



Innovations—Biotechnology: Baculovirus Vectors as Gene Transfer Vectors for Mammalian Cells: Biosafety Considerations

Thomas A. Kost and J. Patrick Condreay

GlaxoSmithKline, Research Triangle Park, North Carolina

The Innovations section illuminates new and emerging technologies, methodologies, products, etc., that have applicability to the needs and interests of the ABSA membership. These may include rapid laboratory or field diagnostics, innovative research vectors, or perhaps new and unique clinical or environmental monitoring or measurement instruments. I will advise readers of the latest technical and biotechnical innovations impacting the field of biosafety. I welcome feedback or suggestions for future topics. Please e-mail them to tak4306@gsk.com or to the Editor, Ira F. Salkin, at irasalkin@aol.com.

Introduction

Advances in molecular biology, automated instrumentation, and bioinformatics provided the necessary tools for determining the sequence of the human genome (*Nature* 2001, *Science* 2001). In the near future cDNAs encoding the entire genetic complement of the human genome, as well as other species, will be readily available. In parallel, a wide variety of novel gene transfer vectors have been developed for the routine shuttling of gene sequences between prokaryotic and eukaryotic organisms. Gene transfer vectors fall into two categories: viral and nonviral. Viral vector systems have been developed based on a number of virus families including adenovirus, adeno-associated virus, alphavirus, baculovirus, flavivirus, herpesvirus, lentivirus, retrovirus, and poxvirus (Cid-Arregui & García-Carrancá, 2000; Friedmann, 1999). While these gene

transfer vectors are commonly used as laboratory reagents, we should not lose sight of the fact that they are derived from infectious agents.

The widespread development and application of these vector systems for the expression of recombinant gene products challenges biological research scientists and biosafety professionals to conduct thoughtful risk assessments (Knudsen, 1998) and institute appropriate risk management programs for the safe use of these agents in biological research laboratories.

Recombinant Baculovirus Insect Cell Expression System

Baculoviruses are a family of lytic viruses that are pathogenic primarily for insects (Miller, 1997). The first reports describing the use of vectors based on recombinant baculovirus to express foreign proteins were published in the mid-1980s and have been reviewed (Kost et al., 2000). Since the publication of the initial studies, a number of advantageous properties have established the baculovirus insect cell expression system as one of the most widely used recombinant protein production systems currently employed in biotechnology research laboratories. The ease of use and low risk biosafety profile of recombinant baculoviruses have been major factors in the widespread application of this technology. Nongenetically modified wild type baculoviruses are classified as Risk Group 1 (RG1) agents. RG1 agents are not associated with disease in healthy adult humans (Fleming & Hunt, 2000; NIH, 2002;

Richmond & McKinney, 1999). It is well established that baculoviruses are unable to replicate in mammalian cells (Kost & Condeary, 2002; Miller, 1997). The viral nucleocapsid is surrounded by a lipid envelope allowing work surfaces to be easily decontaminated with a 70% ethanol solution. The budded form of the virus routinely used in the laboratory is noninfectious for the natural insect host, thus decreasing the risk of an inadvertent release of recombinant virus into the environment. In general, BSL-1 containment and work practices provide an appropriate level of risk management for experiments involving the baculovirus insect cell expression system.

Recombinant Baculoviruses as Gene Transfer Vectors for Mammalian Cells

Recently, recombinant baculovirus vectors have been modified to carry mammalian cell active promoters (reviewed in Ghosh et al., 2002; Kost & Condeary, 2002; Löser et al., 2002). As discussed in the cited reviews, a number of reports have now validated the utility of these vectors for successful gene transfer into a wide variety of mammalian cell lines and primary cell cultures. In most instances the recombinant protein is produced transiently; however, it has been demonstrated that inclusion of a selectable antibiotic resistance marker in the vector allows for the establishment of stable expression in the presence of the appropriate antibiotic (Merrihew et al., 2001). Thus, in addition to their utility in the insect cell expression system, modified recombinant baculoviruses are being more widely used as mammalian cell expression vectors. In addition, in certain specific circumstances, these vectors have also been used successfully for *in vivo* gene transfer (Ghosh et al., 2002; Kost & Condeary, 2002; Löser et al., 2002).

These new applications raise the question: What biosafety considerations should one be aware of when working with these viruses? Modified baculovirus vectors, containing mammalian cell-active promoters, possess the unique property that they can be easily produced to high titers in insect cells (10^8 infectious units per ml as measured by infection of insect cells) and used for mammalian cell gene transfer in the absence of replication within the transduced cells. For baculovirus

mediated gene transfer into mammalian cells, the term *transduction* is used rather than *infection* due to the inherent inability of the virus to replicate in mammalian cells. From a biosafety standpoint this is a highly desirable feature, since one does not need to be concerned with the development of virus particles that are replication competent in mammalian cells.

