CHAPTER VIII: VIRAL VECTORS

Viral vectors have become standard tools for molecular biologists. For this reason, it is necessary that researchers using these biological agents are aware of their origins and the consequences of their use.

The following contains pertinent information for commonly used viral vectors at UC:

A. ADENOVIRUS

Virology: Medium-sized (90–100 nm), non-enveloped icosahedral viruses containing double-stranded DNA. There are more than 49 immunologically distinct types (6 subgenera: A–F) that can cause human infections. Adenoviruses are unusually stable to chemical or physical agents and adverse pH conditions, allowing for prolonged survival outside of the body.

Cultivation: Virus packaged by transfecting HEK 293 cells with adenoviral-based vectors is capable of infecting human cells. These viral supernatants could, depending on the gene insert, contain potentially hazardous recombinant virus. Similar vectors have been approved for human gene therapy trials, attesting to their potential ability to express genes *in vivo*. For these reasons, due caution must be exercised in the production and handling of any recombinant adenovirus.

Clinical features: Adenoviruses most commonly cause respiratory illness; however, depending on the infecting serotype, they may also cause various other illnesses, such as gastroenteritis, conjunctivitis, cystitis, and rash-associated illnesses. Symptoms of respiratory illness caused by adenovirus infection range from common cold symptoms to pneumonia, croup, and bronchitis. Patients with compromised immune systems are especially susceptible to severe complications of adenovirus infection that can cause more systemic diseases.

Epidemiology: Although epidemiologic characteristics of the adenoviruses vary by type, all are transmitted by direct contact, fecal-oral transmission, and occasionally waterborne transmission. Some types are capable of establishing persistent asymptomatic infections in tonsils, adenoids, and intestines of infected hosts, and shedding can occur for months or years. Some adenoviruses (e.g., serotypes 1, 2, 5, and 6) have been shown to be endemic in parts of the world where they have been studied, and infection is usually acquired during childhood. Other types cause sporadic infection and occasional outbreaks; for example, epidemic keratoconjunctivitis is associated with adenovirus serotypes 8, 19, and 37. Epidemics of febrile disease with conjunctivitis are associated with waterborne transmission of some adenovirus types. Acute Respirator Distress Syndrome (ARDS) is most often associated with adenovirus types 4 and 7 in the United

States. Enteric adenoviruses 40 and 41 cause gastroenteritis, usually in children. For some adenovirus serotypes, the clinical spectrum of disease associated with infection varies depending on the site of infection; for example, infection with adenovirus 7 acquired by inhalation is associated with severe lower respiratory tract disease, whereas oral transmission of the virus typically causes no or mild disease.

Treatment: Most infections are mild and require no therapy or only symptomatic treatment. Because there is no virus-specific therapy, serious adenovirus illness can be managed only by treating symptoms and complications of the infection.

Laboratory hazards: Ingestion; droplet exposure of the mucous membrane.

Susceptibility to disinfectants: Susceptible to Clidox, 1:10 dilution of household bleach (made fresh weekly), 2% glutaraldehyde, 0.25% sodium dodecyl sulfate.

Source:
www.drs.illinois.edu/bss/factsheets/viralvectors.aspx#adenovirus

B. ADENO-ASSOCIATED VIRUS (AAV)

Virology: Adeno-associated virus is often found in cells that are simultaneously infected with adenovirus. Parvoviridae; icosahedral, 20–25 nm in diameter; single-stranded DNA genome with protein capsid. AAV is dependent for replication on the presence of wild type adenovirus or herpesvirus; in the absence of helper virus, AAV will stably integrate into the host cell genome. Co-infection with helper virus triggers lytic cycle, as do some agents that appropriately perturb host cells. Wild type AAV integrates preferentially into human chromosome 19q13.3-qter; recombinant vectors lose this specificity and appear to integrate randomly, thereby posing a theoretical risk of insertional mutagenesis.

Clinical features: No known pathology for wild type AAV serotype 2.

Epidemiology: Not documented. Infection apparently via mouth, esophageal, or intestinal mucosa.

Treatment: No specific treatment.

Laboratory hazards: Ingestion, droplet exposure of the mucous membrane, direct injection.

Susceptibility to disinfectants: Susceptible to Clidox, 1:10 dilution of household bleach (made fresh weekly), 2% glutaraldehyde, 0.25% sodium dodecyl sulfate.

Source:
http://www.med.upenn.edu/gtp/vectorcore/BiosafetyInformation.shtml

C. EPSTEIN-BARR VIRUS (EBV)

Virology: Double-stranded linear DNA, 120–150 nm diameter, enveloped, icosahedral; types A and B; Herpesviridae (Gammaherpesvirinae). A ubiquitous B- lymphotrophic herpesvirus, EBV has been found in the tumor cells of a heterogeneous group of malignancies (Burkitt's lymphoma, lymphomas associated with immunosuppression, other non-Hodgkin's lymphomas, Hodgkin's disease, nasopharyngeal carcinoma, gastric adenocarcinoma, lymphoepithelioma-like carcinomas, and immunodeficiency-related leiomyosarcoma). EBV is a transforming virus and can immortalize B-cells and cause lymphoma in various animal models.

