Hemorrhagic Fever Viruses: Arbo- and Zoonotic Viruses (West Nile, Dengue, Lassa fever, Ebola, Zika and Marburg viruses)

Medical Microbiology

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Arboviruses and Zoonotic viruses

- Arthropod borne
  - Blood sucking arthropods
    - E.g. mosquitoes, ticks
    - E.g. **Yellow fever, dengue fever,** Japanese encephalitic, St Louis Encephalitis, **West Nile fever,** tick-borne encephalitis, Chikungunya fever, **Zika,** Equine encephalitis etc

- Zoonotic viruses
  - Transmitted by rodents and non-human primates (transmission by contact of body fluids and excretions)
    - Eg Hanatavirus, **Lassa fever, Ebola** and **Marbug**

Human Arboviral Infections

- Humans are mainly accidental host
- May involve non-human vertebrates or primates (zoonoses)
  - Birds, bats, monkeys
- Diseases
  - Fevers
  - Encephalitis
  - Hemorrhagic fevers

Classification of Arbo- and Zoonotic viruses

- Arenaviridae
  - Arenavirus
    - Lassa fever (rodent borne)
- Bunyaviridae
  - Hantavirus
    - Hantavirus pulmonary syndrome (rodent borne)
  - Nairovirus
    - Congo hemorrhagic fever
  - Phlebovirus
    - Rift valley fever
- Filoviridae (Zoonotic)
  - Ebola viruses (Ebola hemorrhagic fever)
  - Marbug viruses (Marbug fever)

Classification of Arbo- and Zoonotic viruses

- Flaviviridae (mosquito and tick borne)
  - Flavivirus
    - Dengue fever
    - Japanese encephalitis
    - West Nile fever
    - Yellow fever
    - St Louis encephalitis
    - Zika fever (GBS and microcephaly)
- Reoviridae
  - Coltivirus (Colorado tick fever)
- Togaviridae (mosquito borne)
  - Chikungunya virus
  - Equine encephalitis

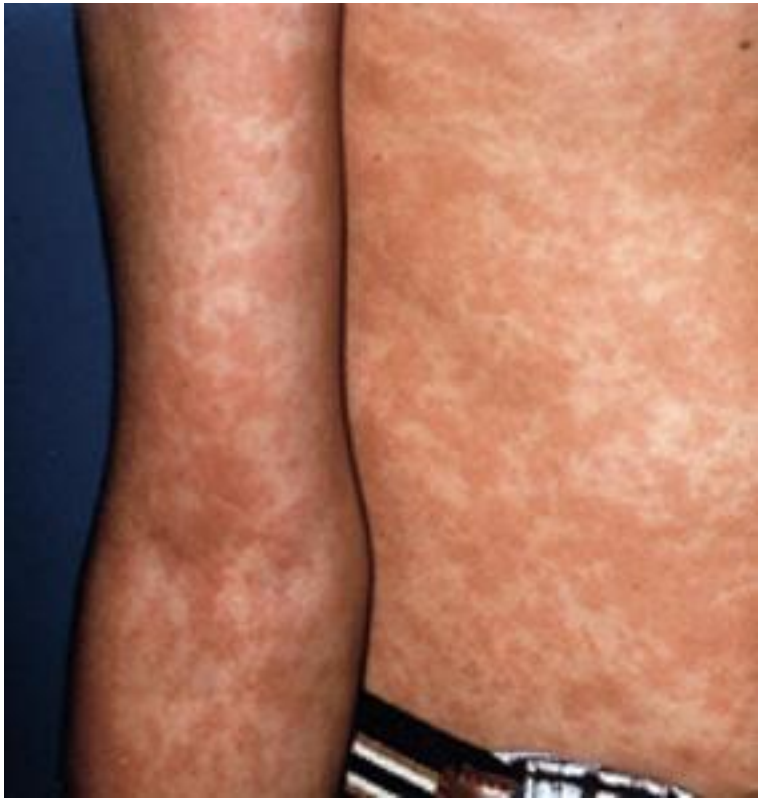
West Nile Virus

- Clinical Features
- Laboratory Diagnosis

  -Serology

  -Molecular
- Treatment

  -Supportive care



Clinical Features

- Mild Infection

  -Most infections are mild and clinically unapparent

  -~20% develop a mild illness (WN fever)

  -Incubation period is thought to be from 3-14 days

  -Symptoms generally last 3-6 days

  -Early outbreaks describe mild WNV infections as a "febrile illness of sudden onset" often accompanied by: malaise, anorexia, nausea, vomiting, eye pain, headache, mylagia, rash, lymphadenopathy

  -Full clinical spectrum of WN fever has not been determined

Clinical Features

- Severe Infection

  -~1/150 infections will result in severe neurological disease

  -Most significant risk factor of neurological disease is advanced age

  -Encephalitis is more commonly reported than meningitis

  -Symptoms among hospitalized patients with severe disease include: fever, weakness, gastrointestinal symptoms, change in mental status

  -Minority of patients with severe disease develop a maculopapular or morbilliform rash involving the neck, trunk, arms or legs

  -Several patients experienced severe muscle weakness and flaccid paralysis

  -Neurological presentations included: ataxia and extrapyramidal signs, cranial nerve abnormalities, myelitis, optic neuritis, polyradiculitis, seizures

  -May also cause myocarditis, pancreatitis, and fulminant hepatitis

Clinical Features

- Severe Infection (Non-specific Lab findings)

  -Total leukocyte counts in peripheral blood is normal or elevated with lymphocytopenia and anemia

  -Hyponatremia (Na+ <135 mmol/L in plasma)

  -CSF shows increase in WBC and proteins are elevated

  -Glucose is normal

  -Tomography and MRIs are often normal

Clinical Features

- Clinical Suspicion

  -Diagnosis of WNV infection is based on a high index of clinical suspicion and obtaining specific laboratory test results

  -WNV, SLE, or other arboviral diseases should be strongly considered in adults >50 years who develop unexplained encephalitis or meningitis in summer or early fall

  -Local presence of WNV enzootic activity or other human cases should raise suspicions

  -Recent travel history is also important

  -Should be considered in persons with unexplained encephalitis and meningitis

Laboratory Diagnosis-CDC

Specimen	1st Choice	Other	Comments
Human serum/CSF	ELISA Plaque Reduction Neutralization	TaqMan/NASBA Virus Isolation	TaqMan (57%) for acute CSF; <10% serum
Human tissue	TaqMan/NASBA	Virus Isolation IHC	Fatal WN cases: TaqMan/NASBA positive ~ 100%
Non-Human	**1st Choice**	**2nd Choice**	
Avian tissue	TaqMan/NASBA Virus isolation	VecTest/ Ag. Cap. ELISA/RT-PCR	Ag.-based tests require 1000 pfu
Mosquito pool	TaqMan/NASBA Virus isolation	VecTest/Ag. Cap. ELISA/RT-PCR	

Laboratory Diagnosis-CDC

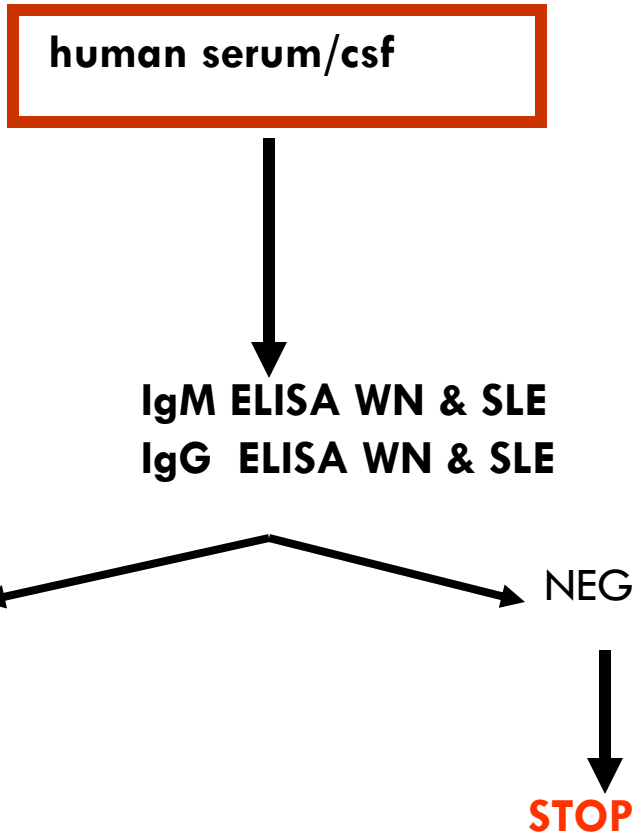
National Case Definition

Confirmed:

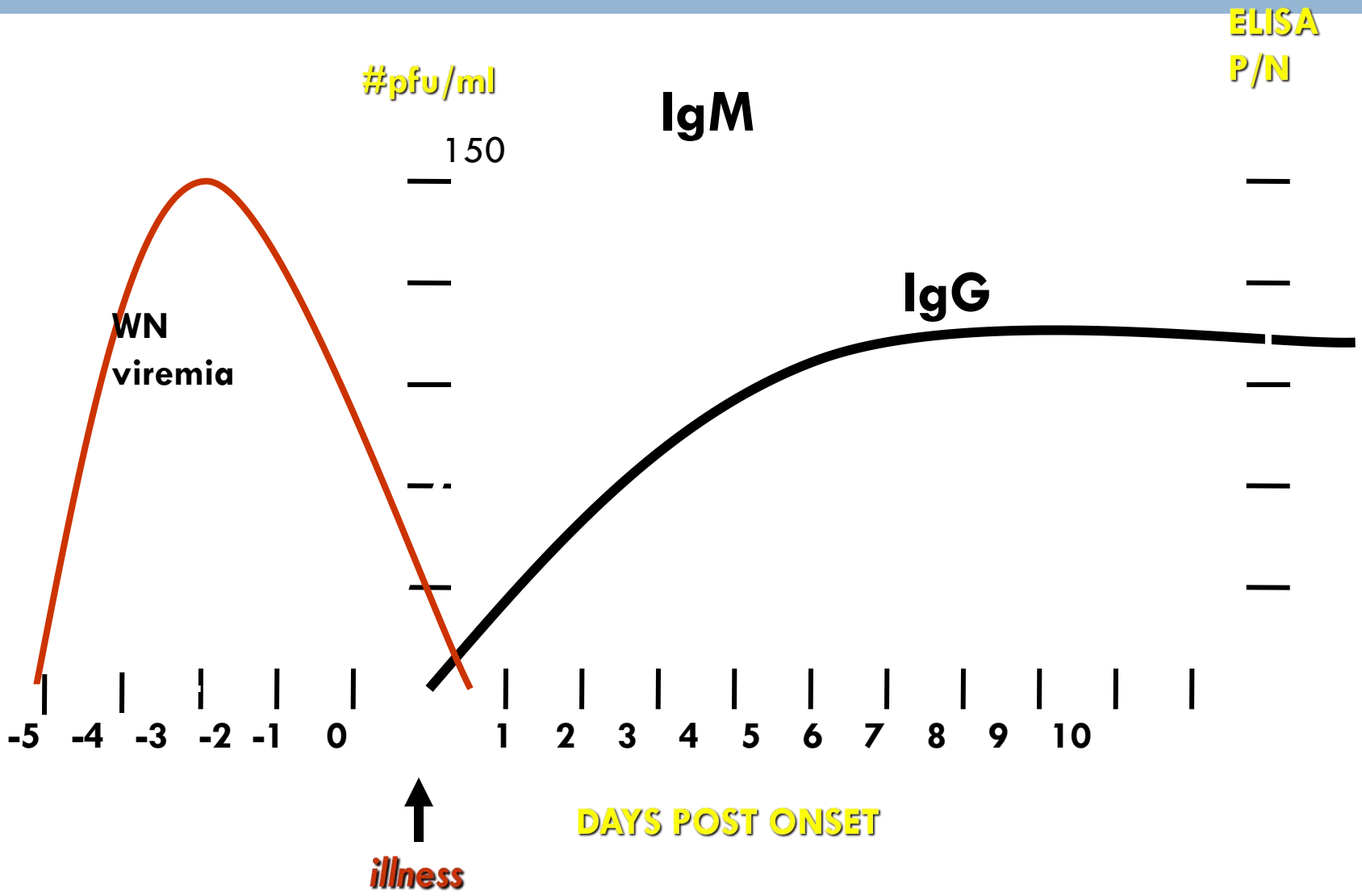
IgM pos csf

IgM pos serum + PRNT

>4-fold increase PRNT titer



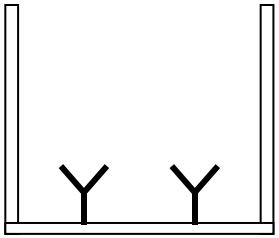
Laboratory Diagnosis



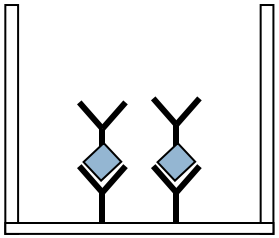
Laboratory Diagnosis

- WNV infection can be suspected in a person based on clinical symptoms and patient history
- Laboratory testing is required for a confirmed diagnosis
- Most efficient diagnostic method is MAC-ELISA (serum collected 8-14 days of illness onset or CSF collected 8 days of illness onset)
- IgM does not cross BBB so presence in CSF is indicative of CNS infection
- Vaccination with related flaviviruses(YF, JE, DEN) may result in positive WNV MAC-ELISA results
- WNV serology cross reacts with JE, SLE, YF, DEN
- PRNT are more specific and should be considered if any of these infections are suspected

