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Baculovirus as Mammalian Cell Expression Vector for Gene Therapy: An Emerging Strategy

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The monopoly of insect cells to host baculovirus *Autographa californica* multiple nuclear polyhedrosis virus (*Ac*MNPV) as a eukaryotic gene expression system has been shattered with the growing evidence that it also infects mammalian cells in culture. Although *Ac*MNPV fails to replicate in vertebrate cells, it does express alien genes with levels of expression that are dependent on the strength of the promoter used to drive transcription of the foreign gene. It also has been reported that the recombinant *Ac*MNPV enters human hepatic cells in culture preferentially and specifically in comparison with the other mammalian cells of different origin and sources. This has resulted in the use of *Ac*MNPV as a potent mammalian cell delivery system as a xenovector for gene therapy, more precisely liver-specific gene delivery *in vitro* and *in vivo*.

Key Words: baculovirus, human gene therapy, AcMNPV, xenovector

The baculovirus *Autographa californica* multiple nuclear polyhedrosis virus (*Ac*MNPV) expression system has been extensively used for expression of a wide variety of mammalian genes, including human ones. In addition to the very high-level expression in insect cells, the baculovirus–insect expression system provides a eukaryotic environment that is suitable for the proper post-translational modifications of the expressed proteins to take place. The expressed proteins are usually post-translationally modified such that they are almost identical to the native proteins [1–5]. *Ac*MNPV enters cells of taxonomically diverse insects and can express genes under viral promoter control.

Baculoviruses, which are also used as biological pesticides [6], traditionally infect the members of the Lepidopteran family. The infection in insect cells is characterized by production of two structurally and functionally distinct types of virions: the occluded or polyhedra-derived virion (PDV) and the budded virion (BV). The PDVs are responsible for primary infection and are embedded within the matrix of proteinaceous structures called occlusion bodies (OBs) or polyhedra. In a natural infection, the larvae ingest the OBs that contaminate their food and the PDVs are released in the alkaline environment of the insect midgut (Fig. 1). The liberated PDVs infect midgut columnar epithelial cells and the infected cells produce the BV type, which is required for secondary infection and systemic spread within insects. This type also infects cells in culture. Cellular entry of BV occurs through adsorptive endocytosis [7]. Following penetration of the plasma membrane, the nucleocapsids move towards the nucleus, the viral DNA is released, and the virus undergoes repeated rounds of transcription and replication. By 12 hours postinfection, the progeny BVs are formed and are released into the extracellular milieu and, soon thereafter, mature PDVs are occluded into OBs. The polyhedrin protein is dispensable for infection in cultured cells. Most baculovirus expression vector systems exploit this phenomenon by replacing the polyhedrin coding region with a foreign gene [1]. However, in nonpermissive insect cells, the host range of *Ac*MNPV is limited not by its inability to enter and uncoat but rather by its ability to replicate and transcribe its genes. A major breakthrough came in 1983 when Volkman and Goldsmith [8] demonstrated that baculoviruses were able to enter certain cell lines derived from vertebrate species and that no evidence of viral gene expression was observed. This revolutionized the manipulation of *Ac*MNPV with regard to its ability to infect mammalian cells [9–11]. Several recent publications have demonstrated gene transfer and expression in mammalian cells mediated by recombinant *Ac*MNPV carrying mammalian gene regulatory elements. Carbonell and Miller [12], using a recombinant baculovirus with a mammalian expression cassette, detected reporter enzyme activity in a human lung carcinoma cell line. It was shown that *Ac*MNPV could infect human hepatocytes and that the reporter gene *luc* (luciferase) was highly expressed from the cytomegalovirus (CMV) immediate early (IE) promoter [13]. The human hepatoblastoma cell line HepG2 and rat primary hepatocytes efficiently expressed a different reporter gene, *lacZ*, under the control of the Rous sarcoma virus (RSV) promoter inserted into *Ac*MNPV [14]. By using a mammalian CAG promoter, derived from chicken β-actin promoter, baculovirus vector-delivered expression of several genes has been observed in human

