Ocular Infection With Herpes Simplex Virus in Several Strains of Rat

Susan M. Nicholls,* Amina Benylles,* Carolyn Shimeld,* David L. Easty,† and Terry J. Hill‡

Purpose. To assess the suitability of the rat for studies of ocular infection with herpes simplex virus (HSV).

Methods. LEW, AO, DA, PVG, and (DAxLEW)F1 X LEW backcross generation rats, 7 to 9 weeks of age, were inoculated with HSV-1 McKrae. The course of primary disease was assessed by clinical observation using a slit lamp. Infectious virus was assayed in ocular and nervous tissue, and the incidence of latent infection was determined.

Results. LEW and AO strains were the most susceptible. All LEW rats died after an inoculum of 4 X 10² plaque-forming units (pfu) and developed severe corneal disease and uveitis. In contrast, all PVG rats survived 10⁴ pfu, 60% survived 4 X 10⁴ pfu, and eye disease was restricted to epithelial lesions, sometimes accompanied by mild stromal haze. This resolved, even in animals that developed central nervous system disease. The DA strain showed intermediate susceptibility. Resistance was dominant because disease in backcross generation (DA X LEW)F1 X LEW rats resembled that of the DA rather than the LEW strain. Resistance appeared to be linked to coat color (P < 0.001) rather than to major histocompatibility complex (MHC) type. Chronic stromal disease did not occur in survivors (DA, PVG, and hybrid strains only).

Conclusions. The susceptibility of rat strains to infection of the cornea with HSV varies, and, as with mice, resistance seems to be controlled by non-MHC genes. Rats may prove useful for immunologic studies. Virus reactivation will be the subject of a future report. Invest Ophthalmol Vis Sci. 1994;35:3260-3267.

The rat has been used less extensively than the mouse for studies with HSV, and its use has usually been restricted to investigations of the pathogenesis and treatment of central nervous system (CNS) disease or for tracing neural pathways. Such studies have frequently involved abnormal routes of inoculation, often directly into the nervous system. Therefore, in comparison with mice, much less is known about the course of disease after primary infection of peripheral tissues or about strain differences in susceptibility to the virus. Our interest in using the rat as a model of HSV disease arose because we wanted to study the recurrence of herpetic disease after corneal transplantation. The rat was chosen in preference to the mouse because the cornea is larger for transplantation purposes and more is known about the characteristics of the allograft response in different rat strains.

In the limited studies that have been performed in rats after peripheral inoculation, the disease resembles that in mice. Thus, corneal inoculation results in stromal keratitis, and cutaneous inoculation induces zosteriform lesions in newborn rats and, in adults, results in latent infection of sensory ganglia. Moreover, in the rat, HSV may recur in the socket after tooth extraction. In mice, there are strain differences in susceptibility to both eye disease and CNS disease, and recurrent disease. Comparable differences may exist in rats. To provide more information about HSV disease in rats and to determine the strain most suitable for latency, reactivation, and transplantation studies, we investigated the course of primary disease and the incidence of latent infection in four readily available inbred strains of different MHC type.
HSV-1 McKrae was the chosen virus strain because it reactivates readily and causes recurrent disease in rabbits and mice.\textsuperscript{16,17} We also undertook a limited investigation of the genetic control of resistance to HSV using the backcross generation from parental strains of differing susceptibility ([DA × LEW]F\textsubscript{1} × LEW).

**METHODS**

**Rats**

Female PVG (RT\textsubscript{1}A), AO (RT\textsubscript{1}U), LEW (RT\textsubscript{1}A), and (DA × LEW)\textsubscript{F}\textsubscript{1} rats were purchased from Harlan Olac Ltd. (Oxford, UK). Backcross generation (DA × LEW)\textsubscript{F}\textsubscript{1} × LEW rats were produced in our own laboratory from purchased stock. All experiments adhered to the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research.

**Inoculation With Virus**

Seven- to 9-week-old rats were anesthetized by intramuscular injection of 0.5 ml/kg fentanyl/fluanisone (Hypnorm; Janssen, Oxford, UK) in one hind limb and 2.5 mg/kg diazepam (Valium; Roche, Welwyn Garden City, UK) in the other and the right corneas were inoculated with HSV-1 strain McKrae by light scarification using a 26-gauge needle. Ten \(\mu\)l of medium 199 containing virus at concentrations varying from \(4 \times 10^{2}\) to \(4 \times 10^{4}\) pfu were placed on the cornea. Scarification lines were made in two directions at right angles through the virus suspension, five in each direction to within 1 mm of the limbus. Care was taken not to penetrate to the corneal stroma.

