune cells; Virus; Topical; Foscarnet; Antiviral

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0166-3542/98/$19.00 
*PII* S0166-3542(98)00003-5

## 1. Introduction

Herpes simplex virus types one and two (HSV-1 and HSV-2) are very common infections in man world-wide producing recurrent orofacial and genital lesions, respectively (Nahmias et al., 1989; Roizman and Sears, 1990). Topical antiviral agents have been used to treat such infections since the introduction of idoxuridine in the early 1960s (Juel-Jensen and MacCallum, 1964) and latterly using formulations of aciclovir (ACV) (Spruance et al., 1984, 1986) or penciclovir (Spruance et al., 1997). The efficacy of topical antiviral therapy is, however, not completely satisfactory and it is very difficult to determine precisely in man, the factors that limit the successful outcome of such therapy.

HSV-1 and HSV-2 readily produce experimental infections in several laboratory animals including mice, guinea pigs, rats or rabbits and, according to the species and route of inoculation, the infection produces patterns of disease that mimic the various syndromes observed in man. However, while it is relatively straightforward to produce an infection model for acute disease, it has been more difficult to obtain reproducible and synchronous recurrences in mice (Wheeler, 1975; Hill et al., 1978; Openshaw et al., 1979; Harbour et al., 1983; Blyth et al., 1984; Stanberry, 1994; Shimeld et al., 1996a) in order to test prophylactic (Blatt et al., 1993) or chemotherapeutic regimens to combat recurrent disease (Douglas et al., 1984; Yang and Datema, 1991). The circumstances in which reactivation of infection occur in the immune individuals leading to recurrent cutaneous lesions are complicated by inflammatory changes. These appear to differ from those which occur during the primary infection and lead to pain and discomfort associated with recurrent herpes labialis, although the presence of infectious virus in the lesion may be transitory (Stanberry, 1994; Shimeld et al., 1996a,b). The present paper describes a new model which is based on a murine zosteriform infection model that was first described by Blyth et al. (1984), which attempts to reproduce at least some of these effects in order to investigate those factors that determine the outcome of topical therapy by specific antiviral agents.

## 2. Materials and methods

### 2.1. Virus

HSV-1 (strain SC16) was originally isolated from a clinical case of herpes labialis (Hill et al., 1975). Virus was grown in baby hamster kidney cells (BHK-21) following established procedures (Field et al., 1995) and stored at $-70°C$. The strain has been used extensively for antiviral studied in several different laboratories

### 2.2. Inoculation of mice

Three days before virus inoculation, female Balb/C mice (16–18 g) were anaesthetised and depilated on the right side of the neck. Inoculation was performed as described by Blyth et al. (1984). Briefly 15 $\mu$l of virus suspension (containing $2 \times 10^5$ plaque forming units (p.f.u.)) was placed on the skin in an area of 1 $cm^2$ at the site of scarification (right side of the ventral surface of the neck, $\approx 0.5$ cm lateral to the ventral mid-line) then 8–10 superficial light strokes were made by a hypodermic needle in a criss-cross pattern parallel to craniocaudal and ventrodorsal axes to produce the scarification.

### 2.3. Adoptive transfer of immunity (ATI)

A group of immune donors were prepared by inoculation of HSV-1 ($10^5$ p.f.u.) into the skin of both left and right ears of anaesthetised female Balb/C mice. The donors were euthanased 7 days post infection. The cervical draining lymph nodes were removed and a suspension of lymph node cells was prepared in phosphate-buffered saline. The cell suspensions were sieved through sterilised muslin cloth; viability was assessed by means of a trypan blue dye exclusion test and recipient mice were given $1 \times 10^7$ live immune cells via the coccygeal vein. The recipient mice had been infected two days previously with HSV-1 by neck scarification (see above). Control recipient mice received no cells. The experimental design is illustrated in Fig. 1.

### 2.4. Disease assessment

#### 2.4.1. Clinical signs

Groups of ten mice were used for clinical observation, zoster score, determination of ear thickness and body weight while further groups of 16–18 mice were used for virus isolation at various times post infection (p.i.). Mice were examined daily for clinical signs at both the primary and secondary sites of virus replication up to day 12 p.i. and then on days 15, 20, 25 and 30 p.i.

