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Genetics

Baculovirus-mediated gene transfer into mammalian cells

(xenovector/liver/gene therapy)

FREDERICK M. BOYCE*† AND NANCY L. R. BUCHER‡

*Department of Neurology, Massachusetts General Hospital, Building 149, 13th Street, Charlestown, MA 02129; and ‡Department of Pathology, Boston University School of Medicine, Boston, MA 02118

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ABSTRACT **This paper describes the use of the baculovirus *Autographa californica* multiple nuclear polyhedrosis virus (AcMNPV) as a vector for gene delivery into mammalian cells. A modified AcMNPV virus was prepared that carried the *Escherichia coli lacZ* reporter gene under control of the Rous sarcoma virus promoter and mammalian RNA processing signals. This modified baculovirus was then used to infect a variety of mammalian cell lines. After infection of the human liver cell lines HepG2, >25% of the cells showed high-level expression of the transduced gene. Over 70% of the cells in primary cultures of rat hepatocytes showed expression of β-galactosidase after exposure to the virus. Cell lines from other tissues showed less or no expression of *lacZ* after exposure to the virus. The block to expression in less susceptible cells does not appear to result from the ability to be internalized by the target cell but rather by events subsequent to viral entry. The onset of lacZ expression occurred within 6 hr of infection in HepG2 cells and peaked 12–24 hr postinfection. Because AcMNPV is able to replicate only in insect hosts, is able to carry large (>15 kb) inserts, and is a highly effective gene delivery vehicle for primary cultures of hepatocytes, AcMNPV may be a useful vector for genetic manipulation of liver cells.**

Current gene transfer vectors are derived from animal viruses that normally may replicate in mammalian cells and must be rendered defective by deletion of one or more essential genes (1). There are several problems inherent in this strategy of using animal viruses for human gene therapy, which include the potential for a preexisting immune response to the virus, for recombination with endogenous viral sequences to form replication competent virus, as well as for "leakiness" of viral late gene expression in the transduced cells (2–4). In addition, most current gene therapy vectors lack the ability to package inserts greater than 10 kb and do not show cell type-specific expression.

The baculovirus *Autographa californica* multiple nuclear polyhedrosis virus (AcMNPV) has been used widely for high-level expression of recombinant proteins in insect cells (5, 6). The normal host range of AcMNPV is limited to lepidopteran insects (7). In principle, its host range could be limited by the ability of the target cell to bind and internalize the virus, to transcribe viral promoters, or to provide host factors for viral replication. Miller and colleagues (8) demonstrated that the viral polyhedrin promoter is not efficiently transcribed in DL-1 cells derived from the fly *Drosophila melanogaster*, which is nonpermissive for viral replication. However, a modified AcMNPV containing the Rous sarcoma virus (RSV) promoter was able to express a chloramphenicol acetyltransferase (CAT) gene in DL-1 cells (8). Thus, in nonpermissive insect cells, the host range of AcMNPV is limited not by its ability to enter and uncoat but rather by its ability to replicate and transcribe viral genes. The AcMNPV RSV-CAT virus failed to produce detectable *de novo* expression in A549 human lung carcinoma cells or in L929 mouse cells (9). Thus, a block to the entry or expression of AcMNPV in mammalian cells was postulated (9).

In this report, we describe the use of the baculovirus AcMNPV as a vector for the delivery of genes into mammalian cells. The human liver tumor line HepG2 and rat hepatocytes in primary cultures were able to express efficiently a reporter gene cassette inserted into the AcMNPV genome. Consistent with the results of prior studies (9), the same virus did not yield efficient expression of β-galactosidase after infection of most other mammalian cell lines. Thus, certain mammalian cells are capable of internalizing and uncoating AcMNPV in a manner that allows the expression of a promoter normally active in mammalian cells. This result raises the possibility that baculovirus-based vectors may prove useful for gene transfer into mammalian liver cells.

