Cytomegalovirus Disease and Prevention

ECCMID
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Professor of Virology
University College London Medical School
CMV is an Important, Underestimated Pathogen

- Congenital infection
  - Commonest virus
- Transplant patients
  - Commonest opportunist
- AIDS patients
  - Significant contributor to mortality
- Intensive care
  - Increased hospitalisation, pneumonia
- Elderly
  - Contributes to immunosenescence
- General population
  - Increased mortality
# CMV Immune Evasion

<table>
<thead>
<tr>
<th>Defence</th>
<th>Response</th>
</tr>
</thead>
<tbody>
<tr>
<td>Complement</td>
<td>CD55, CD46, CD59</td>
</tr>
<tr>
<td>Chemokines</td>
<td>UL146, UL147, UL128</td>
</tr>
<tr>
<td>Chemokine Receptors</td>
<td>US28, US27, UL78, UL33</td>
</tr>
<tr>
<td>Interferon</td>
<td>UL83, UL123, TRS1/IRS1, UL122</td>
</tr>
<tr>
<td>Antibody</td>
<td>UL118 / UL119, IRL11</td>
</tr>
<tr>
<td>Th1 / Th2</td>
<td>UL111.5</td>
</tr>
</tbody>
</table>
Immune Evasion by CMV

[Diagram showing immune evasion mechanisms involving MICA, NkG2D, UL142, UL141, UL16, CD94, CD96, and other pathways related to US3, US6, US2US11, ER, TAP, and PRO.]
CMV pp65 Tetramer-specific CD8 T Cells in Healthy HLA-A2 CMV Seropositive Donor
Congenital CMV

- **Normal**: 7% of cases, 10% mortality, 90% sequelae
- **ASx**: 10% of cases, 0.5% mortality, 50% sequelae
- **Sx**: 13.5% of cases, 10 - 15% sequelae

[References]
Disease Outcome per 1000 Babies with Congenital CMV

Symptoms at Birth?

Yes
127

No
873

5 Deaths 0

122

873

61

118

sequelae

Estimates of Causes of Deafness at Birth and at Four Years in the United States

Morton C, NEJM 354, 2151, 2006
Intervention Against Disease

Why Not Screen for Congenital CMV?

- Important medical problem
- Treatment preserves hearing
- Substantial challenges:
  - Saliva versus blood?
  - PCR versus MS?
  - Magnitude of problem
Annual Cases in USA of 27 Conditions now Screened for in Most States (total 6,618 cases)

<table>
<thead>
<tr>
<th>Condition</th>
<th>Number of Cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>Organic acids</td>
<td>40,000</td>
</tr>
<tr>
<td>Amino acids</td>
<td>38,000</td>
</tr>
<tr>
<td>Fatty acids</td>
<td>36,000</td>
</tr>
<tr>
<td>Haemoglobin</td>
<td>32,000</td>
</tr>
<tr>
<td>Hypothyroid</td>
<td>28,000</td>
</tr>
<tr>
<td>Others</td>
<td>26,000</td>
</tr>
<tr>
<td>Others</td>
<td>24,000</td>
</tr>
<tr>
<td>Others</td>
<td>22,000</td>
</tr>
<tr>
<td>Others</td>
<td>20,000</td>
</tr>
<tr>
<td>Others</td>
<td>18,000</td>
</tr>
<tr>
<td>Others</td>
<td>16,000</td>
</tr>
<tr>
<td>Others</td>
<td>14,000</td>
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<tr>
<td>Others</td>
<td>12,000</td>
</tr>
<tr>
<td>Others</td>
<td>10,000</td>
</tr>
<tr>
<td>Others</td>
<td>8,000</td>
</tr>
<tr>
<td>Others</td>
<td>6,000</td>
</tr>
<tr>
<td>Others</td>
<td>4,000</td>
</tr>
<tr>
<td>Others</td>
<td>2,000</td>
</tr>
<tr>
<td>Others</td>
<td>0</td>
</tr>
</tbody>
</table>

DBS Screening

- Rapid DEAFF of saliva
- PCR run with:
  - Single primer set
  - Dual primer set
- 11,422 neonates single primer:
  - 17/60 (28%) sensitivity
- 9,026 neonates dual primer:
  - 11/32 (34%) sensitivity