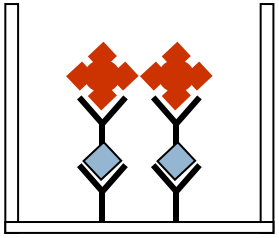
MAC-ELISA



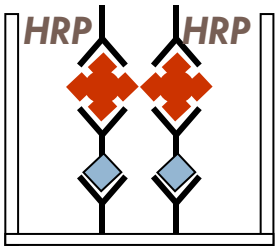
1. Coat With Goat anti-Human IgM
   - 4° Overnight



2. Add Patient Serum @ 1:400
   - 37° 1 Hour



3. Add West Nile Recombinant Antigen
   - 4° Overnight

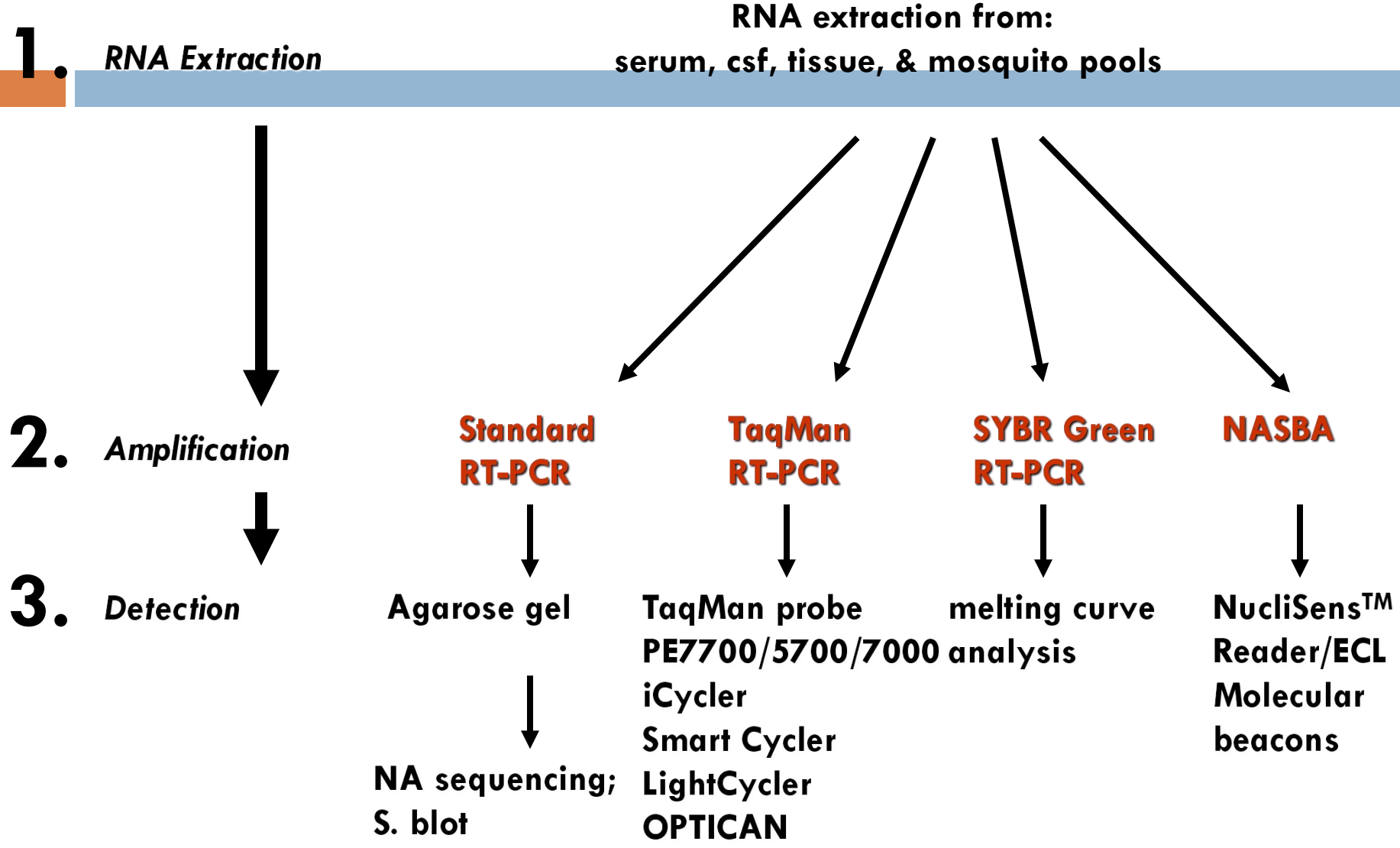


4. Add HRP anti-Flavivirus McAb
   - 37° 1 Hour

Laboratory Diagnosis

- PRNT can be used to help sort false-positives from MAC-ELISA, IFA, and HI
- IgM persists for 6 months or longer, so those in endemic areas may have persistent IgM Ab from previous infection unrelated to current illness
- Some times there are cross-reactivity issues with PRNTs
- RT-PCR can be used but has limited usefulness because of transient and low viremias (50% of those with WN meningoencephalitis tested negative for CSF)
- **Combination of serology and molecular diagnostic is important**
- Virus culture (i.e. Vero cells) is the **gold standard** but is rarely positive except in autopsy material
- Serum and CSF can be refrigerated or frozen prior to testing
- Significant increase in WN-specific neutralizing Ab titer between acute and convalescent-phase serum specimens confirms acute infection

Molecular Diagnosis

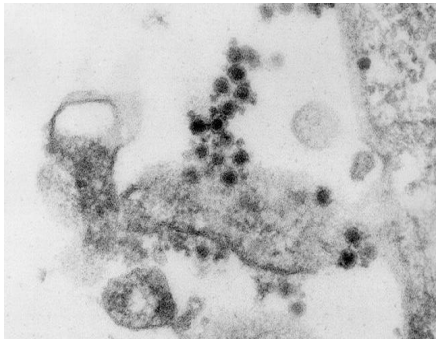


Treatment

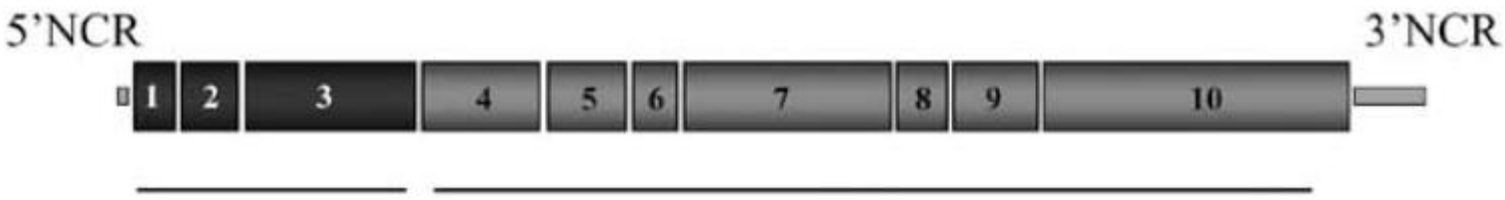
- No specific treatment is available
- In severe cases treatment is supportive care (hospitalization, IV fluids, respiratory support)
- Ribavirin in high doses and INF-α2b were found to have some activity against WNV (in vitro)
- No controlled studies have been completed to look at other medications (steroids, antiseizure drugs, or osmotic agents) for management of WNV encephalitis

General Characteristics

- **Family:** Flaviviridae
- **Genus:** Flavivirus (Japanese Encephalitis Antigenic Complex)
- **Complex includes:** Alfuy, Cacipacore, Japanese encephalitis, Koutango, Kunjin, Murray Valley encephalitis, St. Louis encephalitis, Rocio, Stratford, Usutu, West Nile, and Yaounde viruses.
- 40-60nm
- enveloped, icosahedral nucleocapsid
- positive-sense, single stranded RNA
- 10,000-11,000 bases



(Image courtesy of Bruce Cropp, Microbiologist, Division of Vector-Borne Infectious Diseases, University of Hawaii, Honolulu)



Structural genes

1. Capsid
2. Pre-membrane/Membrane
3. Envelope

Non- structural genes

4. NS1
5. NS2A; 6. NS2B
7. NS3
8. NS4A; 9. NS4B
10. NS5

- 10 major proteins in a single long open reading frame
- three structural, and seven non-structural proteins
- 5' and 3' noncoding regions that contain conserved secondary structures that play important roles in replication and may also be enhancers of protein translation

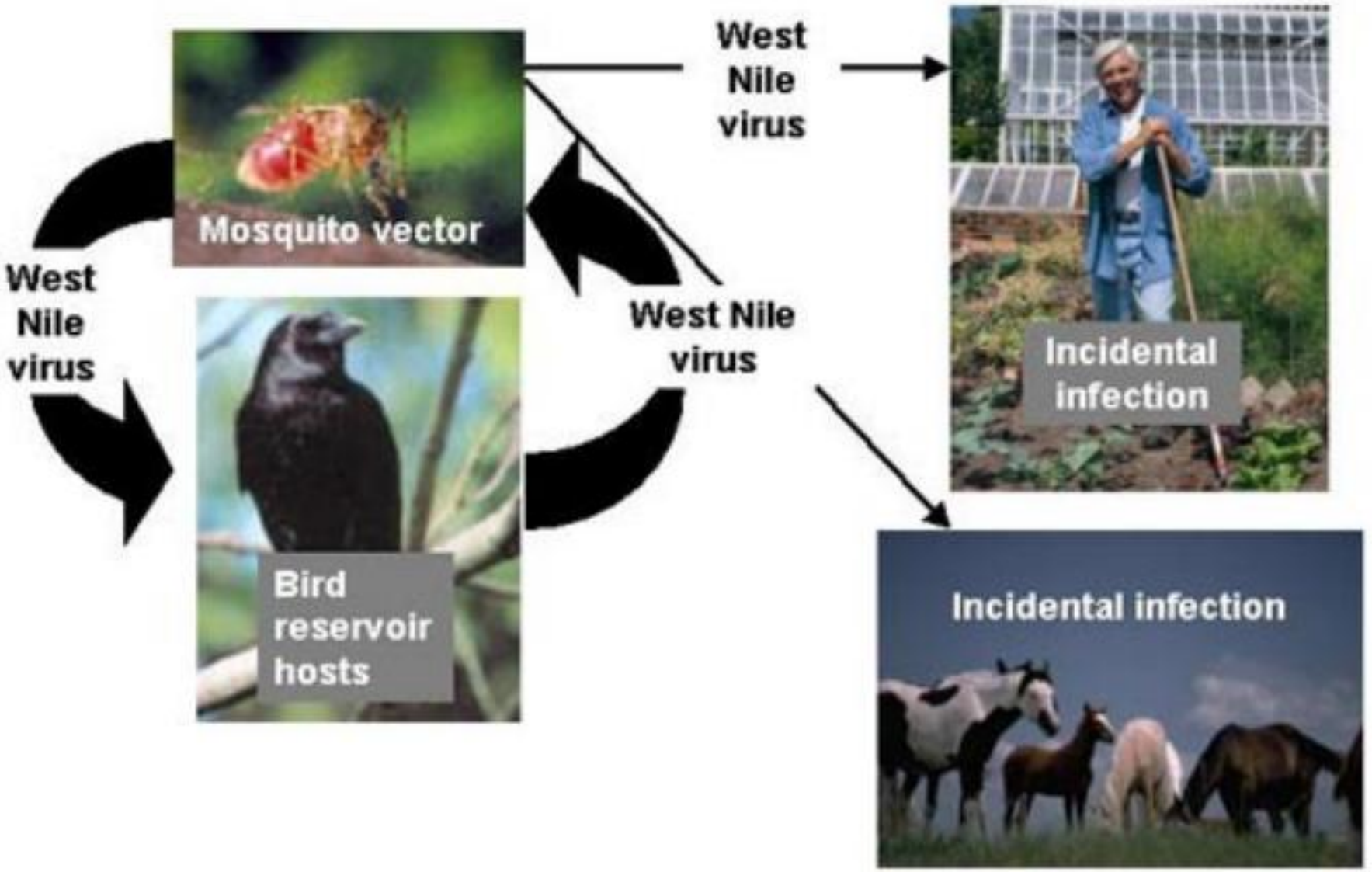
WNV Replication Cycle

- **attachment** to a host cell receptor, or receptors (heparan sulfates and other glycosaminoglycans, integrin)
- **Entry** *via* clathrin-coated pits and actin filaments
- Acidification of endosome leads to fusion of virus and host cell membranes and **uncoating**into cytoplasm
- **Synthesis** of negative strand copies of the genome
- Protein synthesis
- Assembly
- Budding

WNV Transmission

- WNV is maintained in birds and mosquitoes
- *Culex spp. mosquitoes are the main* vectors
- (http://www.cdc.gov/ncidod/dvbid/westnile/mosquitoSpecies.htm)
- Principal vector(s) vary depending upon location
- 284 different bird species (crows and bluejays)
- Transmission to humans and equines are “dead ends”
- lack of significant viremia negates further transmission

