Dengue Virus, Dengue Fever & Dengue Haemorrhagic Fever

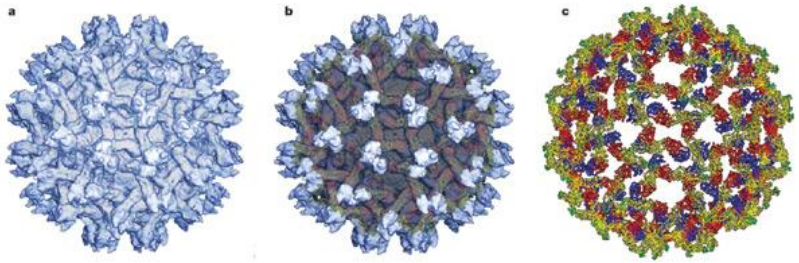
Gavin Screaton

Dengue Virus (DENV)

- *Flavivirus* genus; *Flaviviridae* family

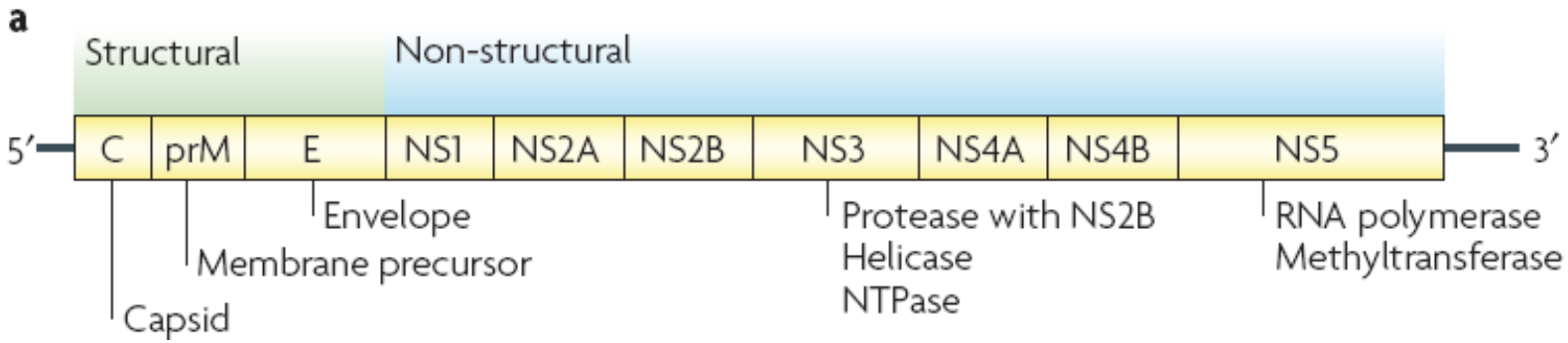
- Other flaviviruses include West Nile, Japanese Encephelitis, Yellow Fever

- ssRNA, positive sense genome, 10.8kb
- 30 nm icosahedral nucleocapsid (NC)
- 40-50 nm spherical virion (NC + envelope)



Dengue Virus (DENV)

- Genome is infectious, mRNA equivalent
- Translated into polyprotein
- Cleaved by viral and host proteases
- 3 structural, 7 non-structural proteins



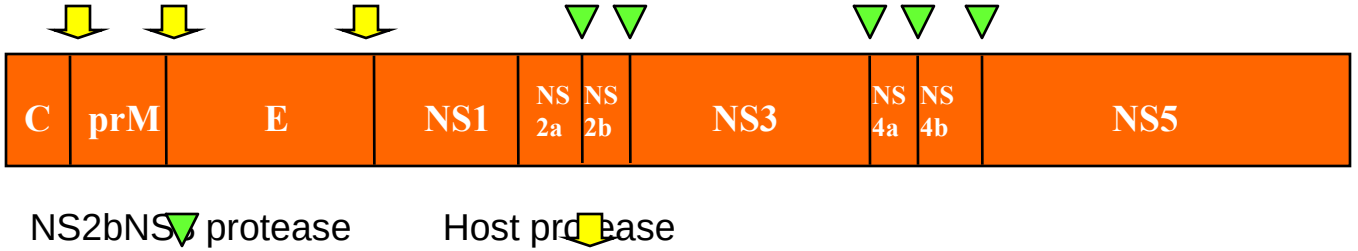
Whitehead et al., 2007

Dengue virus

- Family Flaviviridae
- Enveloped virus containing single stranded RNA genome.

  Structural: capsid (C), premembrane (prM), and envelope (E)

  Non-structural: NS1, NS2a, NS2b, NS3, NS4a, NS4b, and NS5



- Four serotypes: DEN 1, 2, 3, and 4
- % Homology in amino acid sequence among 4 serotypes is 65-70%
- Dengue virus is transmitted to human by mosquitoes

DENV Proteins

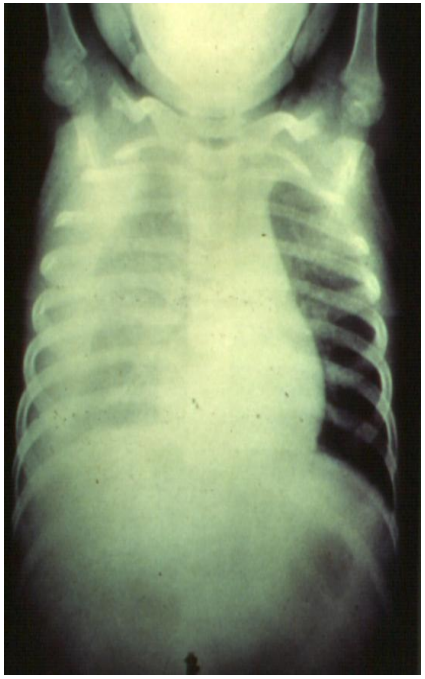
- C (capsid) - encapsidates genome
- M (membrane) – cleaved during maturation
- E (envelope) - glycosylated, binding and fusion
- NS1 - on surface of infected cells, secreted as multimer
- NS3 - protease with NS2B as a cofactor, helicase and NTPase
- NS4B - interferes with STAT1, blocking IFN-β and IFN-γ
- NS5 - RNA polymerase, methyltransferase

