Outbreak

SPECIAL ALERT:
The Truth About the ZIKA VIRUS
MICROCEPHALY

Baby with Microcephaly

Typical head size

Baby with Typical Head Size
How the Zika Virus Spread to the Americas

While studying yellow fever in Uganda in 1947, Alexander Maddow and George Dick identified Zika virus after a fever developed in a rhesus monkey.

Isolated cases were observed between 1977 and 1978 in Pakistan and Malaysia.

The first serious outbreak of Zika virus outside of Africa occurred in April 2007 on Yap, one of the Micronesian islands.

In 1978, a handful of Zika cases were identified in Indonesia, but the disease seemed to disappear without any public health intervention.

The introduction of nonnative mosquitoes, the El Nino climate event, and political and economic crises in Brazil created the conditions for the spread of Zika in the Americas in 2015-2016.

Researchers have suggested the Zika virus arrived in Brazil in 2014 from French Polynesia during major global sporting events.

In May 2015, Zika virus was confirmed as the cause of an outbreak of a dengue-like disease in northern and eastern Brazil.

Expansion in the Americas 2015

Expansion into other African countries 1948

Uganda 1947

Pakistan 1977

Yap 2007

French Polynesia 2014

Indonesia 1978

Sources: CDC, New York Times
Credit: David Foster, Lucie Guerrat, Doug Halsey, Gabriella Melizza
Zika virus transmission cycle

- First infected Aedes mosquito
- In utero transmission
- Liver infection
- Infected red blood cells
- Second infected Aedes mosquito

Zika can be transmitted through blood, but this is an infrequent mechanism. The virus has also been isolated in semen, but person-to-person sexual transmission is unconfirmed.

Source: PAHO/WHO
Zika virus: lack of data in animals models (few studies)

1947
Zika Forest

<table>
<thead>
<tr>
<th>Time</th>
<th>Blood Sample</th>
<th>Normal</th>
<th>Inject blood into brain</th>
<th>Virus passed</th>
<th>ZIKV MR 766</th>
<th>Virus found in Brain/spinal Cord only</th>
</tr>
</thead>
<tbody>
<tr>
<td>T = 40°C</td>
<td>D3 of fever</td>
<td>Normal</td>
<td></td>
<td>From mouse via injection of brain homogenate into brain</td>
<td></td>
<td>Caused paralysis Neuronal degeneration</td>
</tr>
</tbody>
</table>

All mice get sick at day 10

No disease in cotton rats, guinea pigs, rabbits

1976

Infected newborn mice with ZIKV
Infected 4 week-old mice with ZIKV

Mice died: no description

Zika virus microcephaly

Microcephaly – recent case reports confirm Zika as cause

Paper #1 – Two newborn with microcephaly died soon after birth
   Two miscarriages
   
   Results: Brains positive for Zika virus RNA (only brains): (4/4)
             Genetic sequence of Zika virus obtained
             Viral protein seen in brain tissue (2/4)
             Tissue damage only in the brain

Paper #2 – Pregnancy terminated due to severe brain disease of fetus

   Results: Viral protein seen in the brain
             Viral RNA detected and sequenced (complete Zika genome)
             Infection and injury in neurons
             Tissue damage in brain and spinal cord

Zika virus: Unanswered pathogenesis question #1

How does in utero transmission occur and why is Zika virus different than other flaviviruses?

More like hepatitis C virus (5-10%: pre-antivirals)

Risk factors: Viral load in blood

Possible infection of placental cells or trophoblasts

Le Campion Viruses 2012
Zika virus: Unanswered pathogenesis question #2

Does pre-existing **Dengue immunity** affect Zika virus disease severity?

- Anti-Dengue antibodies cross-react with Zika virus
- Anti-Dengue antibodies bind but do not neutralize Zika virus
- Antibody-antigen complex binds to FcγR
- FcγR
- Monocyte
- Zika virus now replicates more efficiently in FcγR expressing myeloid cells

*Adapted from Murphy 2011 Ann Rev Imm*
Zika virus: Unanswered pathogenesis question #3

What is the basis for **Guillain-Barre syndrome** after Zika virus infection?