Clinical Features: Infectious mononucleosis - acute viral syndrome with fever, sore throat, splenomegaly, and lymphadenopathy; one to several weeks, rarely fatal/ Burkitt's lymphoma - monoclonal tumor of B cells, usually involving children's jaw involvement is common; AIDS patients (25%–30% are EBV related) / Nasopharyngeal carcinoma - malignant tumor of epithelial cells of the nasopharynx involving adults between 20 and 40 years.

Epidemiology: EBV infects 80–90% of all adults worldwide; mononucleosis is common in early childhood worldwide, typical disease occurs in developed countries, mainly in young adults; Burkitt's tumor is worldwide but hyperendemic in highly malarial areas such as tropical Africa; carcinoma is worldwide but highest in Southeast Asia and China.

Transmission: Mononucleosis - person-to-person by oropharyngeal route via saliva, possible spread via blood transfusion (not important route); Burkitt's lymphoma - primary infection occurs early in life or involves immunosuppression and reactivation of EBV later, malaria an important co-factor.

Treatment: No specific treatment

Laboratory hazards: Ingestion, accidental parenteral injection, droplet exposure of the mucous membranes, inhalation of concentrated aerosolized materials. Note that cell lines are often immortalized by transformation with EBV.

Susceptibility to disinfectants: Susceptible to many disinfectants – Clidox, 1:10 dilution of household bleach (made fresh weekly), 70% ethanol, 2% glutaraldehyde/formaldehyde.

Source:
http://www.stanford.edu/dept/EHS/prod/researchlab/bio/docs/EBV.pdf

D. LENTIVIRUS

Virology: The genus of the family Retroviridae consists of nononcogenic retroviruses that produce multiorgan diseases characterized by long incubation periods and persistent infection. Five serogroups are recognized, reflecting the mammalian hosts with which they are associated. HIV-1 is the type species.

Available constructs: Most of the lentiviral vectors presently in use are HIV-derived vectors. The *cis*- and *trans*-acting factors of lentiviruses are often on separate plasmid vectors, with packaging being provided *in trans*. The vector constructs contain the viral *cis* elements, packaging sequences, the Rev response element (RRE), and a transgene. The 2nd generation packaging system combine all the important packaging components: *gag, pol, rev,* and *tat* in one single plasmid. The 3rd generation packaging system eliminated the Tat protein and expresses *rev* on an independent plasmid. Even though it is more cumbersome to use, this design provide maximum biosafety by further reducing the probability of replication-competent virus.

Lentiviral Pseudotyping: Replacement of the HIV envelope glycoprotein with VSV-G provides a broad host-range for the vector and allows the viral particles to be concentrated by centrifugation.

Clinical Features: In terms of the pathogenesis of lentivirus, some key properties are:
- **Lifelong persistence.** This is a function both of their ability to integrate into the host chromosome and evade host immunity. This ability to evade host immunity may be related both to the high mutation rates of these viruses, and to their ability to infect immune cells (macrophages, and in the case of HIV, T-cells).
- **Lentiviruses have high mutation rates**. Lentiviruses replicate, mutate, and undergo selection by host immune responses.
- **Infection proceeds through at least three stages**.
    (A) Initial (acute) lentivirus infection is associated with rapid viral replication and dissemination, which is often accompanied by a transient period of disease.
    (B) This is followed by a latent period, during which the virus is brought under immune control and no disease occurs.
    (C) High levels of viral replication then resume at some later time, resulting in disease.

Epidemiology: Transmitted from person to person through direct exposure to infected body fluids (blood, semen), sexual contact, sharing unclean needles, etc.; transplacental transfer can occur.

Laboratory Hazards: Direct contact with skin and mucous membranes of the eye, nose, and mouth; accidental parenteral injection; ingestion; hazard of aerosols exposure unknown.

Please note that if the lentivirus is carrying an oncogene or potential oncogene, an exposure could result in the oncogene integrating into your genome.
A lentivirus harboring an oncogenic transgene is likely one of the most hazardous viral vector constructs used at the University of Chicago

Use of lentivirus at the University of Chicago must be approved by the IBC prior to initiation of the work and requires laboratories operating at Biosafety Level 2 with Biosafety Level 3 practices. Please contact the Office of Biological safety for more information.

Susceptibility to disinfectants: Susceptible to many disinfectants – Clidox, 1:10 dilution of household bleach (made fresh weekly), 70% ethanol, 2% glutaraldehyde/formaldehyde.

Source:
http://www.stanford.edu/dept/EHS/prod/researchlab/bio/docs/Lentivirus.pdf
https://www.addgene.org/lentiviral/packaging/

E. RETROVIRUS (OTHER THAN LENTIVIRUS)

Infectious viruses that integrate into transduced cells with high frequency and may have oncogenic potential in their natural hosts. Retrovirus vectors are usually based on murine viruses. They include ecotropic viruses (infect murine cells only), amphotropic viruses (infect murine and human cells), or pseudotyped viruses, when vector particles express glycoproteins derived from other enveloped viruses (usually can infect human cells). The most common glycoprotein currently used is VSV-G; however, there are newer pseudotypes being derived from viruses such as measles (Rubeola), Ebola, and Marburg.