West Nile Virus Transmission Cycle



WNV Transmission

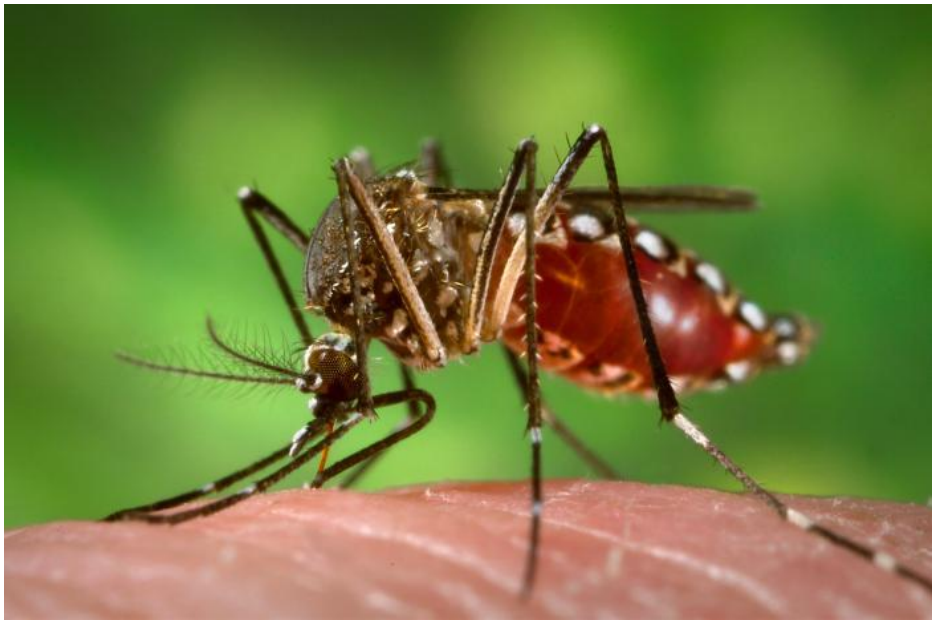
- direct transmission from infected to uninfected individuals
  - Birds - feces or oral secretions
  - *blood transfusion or* organ transplantation
  - laboratory acquired
  - intrauterine transmission
  - transmission *via breast* feeding

Geographic Distribution

- West Nile virus has been described in Africa, Europe, the Middle East, west and central Asia, Oceania (subtype Kunjin), and most recently, North America.
- Outbreaks of WNV encephalitis in humans have occurred in Algeria in 1994, Romania in 1996-1997, the Czech Republic in 1997, the Democratic Republic of the Congo in 1998, Russia in 1999, the United States in 1999-2003, and Israel in 2000. Epizootics of disease in horses occurred in Morocco in 1996, Italy in 1998, the United States in 1999-2001, and France in 2000, and in birds in Israel in 1997-2001 and in the United States in 1999-2002.
- In the U.S. since 1999, WNV human, bird, veterinary or mosquito activity have been reported from all states except Hawaii, Alaska, and Oregon.

Dengue virus

- General Information
- Importance
- Transmission
- Epidemiology
- Biology of the virus
- Virus Replication



Dengue Virus-General Information

- Causes Dengue (DF) and Dengue Hemorrhagic Fever (DHF)
- Flavivirus (type of Arbovirus)
- Transmitted from bite of mosquito vector (*Aedes aegypti* and *Aedes albopictus*)
- Four serotypes (DEN-1, DEN-2, DEN-3, DEN-4); genetically similar but antigenically distinct

Dengue Virus-General Information

- Three Clinical Manifestations: Dengue Fever (DF), Dengue Hemorrhagic Fever (DHF), Dengue Shock Syndrome (DSS)
- 5% death in DSS cases
- More "dangerous" when infected a second time with a different serotype (i.e. DEN-2 then DEN-3)
- Each serotype provides specific lifetime immunity, and short-term cross-immunity
- All serotypes can cause severe and fatal disease
- Genetic variation within serotypes
- Some genetic variants within each serotype appear to be more virulent or have greater epidemic potential

Dengue Virus-Importance

- CDC category A Infectious Disease (Hemorrhagic viruses)
  - -Has potential to cause panic and social disruption
  - -Greatly affects the public health in areas where it is endemic
  - -Easily transmitted
  - -Preparation to combat Dengue demands special action
- Infects 50-100 million people every year
- ½ the world population lives in "hot spots"
- Very hard to create effective vaccine
- Global warming = spread of mosquito vector?
- Urbanization of communities = increased risk?

Dengue Virus-Importance (continued)

- Failed attempts to eradicate in 1950-1970 (yellow fever mosquito campaigns)
- Geographic distribution of previously unestablished serotypes
- Recent outbreaks:

  -Paraguay, 2007

  -India, 2003

  -Hawaii, 2001

  -Taiwan, 2001

  -Puerto Rico, 1994-1995

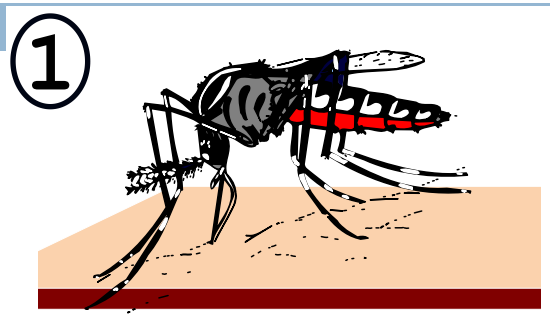
Dengue Virus-Transmission

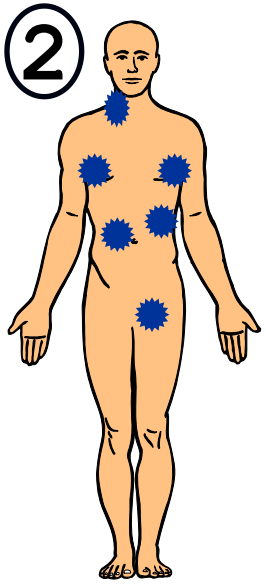
1. Mosquitoes transmit dengue to human dendritic cells

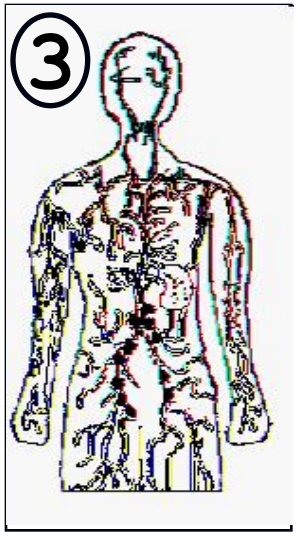
2. Dengue targets areas with high WBC counts (liver, spleen, lymph nodes, bone marrow, and glands)

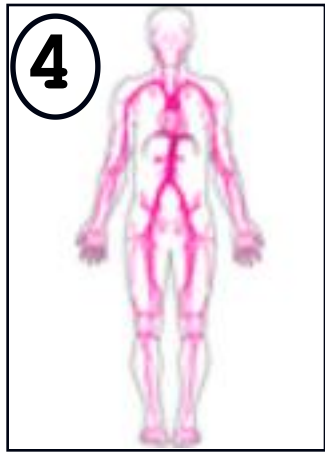
3. Dengue enters WBCs & lymphatic tissue

4. Dengue enters blood circulation



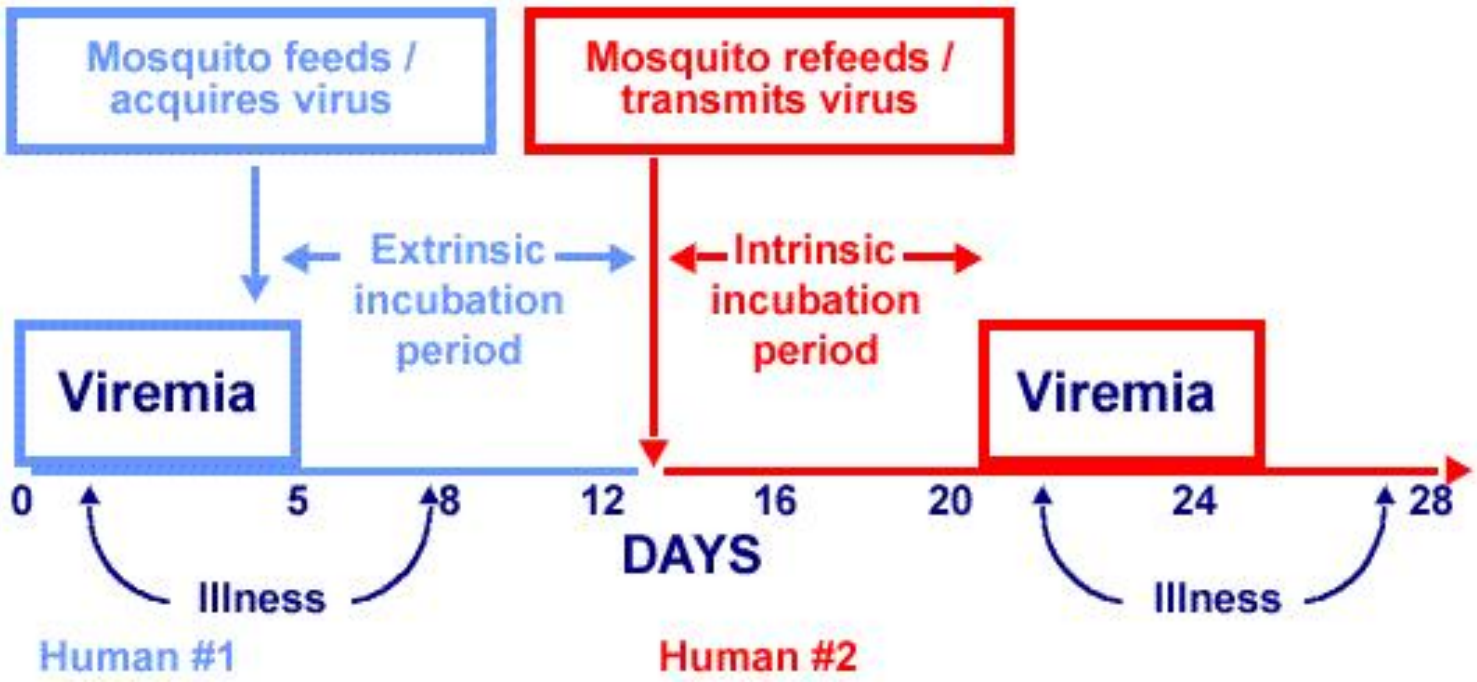




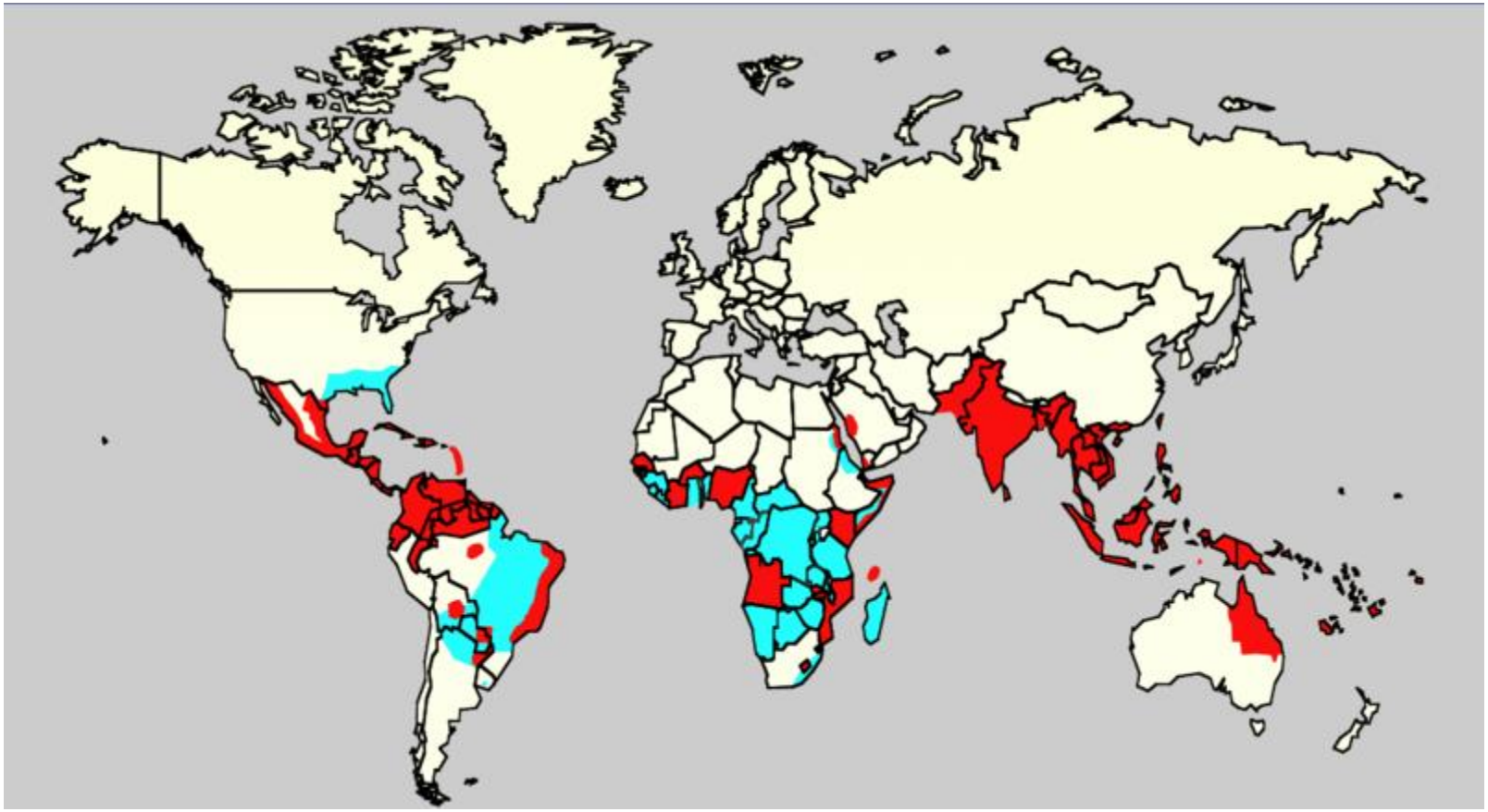


http://phil.cdc.gov/PHIL_Images/08051999/00004/dengue_phf/sld006.htm

Dengue Virus-Transmission (continued)