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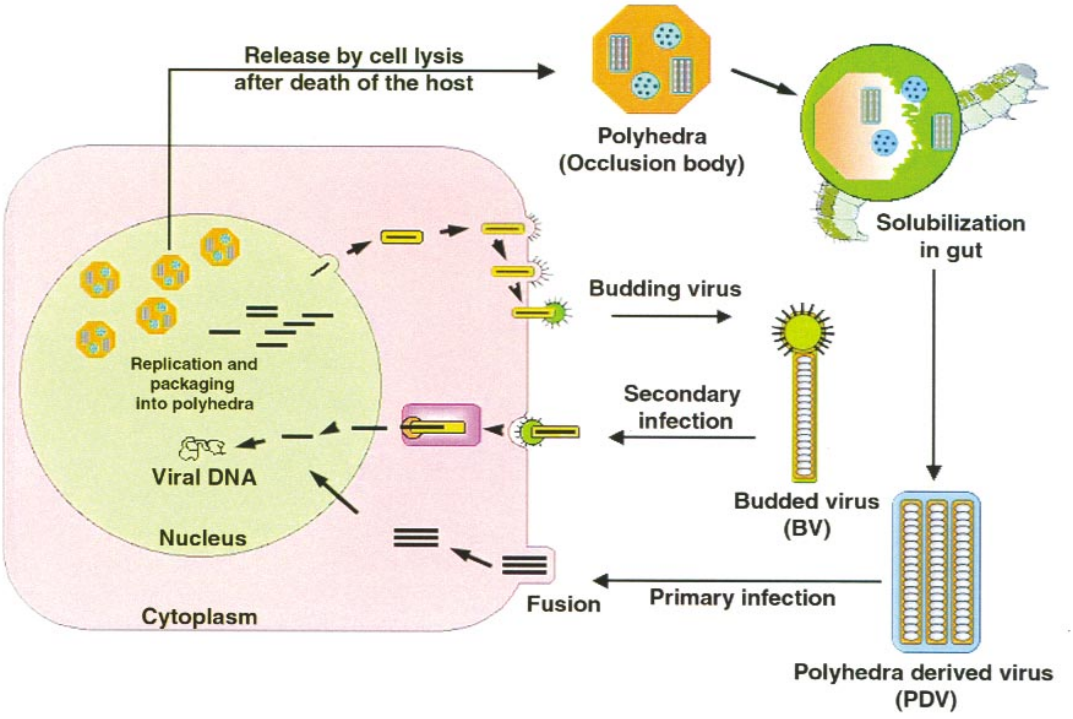

FIG. 1. Life cycle of baculoviruses. Upon ingestion, the OBs or polyhedra are lysed inside the gut of the larvae to release PDVs. Once the PDVs enter the nucleus through primary infection, the viral DNA is uncoated and undergoes replication and transcription, giving rise to BVs that in turn cause secondary infection. Also, the progeny viruses get embedded into OBs which are released upon death of the host.

and porcine cells as well as tumor cell lines from rat, porcine, and human sources [15,16] (Table 1). A recombinant baculovirus containing CMV-IE promoter, directly expressed green fluorescent protein (GFP), and the SV40 early promoter controlling *neomycin phosphotransferase II* have been shown to effectively deliver and express the transgenes in numerous cell types of human, primate, and rodent origin [17]. More recently, Sarkis *et al.* [18] showed that baculoviruses can be used for efficient gene delivery in the central nervous system. Baculoviruses have also been successfully used to deliver genes to rabbit carotid artery [19] and pancreatic islet cells [20]. Previously, Shoji *et al.* [15] constructed the recombinant baculoviruses AcCA39 and AcCA327 carrying hepatitis C virus (HCV) *core* and other structural genes, respectively, and the result indicated that HCV proteins were expressed and processed properly in different mammalian cell lines under the control of a CAG promoter. Subsequently, Delaney and Isom [21] reported a novel transient mechanism for studying hepatitis B virus (HBV) in the well-differentiated human HepG2 cell lines transduced by recombinant *Ac*MNPV carrying the full-length HBV genome under the control of HBV promoter/enhancer with very high expression level. Very recently, the *in vitro* recombinant HBV-baculovirus system has been used to study antiviral drug sensitivity of wild-type and mutant HBV.

Novelty of AcMNPV Based Vector

The ability to efficiently transfer eukaryotic genes for reliable expression in different cell types has been central to the study of the gene of interest, leading to the development of several nonvirus- (physio-chemical) and virus- (retrovirus/adenovirus/adeno-associated virus) based techniques. The nonviral methods are, to varying degrees, deleterious to cells and require a large quantity of DNA and a comparatively low level of expression. The random integration of the retrovirus vectors, genomic rearrangements accompanying integration, immunogenic responses, and stochastic emergence of replication-competent adenoviruses and associated cytopathy remain significant drawbacks of gene transfer mediated by such virus-based vectors. Baculovirus has several striking features that make it an efficient tool as a mammalian cell-directed gene transfer vector. It has a double-stranded, circular DNA 80–200 kb in size. The rod-shaped nucleocapsid structure can accommodate an additional 100 kb or more of foreign DNA. The *Ac*MNPV vectors are helper virus independent and therefore, relatively simpler and faster to construct. Transduction level approaches up to 100% with a very high expression level as compared with the other conventional physio-chemical and viral gene delivery systems. Moreover, it is noncytotoxic even at high multiplicity of infection (MOI).