MATERIALS AND METHODS

Preparation of Virus. An 850-bp *Bgl* II/*Bam*HI fragment containing simian virus 40 (SV40) splice and polyadenylylation sequences was excised from RSVPL9, a derivative of RSV-globin (10), and inserted into the *Bam*HI site of pVL1392 (11). A RSV-*lacZ* cassette was excised from pRSV*lacZ*II (12) using *Bgl* II and *Spe* I and inserted into the *Bgl* II to *Xba* I sites of the modified transfer vector. The resulting transfer vector containing the RSV-*lacZ* cassette and SV40 RNA processing signals was used to generate recombinant virus by cotransfection with linearized AcV-EPA viral DNA (13) into Sf21 cells. Recombinant virus was plaque-purified and amplified on Sf21 cells. Amplified virus was concentrated by ultracentrifugation in an SW28 rotor (24,000 rpm; 75 min) with a 27% (wt/vol) sucrose cushion in 5 mM NaCl/10 mM Tris·HCl, pH 7.5/10 mM EDTA. The viral pellet was resuspended in phosphate-buffered saline (PBS) and sterilized by passage through a 0.45-μm filter (Nalge). AcMNPV was titered by plaque assay on Sf21 insect cells.

Growth of Cells and Viruses. Sf21 cells were provided by N. LeClerc (University of Montreal). Other cell lines used are available from the American Type Culture Collection. Primary cultures of rat hepatocytes were prepared by perfusion with collagenase as described (14).

β-Galactosidase Assays. Colorimetric assay of β-galactosidase enzymatic activity was performed essentially as described (15). Extract protein concentrations were determined using the Coomassie Plus protein assay (Pierce) with bovine serum albumin as a standard. For histochemical staining of β-galac-

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Abbreviations: AcMNPV, *Autographa californica* multiple nuclear polyhedrosis virus; RSV, Rous sarcoma virus; moi, multiplicity of infection; X-Gal, 5-bromo-4-chloro-3-indolyl β-D-galactopyranoside; CAT, chloramphenicol acetyltransferase; SV40, simian virus 40.

†To whom reprint requests should be addressed.

tosidase activity, cells were fixed in 2% (wt/vol) paraformaldehyde/0.2% glutaraldehyde in PBS for 5 min. After several rinses with PBS, the cells were stained by addition of 5-bromo-4-chloro-3-indolyl β-D-galactopyranoside (X-Gal) (0.5 mg/ml) in PBS for 2–4 hr at 37°C.

RESULTS

Susceptibility of Mammalian Cell Lines to Baculovirus-Mediated Gene Transfer. An expression cassette containing the *lacZ* reporter gene under control of the RSV promoter was inserted into a baculoviral transfer plasmid (Fig. 1) and recombined into the AcMNPV genome by cotransfection of Sf21 insect cells with linearized baculoviral DNA. The RSV promoter used in these experiments is known to give strong expression of reporter gene in a variety of mammalian cell types (10). The RSV*lacZ*-modified AcMNPV was then incubated with a variety of mammalian cell lines and baculovirus-mediated gene transfer was monitored by β-galactosidase enzyme activity.

Expression from the RSV-*lacZ* gene in various cell lines was quantified by β-galactosidase enzyme assays of extracts prepared from infected cells. Mock-infected cells were used as a control for background β-galactosidase enzyme activity. Of the 17 cell types assayed, several showed significantly increased activity in infected cells (Table 1). Most dramatically, the human hepatocellular carcinoma line HepG2 exposed to the RSV-*lacZ* baculovirus expressed >80-fold higher levels of β-galactosidase that mock-infected controls. In contrast, the more poorly differentiated human liver tumor line Sk-Hep-1 did not appear to express significant levels of the RSV-*lacZ* cassette. Baculovirus-mediated gene transfer was also detected at a much lower level in three other cell types by this assay. The adenovirus-transformed human embryonal kidney cell line 293 expressed the *lacZ* reporter gene at a level about 4-fold over background. In addition, the PC12 neuronal-like cell line and the human carcinoma line A549 each exhibited ≈2-fold higher β-galactosidase levels after infection with the RSV-*lacZ* baculovirus. These differences were statistically significant ($P < 0.05$). The other cell lines examined, representing a variety of species and cell types, did not yield significantly higher levels of β-galactosidase after infection.