Dengue Virus-Epidemiology



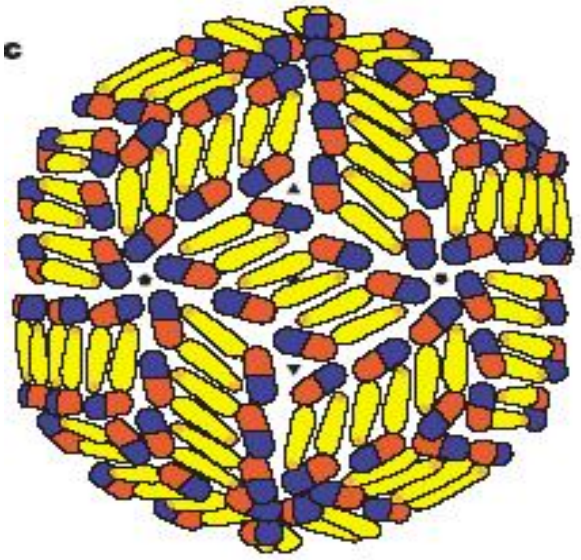
Worldwide distribution of Dengue, 2006 (Red = epidemic dengue, Blue = *Aedes aegypti* vector

Dengue Virus-Biology of Virus

- ssRNA (+) surrounded by icosahedral core
- Icosahedral core is made up of 90 glycoprotein E dimers overlying M proteins
- Protein E is the most important characteristic of Dengue (determines serotype virulence)

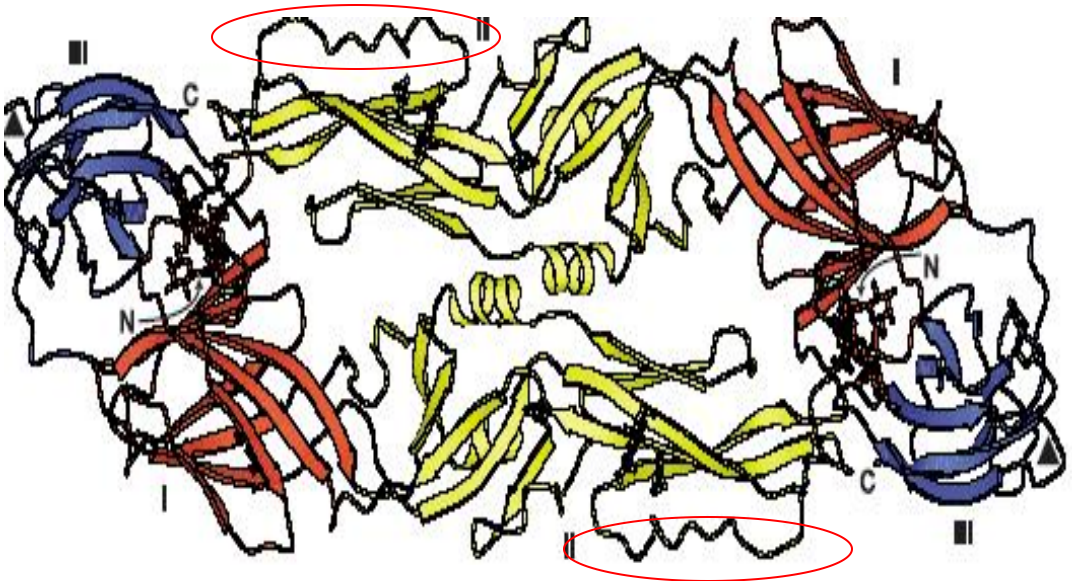
**All you can see are the 90 protein E dimers because they are what overlay the rest of the virus.

**The different colors indicate the three different domains of protein E; domain I is in red, II is in yellow, and III is in blue



Dengue Virus-Biology of Virus

- E protein dimer (pre-fusion w/ cell membrane)
- Note the three domains (I, II, and III) and the fusion loops that are part of domain II, the yellow domain.
- These loops are responsible for membrane fusion once dengue enters the cell



Dengue Virus-Biology of Virus

- Like all flaviviruses, the E protein of dengue is responsible for fusion to the host cell (dendritic and macrophage)
- After fusion with the Fc receptor, it enters an endosome where the pH is low enough to cause a conformational change of the E protein thereby enabling the virus to merge with the endosomal membrane and release the viral RNA into the target cell.
-
- From here, the virus can replicate and ultimately leave the cell to infect other cells

Dengue Virus-Biology of Virus

Dengue infection

Endosome entry & pH change

E protein conformational change

Release of viral RNA into cell

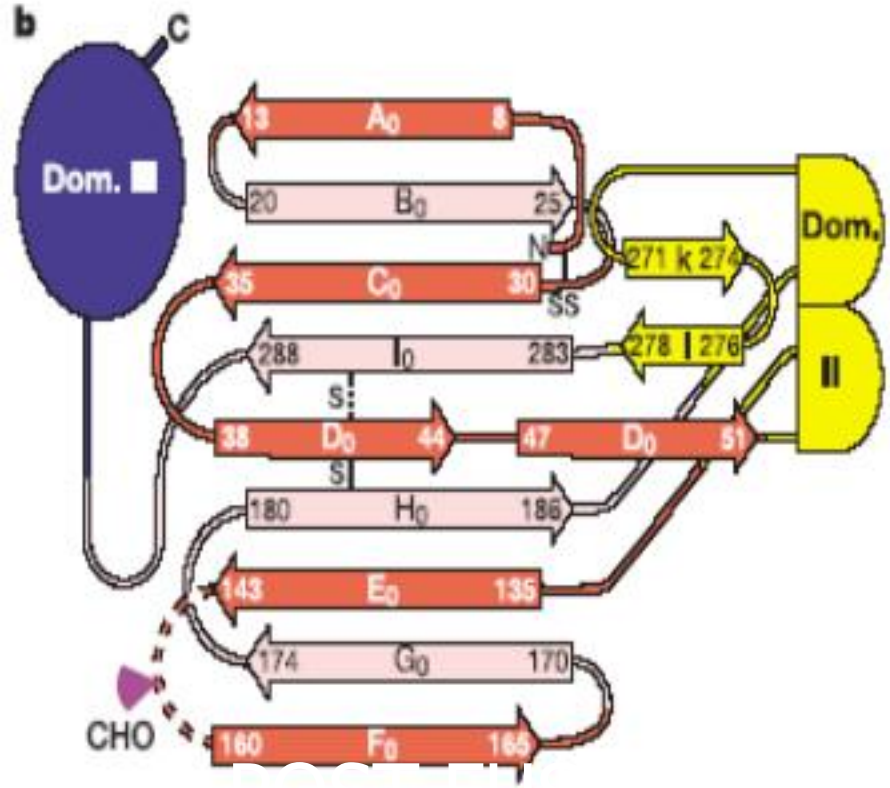
Replication & further infection

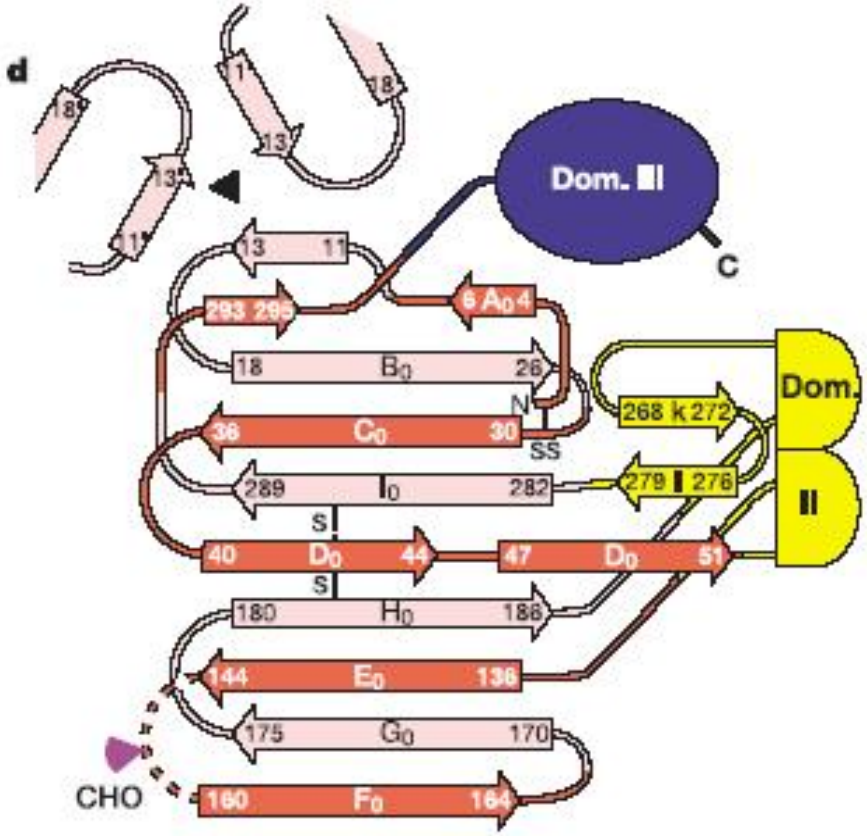
Dengue Virus-Biology of Virus

- When the pH becomes more acidic, the E protein transforms from dimeric form into trimeric form

- Domain III shifts 70 degrees so it is closer to the hydrophobic loops of domain II, and thereby forcing the target membrane and viral membrane together and enabling the virus to release its RNA into the cell and begin the replication process