#### 2.4.2. Ear thickness

The thickness of the ear pinna, ipsilateral to the infection, was measured daily using an Engineers' micrometer (Field et al., 1979). The contralateral (left) ears were measured for comparison.

#### 2.4.3. Lesion severity (zoster score)

The development of lesions on the neck (primary site) and pinna (zosteriform spread) was monitored visually using a magnifying glass. Lesions were scored according to the following scale first at the primary inoculation site then at the secondary site: $-4$ no clinical signs; $-3$ one vesicle and swelling; $-2$ more than one vesicle; $-1$ local erosion; 0 ulceration of the local lesion; 1 primary lesions plus isolated zosteriform lesion; 2 mild; 3 moderate; and 4 severe ulceration of confluent zosteriform lesions (Nagafuchi et al., 1979). Thus the scores $-4$ to 0 describe the lesion development at the site of inoculation (primary lesion), whereas scores $+1$ to $+4$ describe the development of the zosteriform lesion (zoster score) at the secondary site. For reasons of clarity, only positive scores (i.e. those relating to the secondary site where chemotherapy was applied) have been illustrated in the graphs in this communication. The swelling erythema, itching, scratching, pain, and behaviour of the animals at the time of measurements of zoster score and ear thickness were scored by subjective visual assessment.

### 2.5. Histopathology

For histological study, ear samples were fixed in 10% formal saline and stained with haemotoxylin and eosin following standard methods.

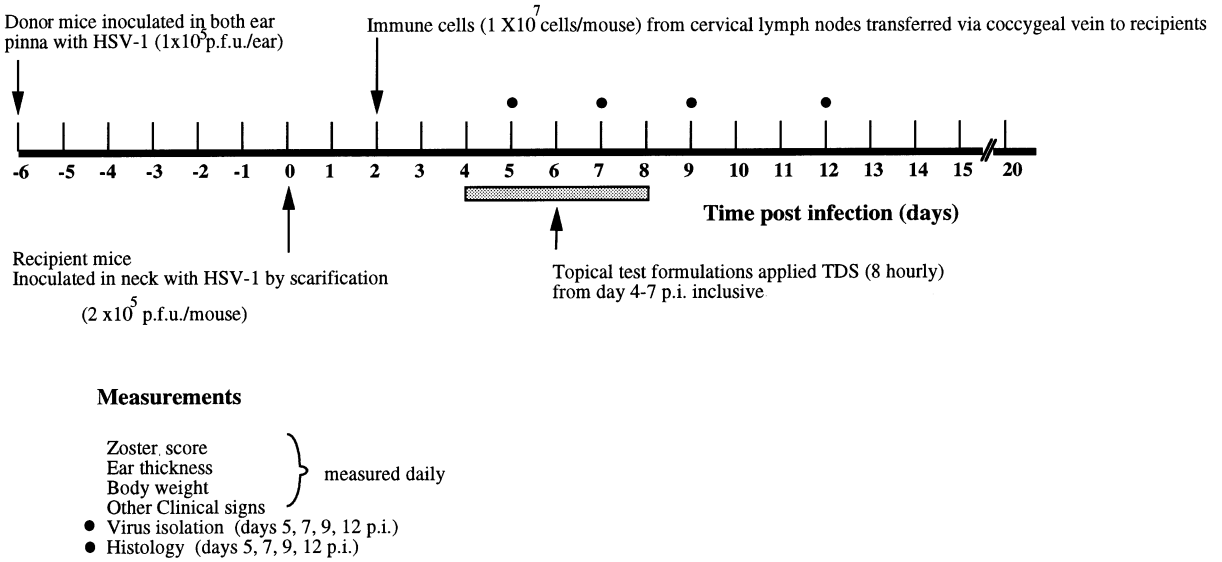


Fig. 1. Experimental design. The relationship between the primary infection and the day of transfer of immune cells (ATI) to recipient mice that had been infected 2 days previously by scarification of the neck with HSV-1 as shown on the temporal scale. Recipient mice were treated by topical application of foscarnet cream TDS at 8 h intervals from day 4 to day 7 p.i. inclusive. Ear thickness, zoster score, body weight and other clinical signs were measured daily while virus titration was carried out on days 5, 7, 19 and 12 p.i.