Histochemical staining using X-Gal was also used to examine β-galactosidase expression in cells exposed to increasing doses of the modified AcMNPV. Stained cells were not found

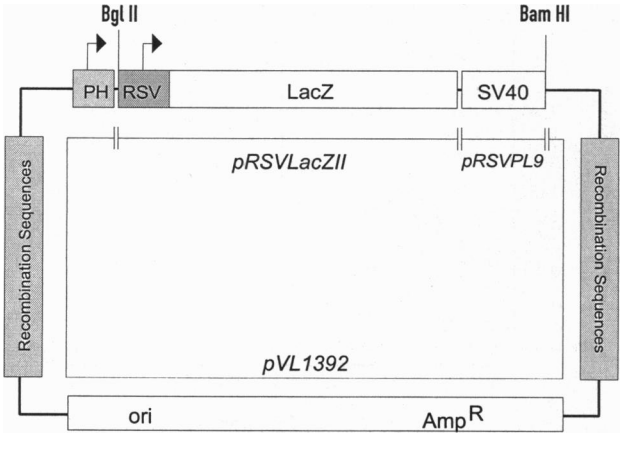

FIG. 1. Baculoviral transfer plasmid containing a RSV-*lacZ* expression cassette. The *E. coli* β-galactosidase gene (LacZ) was placed under the control of the RSV promoter together with SV40 splice and polyadenylylation signals (not drawn to scale). The transfer plasmid was then inserted into the polyhedrin region of AcMNPV by homologous recombination. Transcription initiation sites of the viral polyhedrin (PH) and RSV promoters are indicated by arrows.

Table 1. Baculovirus-mediated expression of RSV-*lacZ* reporter gene in mammalian cell lines

Cell line	β-Galactosidase activity, units/mg (mean ± SD)	
	Mock infected	RSV-*lacZ* virus
HepG2	0.030 ± 0.004	2.628 ± 0.729
Sk-Hep-1	0.019 ± 0.003	0.019 ± 0.004
NIH 3T3	0.026 ± 0.003	0.023 ± 0.005
CV-1	0.075 ± 0.034	0.094 ± 0.026
A549	0.022 ± 0.005	0.046 ± 0.011
WI-38	0.024 ± 0.004	0.036 ± 0.009
HeLa	0.034 ± 0.009	0.036 ± 0.005
CHO/dhfr$^-$	0.020 ± 0.002	0.026 ± 0.005
293	0.092 ± 0.014	0.384 ± 0.024
COS-7	0.029 ± 0.002	0.032 ± 0.007
Ramos	0.008 ± 0.002	0.011 ± 0.004
Jurkat	0.012 ± 0.004	0.007 ± 0.001
HL-60	0.042 ± 0.039	0.014 ± 0.015
K-562	0.018 ± 0.006	0.017 ± 0.002
C_2C_{12} myoblast	0.015 ± 0.001	0.014 ± 0.003
C_2C_{12} myotube	0.049 ± 0.011	0.042 ± 0.004
PC12 (+NGF)	0.019 ± 0.005	0.033 ± 0.004

Standard conditions for infection utilized 2×10^6 cells and RSV-*lacZ* AcMNPV at a moi of 15. Adherent cell lines were seeded 1 day prior to infection. Cells were exposed to virus in 2 ml of medium for 90 min, and then the virus-containing medium was removed and replaced with fresh medium. Mock-infected cells were treated with 2 ml of medium lacking the viral inoculum. Extracts were prepared from cells 1 day postinfection and assayed for β-galactosidase and protein content. Values are expressed as units of β-galactosidase activity per mg of protein. HepG2, A549, 293, and PC12 cells show significantly ($P < 0.05$; Student's *t* test) higher activity after exposure to the virus. Species and cell type of origin of cell lines are as follows: HepG2, human liver; Sk-Hep-1, human liver; NIH 3T3, mouse embryo fibroblast; CV-1, monkey kidney; A549, human lung; WI-38, human lung fibroblast; HeLa, human cervix; CHO/dhfr$^-$, hamster ovary; 293, human kidney; COS-7, monkey kidney; Ramos, human B-cell; Jurkat, human T-cell; HL-60, human promyelocyte; K-562, human myelocyte; C_2C_{12}, mouse muscle; PC12, rat adrenal.