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Mechanism of AcMNPV Entry into Mammalian Cells

Uptake of baculoviruses by mammalian cells is considered to be a general phenomenon. The budded form of *Ac*MNPV (Table 2) normally enters cells by endocytosis followed by low pH-facilitated fusion of the viral envelope (glycoprotein, gp64) with the endosomal membrane, thus allowing viral entry into the cytoplasm [22]. It has been shown that electrostatic interactions may be necessary for baculovirus binding to the mammalian cell surface. The data suggest that heparan sulfate moieties on the mammalian cell surface could be involved in binding of the virus [23]. It has been shown that phospholipids on the cell surface might serve as important docking points for baculoviral gp64, facilitating viral entry into mammalian cells [24]. In contrast, van Loo *et al*. [25] reported that such glycoprotein receptors are not necessary for efficient infection. They indicated a role for actin filaments in cytoplasmic transport of capsids preceded by virus entry by endocytosis and release of capsids into the cytoplasm upon an acid-induced fusion event. *Ac*MNPV is internalized by mammalian cells of different origin [17], including human and mouse hepatic cells [13], with varying degrees of efficiency. The observed high efficiency and preferential gene transfer into hepatocytes is thought to be due to the presence of a particular receptor on the hepatic cell membrane. Inhibition of gene expression by blocking endosomal maturation suggests involvement of the endocytic pathway in the uptake route used by baculovirus in hepatocytes [13]. One insight into the mechanism of why certain cells are more efficiently transduced is suggested by Barsoum *et al*. [26], who used a pseudotyped baculovirus with vesicular stomatitis virus G glycoprotein and compared its transduction efficiency into HeLa cells with unmodified virus. Their data suggest that inefficient gene expression in less susceptible cells is not due to a block in entry of the baculovirus into a cell, but rather, it is due to the inability of the virus to escape endosomes. However, the presence of a hepatocyte receptor in hepatic cells for binding of baculovirus cannot be ruled out.

TABLE 1: Mammalian cell lines transduced with recombinant *Ac*MNPV

Monkey	Hamster
COS7 (Kidney cell)	CHO (Ovary)
CV-1 (Kidney cell)	
	Human
Porcine	HepG2 (Hepatoblastoma cell)
CPK (Kidney cell)	HuH7 (Hepatoblastoma cell)
FS-L3 (Kidney cell)	sK-Hep-1 (Hepatoblastoma cell)
	HeLa (Cervical carcinoma cell)
Rat	W12 (Keratinocyte)
RGM1 (Gastric mucosal cell)	A 549 (Lung)
PC12 (Adrenal cell)	WI-38 (Lung fibroblast)
	239 (Kidney)
Mouse	Ramos (B-cell)
NIH3T3 (Embryo fibroblast)	Jurkat (T-cell)
C2C12 (Muscle)	HL-60 (Promyelocyte)
	K-562 (Myelocyte)

Insertion of Mammalian Regulatory Signals and Stable Expression of Foreign Genes

The activity of the strong baculoviral promoter *Ppolh*, commonly used to transcribe transgene in insect cells, is silent in mammalian cells despite the presence, also in mammalian cells, of a host factor (polyhedrin promoter binding protein) which is required to nucleate the polyhedrin promoter-driven transcription machinery [27]. Therefore, the major prerequisite for constructing a recombinant *Ac*MNPV for mammalian cell-directed gene transfer is the insertion of a strong mammalian gene regulatory element (Table 3) into the viral genome. However, HBV-expressing hepatocytes containing strong heterologous promoter elements from RSV and CMV, proximal to the HBV genome behaved differently in terms of HBV transcription and replication *in vitro* compared with natural infection *in vivo* [21]. This strongly suggests that endogenous regulatory elements are acting during delivery and expression of the gene of interest in native cells, specifically hepatic cells.

In addition to driving transient expression in mammalian cells, recombinant baculoviruses can also be used to generate stably transduced cell lines [15]. This occurs in a way that is similar to HBV infection in that, although the viral DNA does not integrate into the human genome as part of its life cycle, an integration event that enables the transduced hepatic cells to stably express some of the HBV genes occurs during persistent viral infection. Using a dominant selective marker in an expression cassette, they could obtain stable derivatives that maintained stable expression of a green fluorescent reporter protein (GFP) for multiple passages in culture. Analyses of cellular DNA revealed the presence of at least 12 kb of DNA derived from the viral input in Chinese hamster ovary (CHO) cells [17]. Another approach was used by Palombo *et al*. [28], who used a chimeric baculovirus-AAV (adeno-associated virus) where the expression cassettes, controlled by mammalian promoters, were flanked by ITR (inverted terminal repeat) to exploit the ability of AAV to integrate into the genome of host cells. The ITR flanked cassettes integrated into a specific site on chromosome 19, which is characteristic of AAV integration.