Dengue Virus-Biology of Virus

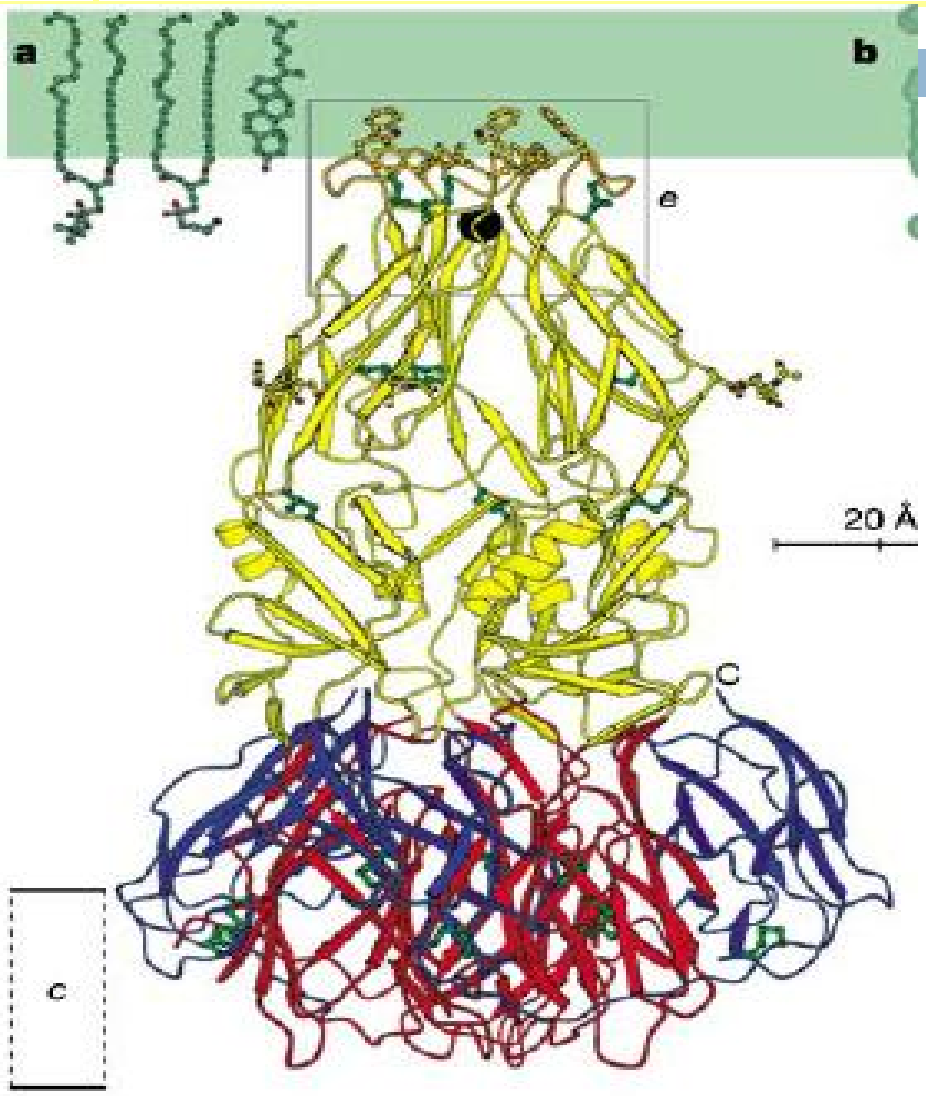


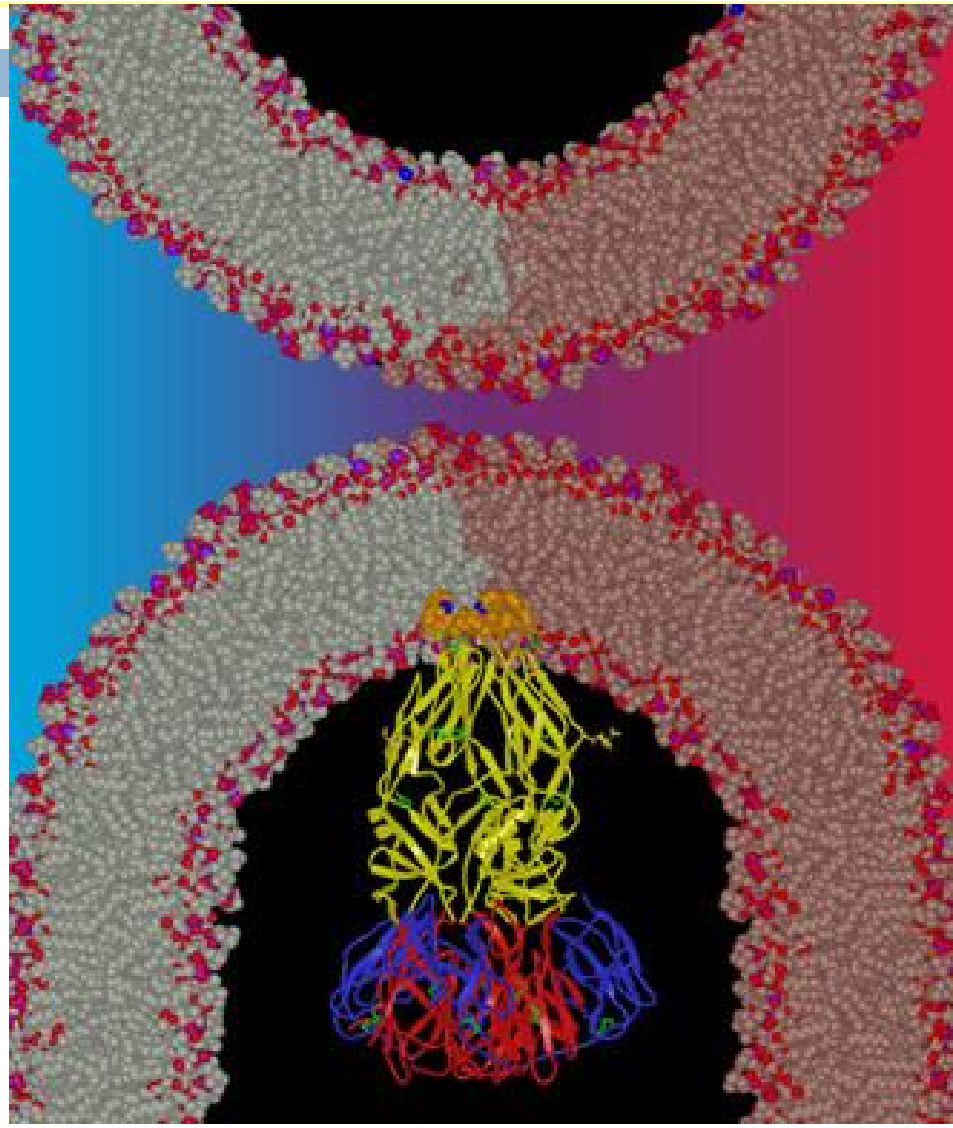


Dengue Virus-Biology of Virus

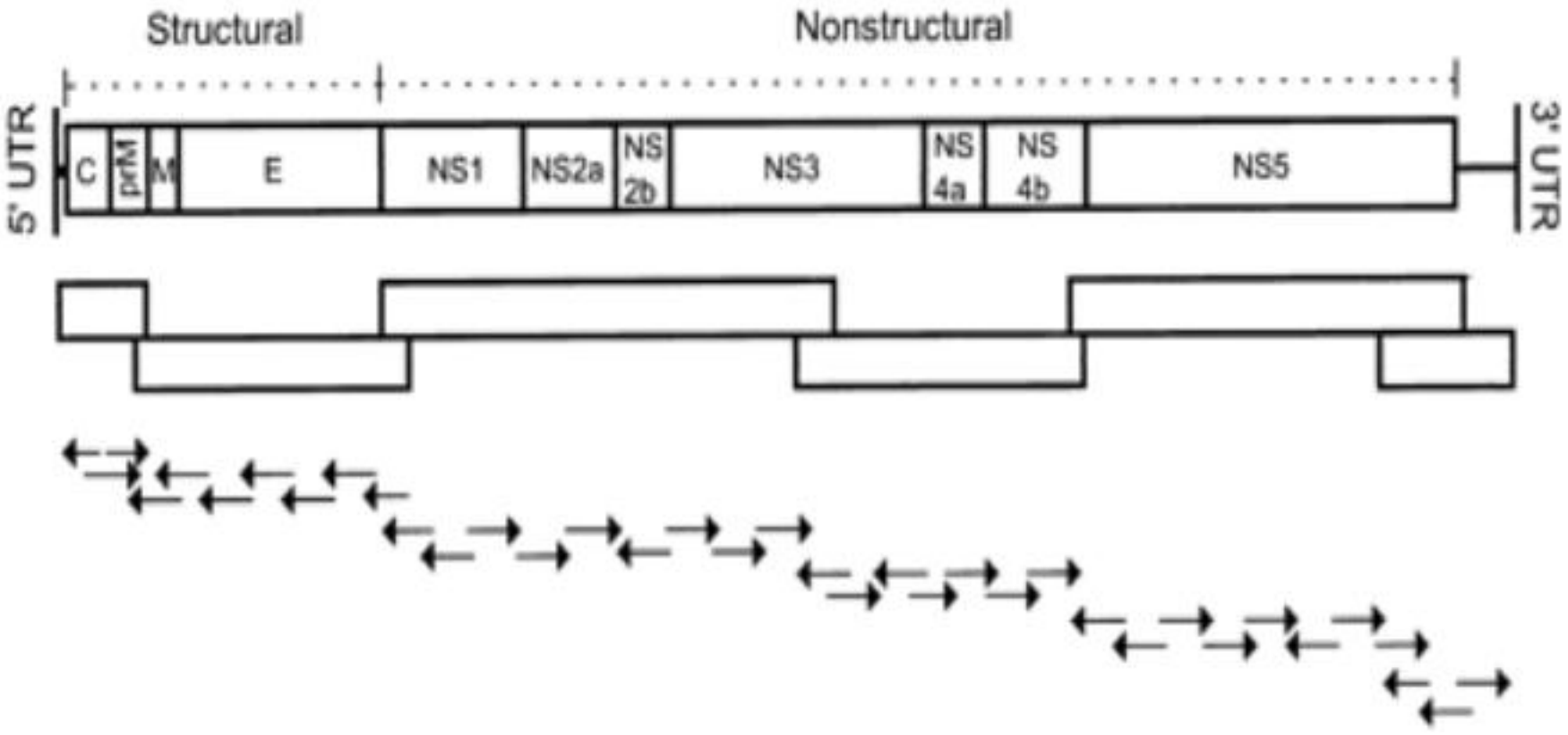
- Protein E after membrane fusion (next slide)
- Three residues in dengue (and in all flaviviruses) on the fusion loops that are exposed to the cell membrane post-conformational change (Tryptophan-101, Leucine-107, and Phenylalanine-108)
- Together they form a hydrophobic rim that enables the protein to insert itself into the membrane
- To stay in the membrane, there is an aromatic (hydrophobic) anchor made up of Trp 101 and Phe 108
- The black dot near the fusion loops is a chloride ion

Dengue Virus-Biology of Virus





Dengue Virus-Genome



Long-Range RNA-RNA Interactions

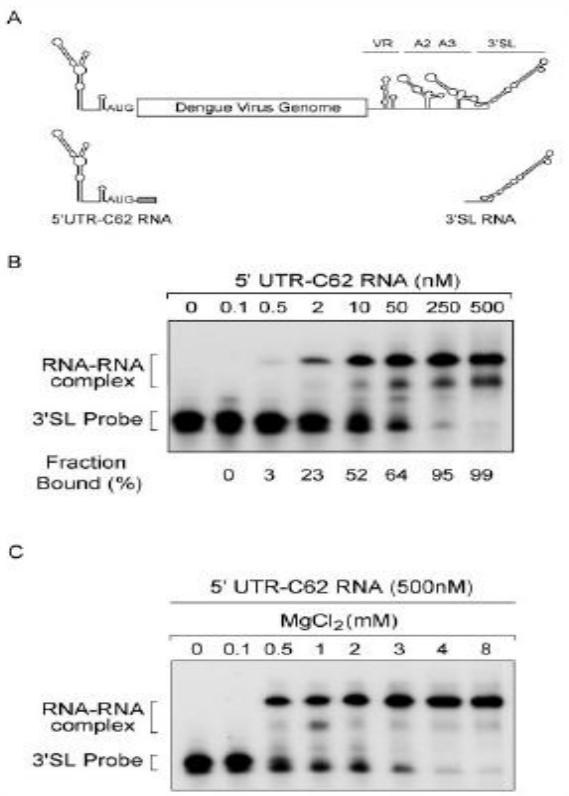


FIG. 1. Biochemical assays reveal RNA-RNA complex formation between the end sequences of dengue virus RNA. (A) Schematic representation of the dengue virus genome showing the predicted secondary structures at the 5′ and 3′ UTR. The four domains of the 3′ UTR, variable region (VR), A2, A3, and 3′ stem-loop (3′ SL), are indicated. The schematic representation of RNA molecules used for binding assays, 5′ UTR-C62 and 3′ SL, are also shown. (B) Mobility shift assays showing RNA-RNA associations. Uniformly labeled 3′ SL RNA, corresponding to the last 106 nucleotides of dengue virus type 2, was incubated with increasing concentrations of the 5′ UTR-C62 RNA corresponding to the first 160 nucleotides of the viral genome. The 5′ UTR-C62 RNA was used from 0 to 500 nM as indicated on the top of the gel. The locations of the 3′ SL probe (Probe) and the RNA-RNA complex are shown. Quantification of the fraction of probe bound for each concentration of the RNA is also indicated at the bottom of the gel. (C) RNA-RNA complex is formed only in the presence of Mg^{2+}. The 3′ SL probe was incubated with an excess of the 5′ UTR-C62 RNA (500 nM), and complex formation was examined in the presence of increasing concentrations of Mg^{2+}, from 0 to 8 mM.

Long-Range RNA-RNA Interactions

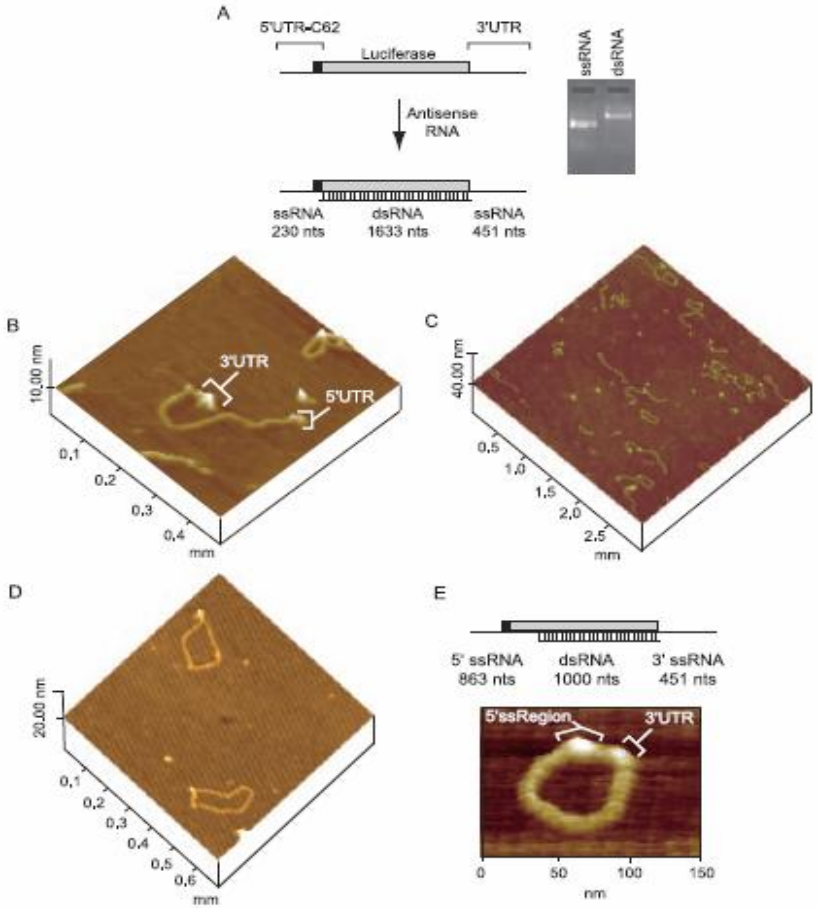


FIG. 2. Single-molecule analysis reveals cyclization of an RNA molecule carrying the 5′- and 3′-end sequences of dengue virus. (A) Schematic representation of a model RNA molecule of 2.3 kb showing the 5′ and 3′ dengue virus sequences flanking the luciferase coding sequence. Annealing of an antisense RNA of 1,633 nucleotides is shown. The resulting molecule bears single-stranded overhangs in the 5′ and 3′ ends of 230 and 451 nucleotides, respectively. On the right, purified single-stranded RNA (ssRNA) and double-stranded RNA were resolved on a 1% agarose gel and visualized by ethidium bromide staining. (B) Visualization of the model RNA molecules by AFM. A single RNA molecule is shown in a linear conformation. The double-stranded RNA region is flanked by single-stranded regions corresponding to the 5′ UTR-C62 and 3′ UTR of dengue virus. (C) An image of a representative field of RNA molecules deposited on mica obtained by tapping-mode AFM. Circular, linear, and head-to-tail dimers were observed. (D) Image of individual RNA molecules in circular conformation is shown. Contacts between the 5′ and 3′ single-stranded regions of the molecules can be observed. (E) Schematic representation of the same RNA molecule shown in A hybridized with an antisense RNA molecule of 1 kb. The double-stranded region of 1 kb is flanked by a 5′ single-stranded region of 863 nucleotides that contained the 160 nucleotides of the 5′ end of dengue virus and a 3′ single-stranded region that corresponds to the 3′ UTR of dengue virus. At the bottom, a representative image of an individual molecule with a double-stranded region of 1 kb is shown in circular conformation.