### 2.6. Titration of tissues

On days 5, 7, 9 and 12 p.i. four mice from each experimental group were euthanased and the ear ipsilateral to the inoculation site was removed, placed in 1 ml Eagle's minimal essential medium (EMEM), and stored whole at −70°C. With topical treatment at the infection site, there is a possibility that the antiviral drug may be present on the ear samples for virus isolation. The skin was therefore washed thoroughly before virus titration. The theoretical maximum concentration of foscarnet in the titration was not considered high enough to affect the plaque counts obtained. To test for infectious virus, skin samples were homogenised in a glass homogeniser and titrated in BHK-21 cells as described by Field et al. (1995). Virus plaques were stained and counted after 3 days incubation.

### 2.7. Application of topical foscarnet

Mice were treated topically three times a day (TDS) at 8 h intervals i.e. 08:00, 16:00 and midnight with topical foscarnet cream (3% foscarnet in a cream base) or placebo cream (base without foscarnet). Approximately 25 mg of cream was applied on both surfaces of the ear flap of the ear ipsilateral to the infection site, and rubbed thoroughly with thumb and index finger. The topical treatment was started at day 4 p.i. and continued to day 7 p.i. (Fig. 1). For treatment, mice were divided into four groups (1–4). Groups 1 and 3 received no ATI while 2 and 4 received ATI. Groups (1) and (2) were treated with placebo while groups (3) and (4) were treated with foscarnet cream. In order to avoid cross-contamination, placebo cream was applied first and gloves were changed between each experimental group.

### 2.8. Statistical tests

Tabulated values were tested by the Student's $t$-test (two tailed test for paired data) and differences were considered significant when $P < 0.001$.

### 2.9. Results

#### 2.9.1. Inoculation of mice without ATI

Two to three days after inoculation, vesicles developed at the inoculation site on the skin of the right ventral side of the neck. Initially the lesions were difficult to see with the naked eye but were readily visible under a magnifying glass. Inflammation was also observed in the ipsilateral (right) ear pinna. The first sign of erythema was observed at the base of the ear at 4–5 days p.i. On days 5 and 6 p.i. further vesicles developed with a zosteriform distribution from the neck to the ear pinna. Numerous small vesicles were noted at first which fused to form large vesicles over a period of 2–3 days. The distribution of lesions was highly reproducible among the individual mice. Concurrently, a marked increase in the ear thickness was evident starting from day 5 to 6 p.i.

#### 2.9.2. Inoculation of mice with ATI

The transfer of immune lymphocytes (ATI) that had been obtained from Balb/C mice inoculated 7 days previously in the ear pinna with HSV-1 had a marked effect on the pathogenesis of the primary infection in recipient mice evidenced by increased ear thickness, zosteriform score and histopathological changes.

#### 2.9.3. Ear thickness

Mice given ATI consistently showed an increase in ear thickness from day 3 p.i. (24 h after ATI) compared with mice that did not receive ATI (Fig. 2a). This increase in ear thickness in mice that received ATI was greatest between days 5 and 10 p.i. and was statistically significant on days 5, 6, 7, 8 and 9 p.i. ($P < 0.001$), although a trend to increase a thickness was observed at other time points. No increase in the ear thickness of the contralateral ears (with or without ATI) was observed during the experiment.

#### 2.9.4. Zoster score

In groups of mice given ATI, higher zosteriform scores were recorded from 1 day after the transfer of immune cells (day 3 p.i.) at the pri-

