ANTIBODIES DETERMINE VIRULENCE IN DENGUE

Scott B HALSTEAD, M.D.
DIRECTOR, Research
Pediatric Dengue Vaccine Initiative
IVI, Seoul, Korea
Global Spread of Dengue

50-100 million infections/year

Countries with active dengue + *Aedes aegypti*
Pathogenicity vs Virulence

Virulence, a quantitative term: Severe disease/total infections
Dengue hemorrhagic fever/dengue shock syndrome has occurred in some (but not all) dengue epidemics since the 1950s.

WHY?
Intuitive answer –

Differences in virus virulence
IN 1950s HAMMON DISCOVERED NEW, “VIRULENT” DENGUE VIRUSES THOUGHT TO BE HF-ASSOCIATED

PHILIPPINES DENV 3, 4:
Hammon WMcD et al Science 131:1102-3, 1960

THAILAND DENV 5, 6:
Hammon WMcD et al AJTMH 13:629-41, 1964
Virulence hypothesis is very much alive.

Selection for virulent dengue viruses occurs in humans and mosquitoes.

Cologna R, Armstrong PM, Rico-Hesse R.

Department of Virology and Immunology, Southwest Foundation for Biomedical Research, 7620 NW Loop 410, San Antonio, TX 78227. rricoh@sfbr.org.
WHAT FACTORS CONTROL SEVERITY OF DENGUE?
INTRINSIC HOST FACTORS (innate immunity)

- Humans: susceptibility resistance.
  - Race\(^1\): Caucasian/Asian African
  - HLA\(^2\): HLA-A*0207 HLA-A*0203
    HLA-B*52 HLA-B*51
  - Age\(^3\): Children Adults
  - Nutrition\(^4\): Well nourished Malnourished

## EXTRINSIC FACTORS

### VIRUS

<table>
<thead>
<tr>
<th>“VIRULENT”</th>
<th>“NON-VIRULENT”</th>
</tr>
</thead>
<tbody>
<tr>
<td>DEN 1, 3, 4</td>
<td>DEN 2 (American)(^1)</td>
</tr>
<tr>
<td>DEN 2 (Asian)</td>
<td></td>
</tr>
</tbody>
</table>

CRITICAL ROLE OF ANTIBODIES
DHF
BANGKOK CHILDREN'S HOSPITAL

AGE (YEARS)

1973-79
1980-89
1990-99

<1  1  2  3  4  5  6  7  8  9  10  11  12  13  14  15  16

0  100  200  300  400  500  600  700
DSS in a 6 month-old infant with primary dengue infection. Vietnam
RELATION OF DHF / DSS IN INFANTS TO LOSS OF MATERNAL ANTIBODY

CATABOLISM OF MATERNAL ANTIBODY LOG 2

ANTIBODY

PROTECTION

ENHANCEMENT

DHF / DSS HOSPITALIZATIONS / 1000

AGE (MONTHS)
Antibody mediated infection of macrophages (ADE)

Macrophage infection in dengue (I)

- IL-1β
- IL6
- TNFα
- PAI-2
- INFα
- Monokines
- Histamin

INFγ + Ab
**In vitro ADE model**

- % Infection without serum
- Ratio of % infection at PENT/ Control
- Peak enhancement titer = titer at maximal % infection for the serum tested

WHAT ABOUT VIRAL VIRULENCE?
NON-VIRULENT DENGUE VIRUS: IQUITOS OUTBREAK

• School children cohorts followed from 1990 until now.
• DEN 1 transmitted in 1990 - 1994.
• DEN 2 transmitted from 1995.
• In 1995, secondary DEN 2 infection rate estimated at 60.5%
NO DHF with secondary DEN 2 (American genotype) infections

- Total population, 5 - 14 yrs-old = 81,479.
- Total 2ndary DEN 2 infections = 49,266.
- Expected hospitalized DHF = 887 - 10247.
- Expected deaths = 18 - 204.
- DHF cases observed = 0