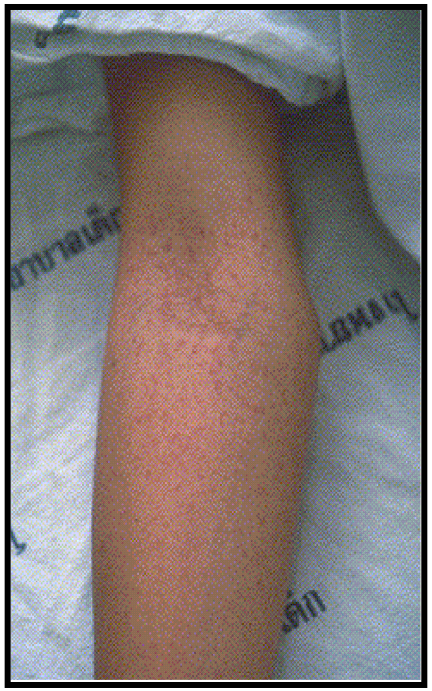
Dengue Fever (DF) & Dengue Haemorrhagic Fever (DHF)

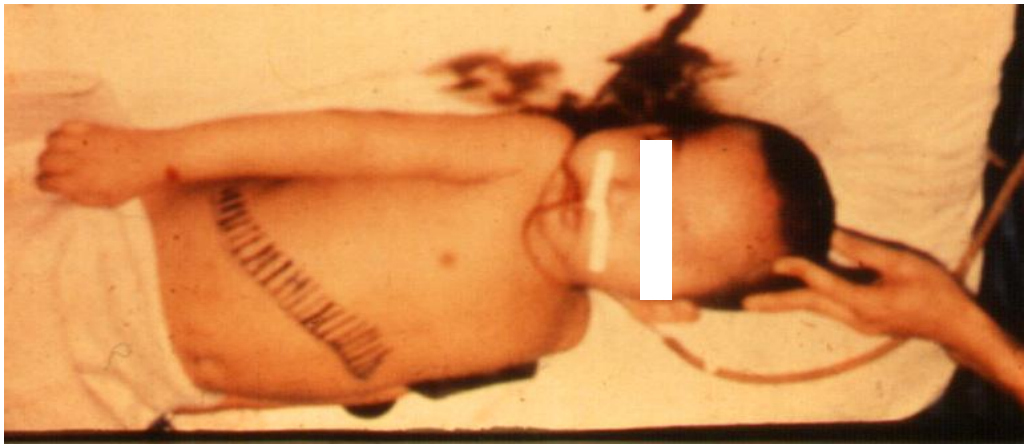
- Asymptomatic or undifferentiated fever
- Dengue fever (DF)
  - Virus incubates for 2-7 days then fever
  - headache, muscle & joint pain, self limiting, 1wk
- Dengue haemorrhagic fever (DHF)
  - 4 grades
  - capillary leakage, thrombocytopenia, altered haemostasis, liver damage
- Dengue shock syndrome (DSS); DHF3,4
  - Fluid loss (pleural effusion) can result in shock

DHF/DSS a unique clinical syndrome

Sudden Onset:
Shock
Pleural Effusion
Ascites
Bleeding
Hepatomegaly





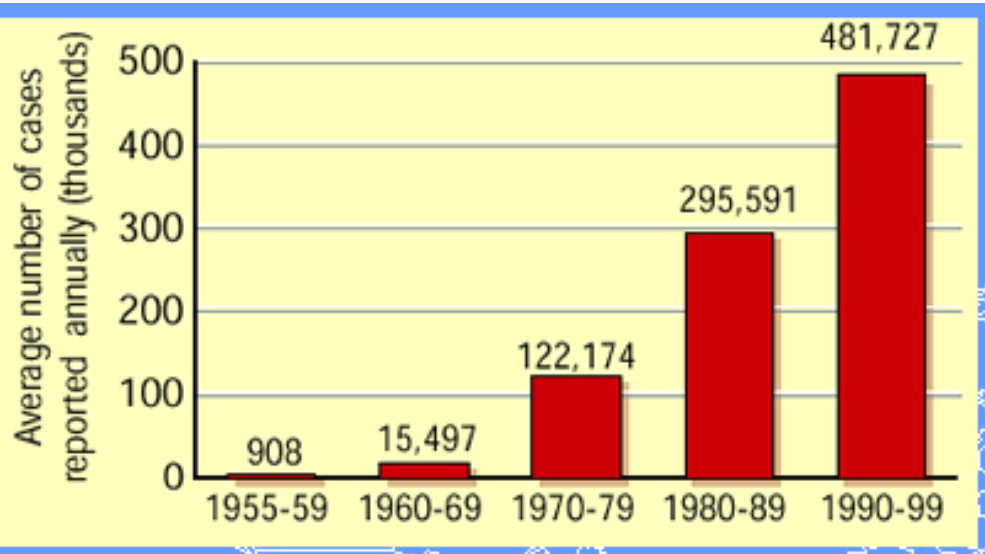


A spectrum of dengue illness

Dengue virus infection

- Asymptomatic
- Symptomatic
  - Undifferentiated fever
  - Dengue fever
    - Without hemorrhage
    - Unusual hemorrhage
  - Dengue hemorrhagic fever
    - No shock — DHF grade 1 & 2
    - Shock — DHF grade 3 & 4

World Distribution of Dengue - 2000



Areas infested with *Aedes aegypti*

Areas with *Aedes aegypti* and dengue epidemic activity


Laboratory-Confirmed DHF in the Americas
Prior to 1981 vs. 1981 - 2003
Prior to 1981
1981 - 2003

Dengue Fever (DF) & Dengue Haemorrhagic Fever (DHF)

- 50-100 million cases of DF each year
- 500,000 cases of DHF
- 95% DHF in children under 15
- 1-20% fatality rate depending on fluid replacement

Dengue Fever (DF) & Dengue Haemorrhagic Fever (DHF)

- *Aedes aegypti* and *Aedes albopictus* - urban
- Increased population, urbanisation, poor sanitisation
- Humans are natural hosts
- Sylvatic cycles do exist in Asia and Western Africa but the contribution of enzoonotic transmission is believed to be minimal

