Prospects for Vaccination Against Congenital Cytomegalovirus Infection

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Congenital CMV Infection

- Most common congenital viral infection in the developed world
- Incidence of congenital infection: 0.5-2% of all pregnancies
- A major cause of mental retardation, developmental disabilities, hearing loss
- Compelling need for a vaccine (Stratton et al., 1999)
CMV Vaccines: Strategies

- Envelope glycoproteins: humoral targets
- Tegument proteins and regulatory proteins: CMI and CTL targets
- Expression techniques: adjuvanted recombinant expression systems, vectored approaches
- Live, attenuated vaccines
Animal Models are Necessary to Study CMV Vaccines

- CMVs highly species-specific
- Murine CMV - no transplacental transfer
- Rat CMV - transplacental transfer reported but no vaccine studies reported to date
- Rhesus CMV - highly relevant to humans
- Guinea Pig CMV - only rodent model of transplacental transfer to placenta and fetus
Guinea Pig Cytomegalovirus is Transmitted \textit{In Utero}, Leading to Disease in Newborn
Evidence that Preconception Vaccination Protects the Fetus in the Guinea Pig Model

- Live, attenuated vaccines (Bia et al., 1980)
- Adjuvanted native glycoprotein vaccines (Harrison et al., 1995; Bourne et al., 2001)
- Passive antibody (Bratcher et al., 1995; Chatterjee et al., 2001)
- Are recombinant expression technologies effective vaccines in guinea pig model?
- GPCMV genome: ~232 kbp
- Strong conservation of CMV genes important in protective immunity
- Putative vaccine candidates in GPCMV model
Table 1. Pup mortality after maternal inoculation with guinea pig cytomegalovirus

<table>
<thead>
<tr>
<th>Group</th>
<th>Dams</th>
<th>Pups</th>
<th>Dead pups</th>
<th>Mortality %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>10</td>
<td>39</td>
<td>13</td>
<td>33</td>
</tr>
<tr>
<td>UL83 vaccine</td>
<td>11</td>
<td>38</td>
<td>13</td>
<td>34</td>
</tr>
<tr>
<td>gB vaccine, ELISA titer</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt;3.4 log</td>
<td>4</td>
<td>13</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>≥3.4 log</td>
<td>8</td>
<td>28</td>
<td>14</td>
<td>50</td>
</tr>
<tr>
<td>Overall</td>
<td>12</td>
<td>41</td>
<td>14</td>
<td>34</td>
</tr>
</tbody>
</table>

Data are no. of guinea pigs, unless otherwise noted. gB, glycoprotein B.

P < .05, vs. control

Table 2. Vertical transmission rates of guinea pig cytomegalovirus (GPCMV) and viral load analyses of infected pups, after third-trimester maternal GPCMV challenge.

<table>
<thead>
<tr>
<th>Tissue virus load, mean ± SD, log genomes/mg of tissue</th>
<th>Group</th>
<th>Liveborn Litters, no.</th>
<th>GPCMV-infected pups, no. (%)</th>
<th>Liver</th>
<th>Spleen</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>pups, no.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>8</td>
<td>26</td>
<td>20 (77)</td>
<td>3.8 ± 1.9</td>
<td>4.0 ± 2.0</td>
</tr>
<tr>
<td>UL83 vaccine</td>
<td>8</td>
<td>25</td>
<td>17 (68)</td>
<td>1.4 ± 1.4</td>
<td>1.3 ± 0.9</td>
</tr>
<tr>
<td>gB vaccine</td>
<td>10</td>
<td>27</td>
<td>11 (41)</td>
<td>1.8 ± 1.4</td>
<td>1.3 ± 0.5</td>
</tr>
</tbody>
</table>

NOTE: Liveborn pups were killed within 72 h of delivery, and liver and spleen were evaluated for presence of GPCMV DNA by quantitative competitive polymerase chain reaction.

a P < .05, vs. control (Fisher’s exact test).
b P < .005, vs. control (Student’s t test).
c P < .01, vs. control (Student’s t test).
Adjuvanted Recombinant gB Vaccines in the GPCMVM Model