**Clinical Examination and Isolation of Virus**

Rats were anesthetized and were examined daily for signs of eye disease using a slit lamp. Shedding of virus in the tear film was detected by washing the eye of the anesthetized animal with 50 \(\mu\)l of medium 199 and incubating the eye washings on Vero cell monolayers for 2 days at 37°C. Animals showing signs of CNS infection were immediately killed. Titters of infectious virus in eyes, trigeminal ganglia, and brains of selected animals were determined at various times during the primary infection. After removal, tissues were rapidly frozen and stored at -80°C in medium 199. When assayed, they were subjected to two further cycles of rapid freezing and thawing. Then they were homogenized, and the homogenates were titrated by plaque assay on Vero cell monolayers.

**Detection of Latent Infection**

At least 6 weeks after inoculation, animals were killed and the left and right trigeminal ganglia were divided in situ into three parts, corresponding approximately to the ophthalmic, maxillary, and mandibular divisions.\textsuperscript{18} Both superior cervical ganglia were also removed. In early experiments (involving doses of virus of \(4 \times 10^{2}\) and \(4 \times 10^{3}\) pfu in PVG animals), the pieces of tissue were placed in 0.5 ml medium containing 5% fetal calf serum and incubated at 37°C in 5% CO\textsubscript{2} for 5 days. They were homogenized and put onto Vero cells in 25 cm\textsuperscript{2} plastic flasks examined for cytopathic effect daily for 1 week. To improve sensitivity, in later experiments tissue pieces were co-cultivated for at least 26 days with Vero cells in 25 cm\textsuperscript{2} flasks or until cytopathic effect appeared.

**Tissue Typing**

Because rat erythrocytes express class I (RT1A) antigens, MHC allotype could be identified by indirect hemagglutination assay. A monoclonal antibody, MN4-91-6 (the kind gift of Professor J. Fabre),\textsuperscript{19} was used to identify the RT1A\textsuperscript{+} antigen, as previously described.\textsuperscript{20}

**Statistical Analysis**

Differences in survival and severity of eye disease in backcross generation animals were analyzed using the Mann-Whitney test.

**RESULTS**

**Central Nervous System Disease**

Signs of CNS disease included listlessness, abnormal gait, and weight loss. At this stage, animals were killed or they became moribund within 24 hours, sometimes suffering seizures. The LEW and AO strains were markedly more susceptible to CNS disease than the PVG strain. LEW rats were all killed by doses as low as \(4 \times 10^{3}\) pfu, whereas all PVG animals survived \(10^{4}\) pfu (Table 1). At the highest dose of virus (\(4 \times 10^{4}\) pfu), all LEW and most AO animals exhibited CNS disease by day 6, whereas such disease occurred in only 40% of PVG animals and not until days 7 to 13 (Fig. 1). Data for the PVG strain are from three separate experiments; mortality in different experiments varied from 18% to 50%, possibly reflecting variation in the severity of scarification at inoculation. Animals that survived the primary infection remained healthy until killed 2 to 7 months after inoculation. DA animals showed intermediate susceptibility to CNS disease and death. To determine the spread of virus within the nervous system, groups of animals were killed at various times after a dose of \(4 \times 10^{3}\) pfu, and virus was assayed in homogenates of eyes, whole trigeminal ganglia, and right and left sides of the brain. In the first experiment, comparisons were made at the onset of signs of CNS disease (day 6 in LEW, days 6 to 7 in AO and days 8 to 11 in PVG animals) (Fig. 2). In all individ-

Downloaded From: https://iovs.arvojournals.org/pdfaccess.ashx?url=/data/journals/iovs/933406/ on 08/28/2018
TABLE 1. Susceptibility of Different Rat Strains to HSV Infection After Corneal Inoculation