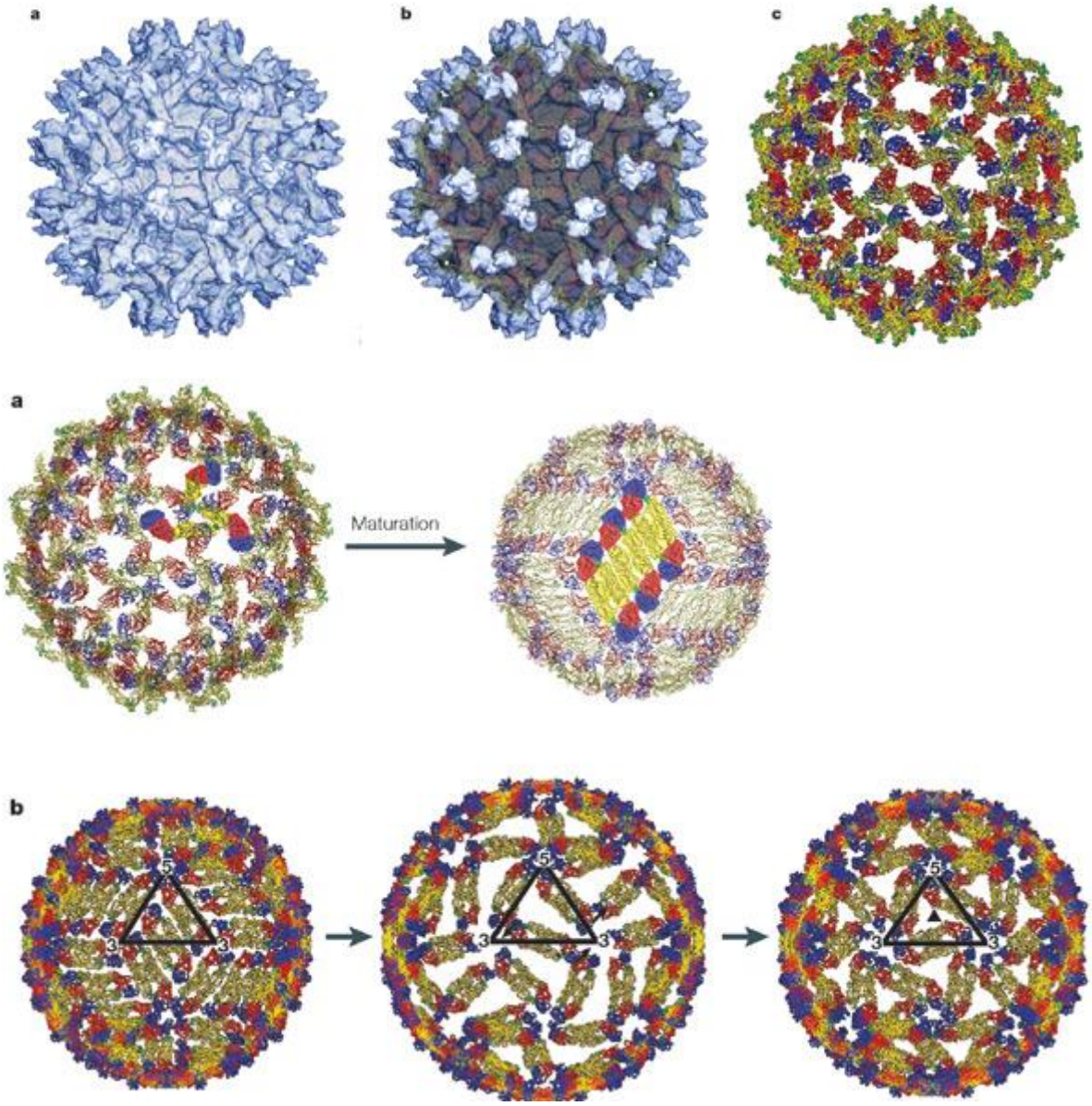
- DENV 1,2,3,4 evolved independently in primates
- Each of the primate viruses then emerged into humans about 500 years ago

