Zika Virus Infects Cytotrophoblasts & Hofbauer Cells in Chorionic Villi from First-Trimester Human Placentas & Downregulates Cellular Markers

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Branching of chorionic villi

Villus branching increases exchange of nutrients, gases & IgG for passive immunity

Maternal blood space
Branched microvilli increase perfusion
Underlying villus cytotrophoblasts
Human chorionic villus explant of placenta 11 wk GA infected with prototype MR766 or epidemic strains from Nicaragua Nica1 and Nica2

Plated on Matrigel-coated permeable filters, 3 dpi
Anchoring Villus Explant Embedded/Sectioned
Section of anchoring villus explant shows CTBs immunostained for cytokeratin (CK)
ZIKV spreads in maternal blood space, infects primary cells, chorionic villi, progeny produced.
First-trimester human chorionic villi

• Patterns of CTB and Hofbauer cell infection - consistently reproducible.
• Functional differences manifested in CTBs infected by Nica and MR766.
• ZIKV-infected CTBs downregulate a marker of cell proliferation.
• Villus explant model useful for testing drugs to ZIKV suitable for pregnancy.
ZIKV infects CTBs in proximal cell columns

CTBs express cytokeratin, CK

Proliferation Ki67+
Nica-infected CTBs migrate/invoke, whereas MR766-infected CTBs are impaired
Nica infects Hofbauer cells in villus core
### Consistently reproducible patterns of Nica ZIKV & MR766 infection in chorionic villi

<table>
<thead>
<tr>
<th>Placenta</th>
<th>1</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>TOTALS</th>
</tr>
</thead>
<tbody>
<tr>
<td>ZIKV strain</td>
<td>Nica</td>
<td>Nica</td>
<td>MR766</td>
<td>Nica</td>
<td>Nica</td>
<td>MR766</td>
<td>MR766</td>
</tr>
<tr>
<td># Chorionic villi</td>
<td>41</td>
<td>10</td>
<td>8</td>
<td>42</td>
<td>150</td>
<td>49</td>
<td>11</td>
</tr>
<tr>
<td>Infected cell columns</td>
<td>13/36 (36%)</td>
<td>1/4 (25%)</td>
<td>5/10 (50%)</td>
<td>0/30 (0%)</td>
<td>3/69 (4%)</td>
<td>4/34 (12%)</td>
<td>8/11 (73%)</td>
</tr>
<tr>
<td>Villi with infected invasive CTBs***</td>
<td>31/35 (89%)</td>
<td>2/4 (50%)</td>
<td>6/8 (75%)</td>
<td>16/24 (67%)</td>
<td>7/38 (18%)</td>
<td>4/23 (17%)</td>
<td>7/11 (64%)</td>
</tr>
<tr>
<td>Infected Hofbauer cells near proliferating CTBs</td>
<td>6/34 (18%)</td>
<td>4/6 (67%)</td>
<td>0/11 (0%)</td>
<td>19/36 (53%)</td>
<td>24/67 (36%)</td>
<td>10/28 (36%)</td>
<td>1/10 (10%)</td>
</tr>
</tbody>
</table>

** *** Migration of infected CTBs varies widely between Nica and MR766 infection as detailed in Figure 2.

Placentas 8-11 wks GA (n=6); anchoring villi and villus sprouts and villus cores, immunostained for cellular markers and ZIKV NS3 and E proteins
Nica-infected CTBs migrate further forming anchoring villi, but not MR766-infected cells.
MR766-infected CTBs exhibit fragmented nuclei in cell columns suggesting apoptosis.
Proliferating CTBs and Hofbauer cells in mitosis express phospho-Histone H3
ZIKV-infected, mitotic CTBs downregulate nuclear protein Ki67, a proliferation marker.
ZIKV infects CTBs & Hofbauer cells releasing infectious progeny - onset of prolonged viremia
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