Occupational Health Considerations for Working with Viral Vectors in the Research Laboratory

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Outline

- Introduction
- Viruses and Vectors
- Specific viruses:
  - Retroviridae
  - Adenoviridae
  - Poxviridae
  - Parvoviridae (AAV)
  - Herpesviridae

- Occupational Health:
  - Clinical presentation
  - Pathogenesis
  - Diagnostic Tests
  - Transmission
  - Epidemiology
  - Treatments
  - Prevention

- Conclusion
Occupational Health – Ideal World

- Laboratory workers visit the occupational physician prior to beginning work.
- They complete a confidential medical questionnaire to identify any relevant medical conditions.
- Workers are sent back to employer with a list of restrictions, if any, that does not identify their medical condition.
- Workers are offered pre-exposure immune surveillance and vaccinations as appropriate.
- Emergency plans are in place and workers are trained on how to handle an exposure event, including PEP.
Laboratory workers never see an occupational physician or are denied access to one.

Workers complete a detailed medical history and fax it to their EHS office.

Appropriate medical restrictions not identified.

Workers not offered pre-exposure immune surveillance or vaccinations.

No emergency plans, miss opportunity for post-exposure prophylaxis, wait in local emergency room for 4 hours.
Post-Exposure Prophylaxis

- **Definition**: Treatment to prevent disease after an exposure has already occurred.

- Has historically worked well with viruses that have a long incubation period, e.g. *Rabies virus, Hepatitis B virus.*
Virus

- Obligate, intracellular parasites. They are unable to grow and divide on their own. Cannot generate energy.

- Have a variety of methods to commandeer the functions of the host cells to direct their own replication scheme, often at the expense of the host cell.

- Researchers attempt to harness the power of these organisms to deliver nucleic acids to turn them into gene delivery tools.
Replication Defective Viruses

- To avoid potential pathogenicity of viral vectors, it is desirable to disable them so they cannot replicate in target cells.

- This is generally accomplished by deleting genes that provide necessary *trans*-acting functions from the vector genome.

- Introduction of these genes and defective vector into cells results in the synthesis of vector genomes and packaging of the defective genomes into virus particles.
Vectors Used in Gene Therapy Trials

- Adenovirus ($n = 400$)
- Retrovirus ($n = 344$)
- Naked/plasmid DNA ($n = 304$)
- Vaccinia virus ($n = 133$)
- Lipofection ($n = 109$)
- Poxvirus ($n = 93$)
- Adeno-associated virus ($n = 75$)
- Herpes simplex virus ($n = 56$)
- Lentivirus ($n = 29$)
- Other categories ($n = 82$)
- Unknown ($n = 55$)

Retroviridae (7 genera)

- Alpharetrovirus
  - ALV, RSV

- Betaretrovirus
  - MMTV

- Gammaretrovirus
  - MLV
  - FeLV, SNV, XMRV*

- Deltaretrovirus
  - HTLV I and II, BLV

- Epsilonretrovirus
  - WDSV

- Lentivirus
  - HIV
  - SIV

- Spumavirus
  - HFV, SFV

*New
Lentivirus is a genus, not a species

- Family: *Retroviridae*
  - Genus: *Lentivirus* (9 species)
    - *Human immunodeficiency virus* 1 (human)
    - *Human immunodeficiency virus* 2 (human)
    - *Simian immunodeficiency virus* (monkey)
    - *Bovine immunodeficiency virus* (cow)
    - *Equine infectious anemia virus* (horse)
    - *Caprine arthritis encephalitis virus* (goat)
    - *Visna/maedi virus* (sheep)
    - *Puma lentivirus* (puma)
    - *Feline immunodeficiency virus* (cat)
Xenotropic Murine Leukemia Virus-Related Virus (XMRV)

- Newly discovered in 2006

- Conflicting Reports:
  - Associated with Prostate Cancer and Chronic Fatigue Syndrome

- Concerns for transplants and blood donation

Retroviral Host Range

- **Ecotropic**: viruses that are *only able* to infect the species originally isolated from.

- **Xenotropic**: viruses that are *unable* to infect the species originally isolated from.