Immunology and Cell Biology (2007) 85, 43–45

www.nature.com/icb

REVIEW

Of cascades and perfect storms: the immunopathogenesis of dengue haemorrhagic fever-dengue shock syndrome (DHF/DSS)

Tikki Pang[1], Mary Jane Cardosa[2] and Maria G Guzman[3]

Phase I clinical trial of TGN1412 in UK

Immunomodulatory drug/Human mAb (CD28 antagonist)

6 Patients hospitalized, 4 with major organ failure

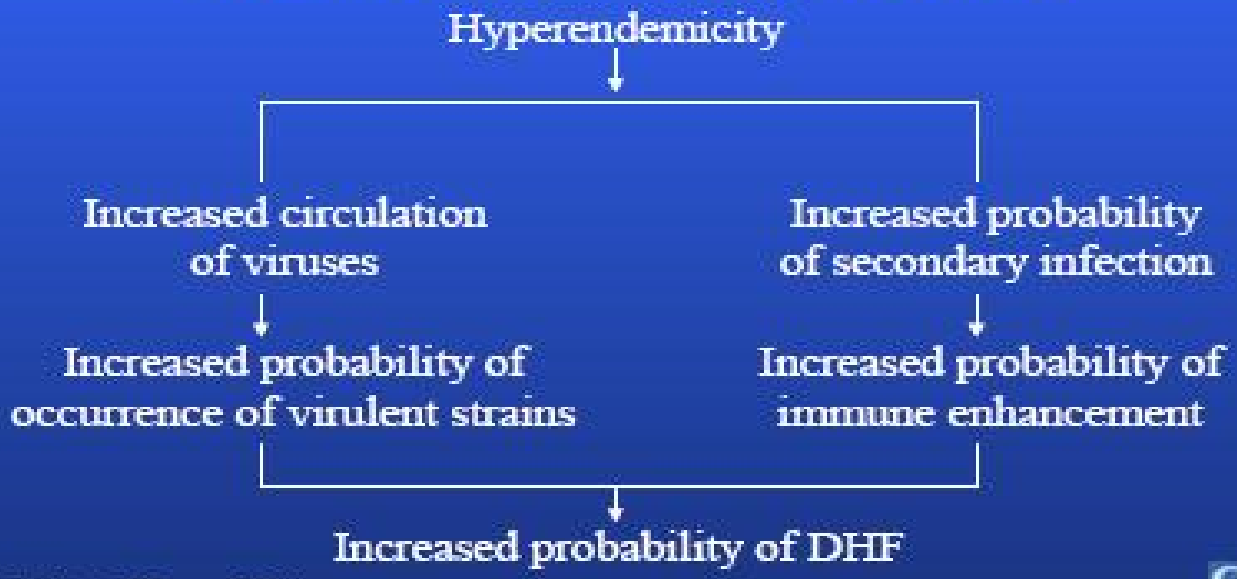
Of cascades and perfect storms

- Dengue hemorrhagic fever (DHF) and Dengue Shock Syndrome have an immunological basis
- Secondary heterologous infection causes severe disease
- Severe disease linked to
  - Memory T-cell activation
  - 'storm' of inflammatory cytokines/other mediators (complement)
  - Activation of T-cells, monocytes/macrophages, endothelial cells
    - Increase in vascular permeability
- Severe disease is
  - Complex
  - Multifactorial
  - Involve various serotypes
  - Involves Interplay of both host and viral factors
  - Genetics, age etc

Risk Factors for DHF

- Higher risk in secondary infections
- Higher risk in locations with two or more serotypes circulating simultaneously at high levels (hyperendemic transmission)

Increased Probability of DHF



Gubler & Trent, 1994

CDC

1. Antibody-dependent enhancement (ADE) and other antibody-mediated events

- During secondary heterotypic dengue virus serotype infection pre-existing, subneutralizing and non-protective levels of dengue antibodies enhance virus replication in FcRγII bearing monocytes/macrophages (in-vitro & in-vivo studies)

- IgG, IgM and complement C3 receptors have been implicated
  - Against major dengue viral envelop (E) Glycoprotein and NS-1

- Confirmed secondary dengue infection is a risk in more than 97% of severe cases

- Enhancing Abs present as long as 20yrs after primary infection
  - Especially during dengue 2 and 3 after dengue 1

1. Antibody-dependent enhancement (ADE) and other antibody-mediated events

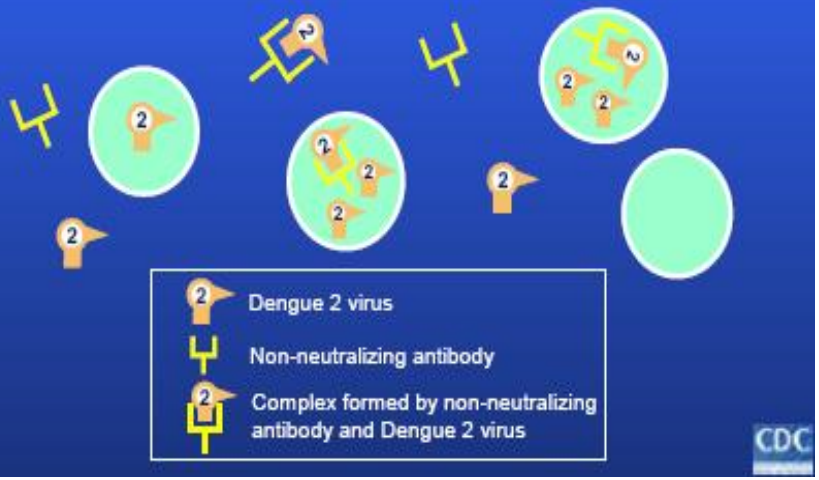
- In addition to ADE, cross reactive antibodies directed against dengue NS-1 protein have been implicated to cause damage to endothelial cells via iNO–mediated apoptosis

- In-vitro studies suggest production of cytokines/chemokines by endothelial cells
  - IL-6, IL-8, MCP-1
  - Autoimmune mechanism like molecular mimicry
  - Abs also cross react with plasminogen (Hemorrhage)
  - Recently, antibody-dependent cell-mediated cytotoxicity (ADCC)

Homologous Antibodies Form Non-infectious Complexes

Heterologous Antibodies Form Infectious Complexes

Heterologous Complexes Enter More Monocytes, Where Virus Replicates



Viral Risk Factors for DHF Pathogenesis

- Virus strain (genotype)
  - Epidemic potential: viremia level, infectivity
- Virus serotype
  - DHF risk is greatest for DEN-2, followed by DEN-3, DEN-4 and DEN-1

CDC

2. T-cell activation

- In-vitro studies have shown T-cell activation following dengue virus infection
  - Involves $CD4^+$ and $CD8^+$ cells
- Following ADE of viral replication in monocytes/macrophages, viral antigens are presented to $CD4^+$ and $CD8^+$ cells
  - This is followed by activation of memory $CD4^+$ and $CD8^+$ cells sensitized during previous infection
    - Leading to proliferation and release of proinflammatory cytokines
      - INF-γ and TNF-α
        - Act on endothelial cells resulting in plasma leakage
- Thai DHF/DSS data in children show massive T-cell activation followed by apoptosis
- 'Original antigenic sin' and cross reactive T cells
  - Maternal antibodies
  - Increased vascular permeability and hemorrhage

3. Cytokine cascades

- Complete picture not known
- Following T-cell activation, cytokine cascade targets endothelial cells creating a 'sieve' effect leading to fluid and protein and leakage.
- Cytokines produced by T-cells, monocytes/macrophages, endothelial cells
  - INF-γ, TNF-α, IL-1β, IL-2, IL-8 and IL-10
  - INF-γ up regulates expression of Fcγ receptors on monocytes/macrophages facilitation ADE
    - Synergy (INF-γ, TNF-α, IL-1)
- Thought
  - Presence of dengue epitopes on endothelial cells is due to deposition of immune complexes rather than viral replication which occurs in monocytes/macrophages

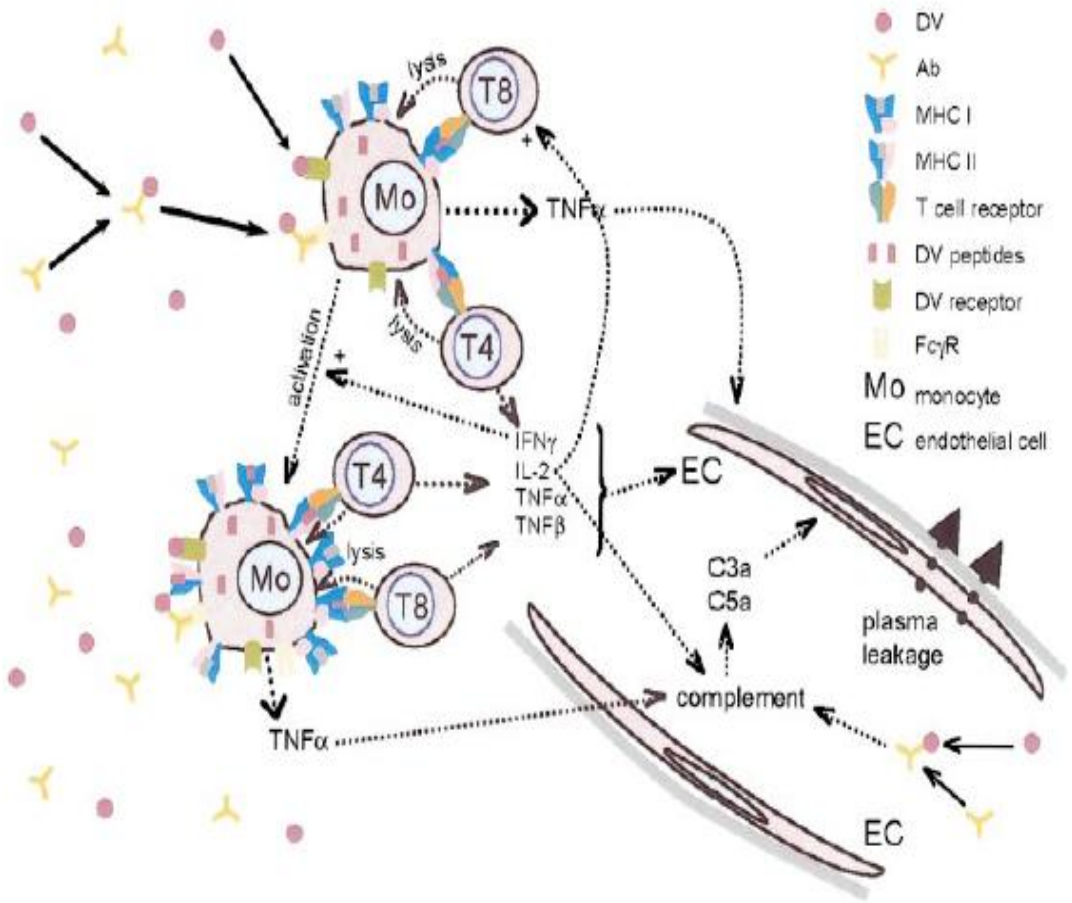
4. Complement and other mediators

- Complement activation has been implicated in DHF/DSS
  - Cause unknown
- Large amounts of dengue NS-1, complement anaphylatoxin C5a, and the terminal complex SC5b-9 were present in pleural fluids of patients with DHF/DSS
  - May contribute to vascular leakage
- Other mediators
  - Histamine, tissue plasminogen activator and macrophage migration inhibitory factor (MIF) have been implicated
    - MIF has been found high in patients who died than those who recovered, potential predictor of disease severity and clinical outcome of disease

Immunopathogenesis of Dengue Hemorrhagic Fever

Alan L. Rothman[1] and Francis A. Ennis

Virology 257, 1–6 (1999)
Article ID viro.1999.9656, available online at http://www.idealibrary.com on IDEAL®



Immunopathological mechanisms in dengue and dengue hemorrhagic fever

Sharone Green and Alan Rothman

Current Opinion in Infectious Diseases 2006, 19:429–436

Effects of immune response to primary dengue virus infection: implications for disease outcome on secondary infection

Pre-existing immune response to primary dengue virus infection	Effector mechanism	Effect on viral burden	Implications for disease severity
Crossreactive neutralizing antibodies	Decrease in antigen presentation to adaptive immune system	↓	↓
Antibodies that mediate enhancement	Increase in antigen presentation to adaptive immune system	↑	↑
Antibodies that mediate ADCC	Decrease in antigen presentation to adaptive immune system	↓	↓
Anti-NS1 antibodies	Molecular mimicry, endothelial cell activation, complement activation	→	↑
Pre-existing low avidity crossreactive T cells Cytotoxicity Cytokine production Proliferaton	Increase in antigen presentation to adaptive immune system; impaired cytotoxicity or altered cytokine response may lead to immune mediated injury or plasma leakage	↑/↓	↑/↓

ADCC, antibody-dependent cell-mediated cytotoxicity.