Dengue Virus Particle

Imature

Mature

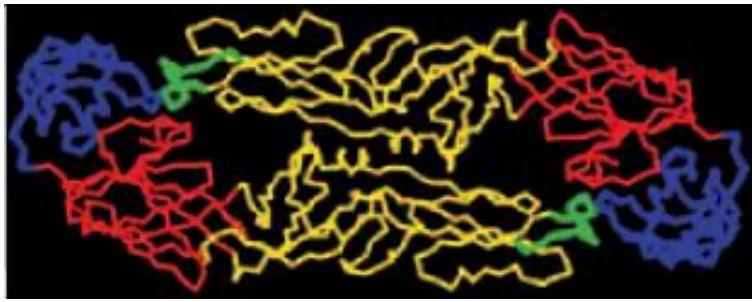
Post fusion



Nat Rev Microbiol. 2005; 3:13

DENV Life Cycle

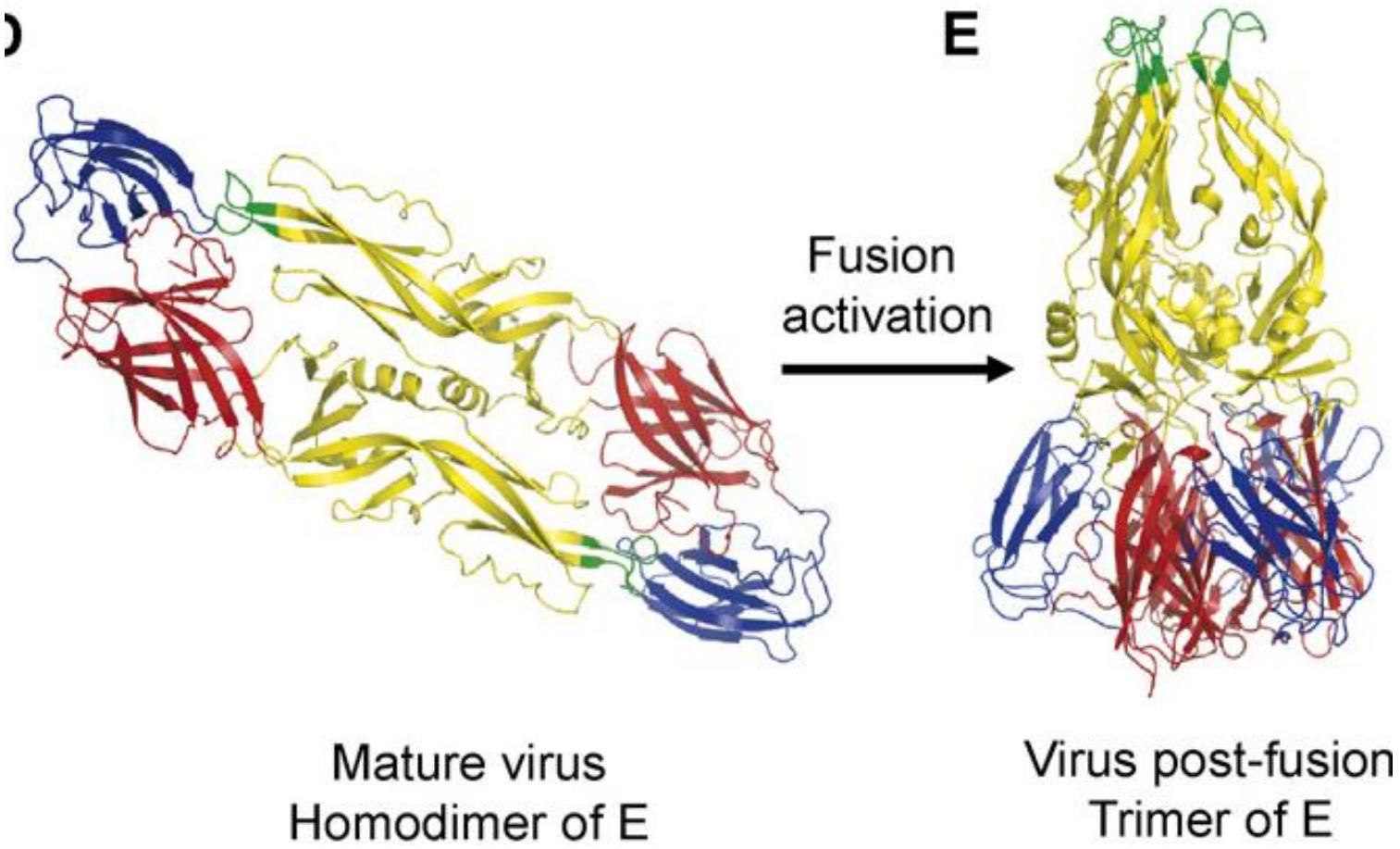
- E dimer binds
  - Sugars?
  - DmIII?
    - Recombinant protein blocks entry
    - Target of neutralising Abs



- Virus is endocytosed
- Acidic environment
- Dimer dissociates into monomers
- Monomers trimerise irreversibly → fusion