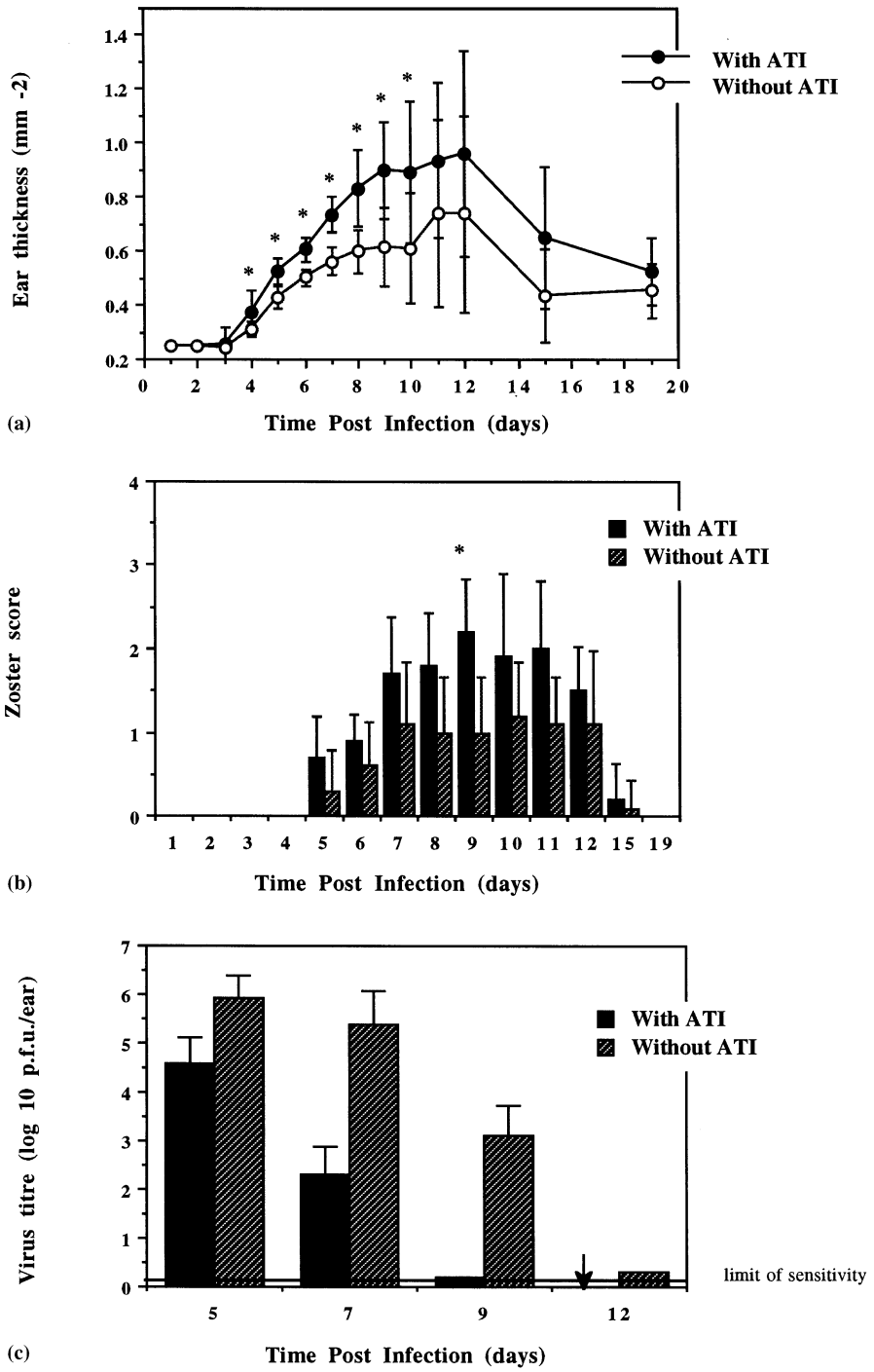


Fig. 2. The effect of transferring immune cells (ATI) on the pathogenesis: ear swelling (Fig. 2a), zosteriform spread (Fig. 2b) and virus isolation (Fig. 2c). Ear thickness and zoster scores were the mean values ( ± S.D.) of ten mice tested at each time while virus titres were the mean values ( ± S.D.) of four mice tested. * Indicates that the values with and without ATI are significantly different ($P < 0.001$). The horizontal line (Fig. 2c) represents the limit of sensitivity of the assay.