- **Amphotropic**: viruses that are able to infect multiple species of cells, *including human*. 
Tropism

- Viral preference for a particular cell type

- Often dictated by the viral envelope or capsid proteins and by receptors on the host cell
HIV-1 Virion
Retroviral Genomes

MLV

LTR

MLV

LTR

HIV

LTR

HIV
Basic Genes

Gag: Nucleocapsid-core (MA, CA, NC)
Pol: RT, protease, integrase
Env: Surface glycoprotein (SU and TM)
Additional Lentivirus Genes

- **Regulatory Genes**
  - tat
  - rev

- **Accessory Genes**
  - vif
  - vpr
  - vpu
  - nef
Clinical Presentation of HIV-1

- Asymptomatic
- Acute infection
- Persistent generalized lymphadenopathy
- Other disease
Pathogenesis of HIV

- AIDS
  - Immunodeficiency
  - Opportunistic infections and cancers
  - Neurological disease (AIDS dementia complex)
Transmission of HIV

- **Wild-type**
  - Restricted tropism
  - Blood products
  - Sexual transmission
  - Vertical transmission

- **HIV vector virus in the laboratory**
  - Broadened tropism
  - Concentrated virus
  - Parenteral
  - Droplet contact
HIV Testing

- **Antibody Testing**
  - Blood, Oral Fluid, Urine
  - ELISA/EIA
  - Western blot

- **Antigen Testing**
  - p24 antigen test, 1996-2003 by blood banks

- **Nucleic Acid Amplification Testing (NAT)**
  - Direct detection, minipool, 2002 by blood banks
  - Viral load assay for patient (prognostic)
Rapid or Point-of-Care Tests

- Cost < $10
- 20-30 minutes
- Several on the market
- Plasma, serum, whole blood, oral fluid
- Require confirmatory test, if positive
Home Tests

- Home Access Express HIV-1 Test
  - 1996, FDA approved
  - Mail-in blood test
Seroconversion—Exposure to a sufficient dose of HIV vector virus would likely score positive on a screening test.

- **Antibody Testing:**
  - Blood, Oral Fluid, Urine
  - ELISA/EIA (Screening)
  - Western blot (Confirmatory)

Modern diagnostic kits generally use recombinant proteins or peptides, including those based on HIV-1 gp160 or gp41, HIV-2 gp36, and HIV-1 p24.
Western Blot

Exposure to a sufficient dose of HIV vector virus could score indeterminate (suggestive)

HIV Testing Issues

- Social Stigma
- Fear
- Medical Privacy
  - For both HIV-negative as well as HIV-positive cases
- State reporting and partner notification
- "Free" HIV testing sites and record keeping
Pre- and Post-Exposure HIV Testing: Points to Consider

- Wild-type HIV vs. vector
- Protecting the institution
- Protecting the worker
- When to establish the baseline?
HIV Post-exposure Prophylaxis (PEP)

- CDC/WHO recommendation:
  - ASAP, but no later than 72 hrs.

- Treatment regime:
  - Consideration of drug-resistant strains

REFERENCES:

CDC. Updated Public Health Service guidelines for the management of health-care worker exposures to HIV and recommendations for postexposure prophylaxis. MMWR 2005;54(No. RR09):1-17.

Research Laboratory PEP for HIV for Highly Concentrated Virus Preps

- Titers now up to $1.4 \times 10^{10}$ TU/ml\(^1\)
- Wild-type or potentially replication-competent preps, goal is to prevent active infection.
- Late generation systems, goal is to prevent integrations, especially with dangerous transgenes, such as oncogenes.
- Need emergency plan that includes on-site, rapid access to anti-HIV medications.

Antiviral Therapy (Drugs)

■ Reverse Transcriptase Inhibitors
  - Nucleoside: AZT, ddI, and 3TC
  - Nonnucleoside: Nevirapine, Foscarnet (Phosphonoformic acid)

■ Protease Inhibitors
  - Ritonavir
  - Indinavir
Antiviral Therapy (New Drugs)

- **Entry Inhibitors**
  - Fuzeon (2003) – Block gp41; injection only
  - Selzentry (2007) – Blocks CCR5

- **Integrase Inhibitors**
  - Isentress (2007)
Traditional Combination Drug Therapy

AIDS "Cocktails" or "HAART"
(Highly Active Anti-Retroviral Therapy)

2 Reverse Transcriptase Inhibitors

plus

1 Protease Inhibitor
Adults and children estimated to be living with HIV | 2011

Total: 34.0 million [31.4 million – 35.9 million]
Prevention

- No Vaccines
- Screening blood supplies
- Universal precautions for workers
- Education about safe sex and drugs
MLV and HIV vector hazards

- Integration (Insertional Mutagenesis, aka "cancer")
- Transgene
- High mutation rate
- High transduction efficiencies
- Broad tropism
- Recombination
- Endogenous retroviruses
- Seroconversion
Genus: *Mastadenovirus*

- *human adenovirus A-G*
  (currently, over 50 serotypes in humans)
Adenovirus Virion

70-100 nm
Adenovirus under Electron Microscope
Generations of Adenovirus vectors

- **First Generation Vectors**
  - Two early genes deleted (E1 or E1/E3)

- **Second Generation Vectors**
  - Three early genes deleted (E1, E3, E4)
  - *He, T.-C. et al. 1998. PNAS. 95:2509-2514*

