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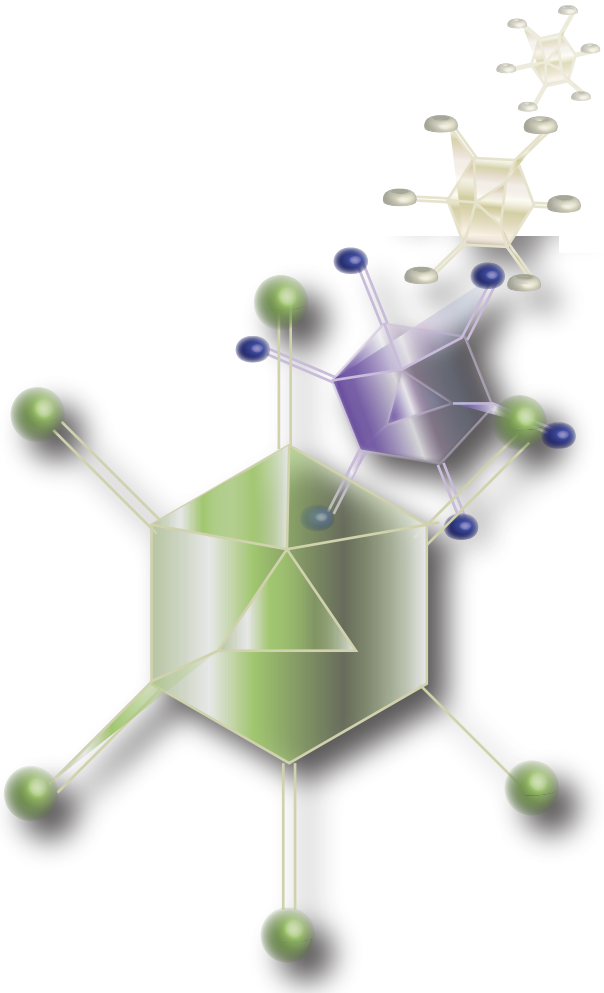
Adenovirus Biosafety Information and Handling Guidelines



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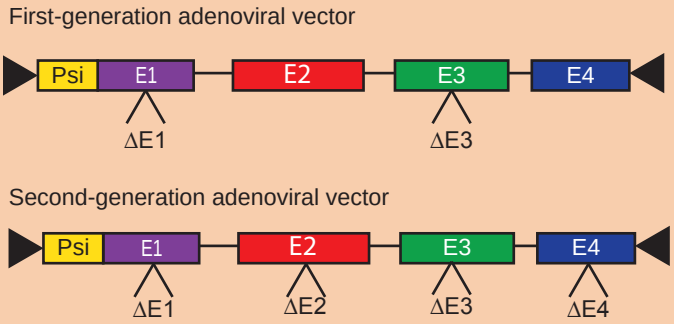
Introduction

Adenovirus is a pathogen that is capable of generating inflammatory immune responses in human. Depending on the type of infection, adenovirus can cause a number of human diseases including respiratory illness and other diseases such as gastroenteritis, conjunctivitis, cystitis and rash. Adenoviruses can typically survive for up to 2 weeks outside the host; however, they have also been shown to survive for up to 8 weeks on environmental surfaces under special conditions.

Due to their ability to infect both dividing and non-dividing cells, adenoviruses have been widely applied in molecular biology as gene delivery vectors [3]. In gene therapy, one concern over adenoviral vectors is their strong immunogenic tendency to illicit both innate and adaptive immune responses in host organisms [2,3]. However, the same trait has made them very suitable as vaccine carriers. Early adenovirus packaging systems arose by construction adenoviral vectors with regions of E1 and E3 early genes deleted [5]. E1 encodes a transcription trans-activator crucial for the expression of other early and late viral genes key to viral replication, while E3 gene products play an important role in subverting host immune defense mechanisms. As a consequence of E1 deletion in first-generation adenoviral vectors, viral growth and replication depend on complementation in trans with the E1 domain present in the genome of packaging cell lines [3]. Although these vectors are replication-defective on their own, a single step of homologous recombination between E1 flanking regions of the vector and the overlapping sequence of host genome may lead to replication-competent adenoviruses (RCA). A major challenge with early generation adenoviral vectors involves transient nature of transgene expression. Furthermore, severe cytoxicity and immune response have been reported in association with their use, including one instance of human death [6].

One reason that contributes to the high level of toxicity of first-generation adenovirus packaging system is the de novo expression of viral proteins [5]. Therefore, additional viral genes have been deleted in the second-generation adenovirus packaging system, giving rise to an improved biosafety profile. Besides E1 and E3 deletion, regions of E2 and E4 early genes are also inactivated in the second-generation adenoviral vectors, further reducing the possibility of RCA generation. E2 gene encondes a DNA-binding protein that is crucial for late gene transcriptional activation and viral replication [1, 2]. Meanwhile, E4 gene regulates several biological functions including cell cycle, DNA transcription and DNA repair. Viruses bearing E4 mutations have been shown to exhibit growth defects and the capability of forming RCA in E1-deleted adenoviral vectors with E4 also removed is further reduced [4]. During production of adenovirus, adenoviral vectors are introduced into engineered cell lines that supply the missing viral genes required for virus assembly and replication. In comparison with the first-generation packaging, adenoviral vectors packaged using the second-generation system show more persisted transgene expression and lower level of cytotoxicity or immune response in vivo.