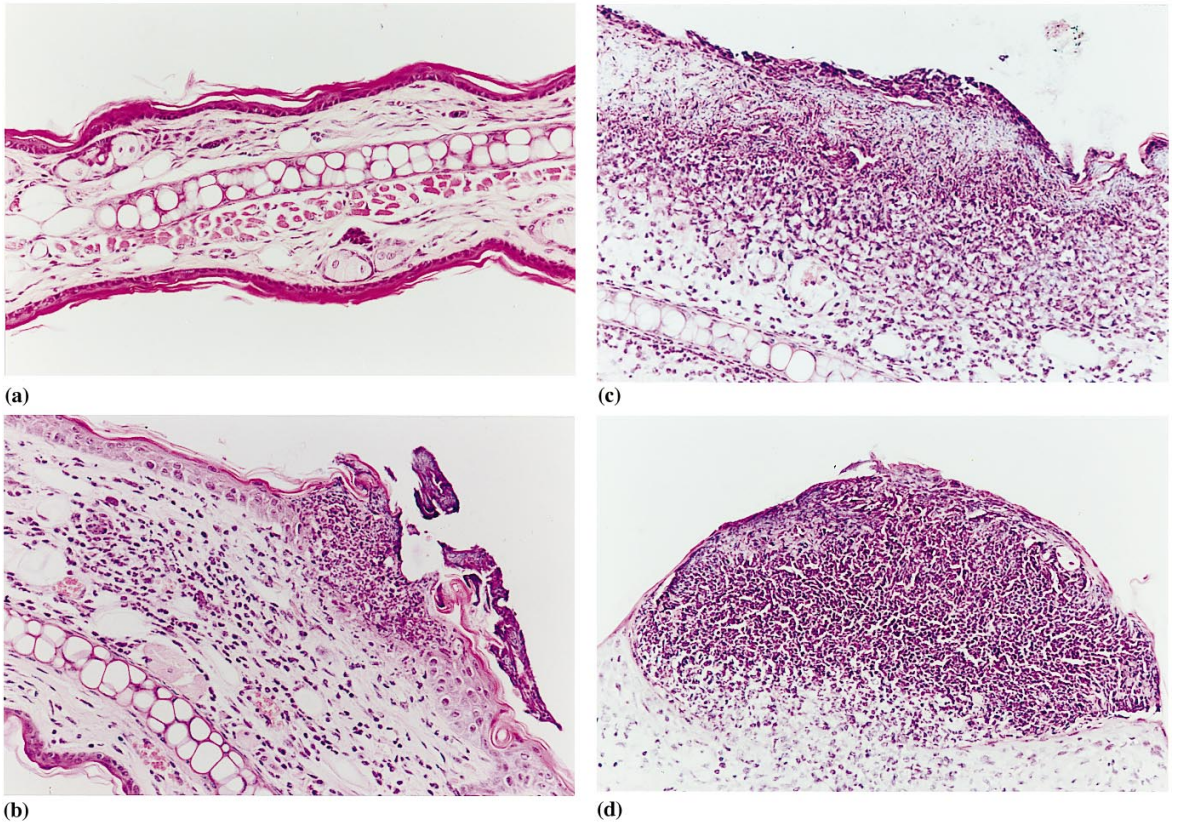


Fig. 3. The effect of transferring immune cells (ATI) on the histopathological changes. Negative control (Fig. 3a): infected ear without ATI on day 7 p.i. (Fig. 3b): infected ear with ATI on days 7 and day 9 p.i. (Fig. 3c,d). Magnification: $\times 260$.

mary site of infection. The increased zoster scores was significant ($P < 0.001$) on day 9 p.i. (Fig. 2b). On days 5–9 p.i. inflammation and apparent pain (as judged by the behaviour of the animal at the time of measurement) were also more pronounced in groups of mice given immune cells. At later times ($> 9$ days p.i.) scratching appeared to increase the severity and duration of lesions compared to non-ATI mice, and healing was delayed (data not shown).

#### 2.9.5. Virus isolation

ATI had a modulating effect on virus titres in the ear pinna with significant reductions seen on days 5 and 7, and virus was cleared by day 9 p.i. Without ATI, infectious virus remained in the ear pinna till days 9 p.i. (Fig. 2c).