<table>
<thead>
<tr>
<th>Rat Strain</th>
<th>Virus Dose</th>
<th>Virus in Eyewashings During Primary Infection</th>
<th>Survivors</th>
<th>Latent Infection in Trigeminal Division</th>
<th>Animals With Latent Infection</th>
</tr>
</thead>
<tbody>
<tr>
<td>LEW</td>
<td>$4 \times 10^2$</td>
<td>/</td>
<td>9/9*</td>
<td>0/9</td>
<td>ND</td>
</tr>
<tr>
<td>LEW</td>
<td>$4 \times 10^4$</td>
<td>13/13</td>
<td>0/13</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>AO</td>
<td>$4 \times 10^3$</td>
<td>12/12</td>
<td>0/12</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>AO</td>
<td>$4 \times 10^4$</td>
<td>18/18</td>
<td>0/18</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>DA</td>
<td>$4 \times 10^3$</td>
<td>7/9</td>
<td>5/9</td>
<td>0/5 1/5 0/5</td>
<td>1/5</td>
</tr>
<tr>
<td>DA</td>
<td>$4 \times 10^4$</td>
<td>10/10</td>
<td>3/10</td>
<td>1/3 1/3 0/3</td>
<td>1/3</td>
</tr>
<tr>
<td>(DA X LEW)×LEW</td>
<td>$4 \times 10^3$</td>
<td>22/24</td>
<td>6/24</td>
<td>3/6 0/6 1/6</td>
<td>3/6</td>
</tr>
<tr>
<td>PVC</td>
<td>$4 \times 10^2$</td>
<td>6/10</td>
<td>10/10</td>
<td>0/10† 0/10 0/10</td>
<td>0/10</td>
</tr>
<tr>
<td>PVC</td>
<td>$4 \times 10^3$</td>
<td>10/11</td>
<td>11/11</td>
<td>0/11 0/11 0/11</td>
<td>0/11</td>
</tr>
<tr>
<td>PVC</td>
<td>$10^4$</td>
<td>10/10</td>
<td>6/10</td>
<td>4/6 3/6 1/6</td>
<td>5/6</td>
</tr>
<tr>
<td>PVC</td>
<td>$4 \times 10^4$</td>
<td>66/68</td>
<td>41/68</td>
<td>18/38 9/38 5/38</td>
<td>23/38</td>
</tr>
</tbody>
</table>

* Number with virus/total tested.
† In PVG rats inoculated with $4 \times 10^2$ and $4 \times 10^3$ pfu, a different method for detecting latent infection was used (see text).
ND = Not done.

In all rats, similar high titers were found in the brain. In susceptible AO and LEW strains, high titers were also found in both trigeminal ganglia and in the contralateral eyes, indicating that spread had occurred via the CNS. In contrast, virus was absent from both PVG ganglia and was minimal in the contralateral eye, despite the fact that titers in brains and ipsilateral eyes were similar to those of the other two strains. In a second experiment, titers were compared in LEW and PVG rats at equivalent time points after inoculation (days 3, 6, and 9) (Table 2). Again, similar titers were found in the inoculated eyes of both strains, but on a given day very little virus was found in the ipsilateral ganglia and brains of PVG compared with LEW animals. By day 6, there was spread to the LEW but not to the PVG, contralateral brain, ganglion, and eye. By day 9, all LEW animals were dead, but the incidence and titers of virus in the ipsilateral brain of the PVG strain had not yet reached the levels seen in the LEW strain on day 3.

Primary Eye Disease

After an inoculum of $4 \times 10^4$ pfu, all LEW, AO, and DA strain animals and 66/68 PVG animals shed virus in the tear film (Fig. 3). For the first 5 days, corneal disease was similar in all strains, consisting of punctate epithelial ulcers that became dendritic in some cases. By day 4, clear differences were apparent. LEW and
TABLE 2. Spread of Virus in LEW and PVG Rats After Inoculation of the Cornea with 4 × 10⁴ pfu HSV-1 McKrae

<table>
<thead>
<tr>
<th>Day After Inoculation</th>
<th>Rat Strain</th>
<th>IE*</th>
<th>CE</th>
<th>ITG</th>
<th>CTG</th>
<th>IB</th>
<th>CB</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>LEW</td>
<td>5/5 (3.5)</td>
<td>0/5</td>
<td>5/5</td>
<td>0/5</td>
<td>3/5</td>
<td>0/5</td>
</tr>
<tr>
<td>6</td>
<td>LEW</td>
<td>5/5 (2.1)</td>
<td>2/5 (2.5)</td>
<td>5/5 (2.9)</td>
<td>4/5 (3.0)</td>
<td>4/5 (4.5)</td>
<td>4/5 (3.8)</td>
</tr>
<tr>
<td>9</td>
<td>LEW</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>PVG</td>
<td>5/5 (1.8)</td>
<td>0/5</td>
<td>0/5</td>
<td>0/5</td>
<td>0/5</td>
<td>0/5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3/4 (3.1)</td>
<td>0/4</td>
<td>0/4</td>
<td>0/4</td>
<td>2/4 (1.7)</td>
<td>0/4</td>
</tr>
</tbody>
</table>

* Number with virus/total tested (mean log₁₀ titer in eyes with virus).

† Abbreviations as in Figure 2.

ND = Not done; no survivors.

