HYPERIMMUNOGLOBULIN and CMV-DNAemia IN PREGNANT WOMEN WITH PRIMARY CYTOMEGALOVIRUS INFECTION

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To avoid fetal rejection (50% allograft) an estrogen-induced immunodepression occurs in pregnancy.

- Decreased NK cells and cytotoxic activity, and functions of neutrophils and phagocytes.
- Thus in pregnancy infections are common but symptoms are less frequent and severe due to a low immunoreactivity.
- The immune response to CMV viremia implies the activation of immune cells (mostly CD8+ T cytotoxic lymphocytes) and production of numerous cytokines.
In CMV-seropositive women, maternal IgG antibodies are increasingly transmitted beginning at 6 weeks’ gestation and provide increased protection to the fetus against CMV.

During primary infection the fetus initially lacks CMV IgG and only some weeks after viremia receives low-avidity maternal antibodies and starts his own IgG production.

Meanwhile maternal stimulated immune cells produce numerous cytokines, which are toxic for fetal cells.

CMV damages the fetus:
- Directly by intracellular viral replication
- Indirectly by activating an inflammatory process leading to the abortion or to immune-mediated diseases mostly due to the aggression of CMV-infected cells from CD8+ T lymphocytes and to the activity of cytokines (some specifically neurotoxic)
- Regulation of the innate and adaptive immune responses is essential in the interplay between infection and immunopathological process

From selected plasma pools of at least 1000 hyperimmune donors identified by screening to have high titer CMV IgG (donors of nonspecific immunoglobulin are not tested)

8 steps of filtration, purification and concentration, including pasteurization

All types of human antibodies
Antiviral activity:
High titer and avidity IgG antibodies, which block CMV antigens including gB and pentamer (gH/gL/UL128/UL130/UL131), preventing the attachment of neutralized viruses to target cells

Immunomodulatory activity:
> Down regulation of interleukin synthesis
> Blockade of Fc receptors
> Specific antibodies towards receptors of T-cells (>cytotoxic CD8+ cells) and cytokines, which cannot be linked to fetal cells

EFFICACY OF ANTI-CMV ANTIBODIES IN ANIMALS: RANDOMIZED STUDIES

• Reduced rate of maternal viremia, fetal deaths and infections, and prevention of placentitis and intrauterine growth restriction by anti-whole virus or anti-gB serum in guinea pigs (Bia et al. JID 1980, Bratcher et al. JID 1995; Chatterjee et al. JID 2001)

• Undetectable virus and lower cerebral inflammation in brains of immune treated newborn mice vs controls (Cekinović et al. J Virol 2008)

• HIG passive infusion provided complete protection against fetal loss in pregnant, CD4+ T cell–depleted, RhCMV-seronegative, rhesus monkeys treated with standard HIG or dose-optimized RhCMV-neutralizing HIG prior to the intravenous challenge with RhCMV (Nelson CS et al. JCI Insight 2017)
HIG DECREASES CMV TRANSMISSION IN PREGNANCY

- Nigro G et al. NEJM 2005: transmission decreased (p=0.02) from 40% (19/47) to 16% (6/37) and no symptoms occurred in the infected infants

- Buxmann H et al. JPM 2012: 23% transmission (vs 40% in controls) and no symptoms in infected infants after HIG

- Revello MG et al. NEJM 2014: transmission decreased from 44% (27/62) to 30% (18/61) (p<0.13)

- Kagan KO et al. UOG 2018: 7.5% transmission from 40 women with primary CMV infection in the 1st trimester after administration of 200 U/kg of HIG every 2 weeks
• 200 U/kg of HIG every 2 weeks until 20 weeks gestation to 40 women with primary CMV infection < 14 weeks gestation.

• 7.5% transmission rate from 40 women.
• 35% transmission rate in untreated controls (p=0.001).

• For HIG treated mothers 1 fetus was infected at amniocentesis, and 2 after stopping HIG at 20 weeks.

• All neonates were asymptomatic.
WHEN MOTHER-TO-FETUS CMV TRANSMISSION REALLY OCCURS?

