The Key Role of FcR Activating Antibodies in Generating "Sterilizing" Immunity against Herpes Simplex Virus

Betsy C. Herold, M.D.
Albert Einstein College of Medicine
**Scope of the Problem**

- HSV-1 and HSV-2 cause **lifelong** recurrent infectious mucocutaneous ulcers
  - HSV-1 predominant cause of oral lesions (gingivostomatitis)
  - HSV-2 more common cause of genital lesions worldwide
    - However, in US and Europe, HSV-1 has emerged as a more common cause of genital herpes

- HSV-1 also leading cause of sporadic fatal encephalitis and corneal blindness

- HIV-HSV-2 syndemic:
  - HSV-2 associated with increased risk of HIV transmission and acquisition

- Both serotypes transmitted perinatally with potentially devastating outcomes to infants even with treatment

- Worldwide prevalence:
  - HSV-1 ~3.7 billion
  - HSV-2 ~417 million
**Prevalence of HSV-2 and HSV-1**

Table 1. Global and regional estimates of the number of existing (prevalent) cases of HSV-2 infection in 2012 by age and sex, in millions (percentage of population with prevalent infection in each age group shown in parentheses).

<table>
<thead>
<tr>
<th>Age group (years)</th>
<th>15–19</th>
<th>20–24</th>
<th>25–29a</th>
<th>30–34</th>
<th>35–39a</th>
<th>40–44</th>
<th>45–49</th>
<th>All ages</th>
</tr>
</thead>
<tbody>
<tr>
<td>Global total (all)</td>
<td>27.6 (4.6%)</td>
<td>48.2 (7.8%)</td>
<td>60.9 (10.5%)</td>
<td>65.7 (12.7%)</td>
<td>69.6 (14.3%)</td>
<td>72.8 (15.6%)</td>
<td>72.7 (17.0%)</td>
<td>417.3 (11.3%)</td>
</tr>
</tbody>
</table>

Table 1. Global and regional estimates of the number of existing (prevalent) cases of HSV-1 infection in 2012 by age and sex, in millions (percentage of population with prevalent infection shown in parentheses).

<table>
<thead>
<tr>
<th>Age group (years)</th>
<th>0–4</th>
<th>5–9</th>
<th>10–14</th>
<th>15–19</th>
<th>20–24</th>
<th>25–29</th>
<th>30–34</th>
<th>35–39</th>
<th>40–44</th>
<th>45–49</th>
<th>All ages</th>
</tr>
</thead>
<tbody>
<tr>
<td>Global total (all)</td>
<td>175 (27%)</td>
<td>353 (57%)</td>
<td>392 (66%)</td>
<td>420 (71%)</td>
<td>453 (74%)</td>
<td>439 (75%)</td>
<td>394 (76%)</td>
<td>378 (77%)</td>
<td>367 (78%)</td>
<td>337 (79%)</td>
<td>3709 (67%)</td>
</tr>
</tbody>
</table>
History of HSV prophylactic vaccines

- Subunit vaccines targeting major envelope glycoproteins gD and gB that elicit high titer neutralizing antibodies dominated the field
  - Neutralizing antibodies presumed to be surrogates of protection

- Two vaccines completed Phase 3 clinical trials with negative outcomes:
  - gD-2/gB-2 + MF59 Phase III (Chiron)
    - Elicited high titer neutralizing antibodies
    - Overall vaccine efficacy was 9% (95% CI, -29% to 36%) (JAMA, 1999)
  - gD-2+ Alum + MPL Phase III (GSK-Herpevac)
    - Elicited high titer gD specific neutralizing Abs (higher than natural infection) and CD4 T cell responses
    - Serodiscordant partners: vaccine protective in ♀ who were doubly seronegative but not HSV-1+ ♂ and not in ♂ (NEJM, 2002)
    - Field study HSV-1/HSV-2 seronegative ♀ 18-30 yrs, not protective against genital HSV-2 disease or infection
      - Efficacy against genital HSV-1 disease was 57% (CI 12 to 80).

- Both vaccines were protective in “standard” murine & guinea pig models
Efficacy of HSV vaccine, −38%; 95% CI, −167 to 29

Belshe, et al., 2012, NEJM
What would happen if instead of focusing on gD as immune target, we vaccinated with ΔgD virus?

- Would we generate immune response against other antigenic targets?
- What would be the functionality of the immune responses elicited?