Boppana SB. JAMA, 303, 1375, 2010.
Saliva PCR Screening

- Rapid DEAFF of saliva
- PCR of saliva without DNA extraction:
  - Transport medium, 4°C
  - Dried swab, room temp
- 17,622 neonates tested liquid PCR
  - 85/85 (100%) sensitivity
  - 99.9% specificity
- 17,327 neonates tested dry PCR
  - 74/76 (97%) sensitivity
  - 99.9% specificity

Boppana SB. NEJM, 364, 2111, 2011.
If We Screened for Congenital CMV

- Case finding
  - Diagnosis and follow-up
  - Compensatory aids/implants

- Consider treatment
  - Symptomatic cases
    - Kimberlin, DW. J Pediatrics, 143, 16-25, 2003
    - = preemptive therapy

- Serial data to assess the effect of a vaccine programme
Institute of Medicine Report

- Costs of CMV disease in:
  - Congenital infection
  - Transplant recipients

- Costs include:
  - Hospitalisation
  - Physician/diagnostic
  - Schooling

- Total $4,000 million pa
- Assumptions about CMV vaccine
  - Development $360m; 3 doses @ $50
  - Efficacy 75%; uptake 50%

- Cost per QALY=minus $50,000
- Top category IOM (cost saving)

Summary: CMV Vaccine Prospects

• Bad News
  • Large genome, 165 genes, all proteins immunogenic
  • CMV has multiple immune evasion genes
  • Immune individuals can be reinfected
  • Direct correlation between seropositive women and incidence of congenital infection
  • A successful vaccine would have to do better than nature


How Vaccines Protect Populations

Immunisation

Transmission

Susceptible cohort → Infectious cohort → Immune cohort

MEASLES/ CMV PRIMARY
How Vaccines Protect Populations

Susceptible cohort → Seropositive cohort → Infectious cohort → Immune cohort

Transmission

Immunisation

CMV REINFECTION
### Age-Specific Prevalence of IgG in Pregnancy


<table>
<thead>
<tr>
<th>Age of women (years)</th>
<th>No. women with antibodies (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>16-17</td>
<td>40</td>
</tr>
<tr>
<td>18-19</td>
<td>253</td>
</tr>
<tr>
<td>20-21</td>
<td>757</td>
</tr>
<tr>
<td>22-23</td>
<td>1188</td>
</tr>
<tr>
<td>24-25</td>
<td>1446</td>
</tr>
<tr>
<td>26-27</td>
<td>1426</td>
</tr>
<tr>
<td>28-29</td>
<td>1522</td>
</tr>
<tr>
<td>30-31</td>
<td>1366</td>
</tr>
<tr>
<td>32-33</td>
<td>1105</td>
</tr>
<tr>
<td>34-35</td>
<td>1026</td>
</tr>
<tr>
<td>36-37</td>
<td>458</td>
</tr>
<tr>
<td>38-39</td>
<td>286</td>
</tr>
<tr>
<td>40-41</td>
<td>164</td>
</tr>
<tr>
<td>&gt;41</td>
<td>75</td>
</tr>
</tbody>
</table>

## Basic Reproductive Numbers

<table>
<thead>
<tr>
<th>Virus</th>
<th>$R_0$</th>
<th>$P_c(%)$</th>
<th>Status</th>
</tr>
</thead>
<tbody>
<tr>
<td>Measles</td>
<td>15 - 18</td>
<td>93 - 94</td>
<td>controlled</td>
</tr>
<tr>
<td>Rubella</td>
<td>8</td>
<td>87</td>
<td>controlled</td>
</tr>
<tr>
<td>Mumps</td>
<td>7</td>
<td>86</td>
<td>controlled</td>
</tr>
<tr>
<td>Polio</td>
<td>6</td>
<td>83</td>
<td>controlled +</td>
</tr>
<tr>
<td>Smallpox</td>
<td>2.3 - 3.4</td>
<td>57 - 70</td>
<td>eradicated</td>
</tr>
<tr>
<td>CMV</td>
<td>2.4 - 2.7</td>
<td>59 - 62</td>
<td>ignored</td>
</tr>
<tr>
<td>CMV</td>
<td>1.7 overall</td>
<td>50</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1.8 females</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

