Immunopathogenesis of Dengue Hemorrhagic Fever

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Etiological Agent

Family: *Flaviviridae*

Genus: *flavivirus*

ssRNA positive genome

Enveloped virus

Four serotypes (D1, D2, D3, D4)
Phylogenetic Relationship of Dengue Virus with other Flavivirus

<table>
<thead>
<tr>
<th>virus</th>
<th>serocomplex</th>
<th>clade</th>
<th>cluster</th>
</tr>
</thead>
<tbody>
<tr>
<td>West Nile</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Kunjin</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Japanese encephalitis</td>
<td></td>
<td>XVI</td>
<td>XIV</td>
</tr>
<tr>
<td>Murray Valley encephalitis</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>St. Louis encephalitis</td>
<td></td>
<td>VI</td>
<td></td>
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<tr>
<td>dengue-1</td>
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<tr>
<td>dengue-3</td>
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<tr>
<td>dengue-2</td>
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<tr>
<td>dengue-4</td>
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<tr>
<td>yellow fever</td>
<td></td>
<td></td>
<td>VII</td>
</tr>
<tr>
<td>Central European encephalitis</td>
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<tr>
<td>Far Eastern encephalitis</td>
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<td>Powassan</td>
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<td></td>
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<tr>
<td>Dakar bat</td>
<td></td>
<td></td>
<td>III</td>
</tr>
</tbody>
</table>

• 2.5 billions people in urban, peri-urban and rural areas of tropics and subtropics at risk

• There are an estimated of 50 million infections yearly, 500,000 hospitalized patients and more than 25,000 fatalities

• Global prevalence has increased dramatically in recent years

• DF/DHF occurred in more than 100 countries

• In South Asia more than 95% of cases are children
Transmission
IS A UNIQUE DISEASE

Clinical Presentation
Dengue Clinical Syndromes

- Undifferentiated fever
- Classic dengue fever
- Dengue hemorrhagic fever
- Dengue shock syndrome
Clinical Case Definition for Dengue Hemorrhagic Fever

4 Necessary Criteria:

- Fever, or recent history of acute fever
- Hemorrhagic manifestations
- Low platelet count (100,000/mm$^3$ or less)
- Objective evidence of "leaky capillaries:"
  - elevated hematocrit (20% or more over baseline)
  - low albumin
  - pleural or other effusions
Hemorrhagic Manifestations of Dengue

- Skin hemorrhages: petechiae, purpura, ecchymoses
- Gingival bleeding
- Nasal bleeding
- Gastro-intestinal bleeding: hematemesis, melena, hematochezia
- Hematuria
- Increased menstrual flow
Risk Factors Reported for DHF

- Virus strain
- Pre-existing anti-dengue antibody
  - previous infection
  - maternal antibodies in infants
- Age
- Host genetics
Virus strain 1981 Cuban DHF/DSS Epidemic
More than 300,000 cases 150 deaths 101 Children

• The virulence of a virus is its capacity, when compared with other closely related viruses, to produce disease in a host.
Risk Factors Reported for DHF

- Virus strain
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  - previous infection
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- Age
- Secondary infections
- Host genetics
**DHF/ DSS Pathogenesis**

**Antibody-dependent enhancement (ADE):** Certain strains of dengue virus, complexed with non-neutralizing antibodies (from previous infection), can enter a greater proportion of cells of the mononuclear lineage, thus increasing virus production.

- **Primary Infection**
  - DEN-2AB
  - Release of vasoactive mediators
  - Monocytes
  - Macrophages

- **Secondary Infection**
  - DEN-2AB
  - Release of vasoactive mediators
  - DHF and DSS

**Mediators:**
- TNFA
- IL2
- IL6
- IL10
- IL18
- INFG
- IL8
Risk Factors Reported for DHF

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- Pre-existing anti-dengue antibody
  - previous infection
  - maternal antibodies in infants
- Age
- Secondary infections
- Host genetics
95% of DHF/DSS occur in children < 15 years of age

> 5% occur in infants
Cytokine Profiles after Dengue infection according age.

INF-\(\gamma\)/IL-10
IL-6
TNF-\(\alpha\)

Vs.

