Secondary Zika virus infection do not support evidences of Antibody-Dependent Enhancement \textit{in vivo} in dengue pre-exposed rhesus macaques
**Experimental Overview**

**Cohort 1**
- DENV infected
- $5 \times 10^5$ pfu by s.c.
- October 2013

**Cohort 2**
- DENV-Naive
- N=4

**Time after DENV Infection:**
- Months 12 | 18 | 30
- ZIKV: Month 34

**ZIKV Infection**
- FP/H/2013
- $1 \times 10^6$ pfu by s.c.
- August 1st 2016

**Bled**
Cross reactivity in ELISA to DENV and ZIKV before ZIKV infection*

DENV1 and DENV2 exposed animals.

*Samples collected up to 1 year after DENV infection, were collected in year 2014, before ZIKV was introduced in LA region.
Observations

• ELISA results include four animals per DENV group (DENV1 and 2). However only two per group were ZIKV-infected.

• All exposed animals showed an increase of DENV antibody titers after DENV infection.

• Only animals exposed to DENV2 showed cross-reactivity to ZIKV-NS1 after 30 days of DENV infection. Cross-reactivity persist in most of the animals up to 2.5 years later.
## Pre-existing immunity to DENV before ZIKV infection

<table>
<thead>
<tr>
<th>ID (infecting serotype)</th>
<th>SAMPLE DATE</th>
<th>TIME POINT</th>
<th>Dengue IgG</th>
<th>ZIKV IgG NS1</th>
<th>DENV-Neut titer</th>
<th>ZIKV-Neut titer</th>
</tr>
</thead>
<tbody>
<tr>
<td>RM1 (D1)</td>
<td>9/24/13</td>
<td>BASELINE</td>
<td>NEG</td>
<td>NEG</td>
<td>D1 &lt;20/&lt;20/&lt;20</td>
<td>&lt;20/&lt;20/&lt;20</td>
</tr>
<tr>
<td>RM2 (D1)</td>
<td>8/1/13</td>
<td>BASELINE</td>
<td>NEG</td>
<td>NEG</td>
<td>D1 &lt;20/&lt;20/&lt;20</td>
<td>&lt;20/&lt;20/&lt;20</td>
</tr>
<tr>
<td>RM3 (D2)</td>
<td>8/14/13</td>
<td>BASELINE</td>
<td>NEG</td>
<td>NEG</td>
<td>D2 &lt;20/&lt;20/&lt;20</td>
<td>&lt;20/&lt;20/&lt;20</td>
</tr>
<tr>
<td>RM4 (D2)</td>
<td>8/14/13</td>
<td>BASELINE</td>
<td>NEG</td>
<td>NEG</td>
<td>D2 &lt;20/&lt;20/&lt;20</td>
<td>&lt;20/&lt;20/&lt;20</td>
</tr>
<tr>
<td>RM1 (D1)</td>
<td>11/4/13</td>
<td>DAY 30</td>
<td>POS</td>
<td>NEG</td>
<td><strong>320/320/320</strong></td>
<td>&lt;20/&lt;20/&lt;20</td>
</tr>
<tr>
<td>RM2 (D1)</td>
<td>11/4/13</td>
<td>DAY 30</td>
<td>POS</td>
<td>NEG</td>
<td><strong>640/640/320</strong></td>
<td>&lt;20/&lt;20/&lt;20</td>
</tr>
<tr>
<td>RM3 (D2)</td>
<td>11/20/13</td>
<td>DAY 30</td>
<td>POS</td>
<td>POS</td>
<td><strong>1280/1280/1280</strong></td>
<td>&lt;20/&lt;20/&lt;20</td>
</tr>
<tr>
<td>RM4 (D2)</td>
<td>11/20/13</td>
<td>DAY 30</td>
<td>POS</td>
<td>POS</td>
<td><strong>1280/1280/1280</strong></td>
<td>&lt;20/&lt;20/&lt;20</td>
</tr>
<tr>
<td>RM1 (D1)</td>
<td>12/3/13</td>
<td>DAY 60</td>
<td>POS</td>
<td>NEG</td>
<td><strong>1280/1280/640</strong></td>
<td>&lt;20/&lt;20/&lt;20</td>
</tr>
<tr>
<td>RM2 (D1)</td>
<td>12/3/13</td>
