Immune correlates of protection from congenital cytomegalovirus after primary infection in pregnancy

Daniele Lilleri
The identification of immune parameters correlated with protection from congenital HCMV would be instrumental to:

i) define immunological end-points for vaccine development and evaluation;

ii) implement prevention strategies by passive immunotherapies;

iii) provide prognostic tools for counseling.
• Antibody response to HCMV infection
  • Avidity maturation
  • Antigen specificity

• T cell response to HCMV infection
Pregnant women developing early IgG antibody with low avidity and with subsequent high increase appear to be at a higher risk of vertical transmission.

Avidity maturation and HCMV transmission to the fetus

Rapid increase in the serum *Cytomegalovirus* IgG avidity index in women with a congenitally infected fetus

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\begin{figure}
\centering
\includegraphics[width=\textwidth]{chart.png}
\caption{Change in IgG avidity index (ΔAI) over 4 weeks in women with and without a congenitally infected fetus.}
\end{figure}

\begin{table}
\centering
\begin{tabular}{|c|c|c|}
\hline
Group & ΔAI (%/4 week) & \textit{p}-value \\
\hline
Women with congenitally infected fetus & 15.7 & <0.001 \\
Women without congenitally infected fetus & 6.5 & \\
\hline
\end{tabular}
\caption{Change in IgG avidity index (ΔAI) over 4 weeks in women with and without a congenitally infected fetus.}
\end{table}
HCMV surface glycoprotein complexes

- gC I: gB omotrimer
- gC II: gM/gN
- gC III: -gH/gL/gO, -gH/gL/pUL128/pUL130/pUL131

- Dispensable for infection of fibroblasts
- Consistently mutated in laboratory adapted strains
- Essential for infection of epithelial, endothelial, dendritic cells and virus transfer to leukocytes
Anti-gB antibodies and HCMV transmission to the fetus

Anti-glycoprotein B IgG antibodies were significantly higher at delivery in transmitters than in nontransmitters. Characterization of the qualitative antibody response revealed lower neutralizing antibody titers in transmitters, suggesting an association between neutralizing activity and intrauterine transmission.

TABLE III. Anti-gB Antibody Response and Outcome of Pregnancy (n = 49)

<table>
<thead>
<tr>
<th></th>
<th>Transmitter</th>
<th>Nontransmitter</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti-gB positive</td>
<td>23</td>
<td>9</td>
</tr>
<tr>
<td>(anti-gB, anti gB/gH)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anti-gB negative</td>
<td>4</td>
<td>13a</td>
</tr>
<tr>
<td>(anti-gH, nonresponders)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>27(55.1%)</td>
<td>22(44.9%)</td>
</tr>
</tbody>
</table>

*a3 nonresponders.
Development antibodies to gB, gHgL and gH/gL/pUL128L and transmission of HCMV to the fetus

Earlier development of IgG to gHgL complexes in non-transmitting women.

NT, non-transmitter T, transmitter

(Lilleri et al PONE. 2013)
Neutralizing vs non-neutralizing antibodies elicited by HCMV glycoproteins after natural infection

Most gH/gL/pUL128-131-specific antibodies are neutralizing, whereas most gB-specific antibodies are non-neutralizing (Kabanova et al, PNAS 2014)
Antibodies anti-gH/gL/UL128-131 and neutralization of epithelial cell infection

• Adsorption of sera with the pentamer complex nearly abolishes ARPE infection neutralizing activity.

• The gB/MF59 subunit vaccine induced epithelial entry-specific neutralizing activities that were on average 15-fold lower than those observed following natural infection (Cui et al., Vaccine 2008).

(Lilleri et al PONE. 2013)
Inhibition of cell-to-cell HCMV spreading in epithelial cells and virus transfer to leukocytes by huMAbs to gH/gL/UL128-131

(Lilleri et al PONE. 2013)
Breadth of the neutralizing response to gH/gL/UL128L and transmission of HCMV to the fetus

NT, non-transmitter
T, transmitter

Broader targeting of neutralization sites on gHgLpUL128L and lower frequency of HCMV in blood early after infection in non-transmitting women.