5. Monocyte activation and cytokine production/Dendritic cells involvement

- Monocytes have been shown to be more permissive to dengue virus infection in-vitro
  - Produce TNF-α and IL-1β (Vasoactive factors)
  - Receptor unknown? FcRγII / ADE

- Dengue virus can infect dendritic cells
  - Via C-type lectin, CD209/DC-SIGN to E-protein
  - Produce cytokines and chemokines
    - Attract cell of the adaptive immunity
    - May also down regulate immune responses (IL-10)
  - Antigen presentation to naïve or memory T-cells, B-cells, NK cells,
    - Cell activation
    - Anti-dengue E and NS-1 antibodies, cytokines, cytolytic granules

Immunopathological mechanisms in dengue and dengue hemorrhagic fever

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Current Opinion in Infectious Diseases 2006, 19:429–436

A proposed model of the immunopathogenesis of dengue hemorrhagic fever: interactions of dengue virus with the innate, humoral and cellular immune systems during secondary dengue virus infection

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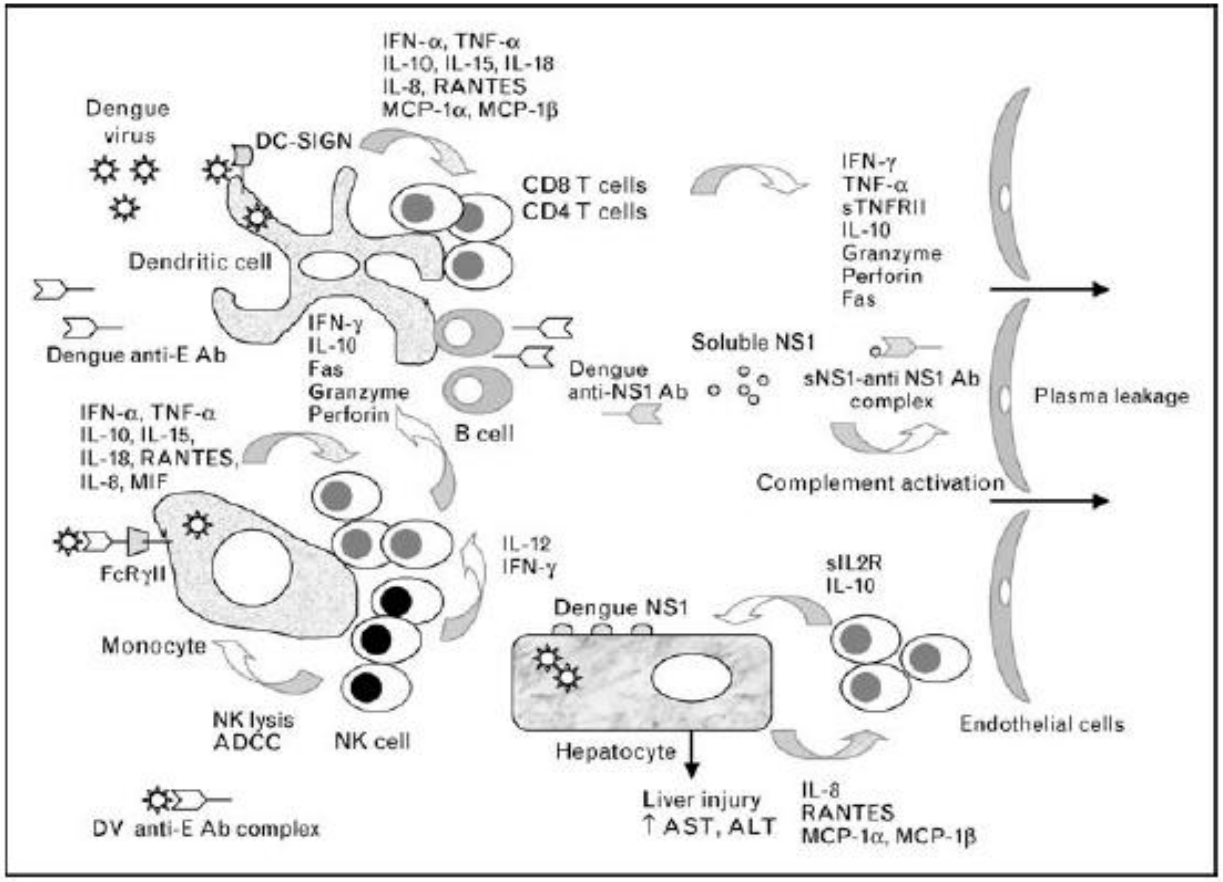
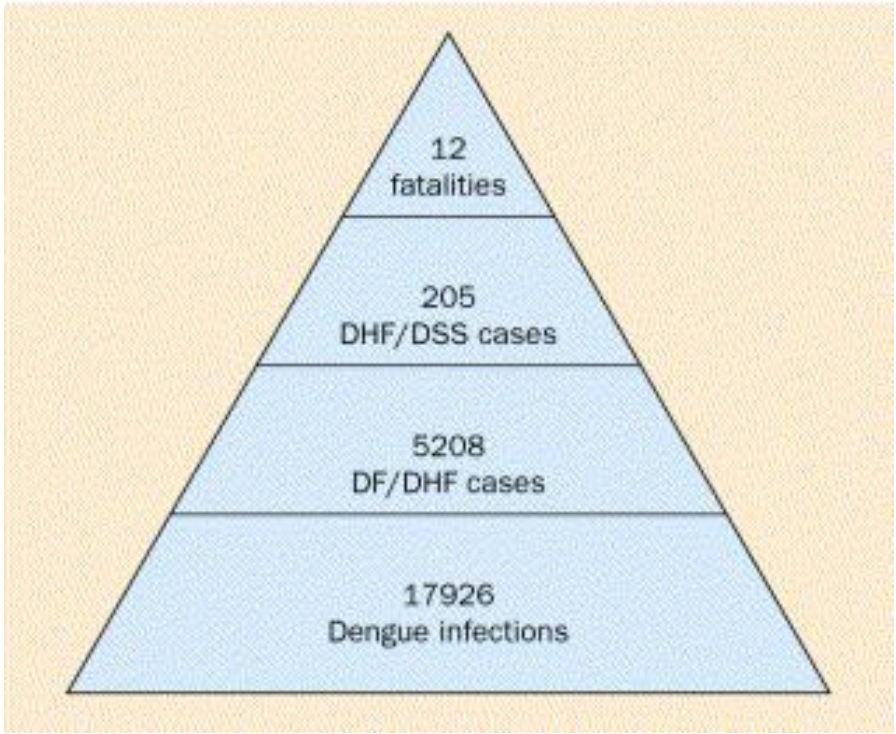


Implications for prevention and control: Vaccine development

- Vector control measures
- Improved disease management
- Better public education awareness
- Public health measures

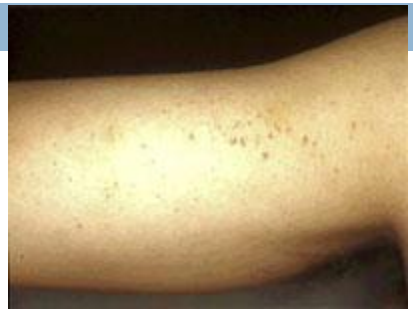
- Challenges
  - Lack of a safe and effective vaccine
    - Long-lasting protection against all the 4 serotypes
      - Incomplete protection could lead to ADE
        - Enhance risk for DHF/DSS
  - Lack of clarity about correlates of protection and absence of a good experimental model for DHF/DSS



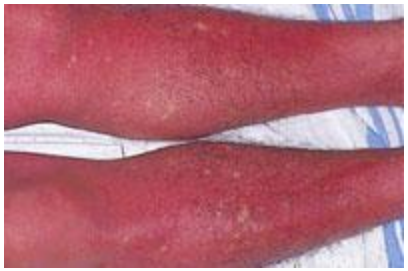
Reported and estimated DF/ DHF and dengue-2 infections during the 1997 DHF Cuban epidemic

Clinical manifestations

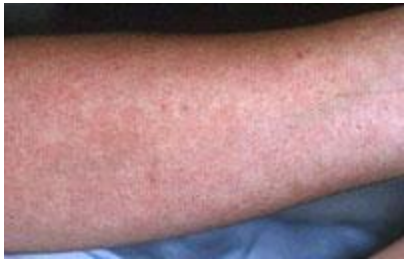
- **Asymptomatic:** ~70%
- **Dengue Fever:**
  - High, prolonged fever (usually lasts for 5 to 7 days)
  - 50% of patients have rash.
  - Severe headache
  - Pain behind the eyes
  - Muscle and joint pains
  - Nausea
  - Vomiting
  - Loss of appetite
  - Fatigue
  - Diarrhea
  - Laboratory: thrombocytopenia, leukocytopenia, and elevated ASTs



Petechial rash



Diffuse erythematous rash



Maculopapular rash

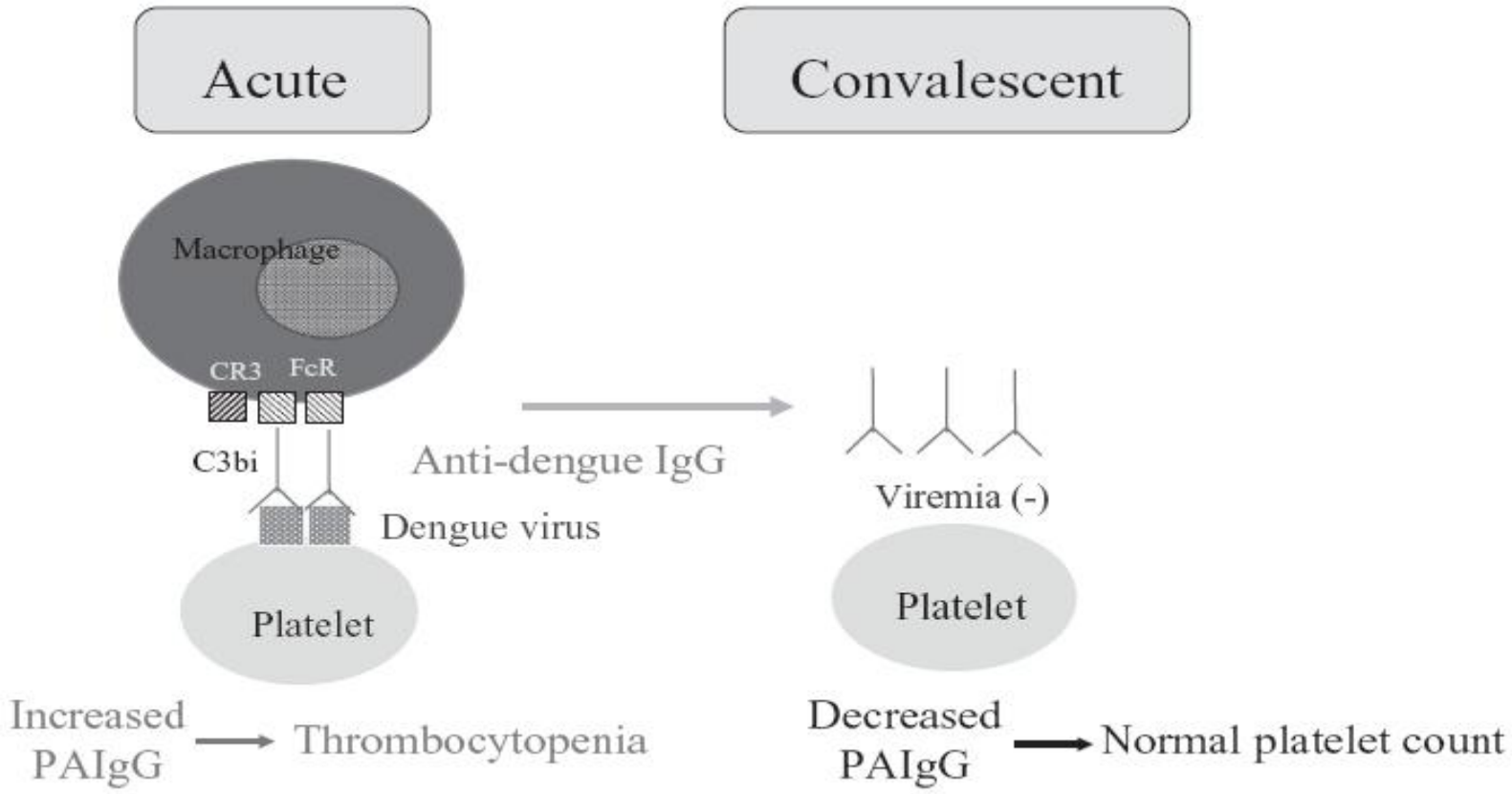
Clinical manifestations

- **DHF**
- Thrombocytopenia (>100 000/µl)
- vascular changes:
  - Capillary plasma leakage
  - increase in hematocrit
  - Pleural effusion
- coagulation disorders:
  - petechiae, purpuric lesions, and ecchymoses
  - tourniquet test
- DHF grades III and IV are dengue shock syndrome

WHO case classifications

- DHF
  - Fever of 2-7 days (100%)
  - Hemorrhagic
    - tourniquet test (27-47%)
  - Thrombocytopenia (>100,000 per ul) 8-94%
  - Haemoconcentration (20% rise in HCT) ~100%
- DHF grade I: + TT
- DHF grade II: bleeding
- DHF grade III: hypotension, weak pluse
- DHF grade IIII: Shock

