

Animal Models of Human Viral Infections for Evaluation of Experimental Therapeutics

Animal Models of Herpes Encephalitis, Disseminated and Cutaneous Infections Utilized for Antiviral Testing

<u>Virus</u>	<u>Species</u>	<u>Route</u>	<u>Disease</u>
HSV-1	BALB/c Mice	i.p. i.n.	Encephalitis Encephalitis
HSV-2	BALB/c Mice	i.p. i.n.	Encephalitis Disseminated infection of newborns and encephalitis
HSV-1	Rat	i.n.	Encephalitis
HSV-1	SKH-1 Mice	i.cut.	Herpes labialis

New compounds are screened initially for HSV activity in BALB/c mice (Charles River Laboratories) inoculated i.p. with HSV-1 or HSV-2. Following i.p. inoculation with HSV-1 or HSV-2, virus replicates in the gut, liver, and spleen and spreads to the CNS by viremia and likely peripheral nerves as well. Virus is detected first in the brain around day five, thus allowing ample time for compounds to demonstrate antiviral effects. This model system has consistently been the most sensitive for determining efficacy of a new antiviral. Although it does not simulate a natural route of infection, it is ideal for screening new compounds to determine optimal dosages and treatment regimens. This is followed by testing in mice inoculated i.n. which more closely simulates human infections. If the experimental drug exhibits activity in mice inoculated i.p., it is next evaluated in mice inoculated by the i.n. route.

I.n. inoculation of three-week-old BALB/c mice with HSV-1 provides a model for herpes encephalitis of humans utilizing a natural route of infection. After inoculation of approximately 10^5 pfu of HSV-1, strain E-377, virus replicates in the nasopharynx and spreads to the CNS by way of olfactory and trigeminal nerves. Untreated animals

generally die by days 6-10. A variety of evidence exists which support the use of i.n. inoculation as a natural route of infection for herpes encephalitis.

I.n. inoculation of three-week-old BALB/c mice with about 4×10^4 pfu of HSV-2, strain MS, provides a model of disseminated neonatal herpes with CNS involvement. After viral inoculation, virus replicates in nasopharyngeal and lung tissue, and disseminates to the liver, spleen, and kidney. In addition, virus spreads to the CNS via olfactory and trigeminal nerves. Acyclovir, ACV, given parenterally or orally is highly effective in all the experimental infections mentioned above and is utilized as a positive control.

It has been estimated that there are over 40 million people in the U.S. with herpes labialis and there is a great need for the development of new antiviral drugs for this disease. We utilize the SKH-1 strain of immunocompetent hairless mice to facilitate scoring of cutaneous lesions. Thus, orofacial inoculation of HSV-1 in these mice provides an appropriate model for testing new antiviral therapies.

In this model, mice are anesthetized with a ketamine/xylazine mixture and injected subcutaneously with an electronic microchip for individual identification. Prior to inoculation, the snout, composed of the triangular shaped area over the nasal bones from the nose bridge to the eyes, is lightly abraded with a #113 tungsten-carbide engraving bit Dremmel tool. This procedure is performed carefully to prevent bleeding. This area is then swabbed for 10 seconds with a dacron swab soaked with HSV-1. Following this procedure, animals are returned to their cages and observed until recovery.

Animals infected with HSV-1 in the orofacial area exhibit lesions that begin to appear on days 4-6 and are usually cleared by day 15. To determine the effect of treatment on cutaneous viral replication, severity of lesions is scored from days 4-21 and swabs of the snout area are taken on days 3-10. The samples are placed in 2.0 mls of media and frozen at -70°C until titrated for HSV-1 on rabbit kidney fibroblast cells in a CPE microtiter plate assay. All experimental drug efficacy studies are placebo or vehicle controlled and also the positive control, Zovirax, is administered topically.

Animal Models of Cytomegalovirus Infections Utilized for Antiviral Testing

<u>Virus</u>	<u>Species</u>	<u>Route</u>	<u>Disease</u>
MCMV	BALB/c mice	i.p.	Disseminated CMV acute, chronic
	SCID-Mice	i.p.	Disseminated CMV acute
HCMV	SCID-hu-Ret	i.oc.	HCMV replication in Retinal tissue
	SCID-hu-thy/liv	i.im.	HCMV replication in thymus/liver tissue

Human CMV does not generally infect laboratory animals and there are no accepted models for disseminated infections available that use the human virus. For this reason, it has been necessary to use surrogate models, that is, a similar but different virus in its natural host. While there are cytomegalovirus strains in a number of animal species, the two that have been studied in the greatest detail have been the murine and guinea pig CMVs. In our laboratory, we have used murine CMV (MCMV) as a model for CMV infections for many years. This model has been predictive of efficacy for antiviral drugs, such as, Foscarnet (PFA), ganciclovir (GCV), and cidofovir (CDV) that have been evaluated in humans,

I.p. inoculation of three-week-old BALB/c mice with approximately 2.0×10^5 pfu of MCMV results in an acute, lethal infection with rapid virus replication in the lung, liver, spleen, kidney, intestine, salivary gland, and other visceral and glandular tissue. Animals die on approximately days 5-7. Since this is a lethal infection, the model can be used for rapid identification of potential antiviral compounds. Reduction of the virus inoculum to 10^4 pfu of MCMV results in a non-lethal, chronic, generalized infection, which has many

similarities to human CMV infections. At various times after inoculation, virus can be readily isolated from blood, lung, liver, spleen, kidney, urine, intestine, and salivary gland. Virus replication persists in these target organs for 45-60 days and in the salivary gland for months. The nature of the chronic infection makes it an ideal model for evaluating long term or maintenance therapy.