CMV Vaccine and Herd Immunity

Griffiths PD, Vaccine 19, 1356, 2001

Ratio of high risk cases after vaccination to before vaccination

Proportion vaccinated
Summary - Maternal Infection

- The fetus can be infected by:
  - Primary maternal infection
  - Maternal reinfection
  - Maternal reactivation

- Primary is most pathogenic to the fetus
- Reinfection is worse than reactivation

Boppana S NEJM 2001, 344, 1366

- Same sequence as in solid organ transplants
  D+ R-  >  D+ R+  >  D- R+

- Immunisation should preferentially control the most severe congenital infection
## BOTE Analysis

<table>
<thead>
<tr>
<th>Reference</th>
<th>Incidence congenital CMV Proportion primary/non-primary</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wang, D. CID, 52, e11-e13, 2011.</td>
<td>40% reinfections</td>
</tr>
<tr>
<td>Yamamoto, AY. AJOG, 202, 297-298, 2010.</td>
<td>12.7% symptomatic</td>
</tr>
<tr>
<td>Dollard, SC. RMV, 17, 355-363, 2007.</td>
<td>50% sequelae</td>
</tr>
<tr>
<td>Staras, SA. STD, 35, 472-479, 2008.</td>
<td>13.5% asymptomatic get disease</td>
</tr>
<tr>
<td>Vaccine</td>
<td>100% efficacy and uptake</td>
</tr>
</tbody>
</table>

- **Boys and girls aged 12 years Toddlers**
Babies With Congenital CMV Born to Women who Reactivated Virus

Vaccination as teenager

Vaccination as toddler

Number of cases of congenital CMV

Years since vaccination started
Babies with Congenital CMV Born to Women with Primary Infection

Number of cases of congenital CMV

Years since vaccination started

0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24

Infected from Partner

Infected from Children
Babies with Congenital CMV Born to Women who were Reinfected

Number of cases of congenital CMV

Years since vaccination started
Congenital CMV Infection and Disease

Number of cases of congenital CMV

Years since vaccination started

- Reactivated
- Reininfected
- Primary
- Infection
Which CMV Antigens?

- Be guided by virus genome
- Multiple immune evasion genes
  - *Immune responses must be important*
- Induce these before challenge with virus
- Avoid antigen presentation in context of immune evasion genes
  - *Not live virus*
- Present with adjuvant
- Start with proteins that are immunodominant
  - *gB, pp65, Major Immediate Early Antigen*
CMV gB MF59 Vaccine in Selected Clinical Trials

Phase I in normal, healthy, seronegatives

- Pass R. JID 1999, 180, 970.
- Frey S. JID, 1999, 180, 1700.

Phase II

- Seronegative women child-bearing age
  - Pass R.
- Seronegative adolescents
  - Bernstein D.
- Allograft candidates
  - Griffiths P.

0, 1, 6 months
20 μg gB
Plus MF59
Design of gB Vaccine Trial

- Post-partum women (at least 6w pp)
- Seronegative, negative pregnancy test
- Randomised (1:1) to 0, 1, 6 months vaccine/placebo
- 17 follow-up visits over 42 months
- Saliva, urine, vaginal swab by PCR and culture
- Primary endpoint: seroconversion by gB-absorbed EIA
- >80% power ($\alpha=0.05$) for 50% efficacy

CMV gB Vaccine Results

- Blinded follow-up duration modified by DSMB at second planned interim analysis
- 441 women ITT analysis
- Average age 20 yrs (14 - 40)
- African-American 73%
- 51 seroconversions by May 2007
Probability of Remaining Free of CMV Infection

No. at Risk
Vaccine
Placebo

P = 0.02
Allografts: Strategies for Antiviral Therapy

- Pre-emptive therapy
- Prophylaxis
- Transplant
- Clinical Symptoms
- Late onset disease, resistance