Virology [Moloney Murine Leukemia Virus (MoMuLV), Murine Stem Cell Virus (MSCV), etc.]: Retroviridae; subfamily oncovirinae type C, enveloped, icosahedral core, virions 100 nm in diameter, diploid, single-stranded, linear RNA genome. MoMuLV integrates into the host genome and is present in infected cells as a DNA provirus. Cell division is required for infection.

Virus is not lytic. Data suggest a pathogenic mechanism in which chronic productive retroviral infection allowed insertional mutagenesis leading to cell transformation and tumor formation. The nature of a transgene or other introduced genetic element may pose additional risk.

The host range is dependent upon the specificity of the viral envelope. The ecotropic *env* gene produces particles that infect only rodent cells. The amphotropic *env* gene allows infection of rodent and non-rodent cells, including human cells.

VSV-G envelope allows infection in a wide range of mammalian (including human) and non-mammalian cells.

Clinical features: None to date.

Epidemiology: MoMuLV infects only actively dividing cells. In mice, the virus is transmitted in the blood from infected mother to offspring. Transmission may also occur via germ-line infection. *In vivo* transduction in humans appears to require direct injection with amphotropic or pseudotyped virus.

Treatment: No recommended treatment.

Laboratory Hazards: Contact with feces or urine from infected animals for 72 hours post-infection. Contact with tissues and body fluids of infected animals. Direct injection.

Susceptibility to disinfectants: Susceptible to many disinfectants – Clidox, 1:10 dilution of household bleach (made fresh weekly), 70% ethanol, 2% glutaraldehyde/formaldehyde.

Source:
http://www.stanford.edu/dept/EHS/prod/researchlab/bio/docs/Moloney_Murine_Leukemia_Virus.pdf

F. POXVIRUS/VACCINIA

Poxvirus vectors include avian viruses (avipox vectors) such as NYVAC and ALVAC, which cannot establish productive infections in humans, as well as mammalian poxviruses, which can productively infect humans such as vaccinia virus and modified vaccinia viruses [e.g., modified Ankara strain (MVA)]. Poxviruses are highly stable, and vaccinia virus can cause severe infections in immunocompromised persons, persons with certain underlying skin conditions, or pregnant women. Such individuals should not work with vaccinia virus.

Virology: The poxviruses are the largest known DNA viruses and are distinguished from other viruses by their ability to replicate entirely in the cytoplasm of infected cells. Poxviruses do not require nuclear factors for replication and, thus, can replicate with little hindrance in enucleated cells. The core contains a 200-kilobase (kb), double-stranded DNA genome, and is surrounded by a lipoprotein core membrane.

Recombinant Vaccinia vectors: Vaccinia virus can accept as much as 25 kb of foreign DNA, making it useful for expressing large eukaryotic and prokaryotic genes. Foreign genes are integrated stably into the viral genome, resulting in efficient replication and expression of biologically active molecules. Furthermore, post-translational modifications (e.g., methylation, glycosylation) occur normally in the infected cells.

Vaccinia is used to generate live recombinant vaccines for the treatment of other illnesses. Modified versions of vaccinia virus have been developed for use as recombinant vaccines. The modified Ankara strain (MVA) of vaccinia virus was developed by repeated passage in a line of chick embryo fibroblasts. NYVAC is another attenuated form of the vaccinia virus that has been used in the construction of live vaccines. NYVAC has a deletion of 18 vaccinia virus genes that render it less pathogenic.

Clinical Features: Virus disease of skin induced by inoculation for the prevention of smallpox; vesicular or pustular lesion; area of induration or erythema surrounding a scab or ulcer at inoculation site; major complications—encephalitis, progressive vaccinia (immunocompromised susceptible), eczema vaccinatum, fetal vaccinia; minor complications—generalized vaccinia with multiple lesions; autoinoculation of mucous membranes or abraded skin, benign rash, secondary infections; complications are serious for those with eczema or who are immunocompromised.

Epidemiology: Communicable to unvaccinated contacts via contact with mucosal membranes or cuts in skin.

Treatment: Vaccinia immune globulin and an antiviral medication may be of value in treating complications.

Susceptibility to disinfectants: Susceptible to Clidox, 1:10 dilution of household bleach (made fresh weekly), 2% glutaraldehyde/formaldehyde.

Source:
http://emedicine.medscape.com/article/231773-overview

G. BACULOVIRUS

Non-mammalian viruses that usually infect insects. They can be very stable, lasting in the environment for years. Able to transduce mammalian cells, but cannot usually replicate within them. Work is usually done at BSL-1.
Note: Even though this vector is nonpathogenic it must still be inactivated by heat or chemical methods following use because it is a recombinant agent.

UC Biosafety Management of Viral Vectors

To determine what biosafety level to use and what method of viral vector testing for replication competent virus that is mandated by the UC IBC, please go to this link: http://ibc.uchicago.edu/docs/ibc_Testing_Requirements_Viral_Vectors.pdf