- **Third Generation Vectors**
  - “Gutless vectors”
  - All viral genes deleted; only essential *cis*-acting sequences retained

![Diagram of Adenovirus Vectors](image-url)
Pathogenesis

- Respiratory infection
  - Particularly seen in children
  - Acute respiratory disease in military recruits
  - Pneumonia in children, recruits, and AIDS
- Eye infection
- Urinary tract infection
- Gastroenteritis
- Cancer
  - Adenovirus Type 12 can cause cancer in hamsters (nonpermissive cells), but not humans
Diagnosis

- Laboratory Analysis

  - **Specimens**: Feces, pharyngeal swab, nasopharyngeal aspirate, transtracheal aspirate, bronchial lavage, conjunctival swab, corneal scraping, tears, genital secretions, urine, biopsy tissue (liver, spleen, lung, brain)

  - **Tests**: Immunoassay, virus isolation, immunofluorescence to detect antigen
Epidemiology

- Estimated to cause ~10% gastroenteritis in children
- Causes ~5-10% of respiratory infections
- Transmission by oral-fecal route most common
- Also transmission by aerosol and water-borne routes
Highly prevalent serotypes:
- Ad1, Ad2, Ad3, Ad5, and Ad6
- These types infect ~80% of people early in life

Other adenovirus types show an epidemic epidemiology, infecting anyone not previously immune.
Control and Prevention

- Personal hygiene
- Live vaccine given to military recruits
  - Capsule form by-passes throat
  - Grows in gut and generates immunity
Adenovirus vector hazards

- Recombination with wild type strains
- Recombination during virus production
- Contamination with helper virus (gutless)
- E1A deficiency complemented in vivo
- Transgene
- Immune response
- Viral protein toxicity
- Difficulties in detecting exposure (prevalence)
- Altered tropism – capsid and fiber proteins
AAV species

Parvoviridae Family

- Genus *Parvovirus* – cats, dogs, mink
- Genus *Erythrovirus* – human; B19
- Genus *Dependovirus* – human; need helper
  (adeno-associated virus; AAV; at least 9 serotypes)
Arrow points to an adeno-associated virus

surface projections

100 nm
Features of AAV

- ssDNA (+ or -), 5 kb genome, non-enveloped
- Replicate in nucleus of dividing cells (using cellular enzymes and helper virus)
- Generally unable to replicate on its own
- Relatively stable virion
- Can integrate at 19q13.4 and replicates as a provirus (Rep-protein dependent) *in vitro*
- Not observed to integrate *in vivo*
Features of AAV (continued)

- Not associated with disease (RG1)
- Can infect all cells tested so far
- Can remain latent in an episomal state (in absence of helper)
- High transduction frequency
- Only requires 145 nt ITR for integration
- No superinfection immunity
AAV Genome
AAV Vector Hazards

- Helper virus contamination
- Specific integration requires Rep protein
- Insertional mutagenesis/cancer
- Deletion/rearrangement during integration
- Multiple copies can integrate
- Reactivation
- Helper viruses: Adeno, herpes, pox
- Evidence of autonomous growth
**Poxviridae**

- *Variola virus* (smallpox virus)  
- *Molluscum contagiosum virus*  
- *Vaccinia virus*  
- *Monkeypox virus*  
- *Cowpox virus*  
- *Fowlpox virus*  
- *Canarypox virus*  

*Used as viral vectors*
Vaccinia Virus Vectors

- RG2 agent
- Wide host range
- Extensively used as a recombinant protein expression vector
- Primary *in vivo* use: expression of antigen to elicit an immune response
- *Vaccinia virus* used as a live vaccine for protection against smallpox
- Attenuated strains (Modified Vaccinia Ankara, MVA)
- Replication defective strains (dVV-L)

Vaccinia Virus Genome

Inverted Repeat 10kbp

Unique Sequences 160kbp

Inverted Repeat 10kbp

Tandem Repeats 13 18
(0.9kbp)(1.3kbp)

Tandem Repeats 18 13
(1.3kbp)(0.9kbp)
Epidemiology

- Smallpox eradicated in 1977
- Molluscum contagiosum virus
  - Common in Zaire, Papua, and New Guinea
- Zoonoses rare
- Transmission by direct or droplet contact
  - Indirect contact (ex. bedding) less common
  - Rarely via air in enclosed settings
  - Relatively stable in environment
Control

- No treatments for smallpox prior to its eradication.

Treatments for Vaccine Complications and Experimentals:

- **Methisazone** – Blocks translation of late mRNAs of poxviruses; used prophylactically against smallpox.
- **Vaccinia Immune Globulin (VIG)**
  - Antibodies from vaccinated people, 1960’s
- **Surgery** to remove mass (reduce amount of virus)
- **Eyes**: Interferon, vidarabine, trifluridine or acyclovir
- **Cidofovir**: Used for CMV; Has shown broad spectrum antiviral activity against all DNA viruses.