INF-\(\gamma\)/IL-10

Thanh Hung et al. JID, 2004:189 (221-232)
Risk Factors Reported for DHF

• Virus strain

• Pre-existing anti-dengue antibody
  – previous infection
  – maternal antibodies in infants

• Age

• Secondary infections

• Host genetics
Primary DENV-1 infection

DENV-1-specific memory T cells

DENV-naive T cells
DENV-2 > DENV-1

DENV-1 > DENV-2

Primary DENV-2 infection

Secondary DENV-2 infection

↓ Lysis of infected cells
↓ Antiviral cytokines
↑ Inflammatory cytokines

DENV-2-specific memory T cells
Aberrant Immune Activation

**DHF/DSS**

- CD4/CD8 <1
- Monocytosis (days 5-6)
- Atypical Lymphocytosis (days 8-10)
- Early activation of mononuclear cells by expression of CD69 (starting day 4)
Risk Factors Reported for DHF

• Virus strain

• Pre-existing anti-dengue antibody
  – previous infection
  – maternal antibodies in infants

• Age

• Secondary infections

• Host genetics
<table>
<thead>
<tr>
<th>Association</th>
<th>$\chi^2$ (Mantel-Haenszel test)</th>
<th>$p$</th>
<th>$p_c$</th>
<th>OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A*31 in all DHF vs controls</td>
<td>25.62</td>
<td>0.000001</td>
<td>0.000004</td>
<td>13.39 (3.63-53.75)</td>
</tr>
<tr>
<td>A*31 in DF +DHF vs controls</td>
<td>16.17</td>
<td>0.00005</td>
<td>0.0002</td>
<td>7.60 (2.30-27.70)</td>
</tr>
<tr>
<td>B*15 in all DF vs controls</td>
<td>14.37</td>
<td>0.0001</td>
<td>0.0004</td>
<td>4.77 (1.88-12.21)</td>
</tr>
<tr>
<td>B*15 in all DHF vs controls</td>
<td>9.65</td>
<td>0.001</td>
<td>0.004</td>
<td>4.07 (1.46-11.36)</td>
</tr>
<tr>
<td>B*15 in DF +DHF vs controls</td>
<td>16.28</td>
<td>0.00005</td>
<td>0.0002</td>
<td>4.46 (1.96-10.29)</td>
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<tr>
<td>DRB1*07 in all DF vs controls</td>
<td>10.34</td>
<td>0.001</td>
<td>0.004</td>
<td>0.24 (0.08-0.63)</td>
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<tr>
<td>DRB1*07 in all DHF vs controls</td>
<td>7.46</td>
<td>0.006</td>
<td>0.02</td>
<td>0.27 (0.09-0.77)</td>
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<td>DRB1*07 in DF +DHF vs controls</td>
<td>14.35</td>
<td>0.0001</td>
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<td>0.25 (0.11-0.55)</td>
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$p = p$ value derived from $\chi^2$ test; $p_c = corrected \ p$ value; OR = odds ratio; CI = confidence interval; DF = dengue fever; DHF = dengue hemorrhagic fever.
Why DHF/DSS

leaky capillaries/
Incremento de la permeabilidad capilar

- Unlikely to be a single mechanism
- Slow leak suggests not direct cytopathic effects on endothelium
- Antigen driven
- Complement Histamine
- Cytokine mediated
# General Mechanism of Dengue Pathogenesis

<table>
<thead>
<tr>
<th>Platelets</th>
<th>Antibodies complement</th>
<th>Monocytes macrophages</th>
<th>T lymphocytes</th>
<th>Endothelial cells</th>
</tr>
</thead>
</table>

- **Platelet activation**
- **Complement activation**
- **Platelet-activating factor (PAF)**
- **C3a, C5a**
- **TNFα, IL-1, PAF**
- **IL-6, histamine**
- **IL-2**
- **TNFα, IL-6, IFNγ**
- **TNFα, IL-1, PAF**
- **IL-6**

**Vascular endothelial cells**

**Capillary leak syndrome** → **Dengue hemorrhagic fever**
Thrombocytopenia

Platelet immune-destruction

NS1
An Integral Hypothesis

Individual Factors

Age
Gender
Race
Nutritional status
Chronic Diseases
Host Response
Genetic Factors

Epidemiological Risk Factors

Number of susceptibles
Vector High Density
Wide Viral Circulation
Hyperendemicity

Viral Factors

Viral virulence
Serotypes, sequence

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