AcMNPV as a Xenovector for Gene Therapy

One of the most important target organs for gene therapy is the liver, where many inherited metabolic disorders

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TABLE 2: Morphologically and biochemically distinct forms of *Ac*MNPV

Budded viruses (BVs) are forms of nuclear polyhedrosis viruses (NPVs) released by budding from the plasma membrane. The rod-shaped nucleocapsid of BV is surrounded by a thick lipid bilayer membrane, the envelope of which is rich in viral-encoded glycoprotein (gp64) at one end. This gp64, during secondary infection, is intimately involved in virus entry into cells.

Occluded viruses (OVs) are formed in the nucleus and comprise enveloped nucleocapsids embedded in a crystalline protein matrix called polyhedrin. Polyhedral inclusion bodies carry several viruses occluded in the proteinacious matrix. Enveloped virions liberated from occlusion bodies enter cells by a different route.

like hemophilia, phenylketonuria, familial hypercholesterolemia, and organic acidemia, as well as viral and autoimmune hepatitis, are manifested. Various methods for transfer of therapeutic genes into hepatocytes *in vitro* and *in vivo* have been developed so far. Generally they include viral and nonviral delivery strategies and both have advantages and limitations in their use for liver-directed gene therapy [29,30]. The retrovirus- and adenovirus-based vectors have been shown to deliver genes efficiently into hepatic cells *in vitro* and *in vivo*. However, these vectors do not allow for the stable expression of the therapeutic gene in the liver, which would be highly advantageous for *in vivo* gene therapy strategies. None of the generally used viral vectors is able to target the liver specifically, and the nonviral vectors based on receptor-mediated uptake indeed achieve liver-specific gene delivery [29,31] but are usually less efficient than viruses with regard to gene transfer and expression level. A liver-specific gene delivery system based on Sendai virus fusion protein-mediated targeting has been described [32]. In contrast, the genetically engineered *Ac*MNPV has been demonstrated to efficiently deliver transgenes into cultured mammalian cells with a very high expression level and to show a strong preference for hepatocytes of different origin, specifically human hepatocytes *in vitro* [21,33]. However, a drawback is that gene transfer is strongly reduced in the presence of native sera, providing an explanation for the failure of direct application of the baculovirus *in vivo* [34]. The complement system appears to be the potent primary barrier for *in vivo* gene therapy as enhanced gene transfer into hepatocytes of complement-deficient mice has been reported [35]. Heat treatment of the sera inactivates the viral neutralizing, heat-labile components (C3 and C4) of the classical complement cascades and facilitates virus entry into cells in culture. It has been demonstrated that baculovirus-mediated gene transfer into perfused liver tissues is also feasible, suggesting that inactivating components can be excluded [33]. Serum treated with anti-C5 antibody or with cobra venom factor to deplete C3 does not fully inactivate the virus, suggesting attractive strategies to alleviate this problem. An additional strategy could be to use recombinant soluble complement receptor type I to inhibit both pathways of the complement cascade [36]. Use of baculovirus in tissues such as those of the central nervous system, in which the complement system is absent, can result in very efficient gene delivery [18]. The complement system also can be avoided by using a physical barrier, as demonstrated by Airenne *et al.* [19], for gene delivery in rabbit carotid artery. They applied engineered baculoviruses in a Silastic collar placed around the arteries that prevented virus contact with blood components.

The cutting edge in baculovirus gene therapy is the recent development of complement-resistant baculovirus [37]. In this novel approach, the human decay accelerating factor gene (DAF), which blocks the complement system at the central step of formation of both classical and alternative pathways by increasing rate of decay of C3bBb and C4b2a complex, is fused in-frame with baculoviral *gp64* gene. DAF–gp64 is driven by the polyhedrin promoter, and the fusion protein is efficiently displayed on the surface of the complement-resistant baculovirus. The complement resistant baculoviruses allowed enhanced gene transfer in complement sufficient neonatal rats.

Another drawback to the use of baculoviruses as gene delivery vehicles is their production, purification, and concentration. The virus tends to aggregate upon centrifugation, the current method to concentrate the virus. However, this problem is likely to be solved by using a cationic exchange matrix to concentrate the baculoviruses [38]. Another important feature associated with this viral vector is that expression in mammalian cells appears to be regulated by epigenetic factors. Sarkis *et al.* [18] have shown that inhibitors of histone deacetylases, such as butyrates, can markedly influence levels of expression. This could be important to regulate desired levels of expression.