<td>DAY 60</td>
<td>POS</td>
<td>NEG</td>
<td><strong>320/160/160</strong></td>
<td>&lt;20/&lt;20/&lt;20</td>
</tr>
<tr>
<td>RM3 (D2)</td>
<td>12/17/13</td>
<td>DAY 60</td>
<td>POS</td>
<td>POS</td>
<td><strong>1280/1280/1280</strong></td>
<td>&lt;20/&lt;20/&lt;20</td>
</tr>
<tr>
<td>RM4 (D2)</td>
<td>12/17/13</td>
<td>DAY 60</td>
<td>POS</td>
<td>POS</td>
<td><strong>1280/1280/1280</strong></td>
<td>&lt;20/&lt;20/&lt;20</td>
</tr>
<tr>
<td>RM1 (D1)</td>
<td>10/22/14</td>
<td>1 YEAR</td>
<td>POS</td>
<td>NEG</td>
<td><strong>1280/1280/640</strong></td>
<td>&lt;20/&lt;20/&lt;20</td>
</tr>
<tr>
<td>RM2 (D1)</td>
<td>10/22/14</td>
<td>1 YEAR</td>
<td>POS</td>
<td>NEG</td>
<td><strong>320/160/160</strong></td>
<td>&lt;20/&lt;20/&lt;20</td>
</tr>
<tr>
<td>RM3 (D2)</td>
<td>10/22/14</td>
<td>1 YEAR</td>
<td>POS</td>
<td>POS</td>
<td><strong>1280/1280/1280</strong></td>
<td>&lt;20/&lt;20/&lt;20</td>
</tr>
<tr>
<td>RM4 (D2)</td>
<td>10/22/14</td>
<td>1 YEAR</td>
<td>POS</td>
<td>NEG</td>
<td><strong>1280/1280/640</strong></td>
<td>&lt;20/&lt;20/&lt;20</td>
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<tr>
<td>RM1 (D1)</td>
<td>4/1/16</td>
<td>2.5 YEAR</td>
<td>POS</td>
<td>NEG</td>
<td><strong>160/160/80</strong></td>
<td>&lt;20/&lt;20/&lt;20</td>
</tr>
<tr>
<td>RM2 (D1)</td>
<td>4/1/16</td>
<td>2.5 YEAR</td>
<td>POS</td>
<td>NEG</td>
<td><strong>1280/640/640</strong></td>
<td>&lt;20/&lt;20/&lt;20</td>
</tr>
<tr>
<td>RM3 (D2)</td>
<td>4/1/16</td>
<td>2.5 YEAR</td>
<td>POS</td>
<td>POS</td>
<td><strong>1280/1280/640</strong></td>
<td>&lt;20/&lt;20/&lt;20</td>
</tr>
<tr>
<td>RM4 (D2)</td>
<td>4/1/16</td>
<td>2.5 YEAR</td>
<td>POS</td>
<td>NEG</td>
<td><strong>320/320/160</strong></td>
<td>&lt;20/&lt;20/&lt;20</td>
</tr>
<tr>
<td>RM5 (Naïve)</td>
<td>7/5/16</td>
<td>BASE L Before ZIKA</td>
<td>NEG</td>
<td>NEG</td>
<td>&lt;20/&lt;20/&lt;20</td>
<td>&lt;20/&lt;20/&lt;20</td>
</tr>
<tr>
<td>RM6 (Naïve)</td>
<td>7/5/16</td>
<td>BASE L Before ZIKA</td>
<td>NEG</td>
<td>NEG</td>
<td>&lt;20/&lt;20/&lt;20</td>
<td>&lt;20/&lt;20/&lt;20</td>
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<tr>
<td>RM7 (Naïve)</td>
<td>7/5/16</td>
<td>BASE L Before ZIKA</td>
<td>NEG</td>
<td>NEG</td>
<td>&lt;20/&lt;20/&lt;20</td>
<td>&lt;20/&lt;20/&lt;20</td>
</tr>
<tr>
<td>RM8 (Naïve)</td>
<td>7/5/16</td>
<td>BASE L Before ZIKA</td>
<td>NEG</td>
<td>NEG</td>
<td>&lt;20/&lt;20/&lt;20</td>
<td>&lt;20/&lt;20/&lt;20</td>
</tr>
</tbody>
</table>

*Challenged on August 1st, 2016
Observations

• Acute or convalescent samples after DENV infection do not neutralize ZIKV *in vitro*.
Serum Viremia

Viremia

Days after ZIKV infection

DENV1 and DENV2 pre-exposed animals.
Observations

- ZIKV peak viremia is not modified by pre-existing immunity to DENV.