**Hypotheses:**
- Vaccine would be safe
  - gD required for entry and cell-to-cell spread
- Vaccine would elicit polyantigenic response unmasked by removal of the immunodominant gD
- Vaccine might elicit functionally different immune responses reflecting loss of immunomodulatory gD protein
Engineering $\Delta gD$ virus on HSV-1 $gD$ complementing cells

Allelic exchange substrate

HSV-2 DNA $\Delta gD::gfp$

Complementing HSV-1 $gD$ cell line (VD60)

parental HSV-2 (G) DNA

recombinant HSV-2 ($\Delta gD::gfp$) DNA
Engineering ΔgD virus on HSV-1 gD complementing cells

HSV-2 DNA ΔgD::gfp

Complementing HSV-1 gD cell line (VD60)

HSV-2 ΔgD⁻/+gD⁻₁
Engineering $\Delta gD$ virus on HSV-1 gD complementing cells

HSV-2 DNA $\Delta gD::gfp$

Complementing HSV-1 gD cell line (VD60)

HSV-2 $\Delta gD^{-/}gD^{-1}$

Non-complementing Cell line (vero)
ΔgD causes no disease in SCID Mice

Petro and Gonzalez, et al., 2015, eLife
Immunization scheme with HSV-2ΔgD

C57Bl/6

Balb/C

Prime  21 days (sc)

Boost  21 days (sc)

Challenge HSV-2 WT  15 days
ΔgD Protects C57BL/6 and Balb/C Mice against vaginal HSV-2(4674)

Petro and Gonzalez, et al., 2015, eLife
ΔgD immunized mice have no detectable HSV-2 in vaginal at 5 days or neural tissue up to 28 days post-challenge.

**NO** viral reactivation from explanted co-cultured neural tissue

**NO** viral titer in vaginal washes

**NO** viral DNA in excised tissue
Adoptive transfer scheme

1. Prime: 21 days (sc)
2. Boost: 21 days (sc)
3. Challenge HSV-2 WT
4. Pan T cell OR Serum
5. IV, IP, ivag
6. 15 days

Spleen Pan T cells

Serum
Passive transfer of $\Delta gD$ immune serum protects naïve mice from HSV-2 challenge

Petro and Gonzalez, et al., 2015, eLife
ΔgD elicits a robust systemic and mucosal HSV-2 Ab response

Prime
HSV-2 ΔgD−/+ 21 days
Boost
HSV-2 ΔgD−/+ 21 days
Challenge
HSV-2 WT 15 days

Total serum anti-HSV-2 antibodies

Total mucosal anti-HSV-2 antibodies

ΔgD−/+  
Control

1:800,000

D7 p-boost
D4 p-challenge

Log_{10} reciprocal dilution of serum

anti-HSV-2 Ig (OD_{450 nm})
Passive Protection Requires FcR and Transport of Ab to Mucosal Sites
HSV529 (single cycle replication impaired virus) showed reduced efficacy against African HSV isolate in murine model.
HSV-2ΔgD-2 provides complete protection following intravaginal or skin challenge with US clinical isolate with vaccine doses as low as 5x10^4 PFU.
Clinical Isolates are Genetically Diverse

B³x HSV-1 and HSV-2 isolates provided from MMC Clinical Lab
ds90 (African clinical isolate) gift from D. Knipe
Clinical Isolates Exhibit Similar In Vitro Growth Kinetics
But Variable Virulence in Mice

HSV-1 isolates
5x10^5 pfu/mouse

HSV-2 isolates
1x10^5 pfu/mouse

Days post challenge

Percent survival

Skin Disease Score

Days Post Challenge

D a y s  p o s t c h a lle n g e
Immunization with HSV-2-ΔgD protects against most virulent clinical isolates

<table>
<thead>
<tr>
<th>Prime</th>
<th>Boost</th>
<th>Challenge HSV-1 or 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>HSV-2 ΔgD</td>
<td>HSV-2 ΔgD</td>
<td></td>
</tr>
<tr>
<td>21 days</td>
<td>21 days</td>
<td>15 days</td>
</tr>
<tr>
<td>sc</td>
<td>sc</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Mock</th>
<th>SD90</th>
<th>B^3_x2.3</th>
<th>B^3_x1.1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ctrl</td>
<td>ΔgD-2</td>
<td>Ctrl</td>
<td>ΔgD-2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ctrl</td>
<td>ΔgD-2</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Day 4

Day 5

Day 6
Immunization with HSV-2-ΔgD confers protection against virulent HSV clinical isolates

Prime
HSV-2 ΔgD
21 days
sc
Boost
HSV-2 ΔgD
21 days
sc
Challenge
HSV-1 or 2
15 days

SD90
Mock

Day 4

Day 5

Day 6

B^3×2.3

B^3×1.1

Percent survival

Days Post Challenge

ΔgD-2 SD90
ΔgD-2 B^3×2.3
ΔgD-2 B^3×1.1
Ctrl B^3×1.1
Ctrl SD90
Ctrl B^3×2.3
ΔgD immunized mice are protected against 10-100x LD90 of Bx1.1 and SD90.
ΔgD rapidly clears virus and prevents establishment of latency
ΔgD elicits high titer IgG2 response in skin following challenge with clinical isolates