ENGINEERING BACULOVIRUSES FOR IMPROVED FEATURES

Several strategies exist to engineer a baculovirus so that it can infect broad range of cell types. One of them is modifying the viral envelope to enhance the infectivity and tropism of the virus. For example, Barsoum *et al.* [26] showed that pseudotyping the baculoviral envelope with vesicular stomatitis G glycoprotein can enhance infectivity of engineered baculovirus towards the different cell lines tested. Pseudotyped viruses can be a very efficient tool for accurate target tissue-specific gene delivery. Pseudotyped baculovirus vectors with envelopes derived from neurotropic viruses, such as α- or rhabdoviruses, can be used to enhance transduction of nervous tissues. Similarly, pseudotyping with hepatotropic viruses, like

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hepatitis B virus and sendai virus, can be used for targeted gene delivery into hepatic tissue, thus avoiding many of the consequences arising from fatal immune hyperactivations associated with infection with these viruses. Recently, Park *et al.* [39] demonstrated that pseudotyped baculovirus can transfer target genes fivefold more efficiently in hepatocytes when placed under the control of a liver-specific AFP (α-fetoprotein) promoter/enhancer. Another strategy could be the inclusion of suitable gene regulatory elements that can drive expression in specific target tissues in the expression cassette. The promoter can be a limiting factor in the context of the baculoviral backbone. For instance, Shoji *et al.* [15] showed that cells that were not transduced by CMV-*lac*Z baculoviruses can be efficiently transduced by CAG-*lac*Z constructs. Thus, it is of interest to examine different promoters of viral and cellular origin in the baculoviral context in mammalian cells.

Baculoviruses can be engineered to display specific ligand-binding moieties on their surface to facilitate cell-specific delivery of the target gene. This strategy was recently demonstrated by using baculoviral vectors displaying either single-chain antibody fragment (scFv) against carcinoembryonic antigen (CEA) or synthetic IgG binding domains (ZZ) derived from *Staphylococcus aureus*. These moieties were displayed by fusing them with the amino terminus of the baculoviral gp64 protein. The anti-CEA-scFv displaying baculovirus was shown to bind specifically to CEA-expressing cells. Similarly, baculovirus displaying ZZ domain specifically bound to BHK cells and efficiently transduced reporter gene expression [40]. Thus, baculoviruses can be engineered to deliver target genes accurately to the cell type of choice.

Recently, Sollerbrant *et al.* [41] have shown that baculoviruses can be used as novel tools to produce large-scale recombinant adenoviruses (rAAV), thereby circumventing present-day problems associated with rAAV production. Similarly, Cheshenko *et al.* [42] demonstrated that baculoviruses can also be used to generate fully deleted adenoviral vectors (FD-AdVs), which appear to be a promising tool for gene therapy. The contamination of vector preparation by helper viruses currently limits the use of FD-AdVs in gene therapy applications. Baculoviruses can also be used as possible cytotoxic vectors rather than therapeutic gene delivery vectors as demonstrated by Song and Boyce [43], who efficiently killed human osteosarcoma cell lines with recombinant baculoviruses expressing p53. When combined with chemotherapy, greater than 95% of cells could be killed. With a suitable surface display moiety, recombinant baculovirus vectors expressing ribotoxins like restrictocin can be used to specifically destroy tumor cells.

Recombinant AcMNPV and Biosafety

The AcMNPV propagates in insect cells but is inherently unable to replicate in mammalian cells, avoiding any risk of outbreak of the replication-competent virus. However, there is a possibility that regions of the *Ac*MNPV-DNA could become integrated into the cellular genome in the culture. The persistence of large regions of the viral genome in cells raises the possibility that IE viral gene products could be expressed in the transduced mammalian cells. There is one report that one of these gene products, the IE-1, which is a translational transactivator, is involved in the activation of the early promoter *he65* in both insect (TN-368) and mammalian (BHK-21) cell lines [44]. The observation that IE-1 activation of the *he65* promoter depends on the presence of an inverted repeat of *hr4left* sequences suggests a comparable mechanism of IE-1 activation in insect and mammalian cells that might involve a direct interaction of IE-1 with the inverted repeat. However, the exact mechanism behind the regulation of this early viral promoter in mammalian cells is not known. It thus appears that much more work will be necessary to evaluate the safety of *Ac*MNPV as a tool for human gene therapy. Although the virus does not appear to replicate in hepatocytes and the viral *polyhedrin* gene transcription unit remains inactive, further investigations will be required to determine whether any of the viral genes are expressed in mammalian cells [14]. Recent data suggest that baculovirus can enhance expression of inflammatory cytokines like TNF-α, IL1-α, and IL1-β in primary hepatocytes [45]. This is in line with the observation by Gronowski *et al.* [46] who showed that live baculovirus can induce interferons in addition to other cytokines through gp67. Moreover, like any recombinant organisms, recombinant *Ac*MNPVs can be treated as if they were potential biohazards, and biosafety guidelines must be followed.