- Baculovirus expression system (Schleiss et al., 2004)
- Recombinant gB adjuvanted with Freund’s adjuvant showed superior immunogenicity and protection compared to alum
- MPL-based adjuvants superior to Freund’s adjuvant
  - Equivalent ELISA titers following three-dose vaccine series
  - Superior protection against pup mortality
  - Equal magnitude of reduction of maternal viremia
Subunit Glycoprotein Vaccines: Other Candidates

- gM/gN complex
- gO/gH/gL complex
- UL128-131 proteins
<table>
<thead>
<tr>
<th>Vaccine/Adjuvant</th>
<th>Pup Mortality</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Group</strong></td>
<td><strong>Litters</strong></td>
</tr>
<tr>
<td>Negative Control</td>
<td>14</td>
</tr>
</tbody>
</table>
| gB/AS02V             | 10 | 4/39 (10%)^
| gB/AS01B             | 9  | 8/34 (24%)* |
| gB/FreundŐs          | 10 | 12/33 (36%)|
| **Overall Mortality in Vaccine Group** | 24/106 (23%) |

Maternal DNAemia at Day 10 Post-Challenge in Vaccine and Control Groups

p<0.00001 vs. control

*p<0.0005 vs. control

† p<0.05 vs. control

@ p<0.05 vs. FreundŐs adjuvant
- Pass et al, NEJM, 2009
- Three dose series of HCMV gB
- Efficacy of 50% against infection
<table>
<thead>
<tr>
<th>Mother-Infant Parity</th>
<th>Maternal Time of Seronegativity</th>
<th>HIG Administered</th>
<th>Possible Ultrasonographic Evidence of Fetal Involvement</th>
<th>Signs and Symptoms at Birth</th>
<th>Disease at ≤2 Yr of Age</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>7–12</td>
<td>23 IV</td>
<td>Ventriculomegaly, ascites, hepatospleomegaly</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>2</td>
<td>20–24</td>
<td>33 IV</td>
<td>IUGR</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>3</td>
<td>11–25</td>
<td>33 IV</td>
<td>Pyelecasis, megaloureter</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>4</td>
<td>4–11</td>
<td>21 IV</td>
<td>Hepatic echodensities</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>5</td>
<td>10</td>
<td>25 IV</td>
<td>IUGR</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>6</td>
<td>10–21</td>
<td>29 IV</td>
<td>Ventriculomegaly, periventricular echodensities</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>7</td>
<td>9–16</td>
<td>28 IV</td>
<td>IUGR</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>8</td>
<td>11–15</td>
<td>30 IV</td>
<td>IUGR, pyelecasis, intestinal echodensities</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>9</td>
<td>10–18</td>
<td>23 IV</td>
<td>IUGR, microcephaly, periventricular echodensities, ventriculomegaly, hepatospleomegaly</td>
<td>IUGR, microcephaly, periventricular calcifications, lissencephaly, thrombocytopenic purpura</td>
<td>Severe mental and motor retardation: not able to speak or stand</td>
</tr>
<tr>
<td>10</td>
<td>8–21</td>
<td>27 IV</td>
<td>IUGR</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>11</td>
<td>14</td>
<td>24 IV</td>
<td>IUGR</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>12</td>
<td>4–11</td>
<td>23 IV</td>
<td>IUGR</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>13</td>
<td>8–19</td>
<td>22 IV</td>
<td>Ventriculomegaly, intestinal echodensities</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>14</td>
<td>10–15</td>
<td>18 IV</td>
<td>Periventricular and intestinal echodensities, ventriculomegaly</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>15</td>
<td>≤9</td>
<td>23 IV</td>
<td>Intestinal and hepatic echodensities</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>16</td>
<td>11–19</td>
<td>None</td>
<td>IUGR</td>
<td>IUGR, seizures (CMV DNA in cerebrospinal fluid), pneumonia, encephalopathy, atresia</td>
<td>Seizures, hypoaesthesia, right strabismus and right arm hypoplasia, ventriculomegaly and left cerebral hypoplasia</td>
</tr>
<tr>
<td>17</td>
<td>≤8</td>
<td>None</td>
<td>IUGR, microcephaly, ventriculomegaly, intestinal echodensities, hepaticomegaly</td>
<td>IUGR, microcephaly, periventricular calcifications, pyelecasis, liver disease, thrombocytopenic purpura, postnatal death</td>
<td>Not applicable</td>
</tr>
<tr>
<td>18</td>
<td>7</td>
<td>None</td>
<td>Ascites, hepatomegaly</td>
<td>Intraventricular death, CMV inclusion in brain, kidneys, liver, and adrenal glands</td>
<td>Not applicable</td>
</tr>
<tr>
<td>19</td>
<td>5</td>
<td>None</td>
<td>IUGR</td>
<td>IUGR, periventricular calcifications, liver disease, thrombocytopenic purpura</td>
<td>Mental and motor retardation (IQ &lt;70); able to speak a few words, not able to walk</td>
</tr>
<tr>
<td>20</td>
<td>18–26</td>
<td>None</td>
<td>IUGR</td>
<td>IUGR, cerebellar atrophy, ventriculomegaly, hemiparesis</td>
<td>Mental retardation (IQ &lt;70); able to speak a few words; severe left hemiparesis (persistent at 8 yr); seizures</td>
</tr>
<tr>
<td>21</td>
<td>≤9</td>
<td>Ventriculomegaly</td>
<td>Microcephaly, periventricular calcifications, choroiditis, microcephaly, bilateral hypoplasia</td>
<td>Microcephaly, periventricular calcifications, cerebral and cerebellar atrophy, retinopathy</td>
<td>Mental retardation (IQ &lt;70); able to speak a few words; unilateral sensorineural hearing loss (right ear, 30 db; left ear, 50 db)</td>
</tr>
<tr>
<td>22</td>
<td>5–14</td>
<td>None</td>
<td>Periventricular and hepatic echodensities</td>
<td>Periventricular and hepatic calcifications, cerebral and cerebellar atrophy, retinopathy</td>
<td>Mental and motor retardation: able to speak a few words, not able to walk</td>
</tr>
</tbody>
</table>

