Immunogenicity of a trivalent subunit vaccine for genital herpes in Rhesus macaques.

Sita Awasthi, PhD
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Atlanta, December 7-9
Global prevalence of HSV-2 Infection

Looker KJ. Plos One Jan. 2015
Rationale for HSV-2 vaccine

- Prevention of genital herpes
  15% of adult population is infected with HSV-2 worldwide
  20 million new infections are added each year globally
- Treatment of genital herpes
  In US ~50 million people are HSV-2 positive
  Economic burden of treatment is ~billion dollars annually in US
- Curb acquisition and transmission of HIV
- There is no cure or FDA approved vaccine
Rationale for a trivalent genital herpes vaccine

Entry protein

Inhibits complement

IgG Fc Receptor
Glycoprotein C protects virus against human complement

Glycoprotein E protects virus from IgG Fc mediated ADCC and C’activation

WT virus: Antibody bipolar bridging

HSV-2 gE mutant: No antibody bridging

gC2/gD2/gE2 as a prophylactic vaccine

Goal: Elimination of acute and recurrent disease, and asymptomatic shedding of HSV-2 DNA in pre-clinical model.

0-acute
0-recurrent
0-shedding of HSV-2 DNA
High ELISA titers to all immunogens and neutralizing titers to HSV-2
gC2/gD2/gE2 as a prophylactic vaccine

Table. Recurrent disease

<table>
<thead>
<tr>
<th></th>
<th>Mock</th>
<th>gD2</th>
<th>gC2/gD2/gE2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Incidence of recurrent genital disease</td>
<td>7/8 (88%)§</td>
<td>16/25 (64%)**</td>
<td>10/36 (28%)**</td>
</tr>
<tr>
<td>Days with lesions/total observation days (15-60)</td>
<td>85/340 (25%)¶</td>
<td>77/1087 (7%)***</td>
<td>16/1509 (1%)***</td>
</tr>
</tbody>
</table>

Results: Trivalent vaccine highly protective against recurrent disease.
HSV-2 DNA and infectious virus from vaginal swabs

Day 28 to Day 48

GP 1

gD2

GP 9

Trivalent

GP 1

GP 9

No HSV DNA

151-1000

$10^3$ - $10^5$

$\geq 10^5$ HSV-2 DNA

$\geq 10^5$ DNA and Infectious HSV-2
**Summary of efficacy studies in guinea pigs**

**Acute and recurrent disease:** Trivalent vaccine is very effective at preventing acute and recurrent genital disease and comes close to meeting 0 acute – 0 recurrent disease goals.

**Asymptomatic DNA shedding:** Trivalent vaccine reduced vaginal HSV-2 DNA shedding, but did not eliminate it. However, no infectious HSV-2 was recovered from vaginal swab during recurrent phase in trivalent vaccinated guinea pigs.
Translational efforts: Immunogenicity in rhesus macaques

Collaboration with Tulane National Primate Center

Immunizations and assessments

Groups
Mock (CpG and alum) n=2
gC2 (CpG and alum) n=2
gD2/gC2/gE2 (CpG and alum) n=2

Immunogenicity Endpoints
ELISA antibody titers for each antigens, mucosal antibody titers, neutralization titers, cellular immune responses, C3b blocking ability, Fc receptor blocking ability.
# Plasma antibody and neutralization titers in vaccinated rhesus macaques

<table>
<thead>
<tr>
<th>Rhesus #</th>
<th>Treatment</th>
<th>ELISA titers</th>
<th>Neutralization titers</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>gD</td>
<td>gC</td>
</tr>
<tr>
<td>1</td>
<td>CpG/alum</td>
<td>&lt;1:50</td>
<td>&lt;1:50</td>
</tr>
<tr>
<td>2</td>
<td>CpG/alum</td>
<td>&lt;1:50</td>
<td>&lt;1:50</td>
</tr>
<tr>
<td>3</td>
<td>gC2-CpG/alum</td>
<td>NA</td>
<td>1:32000</td>
</tr>
<tr>
<td>4</td>
<td>gC2-CpG/alum</td>
<td>NA</td>
<td>1:32000</td>
</tr>
<tr>
<td>5</td>
<td>Trivalent vaccine</td>
<td>1:32000</td>
<td>1:32000</td>
</tr>
<tr>
<td>6</td>
<td>Trivalent vaccine</td>
<td>1:16000</td>
<td>1:32000</td>
</tr>
</tbody>
</table>
## ELISA and neutralization titers in vaginal mucosa