- Supportive care
Smallpox Prevention

- **Dryvax**, live vaccinia virus in dried form
  - First approved 1931, existing doses 1970's & 80's
  - New 100-dose kit approved October 2002
  - NYC Board of Health Strain, Wyeth Lab

- **ACAM2000**
  - Approved in 2007
  - Live attenuated, clone of Dryvax strain / grown in Vero Cells

- Quarantine and contraindication issues

- Complications, including inflammation in around the heart and brain, others (see next)
Normal Vaccine Timeline
Normal Vaccine Variants

Satellite Lesion

Lymphangitis

Cellulitis

Edema
Vaccine Complication

Generalized vaccinia
Vaccine Complication

Ocular vaccinia
Vaccine Complication

Eczema vaccinatum
Vaccine Complication

Progressive vaccinia (fatal)
Avipox Vectors

- Replication competent.
- Poxviruses that productively infect avian species (fowlpox and canarypox) are replication defective in mammalian cells, will direct early gene expression.
- May be a safer alternative to vaccinia-based vectors for \textit{in vivo} applications.
Herpesviridae

- Human herpesvirus 1 (Herpes simplex virus 1)*
- Human herpesvirus 2 (Herpes simplex virus 2)
- Human herpesvirus 3 (Varicella-zoster virus)
- Human herpesvirus 4 (Epstein-Barr virus)*
- Human herpesvirus 5 (Human cytomegalovirus)
- Human herpesvirus 6
- Human herpesvirus 7
- Human herpesvirus 8 (Kaposi’s sarcoma virus)  

*More commonly used as viral vectors
Herpesviruses

- RG2 agents

- Dual life cycle:
  - Lytic growth in epithelial cells
  - Latent infection in neuronal cells
Herpesvirus Genome
Types of Herpes Virus Vectors

- **Conditionally replicating**
  - Gene deletion renders the virus able to replicate *in vitro* but it is compromised *in vivo*

- **Replication defective**
  - Deletion of essential genes
  - Requires helper function (i.e. complementing cell)

- **Amplicon Systems**
  - Vector contains the minimal *cis*-acting signals for viral genomic replication
  - Requires helper virus or complementing cell
  - Cosmids or artificial bacterial chromosomes also used to produce the virus
Herpesvirus Pathogenesis

- Encephalitis
- Keratitis
- Mucocutaneous Disease
  (Immunocompromised host)

- Primary HSV-1
  Oropharyngeal Herpes
  Recurrent Labialis

- Primary Genital Herpes
  (HIV-2 or HSV-1)
- Recurrent Herpes Genitalis

Neonatal Herpes

FIG. 3. Pathogenesis of HSV infections.
Epidemiology and Transmission

- Overall, herpes viruses are highly prevalent (i.e. high seropositivity in the general population)

- Transmission
  - Contact
  - Saliva
  - Airborne
  - Blood
Control and Prevention

Control
- Acyclovir (and its newer derivatives)
- Ganciclovir
- Foscavir

Prevention
- Vaccine (HHV-3)
NIH Requirements

- **Section II-A-2:** Personnel may need periodic medical surveillance to ascertain fitness to perform certain activities; they may also need to be offered prophylactic vaccines and boosters (see Section IV-B-1-f, Responsibilities of the Institution, General Information).

- **Section IV-B-1-i.** Determine the necessity for health surveillance of personnel involved in connection with individual recombinant DNA projects; and if appropriate, conduct a health surveillance program for such projects. The institution shall establish and maintain a health surveillance program for personnel engaged in large-scale research or production activities involving viable organisms containing recombinant DNA molecules which require BL3 containment at the laboratory scale. The institution shall establish and maintain a health surveillance program for personnel engaged in animal research involving viable recombinant DNA-containing microorganisms that require BL3 or greater containment in the laboratory.
Conclusions

- Occupational health screening for laboratory workers investigating viral vectors in research laboratories has been generally neglected.
- Consider pre-exposure screening policy for viruses not highly prevalent (i.e. HIV).
- Protection works both ways (institution vs. individual).
- Consider the morale issue and creating a culture of safety.
Conclusions (continued)

- Always consider the transgene
- Be aware of the symptoms of the agent being investigated
- Always keep in mind characteristics of the wild-type virus and the potential of the vector to recombine and form replication competent virus
“The difficulty lies, not in the new ideas, but in escaping from the old ones, which ramify, for those brought up as most of us have been, into every corner of our minds.”

John Maynard Keynes (1936)