Another important consideration, from a biosafety perspective, is the sensitivity of baculovirus to human complement. Incubation of baculovirus with human complement for 30 minutes at 37°C has been shown to result in a 99% reduction in virus survival (Hofmann & Strauss, 1998; Sandig et al., 1996). Therefore, in the event of an accidental exposure one would anticipate rapid inactivation of virus particles. It is also important to note that the modifications made to the virus to enable mammalian cell transduction do not alter the physical properties of the virus. Thus, neither insect cell tropism, nor inactivation parameters of the virus by common laboratory disinfectants are altered.

As general guidance it is prudent to submit protocols for the use of recombinant baculoviruses designed for mammalian cell gene transfer applications to the local Institutional Biosafety Committee (IBC) for review. For most experimental situations implementation of either BSL-1 or BSL-2 containment and work practices should be considered appropriate risk management for experiments involving volumes of virus of 1 liter or less. For volumes greater than 1 liter, in most instances BSL-2 containment would be recommended.

For example, one may develop and produce a 500-ml preparation of a virus designed to express an ion channel or nuclear receptor protein in mammalian cells. One ml of such a virus preparation containing 10^8 infectious units per ml may then be added to a 10-ml culture of Chinese hamster ovary cells followed by dispensing into the wells of a 96 well assay microtiter plate. Routinely, these cell and virus manipulations would be conducted in a biological safety cabinet followed by the transfer of the flask or microtiter plates into an incubator. This would occur in a BSL-2-designated laboratory area. Following incubation the microtiter plates can be moved into a BSL-1-designated laboratory area and further manipulated using BSL-1 work practices and appropriate decontamination methods. Taking into account the characteristics of the virus outlined above, it does not appear that this example

presents any extraordinary risks to the user when performed at BSL-1 containment and work practices.

This new use of recombinant baculoviruses as a gene delivery tool for mammalian cells is an example of the types of novel vector systems that are being developed for safe and efficient *in vitro* and *in vivo* gene delivery. IBCs face the unique challenge of reviewing and guiding the implementation of appropriate risk management practices that allow investigators to employ these viral vector systems in a safe manner.

References

- Cid-Arregui, A., & García-Carrancá, A. (2000). *Viral vectors: Basic science and gene therapy*. Natick, MA: Eaton Publishing.
- Fleming, D. O., & Hunt, D. L. (Eds.). 2000. *Biological safety, principles and practices* (3rd ed.). Washington, DC: American Society for Microbiology Press.
- Friedmann, T. (1999). *The development of human gene therapy*. Cold Spring Harbor, NY: Laboratory Press.
- Ghosh, S., Parvez, M. K., Banerjee, K., Sarin, S. K., & Hasnain, S. E. (2002). Baculovirus as mammalian cell expression vector for gene therapy: An emerging strategy. *Molecular Therapy*, 6, 5-11.
- Hofmann, C., & Strauss, M. (1998). Baculovirus-mediated gene transfer in the presence of human serum or blood facilitated by inhibition of the complement system. *Gene Therapy*, 5, 531-536.
- Knudsen, R. C. (2001). Risk assessment for working with infectious agents in the biological laboratory. *Applied Biosafety: Journal of the American Biological Safety Association*, 6(1), 19-26.
- Kost, T. A., & Condreay, J. P. (2002). Recombinant baculoviruses as mammalian cell gene-delivery vectors. *Current Opinion in Biotechnology*, 20, 173-180.
- Kost, T. A., Klein, J. L., & Condreay, J. P. (2000). Application of recombinant baculoviruses in biopharmaceutical research. In M. A. Rubeai (Ed.), *Cell engineering, Vol. 2: Transient expression* (pp. 1-28). Dordrecht, The Netherlands: Kluwer Academic Publishers.
- Löser, P., Huser, A., Hillenberg, M., Kumin, D., Both, G. W., & Hofmann, G. (2002). Advances in the development of non-human viral DNA-vectors for gene delivery. *Current Gene Therapy*, 2, 161-171.
- Merrihew, R. V., Clay, W. C., Condreay, J. P., Witherspoon, S. M., Dallas, W. D., & Kost, T. A. (2001). Chromosomal integration of transduced recombinant baculovirus DNA in mammalian cells. *Journal of Virology*, 75, 903-909.
- Miller, L. K. (1997). *The baculoviruses*. New York: Plenum Press.
- National Institutes of Health. (2002). Guidelines for research involving recombinant DNA molecules (<http://www4.od.nih.gov/oba/guidelines.html>).
- Nature* (2001). Vol. 49, Issue 6822.
- Richmond, J. Y., & McKinney, R. W. (Eds.). (1999). *Biosafety in microbiological and biomedical laboratories* (4th ed.). Washington, DC: U.S. Department of Health and Human Services.
- Sandig, V., Hofmann, C., Steinert, S., Jennings, G., Schlag, P., & Strauss, M. (1996). Gene transfer into hepatocytes and human liver tissue by baculovirus vectors. *Human Gene Therapy*, 7, 1937-1945.
- Science*. (2001). Vol. 291, No. 5507.