**Possible mechanisms**

- Immunopathology due to viral antigen mimicry of a host protein
- Virus targets neurons/glial cells (tropism change compared to African strains?)
- Association with prior or concurrent immune response to Dengue

GBS caused by destruction of myelin around nerves: demyelination

- Paralysis
- Tingling in arms and legs
- Muscle weakness

Damaged myelin

Michael S. Diamond. 2015
Zika virus: Pathogenesis summary

Zika Virus Infection

Aedes Mosquito

Viremia

Fever
Rash
Myalgia
Conjunctivitis

Protective immunity

Keratinocytes

DC

ZIKV

Unanswered Questions

Transplacental transmission?

Sexual transmission?

More severe ZIKV disease?

ADE by ZIKV antibodies?

Interference with DENV vaccine responses?

Guillain-Barré syndrome?

More severe DENV disease?

ADE by DENV antibodies?

Persistence

Teratogenic effects?

Michael S. Diamond. 2015
At the completion of this unit the student will be able to:

1. Explain viral pathogenesis
2. Explain host defense mechanisms to viral infection
3. Explain cellular pathology of viral infection
4. Explain pathology of viral infection
5. Analyze diagnostic techniques for viral infection
6. Illustrate pathogenesis, pathology and diagnostic technique of dengue infection
Outlines

1. General properties and multiplication of virus
2. Viral pathogenesis
3. Host defense mechanisms to viral infection
4. Cellular pathology of viral infection
5. Pathophysiology of viral infection
6. Diagnostic techniques for viral infection
Virus

- An infectious, obligate intracellular parasite
- Package genome in a particle for transmission from host to host
- Viral genome contains information to initiate and complete an infectious cycle within a cell
- All viral genomes are able to establish themselves in a host population so that viral survival is ensured
Viruses are much too small to be seen with any light microscope. The largest smallpox virus is more than 5 times smaller than *E. coli* and 50 times smaller than a human red blood cell.
Viral structure

- Genome; DNA or RNA
- Capsid; protein shell
- Envelope; lipid membrane (presence or absence)

HIV-1

Viral classification:

Baltimore classification system

- **Group VII: dsDNA-RT** (e.g. Hepadnaviruses)
  - **Parovirus**: + DNA → - DNA → ± DNA → + mRNA → - RNA
  - **Hepatitis B virus**
  - **Adenovirus**
  - **Herpes simplex virus**
  - **Reovirus**
  - **Rotavirus**

- **Retrovirus**
  - + RNA → - DNA → ± DNA
  - **Ebola virus**

- **Poliovirus**
  - + RNA → - RNA → ± RNA
  - **Influenza virus**

Infectious cycle

1. Attachment & Entry into target cell
2. RNA synthesis
3. Replication of DNA viral genomes
4. Transcription & RNA processing
5. Reverse transcription & integration
6. Translation
7. Assembly

Viral pathogenesis
Viral pathogenesis

- Pathogenesis is the process of producing a disease
- 2 components of viral diseases:
  - Effects of viral replication on the host
  - Effects of host response on virus
- Virus infections span the range from benign to lethal
  - Acute and persistent infections can be quick or amazing slow – days to year of infection
Mechanisms of viruses cause cell injury

Mechanisms of virus:
1. Attachment & entry into target cell
2. Genome replication and protein synthesis
3. Assembly

Host defense and virus evasion
Natural barrier:

- Integrity of skin and mucosa
- Cilliary epithelium
- pH & Temperature
- Bile
- Enzymes
- Normal flora
- Immune cells
Immune response to viral infection

**Innate immunity**
- Virus
- Antiviral state
- NK cell
- Infected cell
- Killing of infected cell

**Adaptive immunity**
- Type I IFN
- B cell
- Antibody
- Neutralization
- CD8+ CTL
- Infected cell
- Killing of infected cell