BUT, DENV 2 INFECTIONS ARE MODULATED BY DENV 1 ANTIBODIES

34 DEN 1- IMMUNE HUMAN SERA NEUTRALIZE AMERICAN GENOTYPE DEN 2 VIRUSES

<table>
<thead>
<tr>
<th>Virus</th>
<th>DEN-1</th>
<th>AMER</th>
<th>SE</th>
<th>SE</th>
<th>ASIA</th>
<th>ASIA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Strain</td>
<td>Venez</td>
<td>IQT</td>
<td>Venez</td>
<td>Thai</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Strain</td>
<td>9842</td>
<td>2124</td>
<td>8041</td>
<td>16681</td>
<td></td>
<td></td>
</tr>
<tr>
<td>GMT</td>
<td>875</td>
<td>262</td>
<td>32</td>
<td>29</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

ONE-WAY CROSS:

17 DENGUE 2-IMMUNE SERA DO NOT NEUTRALIZE DENGUE-1 VIRUSES

<table>
<thead>
<tr>
<th>Genotype Type</th>
<th>DEN 1 Viruses</th>
<th>DEN 2 Viruses</th>
</tr>
</thead>
<tbody>
<tr>
<td>Strain</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SE Asia</td>
<td>16007</td>
<td>16681</td>
</tr>
<tr>
<td>Venez</td>
<td>OBS 9842</td>
<td>IQT</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2913</td>
</tr>
<tr>
<td>GMT</td>
<td>20</td>
<td>27</td>
</tr>
<tr>
<td></td>
<td>635</td>
<td>417</td>
</tr>
</tbody>
</table>
PLAUSIBLE HYPOTHESIS:

HETEROTYPIC DENGUE ANTIBODIES EITHER PREVENT OR ENHANCE SECOND DENGUE INFECTIONS

<table>
<thead>
<tr>
<th>“NON-VIRULENT”</th>
<th>“VIRULENT”</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neutralizing</td>
<td>Non-Neutralizing</td>
</tr>
</tbody>
</table>

ACTIVELY ACQUIRED (TWO INFECTIONS) or PASSIVELY TRANSFERRED IN UTERO

Another example of viral “virulence”
<table>
<thead>
<tr>
<th></th>
<th>MAY</th>
<th>JUNE</th>
<th>JULY</th>
</tr>
</thead>
<tbody>
<tr>
<td>DHF/DSS</td>
<td>37</td>
<td>132</td>
<td>29</td>
</tr>
<tr>
<td>DEATHS</td>
<td>1</td>
<td>6</td>
<td>5</td>
</tr>
<tr>
<td>CONF DF</td>
<td>705</td>
<td>1785</td>
<td>244</td>
</tr>
<tr>
<td>DHF/DF</td>
<td>5.2</td>
<td>7.4</td>
<td>11.9</td>
</tr>
<tr>
<td>DEAD/T</td>
<td>0.14</td>
<td>0.33</td>
<td>2.04</td>
</tr>
<tr>
<td>CFR</td>
<td>2.7</td>
<td>4.5</td>
<td>17.2</td>
</tr>
</tbody>
</table>

SHIFT FROM THREONINE TO SERINE AT NS1 aa 186

- All patients had secondary dengue infections.
- Based on 5 complete and 19 partial genome sequences.
- Reference strain: DENV 2 Jam 1407/83

Rodriguez-Roche R et al J Gen Virol (in press)
- Increased severity only occurs during secondary dengue infections.
- That is, severity is antibody-dependent.
- Perhaps, due to increased "fitness" for viral replication?
• Infectious immune complexes (IgG + virus) directly regulate viral infections in macrophages.
Th1/Th2 responses in macrophages

- Ag presentation to MΦ ▶ IL-12 causes T cells to produce IFNγ (Th1 response)
- Ag + Ab presentation to MΦ ▶ IL-10 causes T cells to produce IL-4 (Th2 response).