* HIG denotes hyperimmune globulin, IV intravenous, IIA intraamniotic, IUGR intrauterine growth restriction, and IC intra-unbiliical cord.
Does UL83 (pp65) Homolog Provide Protection Against Congenital CMV Infection and Disease in Guinea Pig Model?

- Clone and express GPCMV UL83 (pp65) homolog
- Utilize ‘vectored’ approach: Venezuelan Equine Encephalitis Replicon “Virus-Like Particles” (VRPs)
- Vaccination pre-pregnancy in guinea pigs
  - Immune responses
  - Viremia post-CMV challenge
  - Pup outcomes
  - Congenital infection rates
Replicon RNA

- Packaging Signal
- 26S promoter
- 5' nsP1 nsP2 nsP3 nsP4 GP83 3'

Helper RNAs

- 26S
- 5' Capsid 3'
- 26S glycoprotein
- 5' Glycoproteins 3'

Attenuating mutations

Virus-like Replicon Particle

Capsid protein
- VRP-GP83 or control vaccine (flu HA) administered subcutaneously, 3 doses, 2-month intervals, $1 \times 10^6$ IU
- ELISA and Western Blot assay
- T-cell analyses
- Breed animals and challenge with salivary-gland passage GCPMV in early 3rd trimester of pregnancy
- Compare maternal and pup outcomes
  - Pup mortality
  - DNAemia
Pregnancy Outcomes (Pup Mortality and Pup Weights) after Challenge

<table>
<thead>
<tr>
<th>Vaccine</th>
<th>Litters</th>
<th>Dead/Total (%)</th>
<th>Mean Pup Weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>VRP-GP83</td>
<td>10</td>
<td>4/32 (13%)*</td>
<td>118 g</td>
</tr>
<tr>
<td>VRP-HA</td>
<td>8</td>
<td>12/21 (57%)</td>
<td>96 g</td>
</tr>
</tbody>
</table>

* Significantly different from the VRP-HA vaccine group (p<0.001, Fisher's exact test)

Significantly different from the VRP-HA vaccine group (p<0.05, Student's t-test)
## PCR Analyses of Pup Tissues