Adenovirus is designated by NIH as a Biosafety Level-2 (BSL-2) organism. Occupational exposure to adenovirus is generally through the upper respiratory tract; common routes include aerosol exposure, injection, and fecal-oral



A shematic drawing of the genomic structures of first-generation and second-generation adenoviral vectors. Within the 35 kb length of adenovirus genome, E1, E2 and E4 are early genes required for the transcription and expression of late genes indispensable for viral replication; E3 is a regulator of immune response. First-generation adenoviral vectors feature the deletion of E1 and E3 early genes [5]. However, they are associated with high risks of RCA generation and adverse immunogenic effects. This gave rise to the development of second-generation adenoviral vectors, which are made by further deleting E2 and E4 genes [5]. Psi: packaging sequences. Black triangle: Long terminal repeats.

contact. Individuals with immune deficiencies are especially susceptible to adenovirus exposure and should avoid handling live adenovirus. Because adenovirus is a known human pathogen, all associated experiments require extra caution. The purpose of this document is to provide researchers a roadmap regarding adenovirus biosafety, and to minimize the occupational risk of adenovirus exposure.

Comparison of Biosafety Features of the First vs. Second Generation Adenovirus Packaging:

First-generation system	Versus	Second-generation system
• Two adenoviral early genes, E1 and E3, are deleted • Transgene expression is transient and the vector is quickly lost in cells • Causes high cytotoxicity or immune response • A single step of homologous recombination between the vector and host genome sequence may give rise to RCA generation		• Additional essential genes, E2 and E4 are deleted in addition to E1 and E3 • Transgene is expressed at a higher lever for a longer duration • Diminished cytotoxicity or immune response • Deletion of E4 and E2 genes which are essential for adenoviral growth and replication further reduces the potential RCA generation

Laboratory Practices and Containment Requirements

Containment

All work with adenovirus minimally requires BSL-2 containment. Animals used in adenovirus-related studies must be housed following Animal Biosafety Level 2 criteria (ABSL2).

Personnel training

Prior to working with adenovirus or adenoviral vectors, all relevant personnel should be properly trained by the administrator of Employer Health Programs and Institutional Biosafety Committee at your university or institute. Training should cover topics including hazards of adenovirus manipulation, appropriate working procedures and handling of cages, bedding and husbandry materials of adenovirus-infected animals. Since adenovirus is a biohazard, all vacuum lines must be equipped with HEPA filters and all work involving live adenovirus must be performed in a biosafety cabinet.

Personal Protective Equipment (PPE)

In compliance with BSL-2 safety requirements, laboratory and animal staff must wear proper personal protective equipment to reduce the potential for mucosal or skin exposure:

- Lab coat
- Gloves
- Goggles and surgical mask or face shield
- A respirator for use during procedures that have high risk of generating aerosol

Animal studies

Prior to adenoviral infection, an animal adenovirus working area must be established following the guidelines at your institution and be approved by both Institutional Biosafety Committee and Animal Care Committee. Animals must be housed in ventilated cages under negative pressure supplied with HEPA filters. Adenoviruses may be shed by host animals 3 days after infection, during which time all animal husbandry must be strictly maintained at BSL-2 enhanced standards. We strongly advise that the animal husbandry technician in your lab take responsibility for all animal husbandry practices during the first 72 hours post-infection. Post-infection surgeries, necropsies and harvests must be conducted in a Biosafety Cabinet using BSL-2 enhanced practices.

Alternative animal housing and caging options may be explored and executed on a case-by-case basis; the goal neing to ensure animal welfare and reduce the chance of human exposure. Caution must be exercised to avoid aerosol generation when washing cages or cleaning up waste of adenovirus-infected animals with pressure hoses. Cage bedding and all solid animal waste materials that have been used for adenovirus injection must be immediately bagged, correctly labeled, autoclaved and disposed of as hazardous wastes. Sharps

that have been used for adenovirus injection must be collected in an appropriate sharps container before their final disposal.

Comprehensive guidance on laboratory biosafety criteria and vertebrate animal biosafety level criteria for vivarium research facilities is provided by:

CDC/NIH Biosafety in Microbiological and Biomedical Laboratories (BMBL)

Detailed information on biosafety classification of adenoviruses can be found at:

NIH Guidelines for Research Involving Recombinant DNA Molecules

Disinfection

Adenovirus is susceptible to disinfectant, but can survive for weeks on working surfaces that have not been correctly disinfected. Unlike lentiviruses, adenoviruses are quite stable and still retain their infectious capability even after ether and chloroform extraction. Described below are methods to disinfect different types of adenovirus-contaminated surfaces or wastes:

Working surfaces: 10% bleach is an effective disinfectant against adenoviruses on working surfaces. A minimum of 10 minutes' contact time is required.

Liquid waste: Mix disinfectant with the liquid waste to make a 10% final bleach concentration. Let the mix sit for a minimum of 30 minutes before disposal down a waste drain or otherwise as directed by your institution.

Solid waste: All solid waste including adenovirus-contaminated research materials or animal excrement must be autoclaved and disposed of as hazardous materials.

References

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