### 2.10. Histology

Histological sections showed areas of necrosis and inflammatory changes characteristic of herpesvirus-associated cytopathological changes. Numerous inflammatory cells were present in both ATI and non-ATI mice (Fig. 3a,b). However, the histopathological changes were more pronounced, with more intense infiltration of cells at the site of lesions in the ear sections obtained from mice given ATI compared to non-ATI mice tested on days 5, 7 and 9 (Fig. 3c,d).

### 2.11. Topical chemotherapy with 3% foscarnet cream

The ATI infection model was then used to assess the effectiveness of the topical application

of foscarnet cream. Three parameters (virus titre in the ear pinna, ear thickness and zoster score) were measured.

*2.11.1. Ear thickness*

Application of foscarnet for a period of 4 days (from days 4 to 7 p.i. inclusive) reduced the ear thickness from days 5 to 10 p.i. (Fig. 4a). However, this reduction was not significant. A slight increase in ear thickness was noted in the groups of mice treated with foscarnet in the days following the termination of therapy. Although the increase was recorded at a single time-point only, this observation was reproducible.

*2.11.2. Zoster score*

The application of foscarnet reduced the zoster score but the reduction was not statistically significant compared with placebo-treated mice. Furthermore, inflammation, scratching, apparent pain and zosteriform lesions were not prevented. The development of the zosteriform lesions were not significantly different from placebo-treated animal with ATI (Fig. 4b)

*2.11.3. Virus titres*

Whilst the topical therapy with foscarnet did not reduce the inflammation or zosteriform spread, it showed a marked effect on virus replication on days 5 and 7 with virus titres significantly reduced ($P < 0.001$) compared to placebo. Approximately one $\log_{10}$ reduction was noted on each of days 5 and 7 p.i. and virus was cleared earlier in foscarnet-treated compared to placebo-treated mice (Fig. 4c).

## 3. Discussion

Various laboratory models have been used to investigate the mechanisms of acute herpesvirus infection. In the classical mouse ear model experimental approaches often introduce large amounts of virus deeper in the skin, associated with some degree of tissue disruption. Virus travels to the trigeminal ganglia and establishes latent infection (Baringer and Swoveland, 1973; Hill et al., 1983; Sawtell and Thompson, 1992a,b; Stanberry, 1994). In the present zosteriform infection model, virus is introduced by scarification in an attempt to limit the infection to superficial tissues and the method does not favour deep penetration of virus. Following scarification, zosteriform translocation of HSV through the sensory nerves leads to the formation of lesions at distant areas of the same neurodermatome (Simmons and Nash, 1984; Hill, 1985; Stanberry, 1994).

In mice that recover from primary HSV-1 infection, virus becomes latent in the neurons of the sensory ganglia which can be reactivated by various stimuli (Stevens and Cook, 1971; McLennan and Darby, 1980; Sawtell and Thompson, 1992a,b; Steiner and Kennedy, 1995; Shimeld et al., 1996a,b ). On reactivation, centrifugal spread of virus may give rise to recurrent (asymptomatic) or recrudescent (symptomatic) lesions at or near the original site of infection (Perna et al., 1987; Spruance, 1988; 1992). In man, recrudescence is often associated with burning and painful lesions thought to be associated with virus replication, and infiltration of the inflammatory and immune cells at the site of the lesion (Harbour et al., 1983; Stanberry, 1994; Shimeld et al., 1996a,b). Thus, it appears that the presence of inflammatory cells at the site of lesion contributes to the itching, burning and painful sensation in recrudescent disease. Healing is delayed in patients who experience severe prodromal signs (Spruance et al., 1977, 1990). Studies in both the rabbit and murine models and human subjects have suggested that cell-mediated immune responses contribute significantly to the pathogenesis of HSV disease (Myers and Pettit, 1973; Nash et al., 1981, Simmons and Nash, 1984; Hendricks and Tumpey, 1990; Niemialtowski and Rouse, 1992; Spruance et al., 1995).