Pathogenesis



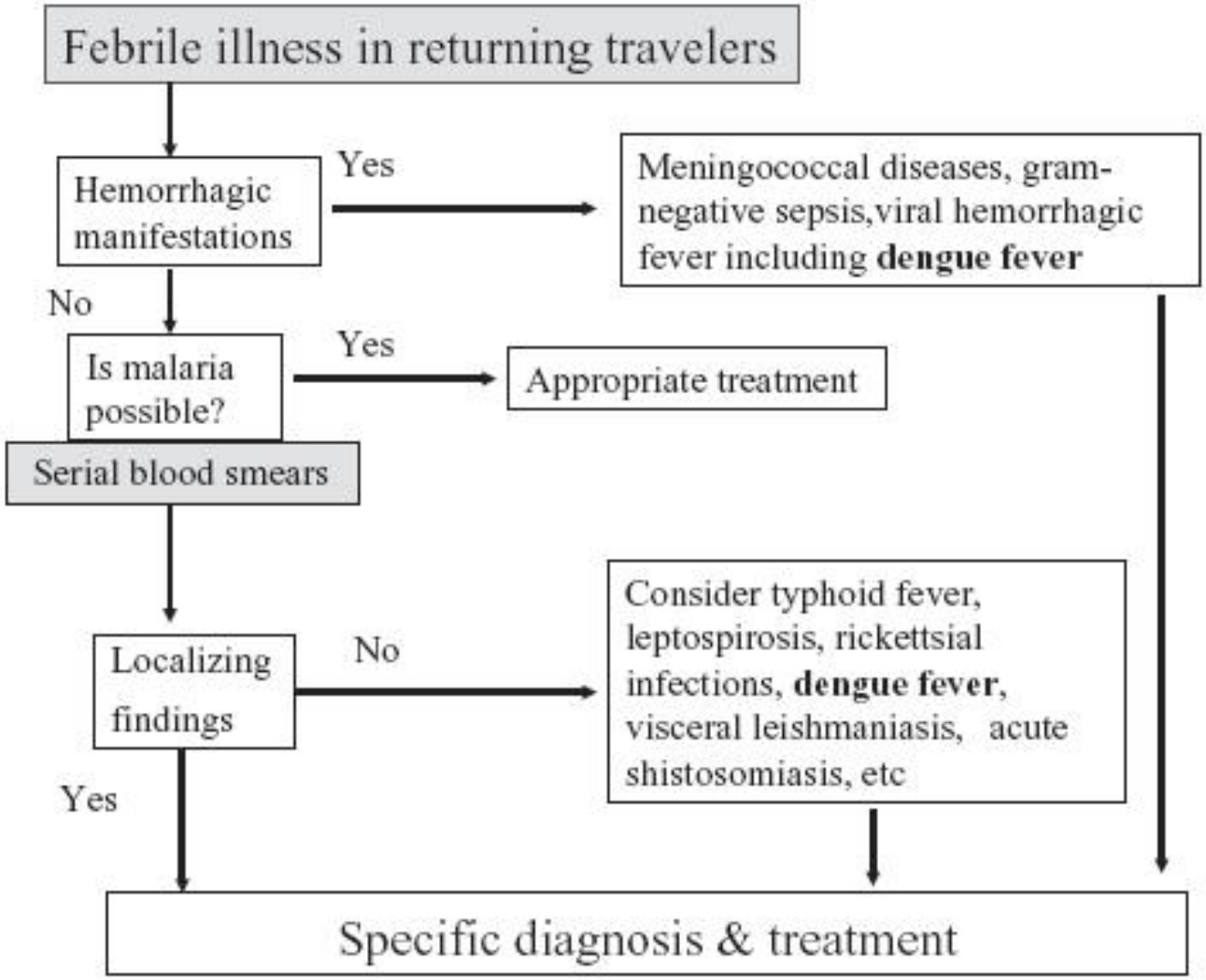
- **Thrombocytopenia:** platelet-associated IgG (PAIgG) in secondary infections
- During the acute phase of viremia, immune complexes of dengue virus with antidengue virus IgG antibodies, which are located on platelets via the direct binding of dengue virus to platelets, and PAIgG formation may result in thrombocytopenia due to platelet clearance by macrophages and/or complement-mediated platelet lysis

Pathogenesis

- **Temporarily altered vascular permeability**
- Indirect endothelial cell activation:
- The activation of immune effector cells → inflammatory cytokines
- NS-1 via the NF-κB pathway can activate endothelial cells
- anti-NS-1 antibody enhanced in the endothelial cell activation
- NS-1 induces complement activation, which is enhanced by an anti-NS-1 antibody
- Complement activation and the apoptosis of endothelial cells by dengue virus infections may lead to increased vascular permeability.
- The clearance of dengue virus-infected apoptotic endothelial cells may also explain the abrupt termination of a dengue virus infection and the increased vascular permeability.

Diagnosis

- WBC, PLT, HCT
- IgM-capture ELISA
- RT–PCR



Lassa fever

- Endemic in west Africa
- Incubation period is 1-3 weeks
- Symptoms
  - Fever
  - Vomiting
  - Mouth ulcers
  - Back and chest pain
  - Severe muscle aches
  - Skin rashes and hemorrhage
  - Pneumonia, heart and kidney damage
  - Deafness

Virology

- Pleomorphic virions with ss RNA genome
- Establish chronic infections in rodents
- Human transmission is through contact of rodent excreta
  - Transmission within rodents is through vertical and horizontal routes

Diagnosis of Lassa fever

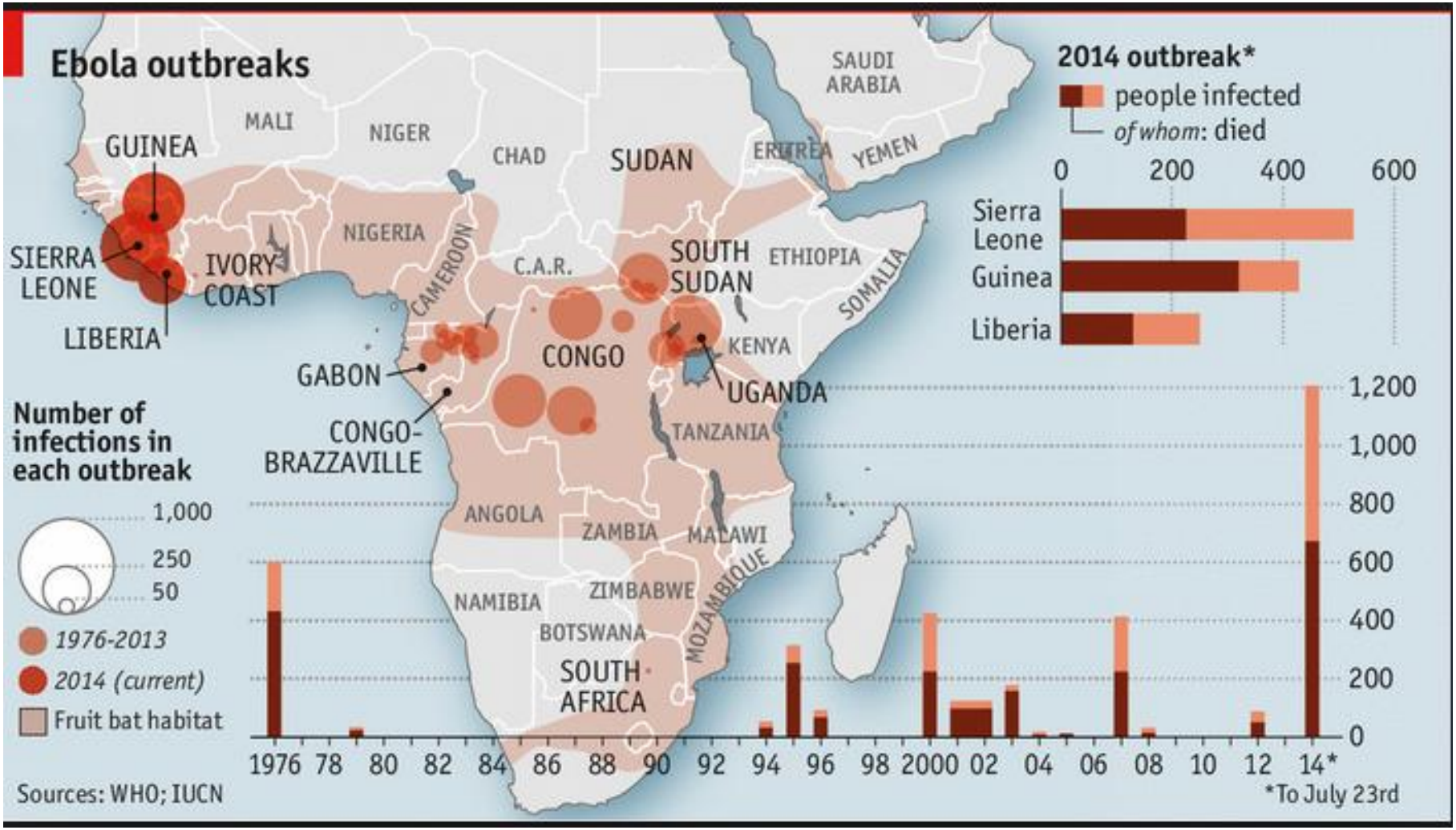
- Detection of IgG and IgM in serum by ELISA
- Immunohistochemistry to detect viral antigens in postmortem tissue
- Detection of viral genome by RT-PCR

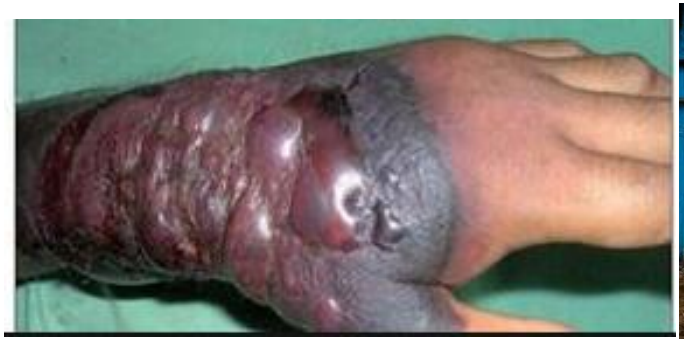
Transmission of Lassa virus

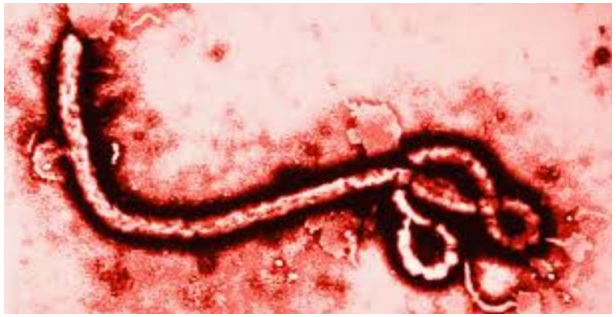
- Rodent reservoir (house mice)
- Virus can be transmitted by human-human contact
- Control: rodent control measures
- **Treatment:** Ribavirin is the drug of choice.

African hemorrhagic fever

- Filoviruses
  - Pleomorphic filamentous virions
  - Ebola viruses and Marbug viruses
  - Negative sense ss RNA genome
  - Virions are released from budding
  - Highly virulent
    - Require Biosafety level 4 containment facilities
    - Natural reservoir hosts are probably bats or rodents (viruses are highly pathogenic in non-human primates to allow for maintenance)


Ebola outbreaks
MALI
NIGER
CHAD
SUDAN
SAUDI ARABIA
ERITREA
YEMEN
GUINEA
SIERRA LEONE
IVORY COAST
LIBERIA
NIGERIA
CAMEROON
C.A.R.
SOUTH SUDAN
ETHIOPIA
SOMALIA
KENYA
CONGO
GABON
CONGO-BRAZZAVILLE
UGANDA
TANZANIA
ANGOLA
ZAMBIA
MALAWI
MOZAMBIQUE
NAMIBIA
ZIMBABWE
BOTSWANA
SOUTH AFRICA
Number of infections in each outbreak
1,000
250
50
1976-2013
2014 (current)
Fruit bat habitat
Sources: WHO; IUCN
2014 outbreak*
people infected
of whom: died
0
200
400
600
Sierra Leone
Guinea
Liberia
1,200
1,000
800
600
400
200
0
1976 78 80 82 84 86 88 90 92 94 96 98 2000 02 04 06 08 10 12 14*
*To July 23rd





Ebola and Marburg viruses

- Highly virulent in humans and non-human primates, infections mainly leading to death (25-90%)
- Incubation period is 3-9 days for Marburg and 2-21 days for Ebola
- Symptoms:
  - Fever, headache, sore throat and muscle pain
  - Abdominal pain, vomiting diarrhea, and rash
  - Internal and external bleeding leading to shock and death

Ebola and Marburg viruses

- Viruses highly tropic for myeloid cells, intestinal fibroblasts and endothelial cells
- High viral titers in the liver, spleen, kidneys and lungs
- Filoviral infections are usually immunosuppressive (impaired humoral immunity)

Diagnosis of Ebola and Marburg viruses

- Viral antigens can be detected in sera by ELISA
  - Sera contains viable virus and so containment is key to prevent transmission.
- RT-PCR can be used to detect viral RNA
- Viral isolation in Vero or monkey Kidney cell lines

Human infection of Ebola

- Introduced by humans infected from reservoir host; bats or rodents
- Followed by person-person transmission via body fluids and excreta
- No effective **treatment** of **vaccines** available!
- Prevention: Biocontainment, avoiding contact with body fluids and excreta.

Zika Virus

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Zika Virus: Why Should We Care? What Do We Do About It?

Commentary

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