in mock-infected cultures (Fig. 2*A*), but when HepG2 cells were exposed to the modified AcMNPV at multiplicity of infection (moi) of 10, 5–10% of the cells stained with X-Gal (Fig. 2*B*). At a moi of 100, 25–50% of the cells were stained (Fig. 2*C*). These data demonstrate that the modified AcMNPV is highly effective at gene transfer into HepG2 cells. In contrast, when the Sk-Hep-1 line was exposed to virus at a moi of 10, no stained cells were observed (Fig. 2*E*). When Sk-Hep-1 cells were exposed to virus at a moi of 100, a few cells were found that stained (Fig. 2*F*). The frequency of stained cells in the Sk-Hep-1 cell line was estimated to be 2000 to 4000-fold less than in HepG2 cells after exposure to equivalent doses of the modified virus. Thus, visualization of β-galactosidase activity by histochemical staining demonstrates low frequencies of gene transfer, which are obscured in enzymatic assays due to endogenous β-galactosidase activity. Other cell types were also stained after reaction with X-Gal, with frequencies in CV-1, COS-7, A549, and 293 cells estimated at 10- to 100-fold less than HepG2 cells (data not shown). The cell type preference demonstrated by the modified AcMNPV varies quantitatively among the cell lines examined, with the well-differentiated human liver tumor line HepG2 being by far the most susceptible to baculoviral-mediated gene expression.

Dose–Response of Baculovirus-Mediated Gene Transfer. The histochemical data presented above suggested that increasing amounts of β-galactosidase are produced after exposure of HepG2 cells to increasing amounts of virus. To quantify the dose dependence of baculovirus-mediated gene expression, HepG2 cells were exposed to increasing doses of virus and assayed for β-galactosidase enzyme activity. The amount