- In general, animals pre-exposed to DENV showed shorter ZIKV viremia compared to DENV-naïve animals.
## Serum Viremia vs. Neut Antibodies

### Viremia<sup>a</sup> (log<sub>10</sub> genome equivalent/ml) during days 1-10 post Zika infection

<table>
<thead>
<tr>
<th>Group</th>
<th>Monkey ID</th>
<th>Day 1</th>
<th>Day 2</th>
<th>Day 3</th>
<th>Day 4</th>
<th>Day 5</th>
<th>Day 6</th>
<th>Day 7</th>
<th>Day 8</th>
<th>Day 9</th>
<th>Day 10</th>
<th>Average Duration (days)</th>
<th>Pre challenge anti-DENV1 Neut&lt;sub&gt;50&lt;/sub&gt;</th>
<th>Pre challenge anti-DENV2 Neut&lt;sub&gt;50&lt;/sub&gt;</th>
<th>Pre challenge anti-ZIKV Neut&lt;sub&gt;50&lt;/sub&gt;</th>
<th>30 days Post challenge anti-DENV1 Neut&lt;sub&gt;50&lt;/sub&gt;</th>
<th>30 days Post challenge anti-DENV2 Neut&lt;sub&gt;50&lt;/sub&gt;</th>
<th>30 days Post challenge anti-ZIKV Neut&lt;sub&gt;50&lt;/sub&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Uninfected controls</td>
<td>RM5</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>6.5</td>
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<td>P</td>
<td>&lt;20</td>
<td>P</td>
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<tr>
<td></td>
<td>RM6</td>
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<td>+</td>
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<td>&lt;20</td>
<td>&lt;20</td>
<td>P</td>
<td>&lt;20</td>
<td>P</td>
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<tr>
<td></td>
<td>RM8</td>
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<td>+</td>
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<td>&lt;20</td>
<td>&lt;20</td>
<td>P</td>
<td>&lt;20</td>
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<td>P</td>
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<td>DENV1 Infected</td>
<td>RM1</td>
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<td></td>
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<td>+</td>
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<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
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<td>P</td>
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<td>P</td>
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<td>DENV2 Infected</td>
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<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
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<td>-</td>
<td>-</td>
<td>+</td>
<td>5.5</td>
<td>ND</td>
<td>1280</td>
<td>&lt;20</td>
<td>P</td>
<td>P</td>
<td>P</td>
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<tr>
<td></td>
<td>RM4</td>
<td>+</td>
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<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>5.5</td>
<td>ND</td>
<td>320</td>
<td>&lt;20</td>
<td>P</td>
<td>P</td>
<td>P</td>
</tr>
</tbody>
</table>

*P: In progress*
Observations

• In animals pre-exposed to DENV, neutralization titers do not correlate with the peak or the magnitude of viremia after infection with ZIKV.
Laboratory Parameters After ZIKV infection

WBC

Lymphocytes

Neutrophils

Monocytes

ALT

AST

DENV2 and DENV1-exposed animals; Naïve animals.
Observations

• Similar to other viral infections, there was an initial decrease in the total WBC count at 7 days after infection in both cohorts of animals and returning to baseline levels by day 15.

• There is a slight increase in the % of lymphocytes that decrease at 7 days after infection both in naïve and pre exposed animals.

• There was an clear initial increase in the % of monocytes at 7 days after infection, more evident in cohort 1. This increase tends to be higher in the animals exposed to DENV2 compared to those exposed to DENV1.

• Both ALT and AST, but particularly ALT showed a strong trend (p = 0.09) to be higher in naïve animals exposed to ZIKV than in animals with previous immunity to any of the two dengue serotypes.

  – These results suggest a protective role to the liver damage by the DENV pre-existing immunity.
Observations

• Generalized Rash in RM1 (DENV1 pre-exposed macaque) seen by day 8 after infection. Rash was still present after 23 days of infection.

• We are evaluating the rash evolution and the meaning in the context of ZIKV infection and dengue-pre existing immunity.

• Other causes of rash are being ruling out.
Flow Cytometry Results. Red and blue squares: DENV2 and DENV1 pre-infected animals.
Observations

• The frequency of B cells activation was significant at 24 and 48 hours after infection compared to the basal level in both cohorts.

• The frequency of activated B cells was statistically higher in the naïve group at 48 hours and 10 days after the infection compared to the DENV pre-exposed animals.

• In both cohorts activated NK16 cells increased at 48 hours after the infection, with a decrease in the frequency of activation by day 7, returning to baseline levels by day 15. However, differences in the frequency of activation was not significant in any cohort.

• CD4+ T cells activation was significantly higher in both cohorts at 24, 48 hours and by day 10. However the frequency and magnitude of CD4+/CD69+ cells showed a trend to be higher in the naïve animals.

• There is a significant increase in the frequency of CD8+/CD69+ cells at 24 and 48 hours after infection in both cohorts. However the magnitude of activation trend to be higher in naïve animals.
Upcoming Results

• ZIKV in Saliva: In Progress

• ZIKV in urine: In Progress

• Serum Cytokines

• Quality and magnitude of ZIKV and DENV Neutralizing Antibodies several times after ZIKV infection

• More Clinical data: Weight, Temperature

• More immunological data: DC, MO, NK8 cells

• *In vitro* ADE using samples before and after ZIKV infection

• Characterization of the epitopes linked to ZIKV vs. DENV neutralization

• Dengue infection of ZIKV-exposed animals
This work is being performed by

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Citing this work:


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