Pathology of viral disease

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Topics for the first lecture.....

General virology
Viral lifecycle
Viral pathogenesis
Laboratory diagnosis
Virus size

Viral Structure
Herpes virus

- Envelope
- Tegument
- Spikes
- Nucleocapsid
- Genome
Viral Structure

Viral Structure

Classification schemes for animal RNA viruses

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<th>Nucleic acid</th>
<th>Symmetry of capsid</th>
<th>Genome architecture</th>
<th>Baltimore class</th>
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Classification criteria:
- Nucleic acid
- Symmetry of capsid
- Genome architecture
- Baltimore class

Family name:
- Reo
- Born
- Calici
- Picorna
- Flavi
- Toga
- Retro
- Corone
- Filo
- Rhahela
- Bany
- Ortho
- Pyrro
- Are

Properties:
- Virion polymerase
- Virion diameter (nm)
- Genome size (total in kb)
Some useful terms

- Plaque
- pfu
- MOI
- Particle to infectivity ratio
- Neutralizing Abs
- Cytopathic effect

Viral life cycle
Pathology of viral disease
Ila R. Singh, M.D., Ph.D.

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Entry into cells

- Binding
- Internalization
- Low-pH induced conformational change
- Uncoating
- Fusion
- Nuclear transport

Viral assembly and release

- Replication of genome
- Nuclear transport
- Protein synthesis
- Assembly
- Budding
- ER
Methods of diagnosis for viral diseases

- Serology
- Cytology or Histology
- Viral growth in cell culture
- Detection of viral genome

I. Serology

- Look for viral antigens or anti-viral antibodies

- A four fold or greater rise in titer between two serum specimens provides a positive diagnosis. Paired sera, the first taken as early as possible in the illness and the second 10 to 14 days after the onset of symptoms.
Serology Methods

◆ ELISA
  ◆ Rapid tests for Flu, RSV
  ◆ Hep B, Hep C etc etc
◆ Western Blots

Serology: ELISA
EIA for RSV

- 93-97% sensitivity and 90-97% specificity when compared to tissue culture
- results in about 6 minutes
- room temperature storage of kit

ELISA

– HIV antigens - from virus or recombinant proteins or synthetic peptides are immobilized on microtitre plates
– Incubate test serum. Wash
– Enzyme-labeled antibody specific for hu-IgG. Wash.
– Substrate changes color
Serology: Western blot

Western blot
II. Histology and cytology

- Inclusion bodies
- Syncitia
- Tzanck test for VZV and HSV
- Negri bodies in rabies
Inclusion bodies

CMV: owl eyed nucleus

Inclusion bodies

Adenovirus: cytomegaly, multinucleate cells, inclusions
Inclusion bodies

Adenovirus: cytomegaly, multinucleate cells, inclusions

Tzanck smear

HSV, VZV: multinucleated giant cells
Negri bodies

Electron microscopy

Rota

Corona
III. Grow virus in culture

- Look for cytopathic effects (CPE) in culture
- Detect viral antigens by Shell vial culture

Cell culture in virology
Cytopathic effect

- Identify virus by type of cell it grows in, time to detection of CPE and morphology of CPE
- Rounding, syncitia, vacuoles etc
- Confirm with fluorescent-labeled antibodies
- Results in days to weeks
Cytopathic effect

Cell and Tissue-types for culture
Screening cells

- Rhesus Monkey Kidney (1°)
  - Myxo-, Paramyxoviruses etc
- Human Embryonic Kidney (1°)
  - Very sensitive for adenovirus and important for lung transplants
- MRC-5 (human embryonic lungs)
  - CMV, VZV, HSV
Cell-types for culture

- African Green Monkey Kidney
  - Rubella grows only on these
- Hep-2
  - RSV
- Vero
  - HSV
- Primary rabbit kidney
  - HSV, enteroviruses

Cell culture plus IF

- Grow virus in culture
  - Detect viral antigens by Shell vial culture
    - Inoculate specimen into many vials (one for each virus to be tested)
    - Stain with specific antibody
    - Results in 1-2 days
Monoclonal antibodies
(commercially available and FDA approved)