Protection against infection

Eradication of established infection
IMMUNE EVASION BY VIRUS

Modulation of surface structure to avoid recognition (e.g., antigenic variability)

- Inhibition of phagocytosis
- Escape from phagosome
- Inhibition of phagosome-lysosome fusion

Hide from immune surveillance; viral latency

- Modulate:
  - Signal transduction
  - Gene expression
  - Cell death

Viral cytokines or soluble receptor homologs

Inhibition of antigen presentation
Acute infection

Wild-type HIV

Infectected CD4^+ T cell

New virion production

Nef-mediated MHC class I downregulation

Gag-specific CTL

Gag peptide

TCR

HLA-B57/HLA-B27

Lysis

Early viral escape mutation in Gag
- Cost to viral fitness, depending on number of mutations
- Control of virus by other CTLs

Poor replicative capacity of virus escape variants

CD4^+

Escape variant 1

Wild-type-virus-specific CTL no longer effective

Escape variant 2

Escape variant 3

Lysis

Goulder PJR and Watkins DI. Nat Rev Imm 2008.
Cytopathic effect (CPE) on viral infection

- refers to damage to host cells during virus invasion

- Cytocidal infection
- Steady-state persistent infection
- Cellular transformation
A. Cytocidal Infection

- Cell death and histological appearance of characteristic CPE e.g. picornaviruses, herpesviruses
- Infected cells usually found cell swelling, shape changes, large nucleus, multinucleated cells, halo, inclusion bodies
Formation of syncytia

Measles virus

Cytomegalovirus infection (CMV)

- **Intranuclear basophilic inclusions**
  - spanning half the nuclear diameter are usually set off from the nuclear membrane by a clear **halo**.
Inclusion bodies (CPE)

- Intracellular structures of viral proteins or Virions

- May result from the clustering of subunits or virions within the nucleus or cytoplasm (e.g., the Negri bodies in rabies infections)

- May contain cell components such as ribosomes (arenavirus infections) or chromatin (herpesviruses)

- Regardless of their composition, these inclusion bodies can directly disrupt cell structure.
B. Steady-State Persistent Infection

- **Infected cells:**
  - Produce and release virus but no CPE, may be found syncytia
  - Can grow and divide but not killed
  - Little destruction of infected cells

- Does not occur with DNA viruses

- Occur with several RNA viruses (Lassa virus, Retroviruses, Rubella, some paramyxoviruses)

- Virus released by cell budding
C. Cellular Transformation

- Viruses produce tumors in animals can transform cell culture
- Virus DNA integrated into host DNA, alter growth and morphology
- Chromosomal abnormalities
- New virus Ag and DNA production
- e.g. Adenovirus, HPV, HSV, EBV, HBV, Sarcoma virus, HTLV1,2

Patient with EBV Infection

Local infection

Systemic infection

SSPE: Subacute sclerosing panencephalitis

EXAMPLE OF VIRAL INFECTION THAT CAUSE OF SYSTEMIC PATHOLOGY
Symptoms of dengue hemorrhagic fever

- Fever
- Rash
- Muscle and joint pain
- Frequent vomiting
- Abdominal pain
- Bleeding under the skin

“HEMORRHAGIC MANIFESTATIONS”
Skin hemorrhages:
- petechiae (Skin rash),
- purpura (Lesions),
- ecchymoses
  (subcutaneous spot of bleeding)
Hemorrhagic Manifestations

- Skin hemorrhages:
  - petechiae (Skin rash), purpura (Lesions), ecchymoses (subcutaneous spot of bleeding)
- Epistaxis (Bleeding from the nose, mouth, or gums)
- Nausea and/or Hematemesis (vomiting of blood)
- Gastro-intestinal bleeding:
  - hematemesis, melena, hematochezia
- Hematuria (Blood in Urine)
The pathology of dengue hemorrhagic fever

Figure 1  Organs from a 2-year-old Thai boy who died from dengue hemorrhagic fever. (A) The eviscerated intestines were markedly edematous and focally hemorrhagic, and there was a hemoperitoneum of 300 mL. (B) The kidneys were edematous with focal hemorrhage and hemorrhage into the calyces and renal pelvis. (C) The liver was swollen with focal hemorrhage. (D) Both lungs were heavy and beefy in consistency and hemorrhagic.
The pathology of dengue hemorrhagic fever