AO rats had developed signs of uveitis (iris hyperemia, infiltration of inflammatory cells, and dilation of the pupil) (Fig. 4a). By day 6, uveitis had become more severe, whereas the cornea was frequently opaque and the lids swollen. Histologic examination of one opaque LEW and one opaque AO cornea on day 6 showed epithelial ulceration with heavy infiltration of cells into the stroma, mainly neutrophils, accompanied by endothelial cell damage. In contrast, eye disease in PVG rats was restricted to corneal epithelial lesions, sometimes with mild stromal haze, but there was no iris or lid involvement (Fig. 4b), even in animals that developed CNS disease. By day 6, cells infiltrating the PVG cornea consisted mainly of neutrophils, as in susceptible strains, but these were less numerous. The epithelial lesions persisted for 11 days in some PVG animals, accompanied by virus shedding into the tear film (Fig. 3); by day 14, corneas had returned to normal. Even at the low dose of 4 × 10² pfu, all LEW rats developed severe eye lesions, including uveitis, whereas only 6/10 PVG rats shed virus, accompanied by only mild epithelial lesions for 3 or 4 days after inoculation. The DA strain showed intermediate susceptibility to eye disease in that cells infiltrated the iris in one-third of animals, but there were no lid lesions (Fig. 4). Persistent stromal lesions did not occur in any animals that survived the primary infection (PVG and DA strains only).

Disease in (DA × LEW)F₁ × LEW Rats

To examine the effect of MHC and non-MHC genes on resistance, disease was examined in (DA × LEW)F₁ × LEW backcross generation animals. This particular strain cross was available in the laboratory, and its use enabled an investigation of the association between disease susceptibility and albinism because approximately 50% of the animals are albino (resembling the parental LEW strain), and the remainder were of varied color (either black, agouti, black hooded, or agouti hooded). We suspected such a linkage because the most susceptible inbred strains were albino. A group of 25 females was tissue-typed by indirect hemagglutination assay. Thirteen were of the heterozygous RT₁α/₁ type and 12 of the homozygous RT₁1/₁ type. They were inoculated with 4 × 10³ pfu virus and monitored daily during primary infection for virus in the tear film and signs of eye and CNS disease. One albino animal and one pigmented animal died during anesthesia (on days 10 and 11, respectively) and were, therefore, excluded from the data on deaths. The overall severity of corneal lesions and the incidence of CNS disease resembled more closely that of the DA than the susceptible LEW strain. Eye disease ranged from mild, with only epithelial lesions and slight stromal haze, to severe, with iris infiltration, pupil dilation, and corneal opacity. Severe eye or CNS disease was correlated with albinism rather than with MHC type. Of six survivors, three were heterozygous RT₁α/₁ and three were homo-

FIGURE 3. Incidence of virus in eyewashings after scarification of the cornea with 4 × 10³ pfu HSV-1 McKrae. The numbers of rats inoculated are shown in brackets.
FIGURE 4. Incidence of eye and CNS disease 4 (a) and 6 (b) days after scarification of the cornea with $4 \times 10^4$ pfu HSV-1 McKrae.

zygous RT1$^{1/1}$ (Fig. 5a). Eye disease was also similar in these two groups ($P = 0.34$ for date of onset of severe eye disease). However, only 1/11 albino animals survived the primary infection compared with 5/12 in the pigmented group. Signs of CNS disease in the albino group occurred significantly earlier (days 6 to 8) than in pigmented animals (days 7 to 11) ($P < 0.001$). Eye disease in albinos was significantly worse than in the pigmented group (Fig. 4b), reaching the severe category (i.e., uveitis) in 10/12 albino and 8/13 pigmented animals and occurring earlier in the albino group ($P < 0.001$).

**Latent Infection**

After inocula of $4 \times 10^3$ and $10^4$ pfu, latent infection was detected in 61% of surviving PVG rats in all divisions of the ipsilateral trigeminal ganglion (Table 1) but not in the contralateral trigeminal ganglion or in the superior cervical ganglia. Cytopathic effect was not seen on indicator cells before day 10 of co-cultivation. Latent infection was also found in the ipsilateral ganglia of some of the few surviving DA and hybrid animals (Table 1).

