The fine specificity and neutralizing function of maternal antibodies predictive of reduced HIV-1 peripartum transmission risk

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Mother-to-Child-Transmission (MTCT) of HIV-1

- ~150,000 new annual pediatric infections despite wide availability of antiretroviral therapy (ART)

- In the absence of ART, only 40% of HIV-infected women transmit the virus to their infant – suggesting the presence of maternal protective factors
Maternal antibodies are transferred to the fetus *in utero*

Naturally protective maternal antibody responses that can block infant HIV could be exploited by a maternal HIV-1 vaccine
Women and Infant Transmission Study (WITS) cohort

• Observational cohort of HIV-1 clade B infected, North American pregnant women
  – Enrolled in 1990s, before AZT

• 248 ART naive mothers: 165 non-transmitters, 83 transmitters

• Multivariable logistics regression model: CD4+ T cell count, viral loads, delivery mode, infant gestational age to define MTCT risk

Maternal humoral immune correlates of protection in the WITS cohort

IgG binding to V3
OR = 0.64 (0.42-0.97) p = 0.04

Neutralization
OR: 0.54 (0.35-0.83) p = 0.005

Responses were correlated and colinear, suggesting they are plasma markers of an underlying mechanism, such as ability to neutralize autologous virus

Permar SR et al. Journal of Clinical Investigation. 2015
Variable loop 3 (V3) is a major neutralizing epitope

HIV Envelope protein

V3 – major target of neutralizing antibodies

Autologous neutralization by V3-specific IgG mAbs from a non-transmitting mother

- 95% viruses neutralized with IC$_{50}$ < 50μg/mL
- Evidence of viral escape from V3-specific mAbs
Aims

• To define the subclass and fine specificity of V3-specific IgG responses associated with MTCT risk

• To define the function of maternal V3-specific IgG responses that associated with reduced MTCT risk

• To determine if the ability to evade maternal plasma neutralization defines infant transmitted variants
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V1V2-antibodies of IgG3 subclass predicted reduced HIV acquisition in the RV144 vaccine trial

Haynes et al. NEMJ 2012, Tomaras et al. STM 2014

Are V3-specific IgG3 antibodies associated with reduced MTCT risk?

Placental transfer efficiency: IgG1 > IgG3 > IgG2 > IgG4
Method used

• IgG total and subclass binding antibody multiplex assay against V3 antigens

PE labelled secondary Ab

IgG1, IgG2, IgG3, IgG4 detectors

Microsphere coupled with antigen of interest

Serum Ab
Higher frequency of V3-specific IgG2, but not IgG3, subclass responses in NT vs T women

Fisher's exact test

N = 248
What is the fine-specificity of the maternal V3-specific antibody associated with reduced MTCT?
Fine-specificity of maternal V3-specific IgG: AA residues flanking V3 loop tip

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<tbody>
<tr>
<td>V3.B wild-type</td>
<td>NNTRKSIHIGPGQAYTGDIIIGDIRQAHC</td>
<td>NNTRKSIHIGPGQAYTGDIIIGDIRQAHC</td>
<td>NNTRKSIHIGPGQAYTGDIIIGDIRQAHC</td>
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<tr>
<td>V3.CRF2</td>
<td>NNTRKSIHIGPGQAYTGDIIIGDIRQAHC</td>
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<td>V3.D</td>
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![HIV Envelope protein](image)
Are AA residues flanking the V3 loop tip important for binding of maternal V3-specific IgG associated with reduced MTCT risk?
A.A. residues to the right flank of the V3 loop tip are important for the binding of potentially protective maternal V3-specific IgG responses.

V3.B K305Q I307T H308T
NNTRQSTTIIGPGRAFYATGDIIGDIRQAHC

V3.B F317L A319T D322R
NNTRKSIHIGPIGRAALYTTEGRIGDIRQAHC

V3.B wild-type:
OR 0.86 (0.75 – 1.00) p = 0.04

V3.B K305Q I307T H308T:
OR 0.88 (0.80 – 0.97) p = 0.01

V3.B F317L A319T D322R:
OR 0.94 (0.86 – 1.03) p = 0.24

N = 248 Multivariable logistic regression
Which V3 residues in the right flank are required for the binding of potentially-protective maternal V3-specific IgG binding responses?
AA residue F317* required for the binding of potentially protective maternal V3-specific IgG binding responses

*N317 also identified in the RV144 viral sieve analysis

\[N = 248\]

Multivariable logistic regression
Aims

- To define the subclass and fine specificity of V3-specific IgG responses associated with MTCT risk
- To define the function of maternal V3-specific IgG responses that associated with reduced MTCT risk
- To determine if the ability to evade maternal plasma neutralization defines infant transmitted variants
Hypothesis: maternal V3 antibodies mediate the neutralization that is associated with reduced MTCT risk