= surveillance blood PCR
Contemporary rt QPCR for CMV

Most infections from recipient, most treatments (previously disease) from donor.
Transplant recipients

CMV peak viral load (Log10)

D+R-

D+R+

D-R+
Duration of viraemia (days)

Liver tx recipients

- D+R- (n=28)
- D+R+ (n=62)
- D-R+ (n=42)

Renal tx recipients

- D+R- (n=29)
- D+R+ (n=83)
- D-R+ (n=47)

*p<0.0001
**Duration of Antiviral Therapy**

- **Liver tx recipients**
  - D+R- (n=26)
  - D+R+ (n=27)
  - D-R+ (n=10)

- **Renal tx recipients**
  - D+R- (n=26)
  - D+R+ (n=35)
  - D-R+ (n=19)

* p=0.0004
** p<0.0001
*** p=0.0005
## Outcomes in SOTx Using Preemptive Therapy

<table>
<thead>
<tr>
<th>Transplant Patient Groups</th>
<th>Viraemia</th>
<th>Anti-viral Therapy</th>
<th>CMV Syndrome</th>
<th>End-organ Disease</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver (n= 321)</td>
<td>136 (42%)</td>
<td>63 (20%)</td>
<td>18 (5.6%)</td>
<td>5 (1.6%)</td>
</tr>
<tr>
<td>Renal (n= 368)</td>
<td>158 (43%)</td>
<td>79 (22%)</td>
<td>18 (4.9%)</td>
<td>3 (0.8%)</td>
</tr>
<tr>
<td>Total (n= 689)</td>
<td>294 (43%)</td>
<td>142 (21%)</td>
<td>36 (5.2%)</td>
<td>8 (1.2%)</td>
</tr>
</tbody>
</table>
Applications to CMV Vaccines

- A preexisting immune response protects against high viral load in transplant patients

- Could vaccine-induced immunity do likewise?
  - Cellular
  - Humoral
    - Viraemia/ donor source/ T-cell immunosuppression

- How potent would a vaccine need to be?
  - Sterilising immunity?
  - Sufficient to prevent threshold effect?

- Proceed to proof of concept study

Growth Rate of HCMV in Immune Naïve and Experienced Hosts

Randomise to vaccine/placebo:
- Seronegatives
- Seropositives

P < 0.001
n = 30

Emery VC. JID 185, 1723, 2002.
140 patients recruited

67 were assigned to receive vaccine
- 35 received 3 doses
- 30 received 2 doses
- 2 received 1 dose
- 12 Excluded
- 14 Awaiting Transplant
- 41 proceeded to transplant and completed follow up

73 were assigned to receive placebo
- 48 received 3 doses
- 20 received 2 doses
- 5 received 1 dose
- 16 Excluded
- 20 Awaiting Transplant
- 37 proceeded to transplant and completed follow up
ELISA gB Titres in Seronegative Recipients

P < 0.001

Log Geometric Mean (95% CI) gB Antibody Titer

Days since first vaccine/placebo

No tested
Vaccine: 35 34 22 16 13 19
Placebo: 34 34 27 26 25 9

Blood Sample Only  Vaccine/Placebo and Blood Sample
ELISA gB Titres in Seropositive Recipients

No tested: 29 29 24
Vaccine: 19 16 16
Placebo: 31 30 27

Log Geometric Mean (95% CI) gB Antibody Titer

Days since first vaccine/placebo

Blood Sample Only
Vaccine/Placebo and Blood Sample

P <0.001

Vaccine
Placebo

No tested: 29 29 24
Vaccine: 19 16 16
Placebo: 31 30 27

Blood Sample Only
Vaccine/Placebo and Blood Sample
## CMV Viraemia & Treatment in Subgroups

