Nieuwe ontwikkelingen in de immunopathogenese van Dengue:

*Belang van moleculaire diagnostiek*

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Modernisering van het Dengue surveillance systeem in Suriname (ZG007)

Uitvoeringsorganisatie Twinning Suriname-Nederland

Dengue: High Impact

According to WHO estimates:

- 2.5 billion people at risk of acquiring dengue
- 50 to 100 million new cases of dengue virus infection each year
- 500,000 – 1,000,000 individuals develop DHF
- ~ 20,000 deaths

“disease of children”
Dengue Virus

- 50-100 million infections annually
- Transmitted by Aedes mosquitoes
- 4 serotypes (DENV 1-4)
- All serotypes can cause 4 clinically defined manifestations
Transmission cycle

1. Transmission

2. Virus replication

3. Dengue virus infects
   - Monocytes
   - Macrophages
   - Dendritic cells

4. Virus released and circulates in blood

5. Second mosquito ingests virus with blood
Clinical syndromes

- Undifferentiated fever
- Classic Dengue Fever (DF) ("knokkelkoorts")
- Dengue Hemorrhagic Fever (DHF)
- Dengue Shock Syndrome (DSS)
Transmission: mosquito-borne disease

- Transmission via mosquitoes
  - *Aedes aegypti* (“tijgermug”) ▶ most important vector for Dengue virus
  - *Aedes albopictus*, *Aedes polynesiensis*

Real size: 5 mm
Dengue

Most common arthropod-borne viral infection

All 4 DENV serotypes circulate in the (sub) tropical regions of the world
Dengue Hemorrhagic Fever

1968-1980
5 countries: 60 cases

1981-2001
28 countries > 93,000 cases
Recent re-emergence of dengue: 
*cocirculation of serotypes*

Several factors are involved in emergence of dengue virus: 

Expectation: 
More frequent and larger epidemics associated with severe disease
Current strategy to combat DENV: Vector Control
Disease and pathogenesis

1\textsuperscript{st} DENV infection

- DENV-1 → DF → recovery → DENV specific antibodies + memory T cells

Homotopic re-infection

- DENV-1 → No disease → Pre-existing antibodies \textit{neutralize} secondary infection

Heterotopic re-infection

- DENV-2 → DF, DHF, DSS → Pre-existing antibodies may \textit{enhance} secondary infection

ADE: Antibody-dependent enhancement
Why is the antibody response to a previous infection responsible for severe disease?

- Infants born to dengue-immune mothers are susceptible to severe disease during a primary infection.
- This susceptibility disappears at approximately 1 year of age.
- This indicates that, in these infants, maternal antibodies are involved in the development of severe disease during a primary infection.
Critical aspects of the immunopathogenesis of DHF

- Pre-existing heterotypic antibodies
- Enhanced uptake of virus by Fc-receptor-bearing cells (monocytes, macrophages and DCs)
- Large infected cell mass and high viral load early in infection
- Strong immune response and release of cytokines
- Endothelial cell damage and hemorrhagic manifestations (primarily plasma leakage and hemoconcentration)
Risk Factors for DHF

- Pre-existing anti-dengue antibody
  - previous infection with another DENV serotype (1-4)
  - maternal antibodies in infants

- Virulence of the virus strain

- Host
  - genetic background (ethnicity)
  - age
Structure of dengue virus:
cryo-EM image reconstruction

(Kuhn et al., Cell, 2002, 108, 717-725)
Cellular life cycle of dengue virus
Assembly of Flavivirus particles

Mature       Mosaic        Immature

Flavivirus-infected cells release a high proportion of particles containing prM
Immature DENV virus

- Furin-deficient LoVo cells secrete fully immature DENV prM content
  - C6/36-derived DENV: 30%
  - LoVo-derived DENV: 100%

- Immature DENV is essentially noninfectious:

  Particle-to-pfu ratio

<table>
<thead>
<tr>
<th>Mature</th>
<th>Immature</th>
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<tbody>
<tr>
<td>100</td>
<td>&gt; 1 x 10^6</td>
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</tbody>
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Immature Dengue Virus: A Veiled Pathogen?