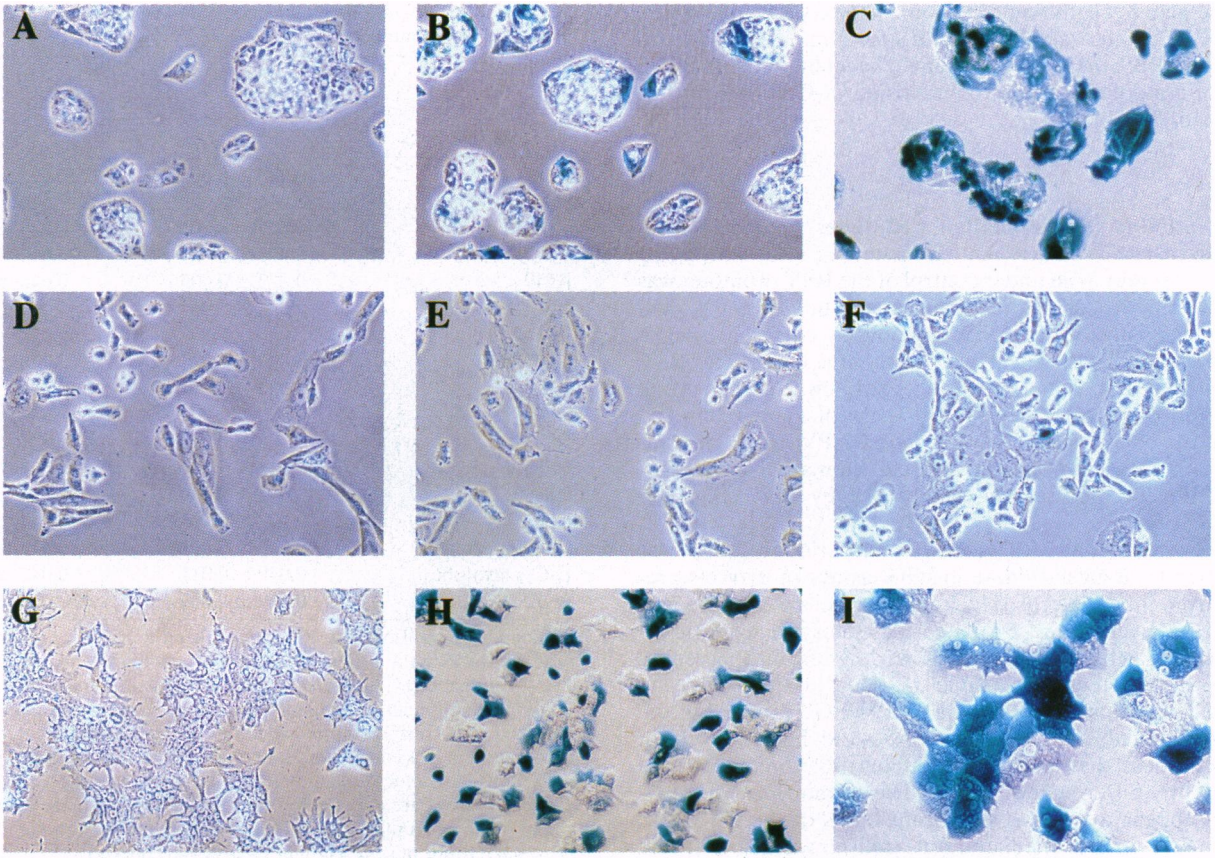

FIG. 2. Histochemical staining for β-galactosidase after baculovirus-mediated gene transfer. Cells were stained blue with X-Gal 1 day postinfection with an AcMNPV virus containing a RSV-*lacZ* cassette. (*A–C*) HepG2 cells. (*D–F*) Sk-Hep-1 cells. (*G–I*) Primary rat hepatocyte cultures. (*A, D,* and *G*) Mock-infected cells. (*B* and *E*) Cells infected at a moi of 10. (*C* and *F*) Cells infected at a moi of 100. (*H*) Low-power view of cells infected at a moi of 430. (*I*) Same as *H* except higher magnification. Morphology of stained cells is characteristic of cultured liver parenchymal cells. (*A–H*, ×10; *I*, ×20.)

of enzyme produced was linearly related to the innoculum of virus used over a wide range of doses (Fig. 3). The maximum dose of virus used was limited by the titer and volume of the viral stock, and no plateau in the amount of expression was observed with higher doses.

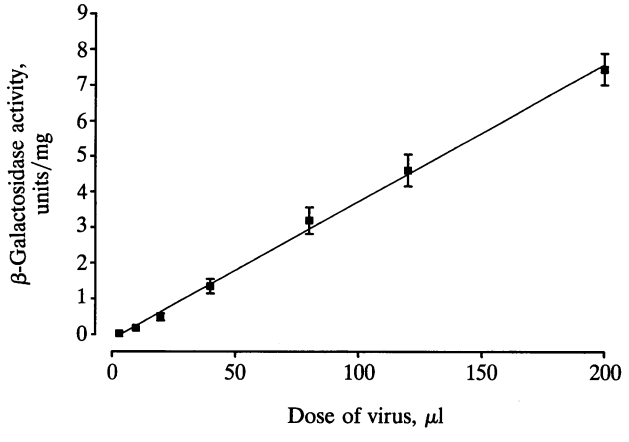

FIG. 3. Dose dependence of baculovirus-mediated gene transfer. 10^6 HepG2 cells were seeded into 60-mm Petri dishes and 1 day later exposed to the indicated dose of an AcMNPV virus containing a RSV-*lacZ* cassette (viral titer, 1.4×10^9 plaque-forming units/ml). One day postinfection, the cells were harvested and extracts were prepared and assayed for β-galactosidase enzyme activity as defined (15). Enzyme activity was normalized for the protein content of each extract. Each point is the average of three independent assays, with the error bars representing SD.

Time Course of Baculovirus-Mediated Gene Transfer. HepG2 cells were exposed to the RSV-*lacZ* virus for 1 hr, after which the cells were harvested at various times and quantitatively assayed for β-galactosidase activity. As shown in Fig. 4, β-galactosidase activity was detected as early as 6 hr after exposure to the virus and peaked 12–24 hr postinfection. As expected for an episomal DNA molecule, expression from the

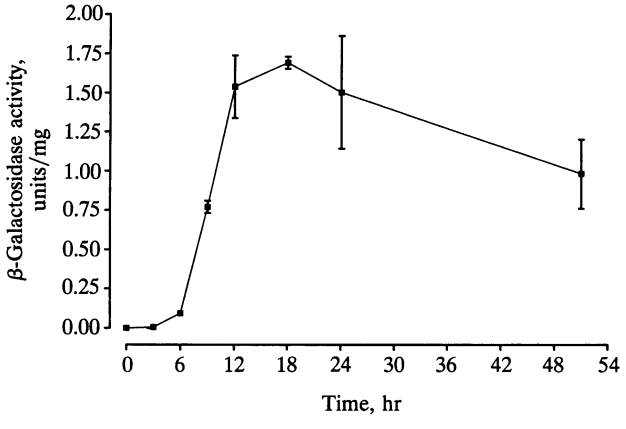

FIG. 4. Time course of baculovirus-mediated expression. HepG2 cells were infected with AcMNPV virus containing a RSV-*lacZ* cassette (moi = 15) at time 0. After 1 hr, the medium containing the virus was removed and replaced with fresh medium. Infected cells were harvested at the indicated time points and assayed for β-galactosidase activity as in Fig. 3. Expression from the virus peaks 12–24 hr postinfection and declines thereafter.