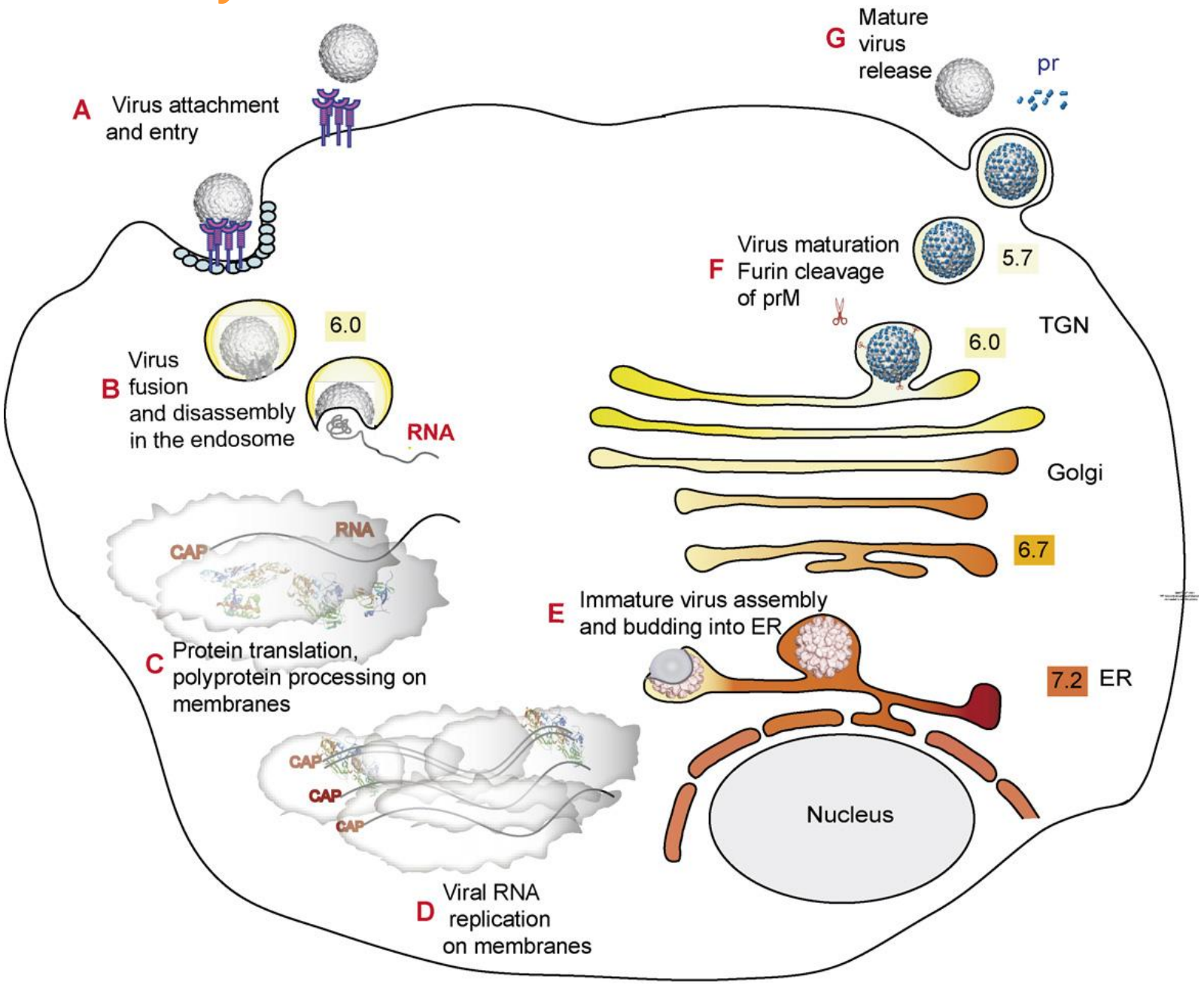
Whitehead et al., 2007

DENV Life Cycle



Whitehead et al., 2007

DENV Life Cycle



Target Cells

- Unknown
- Dendritic cells are infected – bind DC-SIGN
- Monocytes and macrophages may be a major target in vivo
- B cells can be infected *in vitro*
- Vascular endothelial cells and hepatocytes contain antigen *in vivo* but are they infected?

- How do viruses spread?
- Which organs and cells lead to viraemia?

Immune responses to DENV

- E is a major target of neutralising and protective Abs

- NS1 is a target of protective Abs
- NS1-specific Abs lead to ADCC and complement mediated cytotoxicity

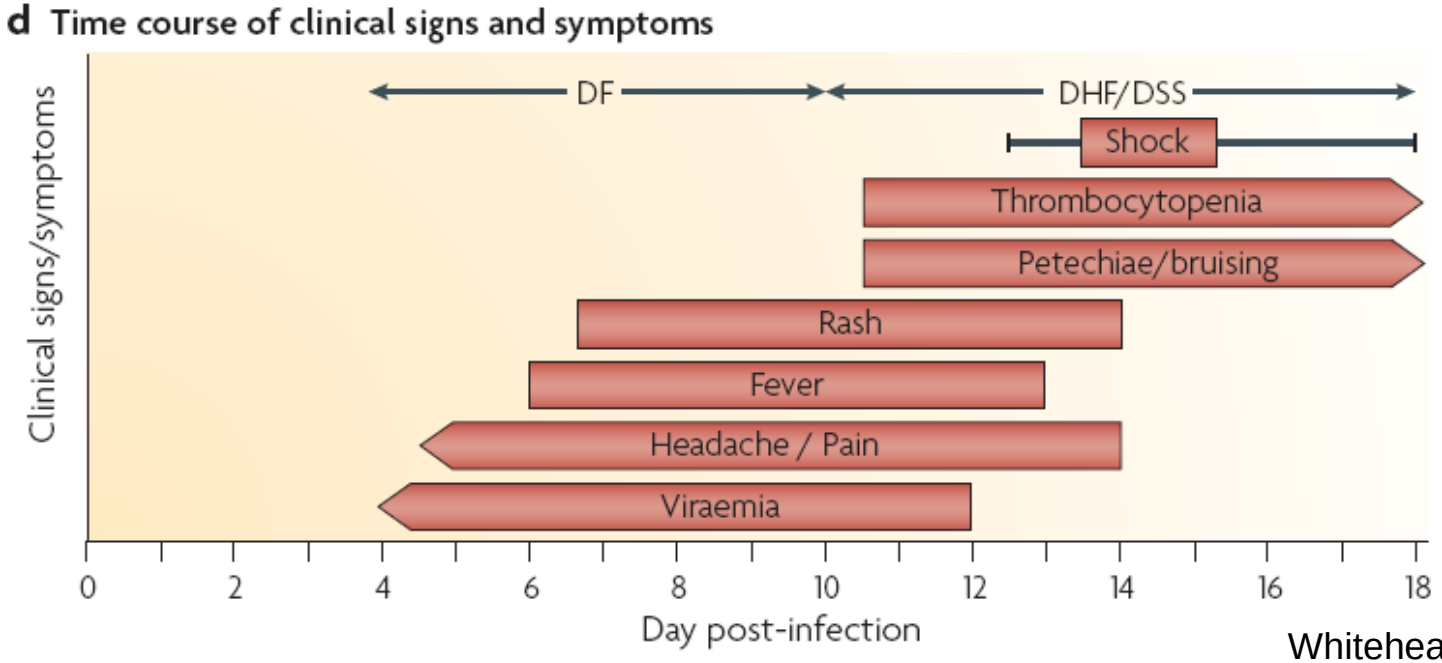
- NS3 is a major target of T cells
  - CD4 and CD8

Immune responses to DENV

- IFN-response is important
- Neutralising Abs are long-lived
- Cross reactive neutralising Abs decline rapidly after infection
- Protection is thought to be life-long for homotypic virus but last only a few months for heterotypic virus
- Serotype-specific and cross-reactive T cells are detected after infection

Dengue Haemorrhagic Fever

- Spectrum of disease. Why?
- Immunopathogenesis?
  - Timing of symptoms, problems after viraemia
- Virus virulence?