Liver biopsy from a 13-year-old Chinese boy from Singapore who presented with dengue hemorrhagic fever and fulminant hepatitis.
The pathology of dengue hemorrhagic fever

Avidin–biotin peroxidase staining with antidengue polyclonal abs for 3 serotypes

A. Liver-Kupffer cells
B. Spleen-white pulp
C. Spleen-MΦ in red pulp
D. Peripheral blood-monocytes
DENGUE VIRUS INFECTION

- Can I get dengue fever after mosquito bites?
- How dengue fever become dangerous (DHF)?
- What is screening test for dengue infection?
DENGUE VIRUS INFECTION

Asymptomatic

Symptomatic

Undifferentiated fever (viral syndrome)

Dengue fever (syndrome)

Dengue Fever

Dengue Haemorrhagic fever

(Plasma leakage)

No Shock

Dengue Shock Syndrome (DSS)

Dengue Haemorrhagic fever
Pathogenesis of Dengue infection

Tourniquet Test

Positive (>20 spots)  Negative (≤20 spots)
Diagnostic technique for dengue infection

- Virus isolation
  - Specimen: serum or plasma at 2-3 day post-hospitalization
  - Culture: mosquito, mosquito larve inoculation, 1-2 day-old mice or cell lines

- Serology: Four-fold rising titer
  - Specimen: serum at first collection and 2 wk after 1°collection
  - Test: Hemagglutination inhibition test, Complement fixation test, ELISA

- Viral nucleic acid: RT-PCR
How to prevent the spread of dengue fever
NOW, I know about pathogenesis, pathology and diagnostic technique of dengue infection!

WHAT ABOUT other viral infection?
Pathology of Viral infection

- **Acute (transient) infection**
  Measles, Mumps, Poliovirus, West Nile virus

- **Chronic (latent) infection (Herpesvirus infection)**
  HSV, VZV, CMV

- **Chronic productive infection**
  HBV

- **Transforming infection**
  EBV
Case 1

A 32-year-old man presents with **high fever, cough, and skin rash of 3 days in duration**. His rash began in the form of **pink papules** behind the ears and spread around his body.

Physical examination shows an extensive maculopapular rash over his face, neck, trunk, and limbs. He displays small white spots on buccal surfaces. This patient’s skin rash is most likely caused by infection with which of the following agents?

(A) *Candida albicans*
(B) Epstein-Barr virus
(C) Measles virus
(D) Mumps virus

**NOTE:**
A maculopapular rash is a type of rash characterized by a flat, red area on the skin that is covered with small confluent bumps.
Case 1

Clinical manifestation:
Face/trunk - blotchy, reddish brown rash
The oral cavity near the opening of Stensen ducts – Koplik spots
Lymphoid organs - follicular hyperplasia, large germinal centers, and randomly distributed Warthin-Finkeldey cells

Koplik spots:
• Necrosis,
• Neutrophilic exudate, and
• Neovascularization.
Case 1

Warthin-Finkeldey cells (grape-like cluster of nuclei)

- Multinucleate giant cells, eosinophilic nuclear and cytoplasmic inclusion bodies.
- Found in hyperplastic lymph nodes early in measles and HIV-infection, Kimura disease, and more rarely in lymphoma and non-neoplastic lymph node disorders
Case 1 - Measles viral infection
Measles Pathogenesis
- Respiratory transmission of virus
- Replication in nasopharynx and regional lymph nodes
- Primary viremia 2-3 days after exposure
- Secondary viremia 5-7 days after exposure with spread to tissues

Measles Clinical Features
- Incubation period 10-12 days
- Prodrome 2-4 days
  - stepwise increase in fever to 103°F–105°F
  - cough, coryza, conjunctivitis
  - Koplik spots (rash on mucous membranes)
- Rash
  - 2-4 days after prodrome, 14 days after exposure
  - persists 5-6 days
  - begins on face and upper neck
  - maculopapular, becomes confluent
  - fades in order of appearance