TABLE 3: Mammalian expression cassettes inserted in *Ac*MNPV

Cytomegalovirus immediate early promoter (CMV-IE)
Rous sarcoma virus-long terminal repeat (RSV-LTR)
Chicken β-actin promoter (CAG)
Simian virus 40 promoter (SV40)
Hepatitis B virus promoter/enhancer (HBV)

Conclusions and Future Prospects

The above reports have illustrated the utility of recombinant baculoviruses as gene delivery vehicles for expression of foreign genes in mammalian cells, significantly expanding the range of such cell types as *in vitro* expression systems as well as for gene therapy. The broad range of susceptible cells, coupled with the relative ease of construction and propagation of the virus, makes the *Ac*MNPV system a useful tool for studying expression and function of human and other mammalian gene products. Baculovirus vectors have thus emerged as novel and promising systems for gene delivery into mammalian cells of different origin, specifical-

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ly human hepatocytes. The recent observation of an enhancer-like element upregulating the expression of genes for polyhedrin and other heterologous promoters through the involvement of specific host factors [27,47–51], which are also present in mammalian cells, has added a new dimension to baculovirus-mediated gene therapy. It is of immense need to further evaluate the potential of *Ac*MNPV-mediated gene delivery and expression of therapeutic proteins in native cells *in vitro* as well as *in vivo*. The identification of *Ac*MNPV binding receptor on the cell membrane and elucidation of its structure would facilitate the understanding of virus–host interaction more precisely at the molecular level. This would enable alteration or modification of these molecules for enhanced uptake of the virus by a broad range of cells. Because *Ac*MNPV is a double-stranded DNA virus, it would be very useful to clone therapeutic genes as well as cDNA of infectious RNA viruses towards antisense therapy approaches. However, the ability of heat-labile complement proteins to block *Ac*MNPV infection of the mammalian cell or organ remains a challenge for the success of *in vivo* gene therapy, and it is necessary to identify these complement component-neutralizing agents and the consequences of post-neutralizing effects on the animal more elaborately. Also, the stable expression of the cloned genes *in vivo* is inevitable for therapeutic approaches, and further investigation will be required to determine exactly how much of the viral genome can be maintained and whether it is integrated into the host genome. Additionally, this system could facilitate an assessment of different promoters for their strength as well as temporal and spatial regulation across mammalian cells of different origin. The recent controversy surrounding gene therapy trials [52] has raised doubts about the safety of the animal virus-based vectors for gene therapy. The hitherto unexploited potential of baculoviruses as safe, efficient, and specific gene delivery vectors holds great promise for the development of a new generation of more efficient gene therapy vehicles in the future.