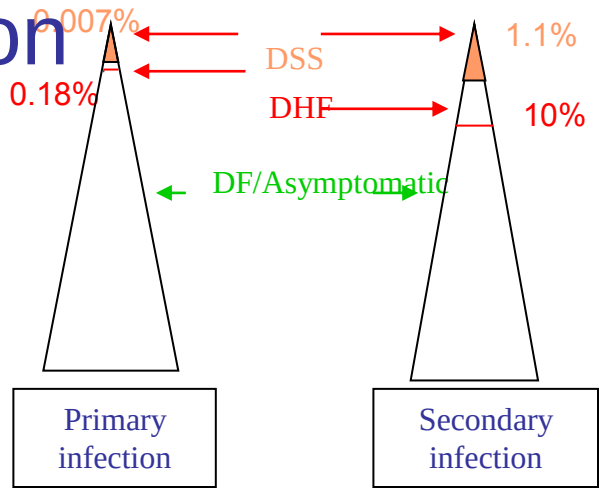


Whitehead et al., 2007

DHF Risk Factors

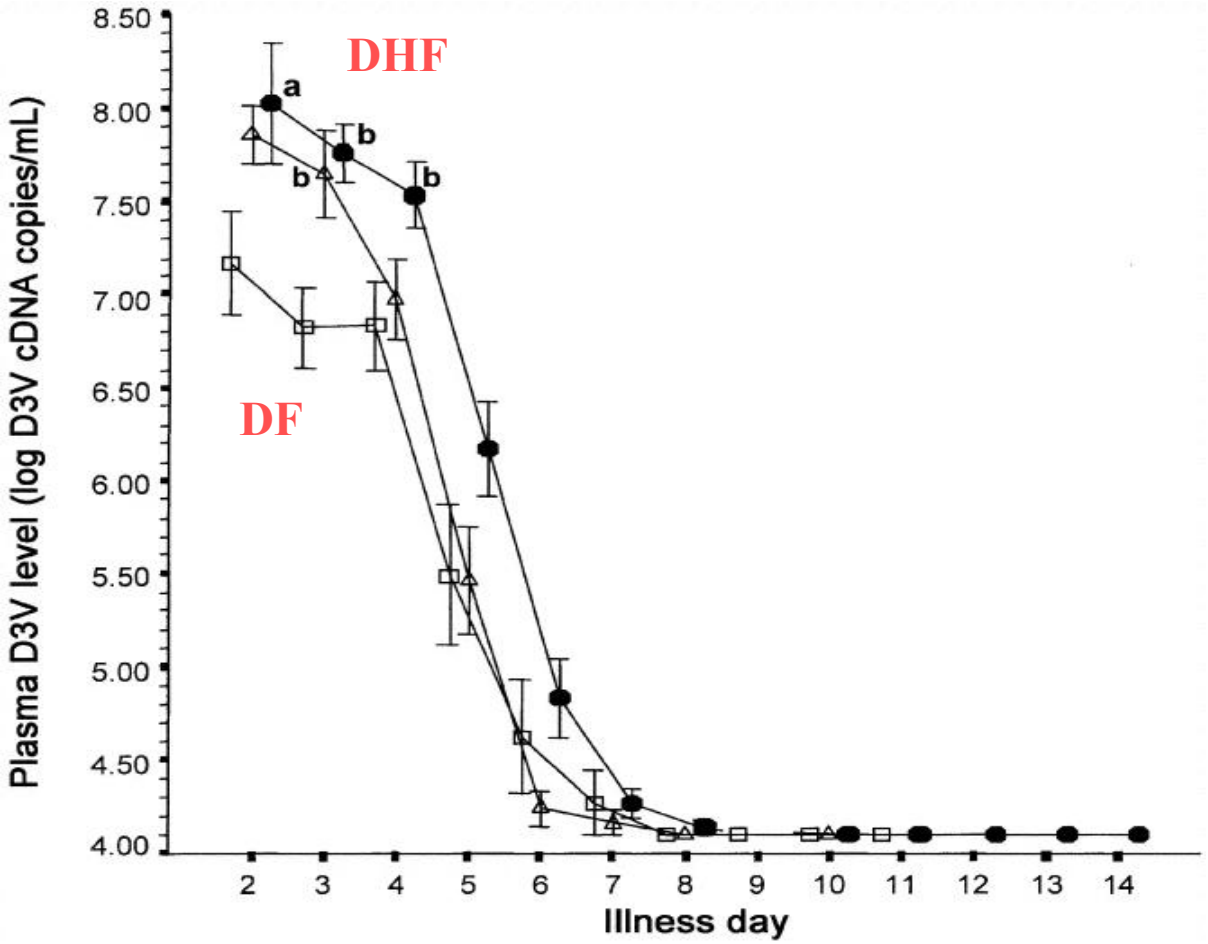
- DHF risk factors include:
- Pre-existing immunity to heterologous serotypes
- Time between infections
- Age
- Ethnicity
- Host genetic background
- Sequence of infecting serotypes
- Viral genotype