Measles Epidemiology
- Reservoir
  - human
- Transmission
  - respiratory Airborne
- Temporal pattern
  - peak in late winter-spring
- Communicability
  - 4 days before to 4 days after rash onset
Varicella zoster virus infection

(a) Initial infection: chickenpox (varicella)
- Latent viral DNA
- Nerve cell in dorsal root ganglion
- Viruses move up peripheral nerve
- Spinal cord

(b) Recurrence of infection: shingles (herpes zoster)
- Activation of viral DNA in nerve cell
- Viruses
- Viruses move down peripheral nerve
- Spinal cord

Images of chickenpox and shingles lesions.
Case 2 Shingles/Zoster (varicella zoster virus infection)

virus infects keratinocytes and causes vesicular lesions, which, unlike chickenpox, are often associated with intense itching, burning, or sharp pain because of the simultaneous radiculoneuritis.
Case 3
Chickenpox

Vesicles

By WashingtonDeceit

www.healthline.com
Case 4

Vesicles

By WashingtonDeceit
Case 4

By WashingtonDeceit
Case 4

INTRANUCLEAR EOSINOPHILIC INCLUSION BODIES
(Cowdry bodies)

Multinucleated cells

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Herpes Simplex Virus (HSV)

Pathogen identification:
Herpes infected cells have homogeneous cytoplasm resembling ground-glass with homogeneous nucleic inclusion bodies (Cowdry bodies). Immunohistochemical findings include antibodies against HSV-1 and HSV-2.

Vesicles

Intranuclear inclusions

Multinucleated cells
Herpes simplex viruses 1 and 2 (HSV-1 and HSV-2) initially infect epithelial cells of the oral or genital mucosa, the skin or the cornea. The virus may enter neurones and may be transported to their nuclei, where they may establish latent infections.

These factors reactivate replication of the virus, resulting in transport of the virus to the surface of the body and causing recurrence of the rash.

These include:
— Ultraviolet radiation;
— Febrile infectious factors;
— Menstruation;
— Emotional irritation;
— Immunosuppression.
Herpes Simplex Virus (HSV)

Morphology: Occasionally infected cells will fuse into multinucleated giant cells. The cytopathic effect of the virus disrupts the contact between the cells, causing formation of epidermal vesicles containing infected cells with replicating viruses.

INTRANUCLEAR EOSINOPHILIC INCLUSION BODIES (Cowdry bodies)
Diagnostic techniques on viral infection based on Pathology
Diagnostic techniques

- DIRECT PATHOGEN IMAGING, gross/microscope
- Hematoxylin and eosin stain (H&E)
- “SPECIAL” (NOT H&E) STAINS, e.g., PAP smear
- Cell / Tissue culture, CPE (CytoPathological Effect)
- ANTIGENS / ANTIBODIES (SEROLOGY)
- PCR / RT-PCR, e.g., viral load
Macroscopic analysis

- Size/Color
- Necrosis
- Abscess
- Hemorrhage
- Rash
Microscopic analysis

- Normal/Abnormal tissue and cell lining
- CPE – inclusion bodies/multinucleated giant cells
- Inflammation (Acute-PMNs vs. Chronic-Lymphocytes / plasma cells)
- HEMORRHAGE
Questions?

FOXTROT By Bill Amend

WHATCHA LOOKING AT?
VIRAL VIDEOS.

LIKE THE "STAR WARS KID" AND THE "NUMA NUMA" ONE?

ELECTRON MICROSCOPE ANALYSIS OF A HUMAN PICORNAVIRUS.

WE MUST GET DIFFERENT E-MAILS.
SEE HOW THE CAPSID IS ICOSAHEDRAL IN SHAPE?
Text books

Robbins and Cotran
Pathologic Basis of Disease
EIGHTH EDITION
KUMAR
ABBAS
FAUSTO
ASTER

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