<table>
<thead>
<tr>
<th>Vaccine</th>
<th>Litters Tested</th>
<th>PCR+/Total</th>
<th>Transmission Rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>VRP-GP83</td>
<td>6</td>
<td>8/17</td>
<td>47%*</td>
</tr>
<tr>
<td>VRP-HA</td>
<td>6</td>
<td>11/13</td>
<td>85%</td>
</tr>
</tbody>
</table>

* p=0.057 compared to the VRP-HA vaccine group, Fisher’s exact test
DNA Vaccines in the GPCMV Model: BAC Vaccine

- Screen colonies from random transposon library of GPCMV BACmids
- Assess whether viral DNA purified from *E. coli* as a BAC plasmid is immunogenic as a DNA vaccine
- Introduction of a premature stop codon in GP48 gene (*UL48* homolog) ORF ensures that immune response is not due to reconstitution of replicating virus *in vivo*
- Vaccination produced overall serum titers comparable to those observed in natural infection
### Vaccine Group

<table>
<thead>
<tr>
<th>Vaccine Group</th>
<th>Live</th>
<th>Dead</th>
<th>Mortality</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (n=12 Dams)</td>
<td>12</td>
<td>23</td>
<td>66%</td>
</tr>
<tr>
<td>GP48 BAC Vaccine (n=10 Dams)</td>
<td>24</td>
<td>10</td>
<td>29%*</td>
</tr>
<tr>
<td>≥ 2.5 log_{10} GPCMV ELISA Titer (n=5 litters)</td>
<td>16</td>
<td>2</td>
<td>11%</td>
</tr>
<tr>
<td>&lt; 2.5 log_{10} GPCMV ELISA Titer (n=6 litters)</td>
<td>8</td>
<td>8</td>
<td>50%</td>
</tr>
</tbody>
</table>

* p<0.005 vs. control group

f p<0.03 vs. high titer group

Schleiss et al., 2006
Live, Attenuated Vaccine Design in the GPCMV Model

- Can live, attenuated vaccines be designed and tested for “proof-of-concept” in the guinea pig model with the aims of:
  - Improved immunogenicity?
  - Decreased reactogenicity?
- Class I homologs
- MIP 1-alpha homolog
Hypothesis: that creating a live attenuated vaccine strain from which some or all NK evasion genes have been removed will increase activation of NK cells, therefore increasing primary T-cell activation, which will generate a more potent long-term memory response.
Preconceptual vaccination with recombinant virus deleted of NK evasins confers protection against congenital CMV infection and disease in newborn pups.

<table>
<thead>
<tr>
<th>Vaccine group</th>
<th>Live</th>
<th>Dead</th>
<th>Mortality (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (n = 12 dams)</td>
<td>26</td>
<td>20</td>
<td>43</td>
</tr>
<tr>
<td>WT (n = 7 dams)</td>
<td>20</td>
<td>6</td>
<td>23</td>
</tr>
<tr>
<td>3DX (n = 7 dams)</td>
<td>21</td>
<td>6</td>
<td>22</td>
</tr>
<tr>
<td>WT + 3DX (n = 14 dams)</td>
<td>41</td>
<td>12</td>
<td>22.6*</td>
</tr>
<tr>
<td>Preconception infection (GPCMV seropositive; n = 5 dams)</td>
<td>10</td>
<td>4</td>
<td>29</td>
</tr>
</tbody>
</table>

* p < 0.02 vs. control Fisher's exact test.
CC CHEMOKINE
EcoRI
CMV MIP
EcoRI
EcoRI
Xba I EcoRI EcoRI

eGFP/gpt

WT 545

HEX HEX HEX
Median Hearing Levels over time by Group

Schraff et al., 2007
What are the Key Correlates of Protective Immunity for the Fetus?

- Magnitude of neutralizing antibody response correlated with reduced pup mortality and decreased congenital transmission
- Adjuvant important in protection
- T-cell target (GP83) is protective
- No strategy eliminates maternal DNAemia or prevents transmission
- Need to include multiple targets in single vaccine?
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NIAID, NICHD, March of Dimes