In recurrent human HSV, the spread of the virus is from ganglion along the nerve to the skin. A vigorous local immune response producing inflammation is rapidly mounted when the virus enters the skin. Previously, a murine zosteriform infection model was reported in which virus travels from the ganglion to the ears of mice previously infected in the neck with HSV-1 (Blyth et al., 1984; Kristofferson et al., 1988). However, zosteriform-spread occurs before the immune response fully developed (Blyth et al., 1984; Sim-

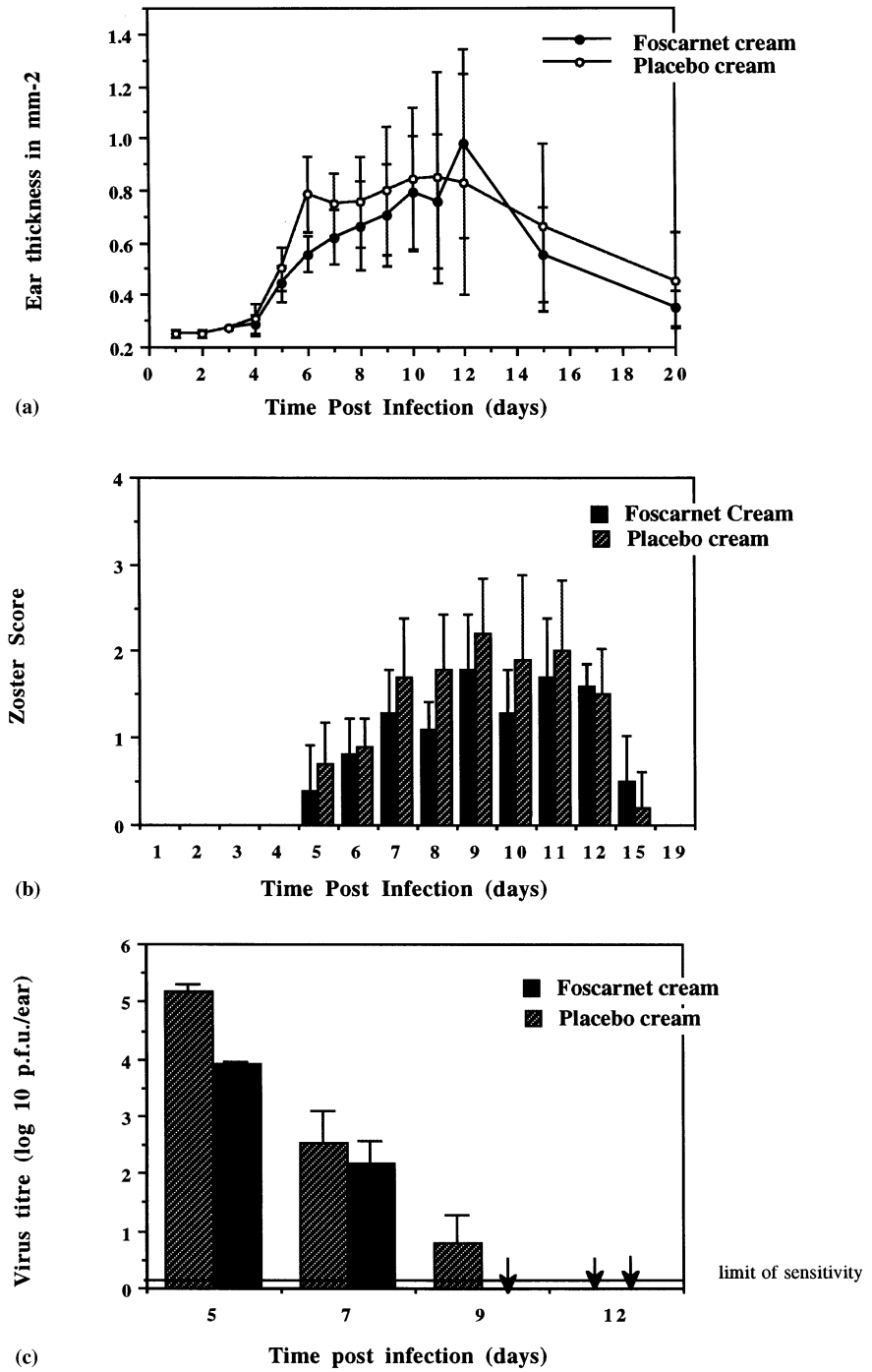


Fig. 4. The effect of topical treatment of foscarnet on the pathogenesis of infection in ATI mice. Ear swelling (Fig. 4a), zosteriform spread (Fig. 4b) and virus isolation (Fig. 4c). The horizontal line (Fig. 4c) represents the limit of sensitivity of the assay.