Methods

• Neutralization of HIV-2 V3 wild-type, and mutant chimeric virus

• Neutralization competition assay with V3 peptides
Maternal V3-specific IgG neutralizing responses associated with reduced MTCT risk target AA 319 and 322

$N = 248$

Multivariable logistic regression
Non V3-specific IgG neutralizing responses also contribute to reduced MTCT risk

N = 248
Multivariable logistic regression
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Selection of mother-infant pairs from Women and Infant Transmission Cohort

- HIV DNA negative at birth and positive >7 days (Peripartum transmission)
- Maternal plasma from birth time-point
- Infant plasma <3 month of age
- 14 Mother-Infant pairs were selected
Methods

- Single genome analysis of the maternal and infant HIV-1 envelope (25-30 single genomes per sample)

- Compared neutralization sensitivity of infant and maternal virus variants with paired maternal serum
Infant infected by 1 or 2 T/F viruses

Infant

Mother

Mother variant closest to Infant T/F

0                     1000                    2000                  3000

Base number

T/F
Are infant T/F viruses resistant to paired maternal serum?
# Maternal plasma neutralization analysis of Infant T/F pseudoviruses

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<tr>
<th>Infant T/F Virus</th>
<th>100307i.2</th>
<th>100052 i.2</th>
<th>101421i.2</th>
<th>100711i.3</th>
<th>102149i.2</th>
<th>102407 i.2</th>
<th>100890i.2</th>
<th>100002i.2</th>
<th>100014i.2</th>
<th>100046i.3</th>
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<td>Paired maternal serum</td>
<td>&lt;20</td>
<td>55</td>
<td>&lt;20</td>
<td>&lt;20</td>
<td>29</td>
<td>49</td>
<td>&lt;20</td>
<td>&lt;20</td>
<td>102*</td>
<td>158</td>
<td>&lt;20</td>
<td>&lt;20</td>
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<td>Virus Tier Sensitivity</td>
<td>1B</td>
<td>1B</td>
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<td>2</td>
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<td>2</td>
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<tr>
<th>Specificity</th>
<th>bNAbs</th>
<th>IC&lt;sub&gt;50&lt;/sub&gt;</th>
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<tr>
<td>anti-CD4</td>
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<tr>
<td>VRC01</td>
<td>0.12</td>
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<td>b12</td>
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<td>NIH 45-46</td>
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<td>CH31</td>
<td>0.06</td>
<td>0.1</td>
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<tr>
<td>gp120</td>
<td>2G12</td>
<td>0.06 0.29 0.22</td>
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<td>gp120 (V1/V2)</td>
<td>2G12</td>
<td>0.06 0.29 0.22</td>
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<tr>
<td>CH01</td>
<td>0.93</td>
<td>2.54</td>
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<td>gp120 (V2/V3)</td>
<td>PG9L_4A293i</td>
<td>0.1 2.97 6.9 0.24</td>
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<tr>
<td>PG16_LL293i</td>
<td>0.03</td>
<td>1.01 22.05 0.27</td>
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<tr>
<td>gp120 (V3 loop)</td>
<td>PGT121</td>
<td>&lt;0.01 0.29 &lt;0.01</td>
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<td>PGT126</td>
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<td>gp41</td>
<td>4E10</td>
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<td>10E8</td>
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<td>DH512</td>
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<td>gp120 (V1/V2) &amp; CD4bs</td>
<td>CH01/31</td>
<td>0.06 0.07 0.14</td>
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* High background neutralization against MLV

**IC<sub>50</sub>**

- <20
- 20-50
- 50-100
- 100-200
- >200

**ID<sub>50</sub>**

- <60
- 61-100
- 101-200
- 201-300
- >300

Joshua Eudailey, Celia Lebranche and David Montefiori
Selection of Maternal Non-Transmitted Variants

- 10-12 maternal variants were selected from 9 mothers
- Pseudovirus prepared using Overlap PCR method
Neutralization Sensitivity of maternal variants and infant T/F to paired maternal serum

ID<sub>50</sub>

Mother
Infant T/F
Maternal variant closest to infant T/F

Mother/ infant Pseudovirus
Neutralization Sensitivity of maternal variants and infant T/F to paired maternal serum

![Graph showing ID50 values for different samples.](image)
Neutralization Sensitivity of maternal variants and infant T/F to paired maternal serum

![Graph showing neutralization sensitivity](image-url)
Conclusions

- V3-specific IgG2 subclass responses are detected in higher frequencies in NT vs. T women

- Potentially-protective maternal V3-specific IgG binding and neutralizing responses associated with reduced MTCT risk target amino acid residues 317, 319, and 322

- Infant T/F variants may be defined by neutralization resistance to paired maternal plasma
Implications

Maternal HIV vaccines that raise levels V3-specific IgG binding targeting the V3 C-terminus and autologous virus-neutralizing antibodies could be important to block peripartum MTCT of HIV
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