• CMV transmission may occur 4-8 weeks after seroconversion (the mean value between the last negative IgG and the first positive IgG detection)

• Seroconversion is concomitant with the detection of only IgM

• Fever or a flu-like syndrome (when present) may be concomitant with CMV viremia

• In fact, viremia precedes CMV IgM by about 2 weeks is also suggested by hypertransaminasemia, even before symptoms first occur
High transaminases (viremia) occurred 2 weeks before pharyngitis, 7 weeks before IgM, 9 weeks before IgG & DNA in AF.
HIG AND THE DECREASE OF CONGENITAL DISEASE (CMV+ at 20 WG)

- Nigro G et al. NEJM 2005: decreased CMV disease after fetal infection and disease (p<0.001)
- La Torre et al. CID 2006: decrease of CMV-induced placentomegaly by ultrasound (p<0.001)
- Nigro G et al. JID 2011: improved outcome in symptomatic children (p<0.001)
- Visentin S et al. CID 2012: decreased symptoms in infants after only one HIG infusion (p<0.01)
- Japanese CCITSG, J Reprod Immunol 2012: decreased number and severity of CMV disease in 12 children
HIG: SERIOUS ADVERSE EVENTS

- Nigro G et al. NEJM 2005: NONE
- Visentin S et al. CID 2012: NONE
- Buxmann H et al. JPM 2012: NONE
- Japanese Congenital Cytomegalovirus Infection Therapy Study Group, J Reprod Immunol 2012: NONE
- Revello MG et al. NEJM 2014:
  **HIG group:** preterm delivery (5 women), IUGR (2), intrahepatic cholestasis (1), postpartum eclampsia (1)
  **Saline Solution group:** abortion, arthralgia, hypertension (1 each)
- Blázquez-Gamero D et al. JMFNM 2017: NONE
Objective of the Study: Association HIG/CMV-DNAemia/outcome

- Study: Large registry database of pregnant women with confirmed primary CMV infection to identify risk factors for fetal infection and neonatal symptoms at birth

- Background: Primary CMV infection in early gestation has the highest rate of fetal and neonatal disease

- Focus: maternal viremia
SUBJECTS

- Women (mostly Italian) who sought consultation for a primary CMV infection during pregnancy between 2010 and 2017
- Each woman agreed to provide clinical data and signed an informed consent.
- Only women whose blood was examined for CMV-DNA (DNAemia) were enrolled
Estimated gestational age at maternal infection:

- Seroconversion half way between seronegative and seropositive serum.

- In women with flu-like symptoms or laboratory abnormalities, the beginning of these was defined as maternal infection

- For women with IgM, IgG, and very low avidity at <2 months gestation, CMV infection was estimated as immediately post conceptional
SUBJECTS: PARAMETERS

- Maternal age at conception
- Gestational age at the time of:
  - maternal CMV infection
  - first CMV DNA detection in blood
  - first HIG infusion
  - CMV DNA detection after HIG or 2nd DNAemia in controls
  - at delivery
- Viral load in the amniotic fluid
- Number of subsequent HIG infusions
- Prenatal manifestations of CMV disease
- Birth weight
- Clinical and laboratory abnormalities in the infants with congenital infection
RESULTS

- 304 women (4 sets of twins) were enrolled
- Maternal blood before and after HIG administration was obtained from 85 women, and from 46 non-HIG treated women.
- Termination of pregnancy (after AF+): 6/161 HIG and 17/147 non-treated women
- 281 live births
- 108 infants CMV+ (42 HIG - 66 non)
- 25 symptomatic infants (1 HIG-24 non)
Analysis of possible predictors of congenital infection

Maternal-fetal transmission

<table>
<thead>
<tr>
<th></th>
<th>Yes</th>
<th>NO</th>
</tr>
</thead>
<tbody>
<tr>
<td>HIG</td>
<td>70%</td>
<td>0%</td>
</tr>
<tr>
<td>DNAemia</td>
<td>60%</td>
<td>40%</td>
</tr>
<tr>
<td>DNAemia 1st and 2nd</td>
<td>40%</td>
<td>60%</td>
</tr>
<tr>
<td>US abnormal</td>
<td>50%</td>
<td>50%</td>
</tr>
</tbody>
</table>

P-values:
- HIG: P=0.0001
- DNAemia: P=0.0001
- DNAemia 1st and 2nd: P=0.0013
- US abnormal: P=0.03
### HIG and Maternal DNAemia as predictors of congenital CMV infection