REFERENCES

1. O'Reilly, D. R., Miller, L. K., and Luckow, V. A. (1992). In *Baculovirus Expression Vectors: a Laboratory Manual* 24–50. W. H. Freeman and Co., NY.
2. Sridhar, P., *et al.* (1994). Baculovirus mediated expression of heterologous genes in insect cells. *J. Biosciences* **19:** 603–614.
3. Nakhai, B., Pal, R., Talwar, G. P., and Hasnain, S. E. (1991). The α subunit of human chorionic gonadotropin hormone synthesized in insect cells using a baculovirus vector is biologically active. *FEBS Lett.* **283:** 104–108.
4. Jones, I., and Morikawa, Y. (1996). Baculovirus vectors for expression in insect cells. *Curr. Opin. Biotech.* **7:** 512–516.
5. Jha, P. K., Pal, R., Nakhai, B., Sridhar, P., and Hasnain, S. E. (1992). Simultaneous synthesis of enzymatically active luciferase and biologically active β subunit of human chorionic gonadotropin in caterpillars infected with a recombinant baculovirus. *FEBS Lett.* **310:** 282–152.
6. Mishra, S. (1998). Baculoviruses as biopesticides. *Curr. Sci.* **75:** 1015–1022.
7. Volkman, L. E., and Goldsmith, P. A. (1985). Mechanism of neutralization of budded *Autographa californica* nuclear polyhedrosis virus by monoclonal antibody: inhibition of entry by adsorptive endocytosis. *Virology* **143:** 185–195.
8. Volkman, L. E., and Goldsmith, P. A. (1983). *In vitro* survey of *Autographa californica* nuclear polyhedrosis virus interaction with nontarget vertebrate host cells. *Appl. Environ. Microbiol.* **45:** 1085–1093.
9. Carbonell, L. F., Klowden, M. J., and Miller, L. K. (1985). Baculovirus-mediated expression of bacterial genes in dipteran and mammalian cells. *J. Virol.* **56:** 153–160.
10. Tjia, S. T., zu Altenschildesche, G. M., and Doerfler, W. (1983). *Autographa californica* nuclear polyhedrosis virus (*Ac*NPV) DNA does not persist in mass cultures of mammalian cells. *Virology* **125:** 107–117.
11. Groner, A., Granados, R. R., and Burand, J. P (1984). Interaction of *Autographa californica* nuclear polyhedrosis virus with two nonpermissive cell lines. *Intervirology* **21:** 203–209.
12. Carbonell, L. F., and Miller, L. K. (1987). Baculovirus interaction with nontarget organisms: a virus-borne reporter gene is not expressed in two mammalian cell lines. *Appl. Environ. Microbiol.* **53:** 1412–1417.
13. Hofmann, C., *et al.* (1995). Efficient gene transfer into human hepatocytes by baculovirus vectors. *Proc. Natl. Acad. Sci. USA* **92:** 10099–10103.
14. Boyce, F. M., and Butcher, N. L. (1996). Baculovirus-mediated gene transfer into mammalian cells. *Proc. Natl. Acad. Sci. USA.* **93:** 2348–2352.
15. Shoji, I., *et al.* (1997). Efficient gene transfer into various mammalian cells, including non-hepatic cells, by baculovirus vectors. *J. Gen. Virol.* **78:** 2657–2664.
16. Yap, C. C., *et al.* (1997). A hybrid baculovirus-T7 RNA polymerase system for recovery of an infectious virus from cDNA. *Virology* **231:** 192–200.
17. Condreay, J. P., Witherspoon, S. M., Clay, W. C., and Cost, T. A. (1999). Transient and stable gene expression in mammalian cells transduced with a recombinant baculovirus vector. *Proc. Natl. Acad. Sci. USA* **96:** 127–132.
18. Sarkis, C., *et al.* (2000). Efficient transduction of neural cells in vitro and in vivo by a baculovirus-derived vector. *Proc. Natl. Acad. Sci. USA* **97:** 14638–14643.
19. Airenne, K. J., *et al.* (2000) Baculovirus-mediated periadventitial gene transfer to rabbit carotid artery. *Gene Ther.* **7:** 1499–1504.
20. Ma, L., *et al.* (2000) Baculovirus-mediated gene transfer into pancreatic islet cells. *Diabetes* **49:** 1986–1991.
21. Delaney, W. E., and Isom, H. C. (1998). Hepatitis B virus replication in human HepG2 cells mediated by hepatitis B virus recombinant baculovirus. *Hepatology* **28:** 1134–1146.
22. Blissard, G. W., and Wenz, J. R. (1993). Baculovirus gp64 envelope glycoprotein is sufficient to mediate pH-dependent membrane fusion. *J. Virol.* **66**: 6829–6835.
23. Duisit, G., *et al.* (1999). Baculovirus vector requires electrostatic interactions including heparan sulfate for efficient gene transfer in mammalian cells. *J. Gene. Med.* **1:** 93–102.
24. Tani, H., Nishijima, M., Ushijima, H., Miyamura, T., and Matsuura, Y. (2001). Characterization of cell-surface determinants important for baculovirus infection. *Virology* **279:** 343–353.
25. van Loo, N. D., *et al.* (2001). Baculovirus infection of nondividing mammalian cells: mechanisms of entry and nuclear transport of capsids. *J. Virol.* **75:** 961–970.
26. Barsoum, J., Brown, R., McKee, M., and Boyce, F. M. (1997). Efficient transduction of mammalian cells by a recombinant baculovirus having the vesicular stomatitis virus G glycoprotein. *Hum. Gene Ther.* **8:** 2011–2018.
27. Ghosh. S., Jain, A., Mukherjee, B., Habib, S., and Hasnain, S. E. (1998). The host factor polyhedrin promoter binding protein (PPBP) is involved in transcription from the baculovirus polyhedrin gene promoter. *J. Virol.* **72:** 7484–7490.
28. Palombo, F., *et al.* (1998). Site-specific integration in mammalian cells mediated by a new hybrid baculovirus-adeno-associated virus vector. *J. Virol.* **72:** 5025–5034.
29. Strauss, M. (1994). Liver-directed gene therapy: prospects and problems. *Gene Ther.* **1:** 156–164.
30. Perales, J. C., Ferkol, T., Beegen, H., Ratnoff, O. D., and Hanson, R. W. (1993). Gene transfer *in vivo*: sustained expression and regulation of genes introduced into the liver by receptor-targeted uptake. *Proc. Natl Sci. Acad. USA* **91:** 4086–4090.
31. Wu, G. Y., *et al.* (1991). Receptor-mediated gene delivery *in vivo*. Partial correction of genetic analbuminemia in Nagase rats. *J. Biol. Chem.* **266:** 14338–14342.
32. Ramani, K., Hassan, Q., Venkaiah, B., Hasnain, S. E., and Sarkar, D. P. (1998). Site-specific gene delivery *in vivo* through engineered Sendai viral envelopes. *Proc. Natl. Sci. Acad. USA* **95:** 11886–11890.
33. Sandig, V., *et al.* (1996). Gene transfer into hepatocytes and human liver tissue by baculovirus vectors. *Hum. Gene Ther.* **16:** 1937–1945.
34. Hofmann, C., and Strauss, M. (1998). Baculovirus-mediated gene transfer in the presence of human serum or blood facilitated by inhibition of the complement system. *Gene Ther.* **4:** 531–536.
35. Hofmann, C., Lehnert, W., and Strauss, M. (1998). The baculovirus system for gene delivery into hepatocytes. *Gene Ther. Mol. Biol.* **1**: 231–239.
36. Hofmann, C., Huser, A., Lehnert, W., and Strauss, M. (1999). Protection of baculovirus-vectors against complement-mediated inactivation by recombinant soluble complement receptor type 1. *Biol. Chem.* **380:** 393–395.
37. Huser, A., Rudolph, M., and Hofmann, C. (2001). Incorporation of decay-accelerating factor into the baculovirus envelope generates complement-resistant gene transfer vectors. *Nat. Biotechnol.* **19:** 451–455.
38. Barsoum, J. (1999). Concentration of recombinant baculovirus by cation-exchange chromatography. *Biotechniques* **26:**834–840.
39. Park, S. W., Lee, H. K., Kim, T. G., Yoon, S. K., and Paik, S. Y. (2001). Hepatocyte specific gene expression by baculovirus pseudotyped with vesicular stomatitis virus envelope glycoprotein. *Biochem. Biophys. Res. Commun.* **289:** 444–450.
40. Ojala, K., Mottershead, D. G., Suokko, A., and Oker-Blom, C. (2001). Specific binding