<table>
<thead>
<tr>
<th>Sub-groups</th>
<th>No.</th>
<th>Received</th>
<th>Viraemia &gt;200 genomes/ml</th>
<th>No. Treated</th>
<th>Proportion of:</th>
<th>Days PCR positive (%)</th>
<th>Days Treated (%)</th>
</tr>
</thead>
<tbody>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>D-</td>
<td>R-</td>
<td>22</td>
<td>Placebo 10</td>
<td>0</td>
<td>0</td>
<td>0/915 (0)</td>
<td>0/915 (0)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Vaccine 12</td>
<td>0</td>
<td>0</td>
<td>0/1204 (0)</td>
<td>0/1204 (0)</td>
</tr>
<tr>
<td>D-</td>
<td>R+</td>
<td>18</td>
<td>Placebo 7</td>
<td>2</td>
<td>0</td>
<td>2/696 (0.3)</td>
<td>0/696 (0)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Vaccine 11</td>
<td>4</td>
<td>0</td>
<td>21/1209 (1.7)</td>
<td>0/1209 (0)</td>
</tr>
<tr>
<td>D+</td>
<td>R+</td>
<td>22</td>
<td>Placebo 15</td>
<td>6</td>
<td>3</td>
<td>119/1489 (8.0)</td>
<td>135/1489 (9.1)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Vaccine 7</td>
<td>4</td>
<td>0</td>
<td>6/803 (0.7)</td>
<td>0/803 (0)</td>
</tr>
<tr>
<td>D+</td>
<td>R-</td>
<td>16</td>
<td>Placebo 5</td>
<td>5</td>
<td>4</td>
<td>339/599 (56.6)</td>
<td>415/599 (69.3)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Vaccine 11</td>
<td>6</td>
<td>5</td>
<td>128/1069 (12.0)</td>
<td>142/1069 (13.3)</td>
</tr>
</tbody>
</table>
Correlates of Immune Protection

- **D-R+ vaccine**
- **D+R+ vaccine**
- **D+R- vaccine**
- **D-R+ placebo**
- **D+R+ placebo**
- **D+R- placebo**

R = 0.62  P = 0.002
Vaccine Conclusions

- Encouraging results
  - Dominant effect appears to be reducing transmission from donor to recipient
  - Humoral immunity important

- 165 proteins reduced to one
  - Epitope specific response to gB?
  - Boosting of old epitopes or recognition of new?
  - Hypothesis-generating for next vaccine studies

- Consider other components of receptor/ internalisation process
Lab Strains vs Wild Type HCMV

- Lab strains lack the UL/b’ region
- This region includes
  - Tropism factors
  - Neutralizing antibody targets
  - Immune evasion genes
- It is lost upon culture in fibroblasts

Working Model for HCMV Entry into Cells


[Diagram showing HCMV entry process with proteins gH/gL/UL131A and UL130/UL128 involved in tethering, docking, and postattachment events.]
Working Model for HCMV Entry into Cells
Direct Proof of Humoral Protection

- Close the intellectual loop: randomise D+R- to mAb versus placebo

- Primary end-point:
  - Interruption of clinically-significant acquisition of CMV from donor
Study Design

CMV +ve

mAb

Placebo

CMV -ve
Characteristics of mAb

- Target protein gB, gH, UL128 etc
- mAb pools?
- Affinity
- On-off rate
- Neutralise / ADCC
- Bind multiple strains
No evidence that transmission of CMV from organ donor to recipient shares steps with maternal virus crossing the placenta to infect the fetus.

No evidence that the steps are distinct.

gB/MF59 vaccine has provided some protection to both patient groups.

If safety and efficacy of mAb established in transplants, a RCT could be conducted in pregnant women with primary CMV.
“No major epidemic of rubella has occurred in the United States since 1964. A retrospective survey of cases of congenital rubella syndrome occurring in the United States during the three-year period ending in 1968 located 491. Comparable figures for CMV congenital syndrome, based on the minimum estimate, would be in excess of 10,000 cases for the same three-year period”


“We should not wait another 20 years, while thousands of additional children are born seriously handicapped”

Acknowledgements

- Clinical Colleagues
  - Prof A. Burroughs & liver Tx team
  - Dr M. Harber & renal Tx team
  - Prof S. Mackinnon & BM Tx team
- Patients
- Research Nurse
  - Emily Rothwell

- Centre for Virology
  - Prof. Vincent Emery
  - Dr Richard Milne
- Diagnostic Virology
  - Laboratory staff
- Biostatistics
  - Dr Colette Smith
- Funding
  - The Wellcome Trust
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