(Lilleri et al PONE. 2013)
Summary (more questions than answers)

- An higher and more sustained viral replication may lead to:
  i) higher risk of virus transmission to the fetus
  ii) more prolonged stimulation of antibody production and a higher avidity maturation

- Could an earlier and broader neutralizing antibody response (to gHgLpUL128L) induce a faster block of HCMV dissemination, and reduce the risk for virus transmission to the fetus?

- May anti-gB antibodies (which are mostly non-neutralizing) facilitate virus transmission?
• Antibody response to HCMV infection

• T cell response to HCMV infection
  • Function
  • Phenotype
  • Antigen specificity
Simultaneous detection of HCMV-specific CD4+ and CD8+ effector T-cells. (Lozza et al., EJI 2005)

1. Generation and culture of monocyte-derived immature DCs

2. 24h infection of immature DCs with the endotheliotropic and dendrotropic HCMV strain VR1814

3. Overnight coculture of infected (and mock-infected) DCs and lymphocytes + Brefeldin A
Detection of HCMV-specific CD4+ and CD8+ T-cells producing IFN-γ and IL-2.

Functionally distinct antigen-specific T-cell subpopulations were described based on their ability to produce IFN-γ and/or IL-2 according to antigen persistence and load. (Pantaleo & Harari, Nat Rev Immunol 2006;6:417-22)

In HIV-patients, control of HCMV required the presence of dual IFN-g and IL-2 producing T cells. (Harari et al., Blood 2004;103:966-72)

4. Detection of HCMV-specific CD4+ and CD8+ T cell activation by flow cytometry analysis of cytokine production
Naïve and memory T cells according to CD45RA and CCR7 expression

1 month after HCMV infection

Naïve T-cells (CD45RA⁺CCR7⁺), after encountering antigen, switch from the CD45RA isoform to CD45RO.

Memory T-cells, according to CCR7 expression, can be divided into:
- “central memory” T-cells (T_CM, CD45RA⁻CCR7⁺)
- “effector memory” T-cells (T_EM CD45RA⁻CCR7⁻)

A proportion of T_EM can revert to the RA isoform of CD45 (T_EMRA, CD45RA⁺CCR7⁻) after the acute phase of infection.

The kinetics of CD45RA re-expression depends on the time-lapse from HCMV disappearance from blood.

12 months after HCMV infection
Cytokine production and surface phenotype of HCMV-specific T-cells in healthy subjects with remote infection. (Lilleri et al., JID 2008)

**Graph A:**
- HCMV-specific T-cells/1 blood
- Plot shows the frequency of CD8⁺ IFN⁺, CD8⁺ IL-2⁺, CD4⁺ IFN⁺, and CD4⁺ IL-2⁺ T-cells.

**Graph B:**
- % HCMV-specific memory T-cell populations
- Plot shows the percentage of CD8⁺ Tcm, CD8⁺ TEM, CD8⁺ TEMRA, CD4⁺ Tcm, CD4⁺ TEM, and CD4⁺ TEMRA T-cells.
HCMV-specific T-cell response and virus transmission to the fetus.

**Proliferation**

**Development of CD4⁺ LPR**

- **NT (median time: 71 days)**
- **T (median time: 246 days)**

**Development of CD8⁺ LPR**

- **NT (median time: 107 days)**
- **T (median time: 283 days)**

NT, non-transmitting mothers
T, transmitting mothers

(Revello et al., JID 2006; Lilleri et al., JID 2007, Proliferation)
HCMV-specific T-cell response and virus transmission to the fetus.

Higher frequency of IL2 producing CD4⁺ T cells 30 days after infection in non-transmitting women.

HCMV-specific T-cell response and virus transmission to the fetus.

IFN-γ

Low Interferon Relative-Response to Cytomegalovirus Is Associated with Low Likelihood of Intrauterine Transmission of the Virus

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HCMV-specific T-cell response in pregnant women and virus transmission to the fetus.

NT, non-transmitting mothers
T, transmitting mothers

Earlier development of $T_{EMRA}$ cells (re-expressing CD45RA) in non-transmitting women.