- HSV 1 and 2
- VZV
- CMV
- Flu A and B
- Parainfluenza 1, 2 & 3
- RSV
- Adeno
- Mumps
- Measles
- Some enteroviruses
- Chlamydia

Detect and analyze viral genomes

- PCR
- RT-PCR
- Quantitative PCR to detect viral load
- Branched DNA
- Hybridization, using microarrays
- Genotyping
- Phenotyping?
Polymerase chain reaction

Detect and analyze viral genomes

- PCR
- RT-PCR
- Quantitative PCR to detect viral load
- Branched DNA
- Southern blots
- Hybridization, using microarrays
- Genotyping
- Phenotyping?
Southern blot

Nucleic acid sample
Separated nucleic acids

Blotting tank
Nucleic acids transferred to nitrocellulose

Develop and fix autoradiograph
Nucleic acid bands visualized

 Autoradiography

MOI = 0.1

HSV-1

1 h 16 h

mock

DNA loaded 1x 1x 0.1x
Hybridization with microarrays

Sensitivity of NAT

- Combination of PCR/Southern blot: 95% confidence intervals
  - HAV, 5-9 copies/ml
  - HBV, 1-2 copies/ml
  - HCV, 3-5 copies/ml

Reduce risk of HCV transmission
From 1:100,000 to 1:500,000-1:1,000,000

Data from National Genetics Institute, Labcorp
Pool testing

8 X 8 X 8
= 512 samples

Other labs

- State Department of Health lab
- Centers for Disease Control
- Other commercial labs
NYDOH lab for viral encephalitis

- Herpes Simplex
- Varicella Zoster
- Cytomegalovirus
- Epstein-Barr Virus
- Enteroviruses
- St. Louis Encephalitis (SLE)
- Eastern Equine Encephalitis (EEE)
- California Encephalitis
- Powassan (POW)
- Rabies
- West Nile Virus

- Tests include: 1) PCR, and 2) ELISA.
- Freeze leftover CSF at -70°C in the event that PCR testing becomes necessary.

CDC

- Small pox, Hantavirus, Ebola etc
- Usually via the State labs
What specimen to collect?
When?

Viremia
HIV infection

What specimen to collect?

When?

- **Throat**
  - first presentation with fever (measles, mumps, rubella, also viral meningitis caused by enteroviruses and neonatal HSV). Vigorous swab, because you need cells.

- **Nasopharyngeal swab or wash**
  - Flu, RSV, Rhino-, CMV (if lots of virus)

- **Rectal**
  - entero- and adenoviruses (meningitis), rotavirus

- **Urine**
  - Adenovirus (hemorrhagic cystitis)
  - MMR, after cleared from throat or sometimes concomitant
  - CMV and HSV (rare)
What specimen to collect? When?

- **CSF**
  - PCR for HSV, VZV, CMV, adeno or flu
  - Rarely can grow coxsackie or echo

- **Lesion**
  - VZV, CMV, measles (scrape for cells)
  - HSV, Tzanck smear

- **Conjunctival**

What specimen to collect? When?

- **Genital**
  - HSV, vulvar swab (not endocervical) in last month of pregnancy

- **Buffy coats**
  - CMV (fresh specimen, <1hr)

- **Bronchial and BAL wash**
  - RSV, Flu, Adeno-, CMV etc

- **Other**
  - Biopsy, autopsy specimens
Transport to lab

- Since we still depend on viral growth for diagnosis, rapid transport to lab is essential
- Specimen on ice
- Refrigerate if delay inevitable, DO NOT FREEZE
- If need to store for more than 6 days, freeze at -70°C
- Transport and store in viral transport medium
- Enteroviruses more stable and will tolerate some delay
- Hand delivery encouraged (also for better communication: viruses suspected, source of material)

Web resources

- www.cdc.gov, get a free electronic MMWR subscription
- www.wadsworth.org
- HIV database: hiv-web.lanl.gov
- All the Virology on the WWW: www.virology.net/garryfavwebindex.html
- Pan-American Society for Clinical Virology: www.virology.org/
- www.specialty.com