RSV-*lacZ* cassette gradually subsided at later times (Fig. 4, data not shown).

Expression Occurs *de Novo* in Mammalian Cells. Several experiments suggest that the observed expression of *lacZ* in HepG2 cells was due to *de novo* synthesis of β-galactosidase rather than from enzyme associated with the viral preparation. First, the RSV-*lacZ* virus innoculum was assayed for β-galactosidase activity and was found to be <10% that expressed after infection of HepG2 cells. Second, HepG2 cells were infected with the RSV-*lacZ* virus and then cultured in the presence of the protein synthesis inhibitor cycloheximide. Inclusion of cycloheximide postinfection inhibited the accumulation of β-galactosidase enzyme activity by >90% (Table 2). Third, HepG2 cells were infected with a baculovirus in which the *lacZ* gene was under control of the viral polyhedrin promoter rather than the RSV promoter (Table 2). The latter virus expresses extremely high levels of β-galactosidase activity in insect cells (data not shown) but fails to give significantly higher enzyme activity than in mock-infected cells (Table 2). In contrast to prior studies of baculovirus interactions with mammalian cells (9), these data demonstrate that *de novo* synthesis of *lacZ* occurs after baculovirus-mediated gene transfer into HepG2 cells.

Baculovirus-Mediated Gene Transfer Is Inhibited by Lysosomotropic Agents. Like other enveloped viruses, the budded form of AcMNPV normally enters cells via endocytosis followed by low pH-triggered fusion of the viral envelope with the endosomal membrane, thus allowing escape into the cytoplasm (16). To determine whether endosome acidification was necessary for baculovirus-mediated gene transfer into mammalian cells, HepG2 cells were infected with RSV-*lacZ* AcMNPV in the presence of chloroquine, a lysosomotropic agent. When 25 μM choroquine was continuously present during and after exposure of HepG2 cells to the virus, *de novo* expression of β-galactosidase was completely prevented (Table 2). This suggests that baculovirus-mediated gene transfer is depended on endosomal acidification. When chloroquine was added to the cells 90 min after exposure to the virus, only a partial inhibition of β-galactosidase expression was observed. Apparently, a portion (≈22%) of the virus was able to proceed through the endosomal pathway during the 90 min of exposure to the virus.

The AcMNPV Genome Can Enter Cells but Not Be Efficiently Expressed. To determine whether the susceptibility to baculovirus-mediated gene transfer results from differential internalization of the virus in various cell types, the entry of the viral genome was compared between highly susceptible HepG2 cells and the relatively refractory Sk-Hep-1 cell line. Each cell line was exposed to equivalent amounts of the RSV-*lacZ* virus and the presence of the viral genome was assayed by semiquantitative PCR of total cellular DNA. After 2 hr of exposure to the virus, both cell lines had entry of ≈10 copies of the AcMNPV genome per cell (Fig. 5*A*, lanes 2 and 5). The viral genome persisted at 24 hr postinfection, although there was a decrease in copy number in both cell lines (lanes 3 and 6). In contrast, RNA transcribed from the RSV-*lacZ* cassette was found in infected insect cells (lane 2) and HepG2 cells (Fig. 5*B*, lane 4) but not in infected Sk-Hep-1 cells (lane 5). No transcription was detected in uninfected cells (lanes 1 and 3). Thus, although the viral genome is able to enter Sk-Hep-1 cells, it does not appear to reach the proper cellular compartment to be transcribed.

Table 2. Baculovirus-mediated gene transfer occurs *de novo*

Virus	Drug during infection	Drug postinfection	β-Galactosidase activity, % of RSV-*lacZ* (mean ± SD)
RSV-*lacZ*	None	None	100 ± 5.8
None	None	None	3.2 ± 0.4
RSV-*lacZ*	None	Cycloheximide	10.3 ± 1.0
BacPAK6	None	None	2.8 ± 0.4
RSV-*lacZ*	Chloroquine	Chloroquine	2.9 ± 0.1
RSV-*lacZ*	None	Chloroquine	25.1 ± 6.2

HepG2 cells were exposed to AcMNPV virus in medium containing or lacking inhibitor for 90 min, and then the virus-containing medium was removed and replaced with fresh medium containing or lacking inhibitors as listed. One day postinfection, the cells were harvested and extracts were assayed for β-galactosidase activity and protein content. Each value represents the average of three independent assays, with the amount of β-galactosidase produced by the RSV-*lacZ* AcMNPV virus in the absence of inhibitors assigned a value of 100%. β-Galactosidase activity was normalized for protein content of each extract. AcMNPV virus with the *lacZ* gene under the control of the viral polyhedrin promoter was BacPAK6 (Clontech) used at an equivalent moi.

