# Animal Models for HSV




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# Endpoints of HSV Vaccine Trials in Humans and Animal Models

Human Studies:

- Genital herpes disease: signs (local lesions) and/or symptoms on the skin or mucosa of the anogenital region and/or buttocks **and** positive HSV culture (or PCR) or HSV seroconversion
- HSV Infection: genital herpes disease **or** asymptomatic seroconversion (or HSV culture)

Animal Studies:

- Mice: survival, acute shedding, latent infection
- Guinea pigs: infection, severity of acute disease, frequency of recurrences, acute and recurrent, shedding, latency

# Ideal Animal Model

- Immune system identical to humans:

  -HLA and other immune cell proteins

- Genital tract anatomy identical to humans:

  -expression of cellular proteins (e.g. nectin-1)

- Cellular proteins affected by HSV immune evasion proteins identical to human cellular proteins
- Spontaneous recurrences occur

# Mouse Model for HSV

- Inoculation by eye, vagina, flank, ear, footpad
- Induces severe disease: fatal encephalitis, hind limb paralysis, urinary retention
- Reactivation can be induced with UV, hyperthermia, severe stress, sodium butyrate, or explant co-cultivation in vitro
- Zosteriform model can be used to mimic recurrent disease: virus disseminates from skin to nervous system and then returns back to skin
- Most studies performed with inbred strains that have different susceptibilities to disease: DBA/2 > Balb/C > C57B6

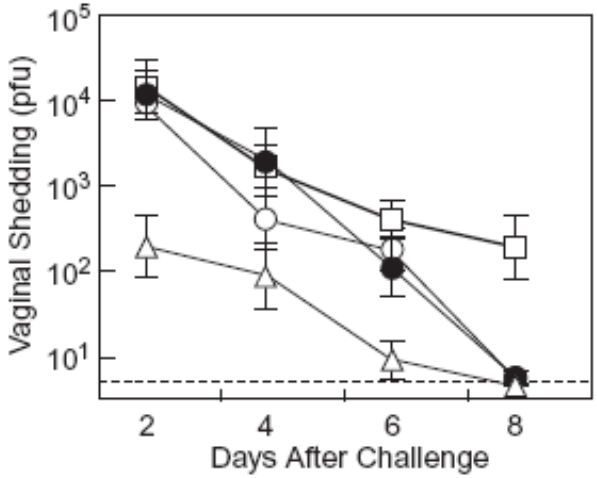
# Rabbit Model for HSV

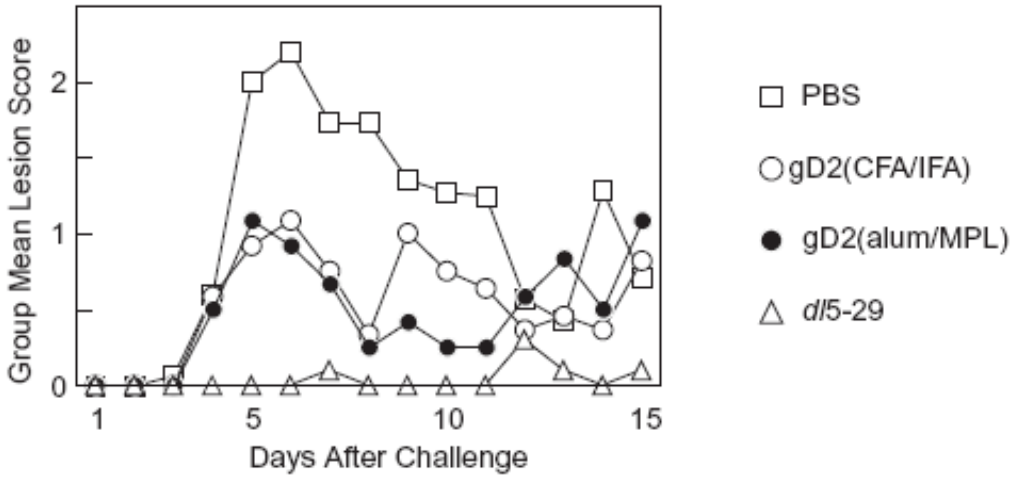
- Anatomy of the eye (conjunctival associated lymphoid tissue) similar to humans
- Slit lamp examination of rabbit eye is easy allowing quantitative measurements
- Corneal inoculation results in HSV keratitis, latency, and spontaneous reactivation of virus in tears
- Reactivation can be induced by epinephrine iontophoresis (90% with HSV-1 McKrae), damage to corneal nerves, or cyclophosphamide/dexamethasone
- Immune system not as well characterized as in mice