• An earlier development of LPR, IL2 producing CD4+ T cells, T_{EMRA} phenotype (CD45RA re-expression) and low IFNγ production by CD8+ T cells appears to be associated to a reduced risk of virus transmission to the fetus.
Dissection of the T-cell response to HCMV proteins

**non-structural:**
IE, immediate early antigen,

**structural:**
pp65, early/late antigen, internal tegument

gH/gL/pUL128-131, late antigen, surface glycoprotein

gB, late antigen, surface glycoprotein

Overlapping peptides (15mers) spanning the entire proteins
Library generation by T cell amplification

Seeding of T cells in multiple wells

Screening of the amplified T cell library

Geiger et al., Exp Med 2009, 206:1525-1534
T cells specific for selected HCMV antigens (Ag) in 25 patients at early (1 month) and late (6-12 months) stages of primary infection

- IE is recognized better by CD8\(^+\) T cells;
- gHgLpUL128L and gB are recognized better by CD4\(^+\) T cells;
- pp65 is equally recognized by CD4\(^+\) and CD8\(^+\) T cells;
- IE- and pp65-specific CD4\(^+\) T cells and pp65 and gB-specific CD8\(^+\) T cells seem to decrease in the late stage of infection.
Ag-specific T cell frequency in pregnant women transmitting (T, n=11) or non-transmitting (NT, n=10) the infection to the fetus

No association between T-cell antigen specificity and transmission of HCMV to the fetus
IL7-receptor (R) positive vs negative CD4\(^+\) and CD8\(^+\) memory T cell subsets

Selective expression of the interleukin 7 receptor identifies effector CD8 T cells that give rise to long-lived memory cells

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IL7-R\(^\text{neg}\): Short Term Effector (antigen-driven persistence)

IL7-R\(^\text{pos}\): Long Term Memory (antigen-independent persistence)
Analysis of the IL7-R\textsuperscript{neg} vs IL7-R\textsuperscript{pos} CD4\textsuperscript{+} and CD8\textsuperscript{+} memory T cell subsets

1 month after HCMV infection

CD4\textsuperscript{+}

IL7R-  IL7R+

CD8\textsuperscript{+}

IL7R-  IL7R+

12 months after HCMV infection

IL7R-  IL7R+

IL7R-  IL7R+
% IL7-R<sup>neg</sup> among HCMV*-specific CD4<sup>+</sup> and CD8<sup>+</sup> total memory T cells at different stages of infection

*considered as the sum of the T cells specific for the tested antigens

A higher percentage of IL7-R<sup>neg</sup> CD4<sup>+</sup> and CD8<sup>+</sup> T cells specific for HCMV is detected during primary infection, subsequently decreasing.
% IL7-R\textsuperscript{neg} HCMV-specific T cells in pregnant women transmitting (T, n=10) or non-transmitting (NT, n=10) the infection to the fetus

A higher percentage of IL7-R\textsuperscript{neg} CD4\textsuperscript{+} T cells specific for HCMV is detected in the early and late stages of infection in women transmitting the infection to the fetus (dark bars).
Factors possibly associated with IL7R\textsuperscript{pos} and IL7R\textsuperscript{neg} T cells and their correlation with HCMV transmission

In kidney transplant recipients, the percentage of IL7R\textsuperscript{neg} HCMV specific CD8+ T cells correlates with peak viral load in the acute phase of infection. (van Leeuwen at al., Blood 2005)

Inflammation Directs Memory Precursor and Short-Lived Effector CD8+ T Cell Fates via the Graded Expression of T-bet Transcription Factor (Joshi et al., Immunity 2007)

The strenght of T cell stimulation determines IL7 responsiveness. (Lozza et al., Eur J Immunol 2008)
Conclusions

• Notwithstanding some correlates between antibody response and protection from congenital infection, the most robust correlates were observed in the CD4$^+$ T cell response.

• A delayed appearance of CD4$^+$ T cell phenotype and functions associated with antigen clearance is observed in transmitting women.

• At the moment, these findings do not permit to define reliable prognostic parameters for use in the clinical setting, nor clarify the causal mechanism of protection.

• Taken together these findings suggest that a rapid control of HCMV viremia by the immune response is associated with a lower risk of virus transmission to fetus and encourage to pursue the analysis of the immune response in view of defining reliable correlates of protection.
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There are more things in CMV and immunity
Than are dreamt of in our virology
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