The majority of severe dengue complications occur upon secondary infection



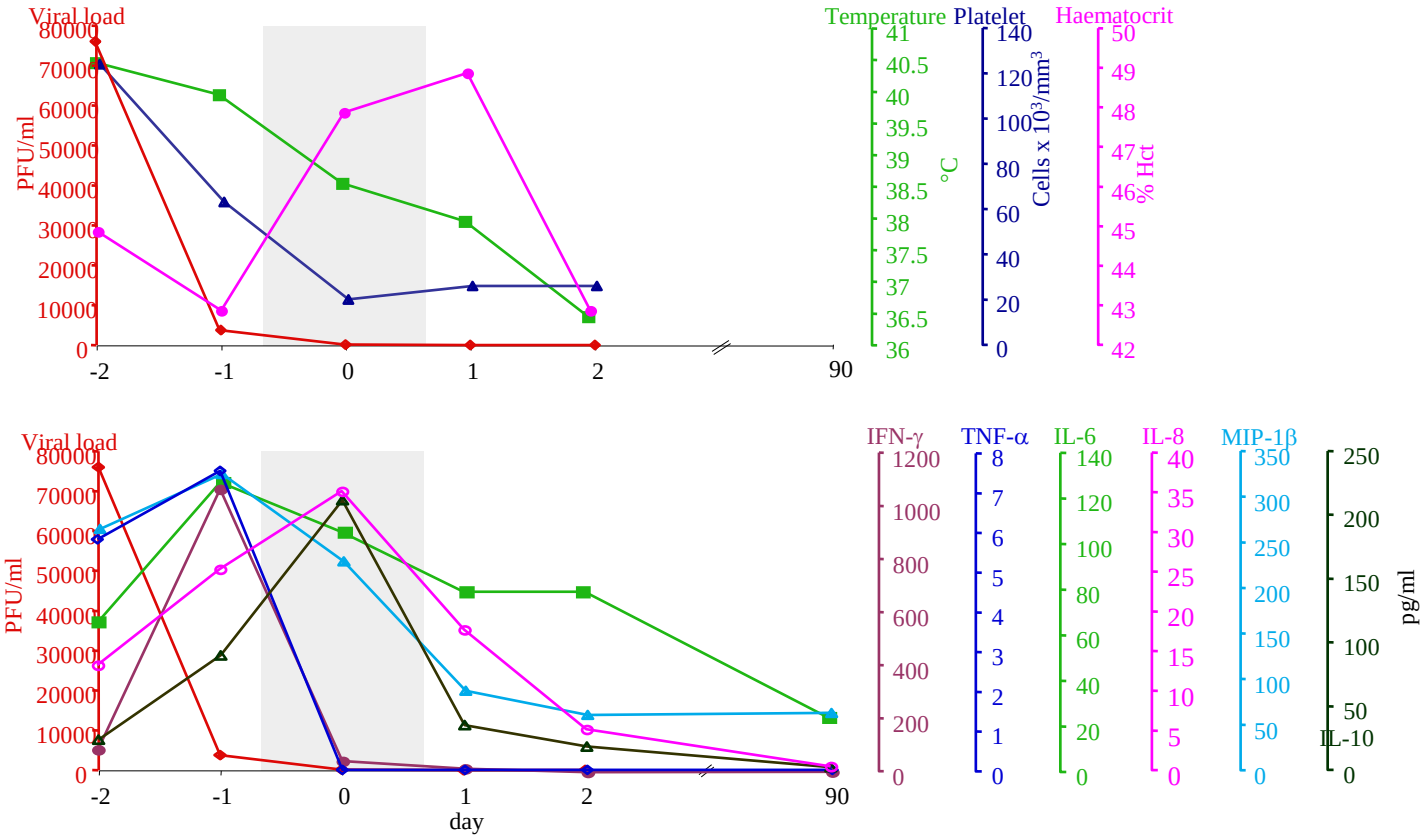
COUNTRY	State/Province	Period:	DF cases	DHF cases	Deaths	DHF/DF+DHF	CFR
Cuba	Den 1	1/1/77 - 1/1/78	477440	No Report	No Report	Not Displayed	No Report
Cuba	Den 1	1/1/78 - 1/1/79	75692	No Report	No Report	Not Displayed	No Report
Cuba	Den 1	1/1/79 - 1/1/80	1497	No Report	No Report	Not Displayed	No Report
Cuba	Den 1	1/1/80 - 1/1/81	169	No Report	No Report	Not Displayed	No Report
Cuba	Den 2	1/1/81 - 1/1/82	344203	10312	158	0.03	0.0
Cuba		1/1/94 - 1/1/95	0	No Report	0	Not Displayed	No Report
Cuba	Den 2	1/1/97 - 1/1/98	3012	205	12	Not Displayed	0.1
Cuba		1/1/99 - 1/1/00	0	0	0	Not Displayed	No Report
Cuba		1/1/00 - 1/1/01	138	0	0	0.00	No Report
Cuba		1/1/01 - 1/1/02	11363	69	2	0.01	0.0

Clue 2. High virus load is associated with worse disease



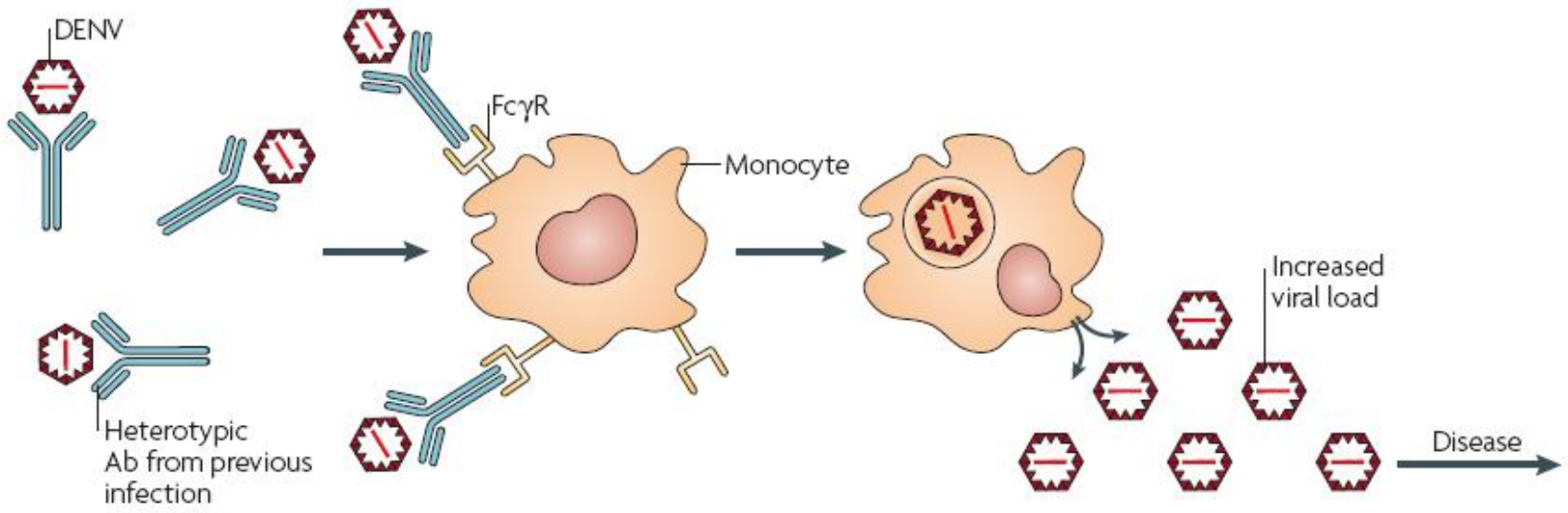
Libraty JID 2002

Illness and a cytokine storm coincide with viral clearance



1. Illness is worse following secondary infection
2. High virus loads predict a severe outcome
3. Severe symptoms occur at point of virus control

Antibody dependent enhancement (ADE)



- Leads to increase in viraemia
  - DHF and DSS are associated with increased viral load
- And/Or leads to alternate cytokine cascades

Whitehead et al., 2007

Antibody dependent enhancement (ADE)

- First observed *in vitro* in 1930s, common *in vitro* to many viruses
- During a primary infection with a given serotype, cross-reactive Abs are induced that will be able to bind to the other serotypes
- During a secondary infection with another serotype, these cross-reactive Abs will be boosted rapidly and bind
- Not neutralising
  - Concentration is too low – declined over time
  - Enhancing Abs recognise different epitopes to strongly neutralising Abs
- Bind to Fc receptors (Fc gamma) on macrophages/DCs and endocytosed

Antibody dependent enhancement (ADE)

- Halstead studied ADE in monkeys
- Sequential infections of different serotypes compared to primary (DV2)
  - Some showed increased (10-fold) viraemia
- Animals given dengue-immune serum and then challenged had higher viraemia than those given non-immune serum

- Good animal model is lacking

Antibody dependent enhancement (ADE)

- In human adults, the presence of pre-existing heterotypic Abs is associated with more severe disease

- In infants (6months-1yr), DHF is observed in primary infections
- Associated with the presence of maternal Abs (IgG)
  - Sub-neutralising concentrations
  - Serum enhances *in vitro*
- Up to 6 months, risk of DHF is low – high neutralising Ab titres
- After one year, risk of DHF is low – degradation of maternal Abs

Endothelium damage?