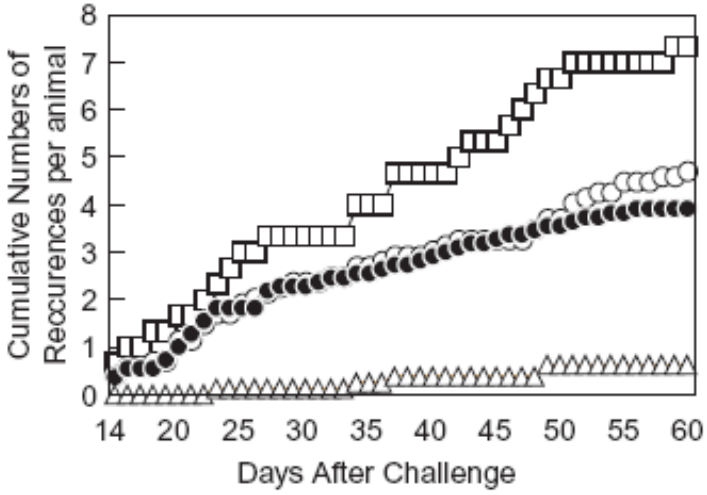
# Guinea Pig Model for HSV

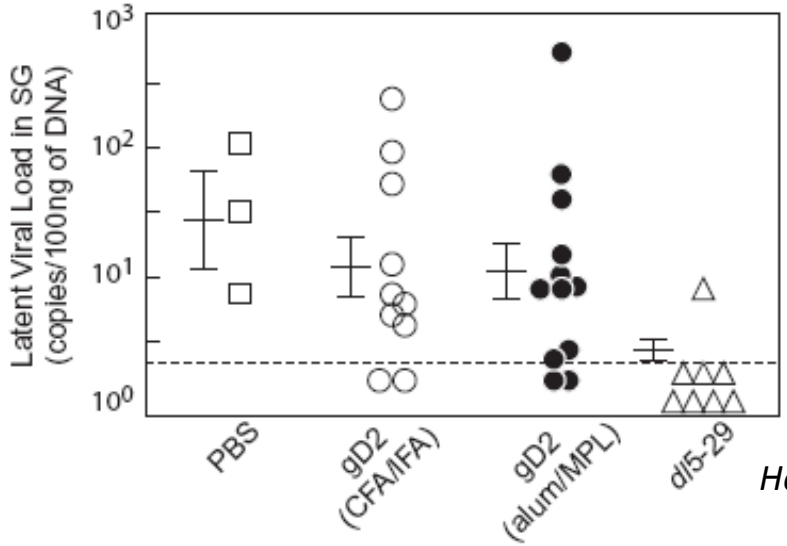
- Vaginal inoculation results in genital lesions
- Associated with severe disease: paralysis and urinary retention
- High frequency of recurrent visible lesions: HSV cultured from 30% of lesions
- Asymptomatic shedding reported
- Spontaneous and UV induced reactivation
- No humanized HLA transgenic animals
- Subjective scoring system
- Reagents for cellular immunity are limited, DTH responses demonstrated, cell mediated cytotoxicity described

# HSV-2 Shedding, Lesion Scores, Recurrence Rates, and Latency Can be Measured in Vaccinated and Challenged Guinea Pigs




*Hoshino et al JID 2009*

# Cotton Rat Model for HSV

- Intravaginal inoculation with HSV-2 results in acute genital herpes without paralysis or death in the absence of medroxyprogesterone *(Yim et al JV 2005)*
- HSV spreads to liver, lung, brain, kidney
- Spontaneous recurrences occur in 60-70% of animals, which can also be induced with corticosteroids
- Virus detected by PCR and culture before and during recurrences
- Pretreatment of animals with topical microbicide (PRO 1000) protects from infection and disease

# Animal Models

| Animal | Advantages | Disadvantages |
|---|---|---|
| Mouse | cheap, ko and transgenics<br>inbred strains, immune<br>reagents, humanized HLA | no spontaneous reactivation<br>severe disease<br>requires medroxyprogesterone |
| Rabbit | spontaneous reactivation<br>humanized HLA | fewer immune reagents available<br>less characterized immune s. |
| Guinea pig | spontaneous reactivation<br>resemble human genital d | few immune reagents<br>less characterized immune s. |
| Cotton rat | spontaneous reactivation | limited reagents, unfriendly |
| Rhesus monkey | immune system similar to humans | no reproducible disease |