Severe combined immunodeficient (SCID) mice, which lack functional T and B cells are extremely sensitive to infection with MCMV and have been utilized as models for CMV infections in an immunocompromised host. SCID mice that are inoculated with a range of $1.0-10^5$ pfu of MCMV, and are left untreated, eventually die in a dose dependent manner. Animals that receive 10^5 pfu have a mean day of death of about 14 days, whereas, those inoculated with 10 pfu survive an average of 25 days. With each \log_{10} increase in virus inoculum, survival time is decreased by about three days. To determine the pathogenesis of MCMV in SCID mice, we inoculated mice with 10 pfu. On each of various days post infection, three mice were euthanized, their tissues removed, homogenized, and assayed for MCMV. Virus was first detected in salivary gland by day six followed by lung, spleen, kidney, adrenals, and pancreas on days 9-12. Liver, which is one of the most permissive organs in normal mice, did not exhibit detectable virus until day 18. In addition, brain was infected by day 18. These data indicate that inoculation of SCID mice with low concentrations of MCMV results in a disseminated infection with viral replication in the same target organs as observed in immunodeficient patients. These animals demonstrate high levels of virus in their tissues for 2-3 weeks, thus allowing adequate time to document an antiviral response in treated animals compared with placebo animals.

Human CMV infections can cause a wide range of clinical manifestations, especially in the immunocompromised host. Presently, there are few models to study HCMV infection since the virus is extremely host-specific and infection and replication are limited to human cells. In these studies, we have utilized a model that involves HCMV infection of fetal human retinal tissue implanted in the eyes of severe combined immunodeficient (SCID) mice. Small fragments of fetal human retinas are implanted into the anterior chamber, and four to six weeks after transplantation are inoculated with

2,000 to 10,000 pfu of HCMV. Animals are euthanized and eyes enucleated at various time points after infection. Eyes are prepared for microscopy by sectioning fixed tissue, or are homogenized for detection of infectious HCMV by plaque assay. The model has now been validated using GCV, CDV, and other antiviral therapies. In addition, this model is also being utilized to study and identify the virulence characteristics of HCMV by examining the growth of various HCMV mutants.

The SCID-hu thy/liv implant model can also be used in these drug efficacy studies. In this model, small fragments of human fetal thymus and liver are implanted under the kidney capsule in the SCID mouse. Approximately 12-16 weeks later, implants that are fully vascularized and quite large (10-50% the size of the kidney) are inoculated with 10^3 - 10^4 pfu of HCMV. At various time points after infection, implants are biopsied and homogenized, and HCMV replication is quantified by plaque assay. As with the SCID-hu mouse ocular model, this model appears to be very useful in determining the efficacy of various antiviral therapies.

Animal Models of Cowpox and Vaccinia Virus Infections Utilized for Antiviral Testing

<u>Virus</u>	<u>Species</u>	<u>Route</u>	<u>Disease</u>
Cowpox Virus (BR)	BALB/c Mice	i.p.	Death – Rapid Liver-Visceral Involvement
	BALB/c Mice	i.n.	Death – Slower Lung-respiratory involvement
	SKH-1 mice SCID Mice	i.d. i.p.	Skin lesions Disseminated disease
Vaccinia Virus (WR)	BALB/c Mice	i.n.	Death Disseminated Disease
Vaccinia Virus (IHD)	BALB/c Mice	i.n.	Death Disseminated Disease
Vaccinia Virus (WR)	SCID Mice	i.p.	Death Disseminated Disease
Vaccinia Virus (NYC)	SCID Mice	i.p.	Death

Drug Toxicity	BALB/c Mice	i.p.	Disseminated Disease Weight loss, Abnormal signs, death
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The causative agent of smallpox, variola virus, cannot be utilized outside a BSL-4 containment area and does not cause disease in adult mice. Various orthopoxviruses can be utilized as surrogate viruses for smallpox including VV and CV. They can be inoculated i.p. or i.n. into SCID mice with an endpoint of death. In normal mice, CV, VV-WR, or VV-IHD, but not VV- Copenhagen Strain, will produce mortality when inoculated by variety of routes. Intranasal inoculation of mice with CV produced an infection with features similar to systemic or disseminated smallpox. Other routes of inoculation such as i.p. or i.v. with VV or CV result in less bronchial involvement and more skin lesions. The IHD strain of VV is less virulent in BALB/c mice than the WR strain. The WR strain of VV produces mortality in BALB/c mice by i.n. inoculation and SCID mice by i.p. inoculation. SKH-1 hairless mice can also be inoculated with VV and CV by inoculation of abraded orofacial areas, similar to the HSV techniques. Mice can be treated systemically or topically with antiviral compounds for evaluation of efficacy against disease (lesion scores) or viral replication (viral titers).