Anti HSV-2 IgG (ELISA)

- ΔgD-2 Day 2
- ΔgD-2 Day 5
- Ctrl Day 5

Relative anti-HSV IgG isotype ratio at 1:100 dilution

Day 2

Log₁₀ reciprocal dilution of skin homogenate

Anti-HSV2 IgG (OD450nm)
Antiviral antibody responses

Response impacted by antigenic target, isotype of Ab, FcR interactions and glycans

Antibody responses

- Retro-orbital or cardiac bleed
- HSV specific ELISA for titer, isotype
- Neutralization assay
- Antigenic targets
- Passive transfer
- Fc receptor activation
Immune serum from vaccinated animals showed little neutralization of HSV *in vitro*.

**Serum neutralizing Ab**

1:5 dilution of serum
$\Delta gD$ elicits predominantly anti-HSV IgG2 antibodies
Immune Serum Elicits Antibody-Dependent Cell Mediated Cytotoxicity

Petro and Gonzalez, et al., 2015, eLife
Serum from ΔgD-vaccinated mice mediate Antibody-Dependent-Cellular phagocytosis (ADCP) \textit{in vitro}

Monocyte/Macrophage

Phagocytosis via FcyR engagement

HSV-coated beads

Dotted- Control serum+HSV

Solid-ΔgD\(^{-/+}\) serum+HSV

Solid-ΔgD\(^{-/+}\) serum+HSV

Red-ΔgD\(^{-/+}\) serum+Cell

\begin{align*}
\text{Phagocytic Score} & \quad \left( \frac{\text{% positive X MFI}}{10^6} \right) \\
\text{Lysate coated beads:} & \quad \text{V} \quad \text{C} \quad \text{V} \quad \text{C} \\
\text{IFN\gamma} & \quad \text{pg/ml} \\
\text{cut off} & \quad \text{pg/ml}
\end{align*}

\begin{itemize}
\item \text{Control serum}
\item \text{ΔgD-2 serum}
\end{itemize}
Mouse Fc Receptors

Lünemann et al, 2015
Measuring Fcγ receptor activation

HSV-1 or HSV-2 infected target cells

Mouse serum

NFAT pathway

NFAT-RE Luc

Glo
ΔgD vaccinated animals have high titer mFcγRIV activating antibodies
Heat inactivated ΔgD not protective
Decrease in inflammatory response in the skin by day 5 post challenge in ΔgD versus Control-vaccinated mice.
Decrease in inflammatory response in the skin by day 5 post challenge in ΔgD versus Control-vaccinated mice
What are the antigenic targets of immune serum?

Western blots of cellular lysates infected with HSV-2(4674) and probed with dilutions of sera from HSV-2 ΔgD vaccinated mice 7 days post-boost.
Conclusions

- Subunit vaccines elicit neutralizing Abs (gD/gB) similar to natural infection
  - NOT sufficient to prevent infection in clinical trials
  - Did not prevent latency in “standard” murine models
  - High neutralization titers are observed in pts with frequent recurrences

- ΔgD vaccine:
  - Safe and immunogenic
  - Elicits high titer FcR activating Abs and CD4 and CD8 T cell responses
    - Optimal protection observed with live, not inactivated, virus
  - Proves protection against array of clinical isolates in vaginal & skin murine models (male and female)
  - Prevents establishment of latency (98/100 mice)
  - Protects guinea pigs from lethal challenge and prevents virus reaching DRG (challenged with clinical isolate)

- Preclinical studies should include evaluation of vaccine responses against multiple clinical isolates of HSV-1 and HSV-2
Speculations

- Rapid recruitment of FcR activating Abs to sites of HSV exposure required for optimal prevention
- FcR activation may provide better correlate of vaccine efficacy
- The presence of gD may skew the immune response towards generation of neutralizing Abs or…
- Absence of gD promotes an IgG2 dominant ADCC antibody response-
  - ? WHY
Acknowledgements

**Herold Lab**
- Clare Burn
- Chris Petro
- Natalia Cheshenko
- Natalie Ramsey
- Naz Khajoueinejad
- Carol Kao

**William Jacobs**
- Brian Weinrick
- Kayla Weiss
- Joseph Dardick
- Bing Chen
- Pablo Gonzalez

**David Knipe**
*Harvard Medical School*

**Kelsoe Lab**
*Duke University*
- Garnett Kelsoe
- Masayuki Kuraoka
- Akiko Watanabe

**GlaxoSmithKline**

**Promega**
- Mei Cong
- Vanessa Ott
- Aileen Paguio