

Recombinant DNA in Animal Models

There are several important issues to consider when assessing the potential hazards associated with introducing recombinant DNA into an animal for research purposes. It is essential that the people doing the experiments (1) know what recombinant DNA sequences are present in the animals and/or cells used in the experiments they are conducting, (2) that all the people who are directly involved with the animals, cells and/or tissues that derive from the experiments have a clear idea of what they are working with, and (3) if there are any potential risks associated with the work. The most important consideration is whether there is the potential for part or all of the recombinant DNA to be transmitted to other animals and/or humans. In general, the fundamental issue is whether part or all of the recombinant DNA can be mobilized as a virus either by itself or by some interaction (complementation and/or recombination) with other viruses, viral vectors or viral segments (either endogenous or exogenous) present in any cells that contact the recombinant DNA in the course of the experiment and the impact this may have for the animal host and/or the humans that interact/care for the animals. If the possibility exists that recombinant DNA can be mobilized, it is also important to consider whether the resulting virus might be potentially pathogenic to the host animals or to humans. With these considerations in mind, the safest experiments involve the introduction of DNA sequences (either directly into the animal or into cells that will be introduced into the animal) that contain no viral sequences. There is little reason to expect DNAs of this type to be mobilized so there is little or no risk. In contrast, experiments with viral sequences and/or viral vectors need more careful consideration.

Overview of possible risks associated with viral elements in animal experiments

- 1) Experiments with minimal DNA segments from viruses that do not normally infect the animal used in the experiments (CMV promoters in mouse cells/mice for example). Although there is some remote possibility that this could lead to recombination, it is unlikely that recombination will occur if such viral promoters are used in mice or other non-primate hosts.
- 2) Experiments that involve minimal DNA segments from viruses that can infect the animal host. The issue here is whether there is any reasonable expectation that the animal host has an endogenous complementing virus or will be infected with a complementing virus. It is particularly important, if the viral sequences are from a retrovirus, to consider the possibility of recombination of a retroviral promoter with a related endogenous virus (for example when a murine LTR promoter is used to express a gene in a mouse or in mouse cells).
- 3) Experiments involving replication-defective or replication-incompetent viral vectors. In planning experiments with viral vectors that are intended to be defective, it is important to consider the possibility of the experimental protocol giving rise to replication competent recombinants. This is obviously an issue with the defective adenovirus vectors that are rendered replication incompetent through the deletions of E1a or E2, E3, and E4. Special consideration needs to be given to determining the possibility of an experiment giving rise to replication recombinants for experiments using retroviral vectors that are generated using complementing segments from a single viral parent or, whenever a retroviral vector is passed through cells that harbor closely related endogenous viruses (i.e. mouse retroviral vectors passed in murine cells). For instance, VSV-G can efficiently pseudotype retroviral vectors. VSV-G pseudotyped retroviral vectors have not been shown to generate replication

competent recombinants provided the retroviral env gene has a substantial (non-reverting) deletion. It should be noted that the use of VSV-G will significantly expand the host range of the viral pseudotype. Additionally, it is also important to remember that the humans who prepare the vector stocks and/or care for the animals can carry viruses related to the vectors (human adenoviruses for example) and may serve as a source of complementing sequences.

Questions to consider when conducting experiments involving viral elements

- 1) Can the resulting virus infect either other animals or humans? It is important to distinguish the generation of viruses that can infect the target animal (and humans) from those that cannot.
- 2) If a virus is produced that can infect either animals or humans, will the resulting virus replicate or be replication defective? This is an important distinction: there are a number of viruses that will infect hosts but they will not replicate. For example the ASLV family of avian retroviruses can be modified so that they will infect mammalian cells; however, even when the mammalian cells are infected, ASLVs do not produce infectious viruses in human cells. ASLV-based vectors can be distinguished on this basis from viruses (like the murine retroviruses) that can replicate in mammalian cells (including human cells). Special care is always warranted when using viral vectors known to be replication competent and to infect a broad range of species, including humans (vaccinia vectors for example).
- 3) Is the unmodified version of the vector known to be pathogenic in either animals or humans? Even though murine retroviral vectors can replicate in human cells, long experience has shown that these viruses do not set up active pathogenic infections in immunocompetent humans. Extra care must be taken with vector systems that are derived from known pathogens that can replicate in humans (like HIV-1).
- 4) Has anything been done to the vector (inserted sequences, enhanced host range, etc.) that might increase its potential pathogenicity, or the potential pathogenicity of viruses (resulting from a recombination event) that could reasonably be expected to derive from the vector? Such experiments can be done safely, but extra care is needed, and careful thought should be given to the best way to minimize potential hazards of this sort.

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