<table>
<thead>
<tr>
<th>Rhesus #</th>
<th>Treatment</th>
<th>Mucosal IgG (ELISA Ab titers)</th>
<th>Mucosal Neutralization titers HSV-2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>gD</td>
<td>gC</td>
</tr>
<tr>
<td>1</td>
<td>CpG/alum</td>
<td>&lt;1:25</td>
<td>&lt;1:25</td>
</tr>
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<td>2</td>
<td>CpG/alum</td>
<td>&lt;1:25</td>
<td>&lt;1:25</td>
</tr>
<tr>
<td>3</td>
<td>gC2-CpG/alum</td>
<td>NA</td>
<td>ND</td>
</tr>
<tr>
<td>4</td>
<td>gC2-CpG/alum</td>
<td>NA</td>
<td>ND</td>
</tr>
<tr>
<td>5</td>
<td>Trivalent vaccine</td>
<td>1:400</td>
<td>1:200</td>
</tr>
<tr>
<td>6</td>
<td>Trivalent vaccine</td>
<td>1:6400</td>
<td>1:1600</td>
</tr>
</tbody>
</table>
Blocking of gE binding to the Fc end of human IgG by vaccinated rhesus IgG

- Plate was coated with human IgG from an HSV-1/2 negative donor
- gE2 was incubated with rhesus IgG (0 or 200ng/µl) at 37°C for 1h then added to IgG-coated wells
- Bound gE was detected with polyclonal rabbit anti-gE2 Ab
- * P<0.001

Blocking of gE2 binding to IgG Fc will enhance neutralizing ability of gD2 antibody
Blocking of gC2 binding to C3b by vaccinated rhesus IgG

- Plate was coated with C3b
- gC2 was incubated with rhesus IgG (3rd bleed) at 37°C for 1 h then added to C3b-coated wells
- Bound gC was detected with polyclonal rabbit anti-gC2 Ab

Antibodies that block gC2 binding to C3b will enhance neutralizing ability of gD2 antibody
Cellular immune response in gC2-immunized Rhesus macaques

- PBMCs were isolated following pre and post-immunizations.
- Cells were stimulated with a pool of overlapping peptides (gC2).
- IFNγ positive CD4 and CD8 cell were measured by surface and intracellular staining, followed by FACS analysis.

gC2 immunized rhesus PBMC had increased IFNγ positive CD4 and CD8 T cells when stimulated with gC2 peptide pool.
Vaccine specific CD4+ T cell responses in rhesus

- PBMCs were isolated following pre and post-immunizations.
- Cells were stimulated with purified gC2, gD2 or gE2.
- IL-2, TNF-α, and IFNγ positive CD4+ cell were measured by surface and intracellular staining, followed by FACS analysis.

Immunogen specific CD4+ T cell responses are induced in vaccinated rhesus.
Summary

• Trivalent vaccine is highly effective in eliminating primary and recurrent disease in guinea pigs.
• High level ELISA titers to gD, gC and gE are detected.
• High level of neutralizing antibody titers are noted in plasma followed by trivalent vaccine immunization.
• Vaccine specific mucosal IgG are detected in rhesus vagina.
• C3b blocking and FcR blocking antibodies are produced in vaccinated rhesus.
• CD4 and CD8 T cell responses are induced in vaccinated rhesus.

Conclusions

A trivalent vaccine that blocks evasion of host immunity is a promising candidate from future human trials.
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GSK

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CD4+ T cell responses

Media Control  gC2  gE2  SEB

Animal IH34
Sample date
4/22/2014