<table>
<thead>
<tr>
<th>Predictor</th>
<th>CMV infected fetuses/infants</th>
<th>CMV uninfected fetuses/infants</th>
<th>Univariate P-value</th>
<th>Multivariate P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>All women (304)</td>
<td>131</td>
<td>173</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. of mothers:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HIG yes</td>
<td>48</td>
<td>109</td>
<td>P=0.0003</td>
<td>P=0.0003</td>
</tr>
<tr>
<td>HIG no</td>
<td>83</td>
<td>64</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. of mothers:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DNAemia pos 148</td>
<td>81</td>
<td>67</td>
<td>P=0.001</td>
<td>P=0.0063</td>
</tr>
<tr>
<td>DNAemia neg 156</td>
<td>50</td>
<td>106</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Resolution DNAemia by HIG administration

Resolution of maternal DNAemia

- HIG YES
- HIG NO

P=0.5

10/26 vs 0/8
P=0.003

DNAemia resolved
DNAemia resolved < 2 wks
<table>
<thead>
<tr>
<th>Predictor</th>
<th>CMV infected fetuses/infants</th>
<th>CMV uninfected fetuses/infants</th>
<th>Univariate P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Birth weight</td>
<td>3094</td>
<td>3299</td>
<td>P=0.0003</td>
</tr>
<tr>
<td>Mean WG when mother was infected</td>
<td>14.26</td>
<td>11.73</td>
<td>P=0.04</td>
</tr>
<tr>
<td>Mean WG of 1st DNA test</td>
<td>20.1</td>
<td>18.5</td>
<td>P=0.03</td>
</tr>
<tr>
<td>Mean WG when 1st HIG was given</td>
<td>22</td>
<td>19</td>
<td>P=0.009</td>
</tr>
</tbody>
</table>
## HIG and Maternal DNAemia as predictors of CMV disease

<table>
<thead>
<tr>
<th>Predictor</th>
<th>CMV symptomatic infants</th>
<th>CMV asymptomatic infants</th>
<th>Univariate P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>All women (281)</td>
<td>25</td>
<td>257</td>
<td></td>
</tr>
<tr>
<td>No. mothers:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HIG yes</td>
<td>1</td>
<td>151</td>
<td>P=0.0001</td>
</tr>
<tr>
<td>HIG No</td>
<td>24</td>
<td>106</td>
<td></td>
</tr>
<tr>
<td>No. of mothers:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DNAemia POS</td>
<td>12</td>
<td>122</td>
<td>P=1.0</td>
</tr>
<tr>
<td>DNAemia NEG</td>
<td>13</td>
<td>137</td>
<td></td>
</tr>
</tbody>
</table>
Early resolution of DNAemia by HIG prevent CMV disease

Resolution of maternal DNAemia

DNAemia resolved

DNAemia resolved < 2 wks

P=0.5

HIG YES

HIG NO

P=0.003

7/8 fetuses aborted

4/8 fetuses: abnormal US
## Factors NOT predictive of congenital CMV infection

<table>
<thead>
<tr>
<th>Predictor</th>
<th>CMV infected fetuses/infants (131)</th>
<th>CMV uninfected fetuses/infants (176)</th>
<th>Univariate P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maternal age (years)</td>
<td></td>
<td></td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>32</td>
<td>33</td>
<td></td>
</tr>
<tr>
<td>Maternal infection &lt;14 WG (179)</td>
<td>73 (56%)</td>
<td>107 (61%)</td>
<td>p=0.1</td>
</tr>
<tr>
<td>&lt;14 WG (125)</td>
<td>58 (44%)</td>
<td>69 (39%)</td>
<td></td>
</tr>
<tr>
<td>Mean doses of HIG for women HIG-treated</td>
<td>3.0</td>
<td>3.4</td>
<td>P=0.22</td>
</tr>
<tr>
<td>Mean copy number of CMV DNAemia</td>
<td>3128</td>
<td>2348</td>
<td>P=0.55</td>
</tr>
</tbody>
</table>
MATERNAL–FETAL CMV INFECTION: PREVENTION OR THERAPY?

- To decrease the rates of primary infection in pregnancy, women should be informed about CMV and hygienic measures.

- To prevent fetal infection and disease, CMV in pregnancy should be diagnosed as soon as possible following at least a double screening (8–12 and 14–18 weeks).

- Positive DNAemia at enrollment may have a negative prognostic value.

- HIG could decrease CMV transmission and fetal disease (also including cortical malformations and deafness if given early).