**DISCUSSION**

In rats, as in mice,$^{11-14}$ there is a marked strain difference in susceptibility to both eye and CNS disease after corneal inoculation with HSV-1. PVG animals are more than 100-fold more resistant than LEW. At the doses given, the AO strain resembles the LEW strain;
all animals suffered CNS disease and severe eye disease involving pupil dilation and infiltration of leukocytes into the iris. The more extensive and more rapid spread of virus in the LEW compared with the PVG strain was confirmed by the much higher titers of virus found in the LEW ipsilateral ganglion and brain on day 3 after inoculation and by high titers in the contralateral brain and ganglion by day 6. In mice, severe eye disease is indicative of more extensive neural spread, resulting in zosteriform spread of virus from the inoculation site via the trigeminal ganglion and/or the central nervous system back to the eye.21,22 Nerve section experiments after cutaneous inoculation of mice have confirmed that a component of the peripheral disease, even at the inoculation site, is caused by such zosteriform spread.23 Hence, the mild disease in the PVG strain may be a consequence of restricted virus replication in the trigeminal ganglion and perhaps in the central nervous system, thus reducing the amount of virus spreading back to the eye. Restricted replication in the sensory ganglion, rather than at the inoculation site itself, is also correlated with resistance in mice.24 The notion of ganglionic restriction in the PVG rat is further supported by the absence of virus in the contralateral ganglion at the time of death, despite high levels in the contralateral brain. However, some spread of virus within the ipsilateral PVG ganglion occurred, presumably via the trigeminal nerve root or brain stem because latent infection was found in the maxillary (II) and occasionally in the mandibular (III) division. Such latency is not indicative of virus replication in the ganglion itself because latency can be established in its absence.25

Chronic stromal disease is a characteristic of mouse ocular infection and occurs even in resistant C57BL/6 animals.11,12 However, it did not occur in rats. In all rats that survived the primary infection (DA, PVG, and hybrid animals only), eyes returned to normal. Even in PVG animals that developed CNS disease, eye disease was never severe and was improving, or had resolved, at the time of death. As with mice, passive immunization of a susceptible strain (AO) with rabbit anti-HSV antibody (data not shown) protected against death, but chronic stromal disease in survivors was again absent.

This limited study of susceptibility of (DA × LEW)F1 × LEW rats suggests that resistance is determined by non-MHC rather than MHC genetic loci. There appeared to be no influence of MHC type either on survival or susceptibility to primary eye disease. The greater susceptibility of the hybrid albino compared with pigmented rats and the fact that the most susceptible inbred strains (LEW and AO) were albino is evidence that susceptibility is linked to the gene for albinism. However, the pattern of disease in the four different inbred strains indicates that there is more than one locus determining resistance, as has been shown in mice.26 The early development of strain differences in virus titers in the ganglion (i.e., day 3) suggests that the resistance observed is due to innate rather than antigen-specific mechanisms, again favoring non-MHC control. However, if further comparative studies were performed using congenic strains on the PVG background so that animals survived long enough for the specific immune response to develop, the effect of MHC-linked mechanisms might become apparent.27

Candidates for non-MHC genes involved in resistance are those controlling cytokine secretion. In mice, higher levels of interferon production at the site of inoculation during the first few hours of infection have been implicated in resistance.28,29 It is reported that spleen cells from AO rats secrete lower levels of interleukin-2 than do those of DA rats when stimulated in vitro by mitogens30 and that this is under genetic control.31 These characteristics may have bearing on the susceptibility of the AO strain to HSV.

Other evidence from mouse studies suggests that B cells may be involved in resistance.24,32 The Igh-1 locus encoding immunoglobulin heavy chain genes has been implicated,33 but the mechanism remains obscure.

Further possible explanations for resistance include innate resistance of macrophages,34 corneal epithelial cells, stromal keratocytes,12 or neurons. Replication of HSV in mouse stromal keratocytes12 and embry fibroblasts31,35 varies in vitro and is correlated with strain susceptibility in vivo. Our observation that at a low virus dose (4 × 107 pfu), 9/9 LEW rats compared with 6/10 PVG rats shed virus in the tear film, the difference evident from the first day after inoculation, is circumstantial evidence of relative resistance of PVG epithelial cells in the early stages of infection. This may be mediated by cytokines such as interferon. Moreover, the limited replication of virus observed in PVG trigeminal ganglia could be mediated by soluble products of corneal cells influencing neurons via their peripheral termini within the cornea. However, an inhibitory effect originating in the cornea might also be due to factors in the tear film and further studies, including more data on titers of virus in the cornea during primary infection, would be required to shed more light on this question.

In summary, we have shown that there are strain differences in susceptibility to corneal inoculation with HSV in rats that parallel those of mice. Rats may prove useful for immunologic studies, particularly in aspects of their response that differ from those of mice. For example, they resemble humans in that they do not develop chronic HSV stromal disease after the primary infection. Moreover, because we have also
shown that latent infection can be established in the trigeminal ganglion, they may be useful for reactivation studies. This will be the subject of a further report.

Key Words
HSV, rat, cornea, pathogenesis, latency

References
Ocular HSV Infection in Rats