• Considerable levels of antibodies against prM are found in Dengue-positive patients’ sera [Se-Thoe, et al., 1999; Cardosa, et al., 2002; Dejnirattisai, et al., 2010; de Alwis et al., 2011]

• The levels of prM antibodies are significantly higher in patients experiencing a secondary infection [Lai, et al., 2008] and are significantly elevated in patients suffering from DHF/DSS [Rai, et al., 2008]
Infectivity of immature DENV in FcR-bearing cells in presence of anti-prM antibodies

Infection of K562 cells

43 hpi

Harvest

Imature DENV + YYY

Complex formation 1h, 37°C

Cells for FACS

Supernatant for plaque assay
Immature virions turn highly infectious in presence of anti-prM antibody

Rodenhuis-Zybert et al., PLoS Pathogens, 2010
Blocking Fc-receptors impedes enhancement of infection

<table>
<thead>
<tr>
<th>anti-prM mAb</th>
<th>anti-CD32 mAb</th>
<th>prM Titer (log₁₀ PFU/ml)</th>
<th>wt Titer (log₁₀ PFU/ml)</th>
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<tr>
<td>-</td>
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<td>n.d.</td>
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</table>
Furin activity in target cell is crucial to render immature virions infectious.
Enhancement of infection in human PBMCs of both immature and wt virus

<table>
<thead>
<tr>
<th>anti-prM mAb (ng/ml)</th>
<th>Titer (log_{10} PFU/ml)</th>
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<tbody>
<tr>
<td>0</td>
<td>n.d.</td>
</tr>
<tr>
<td>4</td>
<td>n.d.</td>
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<tr>
<td>40</td>
<td>n.d.</td>
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<tr>
<td>400</td>
<td>n.d.</td>
</tr>
<tr>
<td>4000</td>
<td>n.d.</td>
</tr>
<tr>
<td>IgG</td>
<td>n.d.</td>
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</table>

**immature**

**wt**

* indicates a significant difference.
Immature DENV becomes infectious in the presence of DENV-immune sera

Sera obtained from Dr. Guillermo Comach, LARDIDEV, BIOMED-UC, Maracay, Venezuela
Visualization of dengue virus entry in living cells: single-particle tracking

- DENV is labeled with the lipophilic fluorescent probe DiD

- DiD surface density
  - High: self-quenching
  - Low: bright fluorescence
Analysis of the cell entry dynamics of prMDENV opsonized with prM antibodies

- Single-particle tracking
  - murine P388D1 macrophage cells

- Treatment of cells with chemical inhibitors indicates that prMDENV-immune complexes infect cells through phagocytosis

Ayala-Nunez et al, unpublished
Immature DENV fuses at a later timepoint than standard DENV

Ayala-Nunez et al, unpublished
Model of immature flavivirus entry to cells

No antibodies (Abs)

- No binding
- Non-infectious

In presence of prM Abs

- Binding
- Phagocytosis
- Fusion
  - Low-pH induces structural change
  - Furin-cleavage of prM to M
  - Release of the pr-peptide
  - Membrane fusion
Disease and pathogenesis

1st DENV infection

DENV-1 → DF → recovery → + memory T cells
DENV specific E, prM, NS1 antibodies

Homotypic re-infection

DENV-1 → No disease → Neutralization: Predominant role for serotype-specific E antibodies

Heterotypic re-infection

DENV-2 → DF, DHF, DSS → Enhancement: Predominant role for cross-reactive prM and/or E antibodies
Perspectieven en Belang van Moleculaire Diagnostiek

- Moderne, snelle, op PCR gebaseerde, moleculaire diagnostiek is essentieel voor een adequate behandeling van patienten en zal uiteindelijke levens redden
- Een state-of-the art surveillance-systeem, gebaseerd op GIS, is cruciaal voor early warning en controle van Dengue-uitbraken
- Kennis op het gebied van de infectiemechanismen en de pathogenese van Dengue, ihb kennis van het mechanisme van ADE, is cruciaal voor de ontwikkeling van effectieve en veilige vaccins en antiviralen middelen en identificatie van predictors of severe disease. Moleculaire diagnostiek speelt hierin een essentiële rol
  - Infectiehistorie van patienten
  - Immuunrespons op infectie
  - Moleculaire karakterisering van de virussen die DHF/DSS veroorzaken
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