ADCC and HVEM: Lessons from an HSV-2 ΔgD vaccine

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Herpes Simplex Viruses

• Predominantly infect epithelial cells; establish latency in peripheral nerves
• HSV-1 and HSV-2 cause painful recurrent oral or genital mucosal lesions
  • Both transmitted perinatally
  • HSV-2 associated with increased risk of HIV acquisition/transmission
• HSV-2: 400+ million people worldwide
• HSV-1: 3.7 billion people worldwide
  • Major cause of genital herpes in the developed world
  • Leading cause of fatal infectious encephalitis
  • Corneal blindness

Looker et al, 2012
HSV vaccine trials

- Dominated by subunit vaccines targeting gB and gD that generate primarily a neutralizing antibody (nAb) response
- Clinical trial results:
  - gB/gD/MF59 (Chiron) elicited high titer nAbs, but did not protect
    - Overall vaccine efficacy 9% (95% CI, -29% to 36%) (JAMA, 1999)
  - rgD2/AS04 vaccine elicited high titer gD nAbs and CD4 T cell responses but failed to protect
    - In discordant partners, protective in ♀ who were seronegative for HSV-1 and 2, but not HSV-1+ (NEJM, 2002)
    - No efficacy against HSV-2 disease or infection in field study seronegative ♀ (-38% [95% CI, -167 to 29]
- DI5-29
  - Replication defective, deleted in 2 genes involved in viral replication (expresses gD)
  - Phase 1 completed, results pending
What if we try something different?

- HSV-2 single-cycle vaccine strain deleted in gD
  - Single cycle virus complemented with HSV-1 gD to allow initial round of infection but prevent recombination

HSV-1

HSV-2 ΔgD-

ΔgD-/-

Complementing HSV-1 gD cell line (VD60)

Non- complementing Vero cell line
What we know about HSV-2 ΔgD

- Protected > 100 mice from lethal challenge (100%)
  - Male and female mice
  - Challenged intravaginally (female) or by skin scarification with different clinical isolates of HSV-2 and HSV-1
  - Prevents establishment of latency
  - Rapidly clears virus
  - Lasting protection (100% out to 6 months post-boost)
- Generates high titer HSV Ab response as well as CD4 and CD8 T cells
  - Abs alone are sufficient to passively protect naïve mice from challenge
  - Abs are NOT neutralizing but activate the FcR to induce ADCC and ADCP
Why does this vaccine do something different?

Approach: Compare different vaccines

ΔgD
DI5-29
rgD-2 AS04
VD60 lysate control

HSV-2 SD90 or HSV-1 B^{3}\times 1.1 10 \times LD90
HSV-2 MS-luc 10 \times LD90

Day 1: Prime
Day 21: Boost
Day 28: Serum
Day 41: Depilate
Day 42: Challenge
Day 56: Harvest

DI5-29: replication defective; deleted in 2 genes involved in viral replication
Gift from David Knipe (Harvard)
HSV-2 ΔgD rapidly clears virus & protects against 10xLD90 HSV-1 & HSV-2: dose dependence

HSV-1 survival

HSV-2 survival

HSV-2 MS-luc

Control

DI5-29

ΔgD

n = 5 - 10/group

Images representative of 2 independent experiments
Dose dependent differences in protection from latency and Ab responses

HSV-1 B^3x1.1
DNA in DRG

HSV-2 SD90
DNA in DRG

Copies of HSV-1 DNA (per mg tissue)

Copies of HSV-2 DNA (per mg tissue)

Total HSV-1 binding IgG

Total HSV-2 binding IgG

\( n = 10/\text{group} \)
Mouse Fc receptors

IgG2a, IgG 2a, 2b

IgG1, IgG 2a, 2b

IgG2a,b

IgG1, IgG 2a, 2b

Lünemann et al, 2015
Antibody functionality differs by vaccination

HSV-1 B³x1.1
Neutralizing titer

HSV-2 SD90
Neutralizing titer

FcγRIV activation

n = 10/group
FcγRIV is necessary for passive protection

Survival

Neurological Disease

n= 5/group
Why does ΔgD evoke FcR response?

• Unmasking other antigens?
• Loss of immunomodulatory effect?
  • gD binds HVEM?

Skews the immune response leading to neutralizing Ab response?
  • Bidirectional 
    costimulatory/coinhibitory signalling molecule
  • Activating and inhibitory ligands
  • Depends on cis/trans
  • Broadly expressed on immune cells

Adapted from Murphy and Murphy, 2010

gD is known to block some of the natural ligands of HVEM
Do gD-HVEM interactions play a role in generating protective responses?

ΔgD (missing ligand)
DL5-29 (expresses gD)
VD60 lysate control

10 x LD90
SD90 (HSV-2)

WT or HVEM-/- (no receptor)

Monitor


Expect that dl5-29 but NOT ΔgD will behave differently in HVEM-/-
Changes to the antibody response in HVEM-/- mice

**Total HSV-binding Ab**

**Neutralization titer**

**FcR activation**

**Survival**

- Control WT
- Control HVEM-/-
- ΔgD WT
- ΔgD HVEM-/-
- dl5-29 WT
- dl5-29 HVEM-/-

**n = 10/group**
Passive Transfer Experiments in HVEM KO Mice to Assess Effector Cell Function

Day -1: Serum (i.p) ΔgD DI5-29 Control

Day 0: HSV-2 challenge - skin (4674)

Monitor for disease

Day 14: Harvest

WT or HVEM-/-
HVEM is involved in mounting Ab response AND effector response

Transfer immune serum from: HVEM-/- → WT

Transfer immune serum from: WT → HVEM-/-

n = 5-10/group
## ΔgD Protection Summary

<table>
<thead>
<tr>
<th></th>
<th>HSV-1 Challenge (B³x1.1)</th>
<th>HSV-2 Challenge (SD90)</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Protection</strong></td>
<td>82/85*</td>
<td>146/152*</td>
<td>228/237 (96.2%)</td>
</tr>
<tr>
<td><strong>Protection from DNA in DRG</strong></td>
<td>56/60</td>
<td>110/117</td>
<td>166/177 (93.8%)</td>
</tr>
</tbody>
</table>

*all deaths (and all but 1 HSV+ DRG) at 8 months post-boost or 5 x10^4 vaccine dose
Conclusions

- FcR activating antibody is a correlate of protection for HSV-1 and HSV-2 clinical isolates in mice
  - High FcR titers ($\Delta gD$) $\rightarrow$ greater active & passive protection
  - Little FcR activity (rgD or HVEM KO) $\rightarrow$ Little active or passive protection
- HVEM signaling modulates type of Ab response

HVEM + natural ligands (e.g. BTLA, LIGHT)

ADCC  Neutralizing
Conclusions

• FcR activating antibody is a correlate of protection for HSV-1 and HSV-2 clinical isolates in mice
  • Higher FcR titers $\rightarrow$ greater active and passive protection
  • Loss of FcR activity $\rightarrow$ loss of active and passive protection
• HVEM signaling modulates type of Ab response

HVEM + natural ligands

+ gD
HSV, dl5-29, rgD-2

ADCC

Neutralizing
Conclusions

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