Suppression of antiviral transcription factors (STAT-1 and NF-κB complexes) by ADE infection of macrophages by Ross River virus.

Mahalingham S & Lidbury BA. PNAS 2002 99: 1392

- Polyclonal mouse anti-RRV used at 10-3
- ADE infectivity of mouse macrophage cell line increased 25-fold by 12 hours.
- Nearly 50% of cells infected by viral RNA.
- RRV plus simultaneous addition of irrelevant immune complex did not increase infectivity.
- Ablated or suppressed: NO production, IFN-1, IP-10 + three IFN factors. IL-10 production increased.
- Transcription factors shut down in bystander cells.
- IL-10 thought to mediate this latter effect.
Dengue virus ADE upregulates the production of anti-inflammatory cytokines but suppresses anti-dengue free radical and pro-inflammatory cytokine production in THP-1 cells.


- Human polyclonal antibodies from 2ndary DENV 3 DHF case incubated at 1:100,000 with 16681.
- DENV 2 infection of individual THP-1 cells increased by 10-fold at 24 hours.
- Suppression of STAT-1 phosphorylation and IRF-1 gene expression may be mechanism by which ADE infection down-regulates innate immunity.
- ADE facilitated production of IL-10 and decreased NO production.
- Transient suppression of TNF \( \alpha \).
Increased number of cells and intrinsic viral production with ADE in Mature DC

Marovich, M. personal communication
IL-10 suppresses secondary cytokine responses via SOCS-3 activity

Ubol S et al. JID 2008
Suppression of IFN synthesis in DENV-ADE infected THP-1

Ubol S et al. JID 2008
Global PBMC gene expression secondary DF vs secondary DHF

1. DHF (enhanced infection) has stronger influence on the gene expression profile than DF (partially protected secondary infection).

2. 17 genes in immune response category are more strongly upregulated in DF PBMCs than in DHF PBMCs. 40% of them are genes of the interferon system.

Ubol S et al. JID 2008
Conclusion

1. Dengue enhancing antibodies not only facilitate entry into target cells but also modify post-entry events.
2. An outcome is the suppression of innate and adaptive intracellular anti-viral mechanisms.
3. These are mediated through IL-10 activity and via the mechanism upstream of MDA-5 and RIG-I activation.
4. Immune complexes directly regulate dengue virus infection productivity.
Sequential and cumulative viral distribution in tissues during dengue infection in 31 rhesus monkeys.

PEAK CELLULAR INFECTION OCCURS AT OR AFTER DEFERVESCENCE

Marchette et al
JID 1973 128:23

Halstead SB
In Schlesinger W
Togaviruses
Academic Press
1980: 107-173
After Rothman, 2001
TAKE HOME MESSAGE

- Intrinsic factors place many humans at reduced risk to severe dengue disease.
- In at risk humans **antibodies** (passive or actively acquired) regulate the severity (virulence) of dengue infections:
  - Homologous antibodies provide complete protection.
  - Heterotypic neutralizing antibodies down-regulate severe disease, usually with inapparent outcome.
  - Enhancing antibodies suppress innate antiviral defenses leasing to increased infected cell mass and increased disease severity.
VIRULENCE = ANTIBODIES

FOR THE LEISHMANIA CS
Virulence = visceral leishmaniasis
Leishmania-macrophage interactions....

*L. amazonensis* amastigotes have host IgG on their surface
Lesion-derived amastigotes are coated with host Ig

LOG FLOURESCENCE

Mosser, D personal communication
IgG Reconstitution of J_H Mice and the Effect of α-IL10R

Mosser, D. pers comm., Miles SA et al JEM 201:747-54, 2005
IL-10−/− (BALB/c) mice control lesion development during *L. major* infection

* euthanized
IL-10 induction following FcγR Ligation

- **LPS**
- **LPS + E-IgG**

IL-10 (pg/ml) vs LPS (ng/ml) graph showing the induction of IL-10 with different concentrations of LPS and LPS + E-IgG.