Arbovirus evolution, vector competence, and virulence models – changing patterns of infection

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A. How have the vector-borne viruses been evolving?

What have they been doing in humans?
- replication and virulence
- transmission by viremia

What have they been doing in mosquitoes?
- replication and dissemination
- transmission by bite

B. How can we test these principles?
- phylogenetics and epidemiology
- primary human cell culture
- humanized mouse models of disease
- mosquito colonies
C. Examples:

Dengue viruses – *Flavivirus*
   RNA genome, 11Kb, 8 proteins
   Four serotypes, ~25% different

Chikungunya viruses – *Alphavirus*
   RNA genome, 12Kb, 10 proteins
   One serotype

Both transmitted to humans via bite of female *Aedes aegypti* and *Aedes albopictus* mosquitoes, which have different biting habits and habitat
Signs of infection

**DENGUE**

**Vascular symptoms:**
- Hypovolaemia
- Low blood pressure
- Shock

**Hepatic injury**
- Fluid pooling in body cavities
- Gall bladder thickening
- Haemorrhaging within organs

**Skin symptoms:**
- Rash
- Bruising
- Petechiae
- Purpura

**Infrequent complications:**
- Encephalitis
- Acute pancreatitis
- Renal failure
- Myocarditis
- Splenic rupture
- Pulmonary haemorrhage

**Headache, fever**

**Bleeding gums, nose and eyes**

**Joint pain**

**Altered haematopoiesis**

**Vomiting**

**Intestinal bleeding**
Phylogenetics of serotype 2
Growth in primary human DCs

a

![Graph showing growth in primary human DCs for different donors.]

b

![Graph showing virus output for different donors.]

P<0.0001
Vectorial capacity
Step 1: Sublethal (0.1Gy) body irradiation

Step 2: Intrahepatic injection of $3 \times 10^6$ human CD34+ cells purified from CORD blood

Step 3: Reconstitution level determined by flow cytometry on peripheral blood

Step 4: Infection by injection or mosquito bites

Reconstitution

24 hrs

6-8 weeks

+ Development of multiple hematopoietic lineages
  - Human T cells education in mouse thymus
Dengue virus in humanized mouse blood
Dengue virus in humanized mouse blood

![Graph showing the log10 genome Eq/mL over time post-infection for mosquito bite and virus injection groups.](image-url)
Rash in humanized mice

Erythema index (O.D.) vs. Day post-infection

- Mosquito bite
- Virus injection

* indicates statistical significance.
Antibodies in humanized mice

- Uninfected bite, total
- Infected bite, total
- Dengue-specific

Day post-infection

Day 4: Mouse ID 740, 664, 665, 666
Day 12: Mouse ID 744, 592, 661, 663, 704
Day 32: Mouse ID 682, 699, 701, 706, 707, 729
Models of 3'UTR RNA folding
Targets of infection
CHIKUNGUNYA
Chikungunya phylogenetics

Thiberville et al., 2013
**Step 1:** Sub-lethal (2Gy) whole body irradiation

**Step 2:** Implantation of 1 mm³ human tissue fragments under renal capsules

- Fetal thymus
- Fetal liver

**Step 3:** Injection of 1—3×10⁶ human CD34⁺ cells isolated from fetal liver

- CD34⁺ hematopoietic stem cells

**Step 4:** Infection by mosquito bite with DENV or CHIKV

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**BLT Mice**

- Robust development of multiple hematopoietic lineages
- T cell education in human thymus, active human NK cells
- Development of a lethal wasting disease (GVHD)

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6-8 week old NOD/SCID/IL2Rγ⁻⁻ mice

Reconstitution 9-16 weeks
Dissemination (A) and transmission (B) efficiencies of two CHIKV isolates and two clones of the respective viral isolates in A. albopictus mosquitoes from Paquetá, Rio de Janeiro, Brazil.

Transmission efficiency of three CHIKV isolates in 35 A. albopictus and A. aegypti populations from 10 American countries at day 7 postinfection.

Dengue viruses:

- More virulent, highly replicating genotypes of DENV-2 and DENV-3 are displacing others
- No recombinants, nor new serotypes coming out of sylvatic cycles

Chikungunya viruses:

- Some adaptation to spread by *Ae. albopictus*, which is more prevalent in temperate zones
- Asian genotype has entered the Americas and is efficiently transmitted by both spp.

Future Aims:

- Mechanisms of pathogenicity remain to be determined, esp. DHF and chronic joint pain
- Need better animal models of disease; humanized mice contain target and bystander cells
- The impact of mosquito saliva proteins needs to be included; not done in most studies