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of baculoviruses displaying gp64 fusion proteins to mammalian cells. *Biochem. Biophys. Res. Commun.* **284:** 777–784.

41. Sollerbrant, K., *et al.* (2001). A novel method using baculovirus-mediated gene transfer for production of recombinant adeno-associated virus vectors. *J. Gen. Virol.* **82:** 2051–2060.

42. Cheshenko, N., Krougliak, N., Eisensmith, R. C., and Krougliak, V. A. (2001). A novel system for the production of fully deleted adenovirus vectors that does not require helper adenovirus. *Gene Ther.* **8:** 846–854.

43. Song, S. U., and Boyce, F. M. (2001). Combination treatment for osteosarcoma with baculoviral vector mediated gene therapy (p53) and chemotherapy (adriamycin). *Exp. Mol. Med.* **33:** 46–53.

44. Murges, D., Kremer, A., and Knebel-Morsdorf, D. (1997). Baculovirus transactivator IE1 is functional in mammalian cells. *J. Gen. Virol.* **78:** 1507–1510.

45. Beck, N. B., Sidhu, J. S., and Omiecinski, C. J. (2000). Baculovirus vectors repress phenobarbital-mediated gene induction and stimulate cytokine expression in primary cultures of rat hepatocytes. *Gene Ther.* **7:** 1274–1283.

46. Gronowski, A. M., Hilbert, D. M., Sheehan, K. C., Garotta, G., and Schreiber, R. D. (1999). Baculovirus stimulates antiviral effects in mammalian cells. *J. Virol.* **73:** 9944–9951.

47. Habib, S. *et al.* US Patent No.08/886,595.

48. Habib, S., and Hasnain, S. E. (1996). A 38-kDa host factor interacts with functionally important motifs within the *Autographa californica* multinucleocapsid nuclear polyhedrosis virus homologous region (*hr1*) DNA sequence. *J. Biol. Chem.* **271:** 28250–28258.

49. Habib, S., *et al.* (1996). Bifunctionality of the AcMNPV homologous region sequence (*hr*1): enhancer and *ori* functions have different sequence requirements. *DNA Cell Biol.* **15:** 737–747.

50. Habib, S., and Hasnain, S. E. (1997). A bifunctional baculovirus homologous region (hr1) sequence: enhancer and origin of replication functions reside within the same sequence element. *Current Science* **73:** 658–666.

51. Ramachandran, A., *et al.* (2001). Novel Sp family-like transcription factors are present in adult insect cells and are involved in transcription from the polyhedrin gene initiator promoter. *J. Biol. Chem.* **276:** 23440–23449.

52. Senior, K. (2000). Gene therapy: a rocky start to the new millennium. *Mol. Med. Today* **6:** 93.

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