- Leakage and haemorrhage – increased vascular permeability – How?
- Capillaries in skin biopsies are altered but not severely damaged
- Endothelial cells seem in tact. Tight junctions ok
- Some endothelial swelling observed in capillaries in skin
- One study identified circulating endothelial cells and increased levels of adhesion molecules (sICAM-1 and sVCAM-1) – indicating damage
- In mouse, endothelial cells undergo TNF-induced apoptosis

Antibody dependent enhancement (ADE)

- Endothelium directly affected?
- Higher virus titres?
- ADE infection stimulate alternate cytokine production from macrophages and DCs?
- Activate different Th cells?

Cytokine production

Cytokines	DF	DHF
Interleukin-1β	N	N
Interleukin -2	MI	I
Interleukin -4	D	MI
Interleukin -6	I	MI
Interleukin -8	D	MI
Interleukin -10	D	MI
Interleukin -12	MI	D
Interleukin -13	D	MI
Interleukin -18	I	MI
Tumour necrosis factor-α	MI	MI
Interferon-γ	MI	I
Transforming growth factor-β	D	MI
Human cytotoxic factor	I	MI

- Massive production of cytokines
  - leads to plasma leakage
  - Interferes with stability of the vascular endothelial cells
- T cells and monocytes
- IL-8 and MCP-1 (reduces tight junctions) are also found in pleural effusion
- How induced?
- How function?

Source: Modified from Chaturvedi *et al*[16]; I, increased; MI, markedly increased; D, decreased; MD, markedly decreased; N, no change; DF, dengue fever; DHF, dengue haemorrhagic fever

Host susceptibility

- No clear, unequivocal associations have been made between a given HLA haplotype and DHF
- HLA associated with protection against DHF
  - HLA Class I:
    - Thai – A*0203, B13, B44, B52, B62, B76, B77
    - Cuban – A29, B14
    - Vietnamese – A33
  - HLA Class II:
    - Mexican – DRB1*04

Host susceptibility

- HLA associated with susceptibility to DHF
  - HLA Class I
    - Thai – A2, A*0207, B46, B51
    - Cubans – A1
    - Vietnamese – A24
  - HLA Class II
    - Brazilians – DQ1, DR1?
  - MICB- recognised by NKG2D-NK cell receptor
  - PLCE1 ?related to vascular permeability

Virus virulence

- Virulence is associated with high virus load and high transmission rates
- Some primary patients do get DHF (~2%)
- Differences between strains

Virus virulence

- American DV2 are rarely associated with DHF cases
- Non virulent – reduced growth in vitro, mosquitoes
- Asian genotypes are associated with DHF

Virus virulence

- But American DV2 isolated in Pacific Islands and Venezuela was associated with DHF/DSS
- Viruses taken from within one population rarely differ between patients of DHF and DF
- Good animal model would be very useful!!

Animal models

- Monkeys – viraemia but no disease
- Immunocompetent mice
  - Different routes of infection
  - Different virus strains
  - Different doses
- STAT1 KO – haemorrhage and leakage
- NOD/SCID reconstituted with human cells/tissues – human-like disease
- Some promising results coming up

Vaccines

- Should be possible to make a vaccine?
- Acute infection
- Viraemia can be controlled after 10 days
- Protection conferred against same serotype
- Also, passively transferred Abs can protect mice and macaques
- Vaccines against other flaviviruses eg YF, JE

Vaccines

- Problematic!!
- Equal protective responses against all 4 virus groups required
- Need all or nothing
- One strong dose for immediate effect
- Boosting needs to ensure that Ab levels do not drop and also that they all decline at equal rates

Vaccines

Vaccines against Dengue and Yellow Fever.*

Developer	Type of Vaccine	Stage of Development
Yellow fever		
7 Manufacturers	17D (live, attenuated)	Licensed
Dengue		
Acambis and Sanofi Pasteur	Live, attenuated chimeric dengue–yellow fever	Phase 2; soon to enter phase 3
WRAIR and GlaxoSmithKline	Live, attenuated	Phase 2
NIH, Biologicals E (India), Panacea (India)	Live, attenuated chimeric dengue–dengue	Phase 1
Mahidol University (Bangkok)	Live, attenuated	Predinical†
CDC, Inviragen, Shantha (India)	Live, attenuated chimeric dengue–dengue	Predinical
Hawaii Biotech	Recombinant, subunit	Predinical
U.S. Navy	DNA	Predinical

Vaccines

- Responses against the live, attenuated vaccines are promising
- Strong and show 80-90% seroconversion against all 4 serotypes
- Often immunodominance of one serotype
- Unknown attenuation can be a problem
- Vector backbones with specifically engineered mutations

Vaccines

- Recombinant vaccines
- Not as immunogenic
- Need several boosts

- Understanding pathogenesis and immune responses to DENV more clearly should enable more rational vaccine design
  - Specific antigens
  - Specific epitopes (neutralising vs enhancing)
- PDVI

Vector Control

- Control mosquito spread
- No stagnant water
- Upside down buckets, tyres
- Spraying programmes
- Wolbachia
- Fish/copeopods (small crustacea)

Anti-Dengue drugs

- None yet available
- Need to be safe and given very early
- Promising results with other flaviviruses e.e. Hep C
- NS3 protease
- NS5 replicase
- Envelope protein?