mons and Nash, 1984; Hill et al., 1984). We have further developed the zosteriform infection model by accelerating the development of cellular immunity so that a more advanced immune response is present when the virus enters the distal sites of replication (ear pinna), which occurs 3–4 days after infection in the neck (Blyth et al., 1984; Simmons and Nash, 1984; Kristofferson et al., 1988).

Previously, the immunity to primary HSV-1 infection in experimental animal has been extensively studied (Nash et al., 1980a,b; 1981; Kapoor et al., 1982; Nash and Wildy, 1983). Simmons and Nash (1984) reported the role of immune lymph nodes in zosteriform infection model in accelerating the clearance of virus from the primary site of replication. They also reported that once ganglionic infection is established the appearance of the zosteriform rash was not prevented by transferring of cellular immunity. To allow a more advanced immune response to be available when the virus reaches the ear, we transferred the cells (ATI) after ganglionic infection has been established i.e. 2 days after infection on the neck of the recipients when virus has reached the ganglia but not on the distal site of secondary replication (the ear pinna) (Blyth et al., 1984; Simmons and Nash, 1984; Kristofferson et al., 1988). Under these conditions we believe that human recurrent disease is closely mimicked, since herpetic lesions result from the local replication of reactivated virus in the presence of heightened immunity (Hill, 1985). ATI reduced the virus titre and caused infectious virus to be cleared one day earlier than the non-ATI controls. The lesions remained in all mice at later time points although virus was cleared more effectively with ATI. However, clinical signs increased including ear thickness, and heightened inflammation and painful sensation at the site of secondary replication were reminiscent of an episode of human HSV-1 reactivation.

An important observation in this paper is that the clinical signs were increased by ATI compared with the standard model although virus titres were lower. Therefore, we argue that the ATI model poses a greater challenge for chemotherapy than the standard model and we demonstrate that the infection parameters in the ATI model can be used to evaluate antiviral efficacy using foscarnet. Without ATI, the clinical signs are less, although virus replication persists for longer. Under these conditions foscarnet was also effective, although the data was not shown in the present communication.

This improved zosteriform infection model with ATI has several advantages for the testing of antiviral preparations compared to previous models. Topical treatment with foscarnet for 4 days from the start of clinical signs (day 4 p.i.) resulted in earlier clearance of virus than in untreated animals (with ATI), though pain, swelling and inflammation were not prevented. This corresponds closely to the observed clinical effects of foscarnet in the clinical trials of topical treatment of labial and genital herpes in man (Barton et al., 1986; Sacks et al., 1987). Increase in the ear thickness after the termination of topical foscarnet therapy was observed in mice with ATI (Fig. 4a) or without ATI (data not shown) suggesting that either there might be a 'rebound' on cessation of therapy or an irritant effect of foscarnet on the skin of mice; this needs further investigation. Increase in ear thickness, itching and apparent pain at the site of zosteriform spread, were not prevented by foscarnet therapy. More recently we have shown that antiviral formulations containing combination of anti-inflammatory and or local analgesics may help to reduce the pain and inflammation as well as virus load (Awan et al., 1998).

In conclusion we report an improved zosteriform infection model with ATI which we believe closely mimics human recrudescent disease. The model also parallels healing time, pain and itchiness associated with the infiltration of inflammatory cells into the lesions and should provide more useful data on the likely clinical effects of topical antiviral treatment for recurrent labial or genital herpes in immunocompetent patients.

## Acknowledgements

ARA and HJF wish to thank Astra Arcus AB Sweden for providing the financial support to

carry out the project. We are thankful to Susanne Strandberg and Per Broberg for their help in statistical analysis; Dr Alf Larsson and Ola Flink for their critical but helpful discussion and the Pharmacy Department of Astra Arcus AB for providing the topical formulation of foscarnet.

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