# Evaluation of HSV Vaccines in Animals

- Sterilizing immunity in mouse typically only achieved with vaginal vaccination
- Most vaccines in the guinea pig do not prevent establishment of latency
- Many vaccines with gD can fully protect mice from primary disease, but less often fully protect guinea pigs from primary disease and do not prevent recurrent disease or shedding
- Most animals are challenged at or near the height of the immune response

# Immune Responses in Mice

Antibody:

- IgG protects mice from encephalitis, and reduces genital disease in mice and guinea pigs, but animals become infected *(Morrison & Knipe Virology 1997; Parr and Parr JV 1997; Bourne et al. JGV 2002; Morrison et al. JV 2001)*

T Cells:

- CD4 and CD8 cells both important in protecting mice from genital disease *(Parr and Parr JV 1998)*
- CD4 cells more important than CD8 in protecting mice from genital disease *(Morrison Virology 2008)* and reducing corneal infection and latency in eye model *(Morrison & Knipe Virology 1997)*
- CD8 T cells reduce ex vivo reactivation *(Hoshino et al. JV 2007)*

# Human vs. Guinea Pig Trials of Herpevac: HSV1-/HSV2- Females

| Trial | Herpevac (Belshe NEJM 2012) | Guinea Pigs (Bourne JID 2005) |
|---|---|---|
| Vaccine | gD2 (20ug) in AS04 x 3 doses | gD2 (5ug) in AS04 x 2 doses |
| Outcomes: | | |
| HSV-2 disease | no efficacy | 0% (0/12) primary disease |
| | | 17% (2/12) recurrent disease |
| HSV-2 infection | no efficacy | 100% (12/12) infected |
| HSV-2 shedding | no efficacy | 100% (12/12) shed |
| Disease definition | signs and/or sx AND + culture or seroconversion | signs |
| Infection definition | disease or seroconversion | +culture |

Discordant results

# Human vs. Guinea Pig Trials of Herpevac : HSV1-/HSV2- Females

| Trial | Herpevac (Belshe NEJM 2012)[1] | Guinea Pigs (Bourne JID 2003)[2] |
|---|---|---|
| Vaccine | gD2 (20ug) in AS04 x 3 doses | gD2 (5ug) in ASO4 x 3 doses |
| Outcomes: | | |
| HSV-1 disease | 58% efficacy | 0% (0/10) primary disease<br>10% (1/10) recurrent disease |
| HSV-2 disease | no efficacy | 10% (1/10) primary disease<br>10% (1/10) recurrent disease |
| HSV-1 infection | 35% efficacy | 67% (10/15) infected |
| HSV-2 infection | no efficacy | 67% (10/15) infected |
| Disease definition | signs and/or sx AND +culture or seroconversion | signs |
| Infection definition | disease or seroconversion | +culture |
| Discordant results | | |

# Differences Between Human and Guinea Pig Studies

| Feature | Herpevac (Belshe NEJM 2012) | Guinea Pigs (Bourne JID 2003) |
|---|---|---|
| Challenge: dose | "low" | $10^6$ pfu |
| Number challenges | multiple | one |
| Time from vaccine | 1-19 months | 15-21 days |
| Attack rate (infection) | 4-5% | 100% |
| Disease definition | symptoms or signs | signs |
| Infection definition | disease or seroconversion | culture |
| Virus evolved with host | yes | no |

More stringent

# Limitations of HSV Animal Models

- Different HLA alleles in small animals vs. humans: HSV CTL epitopes likely to be different in animal models vs. humans
- HSV immune evasion molecules less active in small animals vs. humans
- Higher (less physiologic) doses of virus likely used to infect small animals: > 20 sexual exposures are required for the uninfected partner of an HSV-2 infected partner to become infected and most infections occur during asymptomatic shedding
- Animals usually challenged at the peak of immune responses, while vaccine protection will need to persist for many decades
- Viruses used to challenge animals, which have been passaged in cell culture and often plaque purified, are likely to be more attenuated and less diverse than those that infect humans

# Animal Model Needs

- Humanized immune system in guinea pigs and mice
- Humanized guinea pigs and mice that have cellular proteins that can be modulated by HSV immune evasion genes
- Further evaluation of usefulness of the cotton rat model
- Find or modify an animal so that it is more predictive of human vaccine results

- Evaluate protection against various low passage HSV-2 isolates (European, African strains)
- Standardized procedures for infection and challenge, comparators for candidate vaccines (e.g. gD2 in adjuvant vs. placebo)