Baculovirus-Mediated Gene Transfer into Primary Cultures of Liver Cells. To determine whether the special susceptibility of HepG2 cells to baculovirus-mediated gene transfer is representative of normal liver cells, primary cultures of rat hepatocytes were examined for their ability to express β-galactosidase after infection with RSV-*lacZ* AcMNPV. Hepatocytes were exposed to medium containing the RSV-*lacZ* virus, and after 24 hr the cells were stained for β-galactosidase using X-Gal. No stained cells were observed in mock-infected cultures (Fig. 2*G*). Cell counting indicated that >70% of the infected cells were stained with X-Gal (Fig. 2 *H* and *I*). Thus, baculovirus-mediated gene transfer is an effective method of gene transfer into primary hepatocyte cultures.

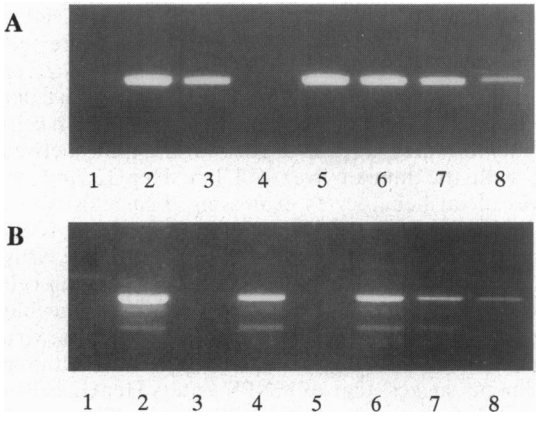

FIG. 5. PCR analysis of viral uptake and expression in HepG2 and Sk-Hep-1 cell lines. (*A*) Uptake of AcMNPV viral genome. Each cell line was exposed to AcMNPV containing a RSV-*lacZ* cassette at a moi of 10 for 2 hr, and then the viral innoculum was removed. Cells were harvested 2 and 24 hr postexposure to the virus and rinsed extensively with PBS; total cellular DNA was harvested with the TurboGen kit (Invitrogen). Purified DNA was used as template for PCR using primers specific for the RSV-*lacZ* cassette; copy number controls were reconstructed with uninfected cellular DNA and the RSV-*lacZ* transfer vector. Lane: 1, uninfected Sk-Hep-1 cells; 2, Sk-Hep-1 cells 2 hr postinfection; 3, Sk-Hep-1 cells 24 hr postinfection; 4, uninfected HepG2 cells; 5, HepG2 cells 2 hr postinfection; 6, HepG2 cells 24 hr postinfection; 7, 10 copies per cell reconstruction; 8, 1 copy per cell reconstruction. Both cell lines show internalization of the virus 2 hr postinfection and persistence of the viral genome 24 hr postinfection. (*B*) Expression of the RSV-*lacZ* cassette. Cell lines were infected with the RSV-*lacZ* virus as described in *A*. Total cellular RNA was prepared and used for reverse transcription PCR using primers specific for the *lacZ* cassette. Lanes: 1, uninfected Sf9 cells; 2, infected Sf9 cells; 3, uninfected HepG2 cells; 4, infected HepG2 cells; 5, infected Sk-Hep-1 cells; 6, same as lane 4; 7, same as lane 6 only cDNA diluted 1:3 prior to PCR; 8, same as lane 6 only cDNA diluted 1:10. Dilutions of the infected HepG2 cDNA were performed to demonstrate the sensitivity of the assay. Transcription of the RSV-*lacZ* cassette is detected in infected insect and HepG2 cells but not in uninfected cells or in infected Sk-Hep-1 cells. Mammalian RNA samples were verified to be intact by hybridization with a control probe (not shown).

DISCUSSION

The baculovirus AcMNPV is widely used as a vector for high-level expression of mammalian genes in insect cells (5, 6). This report describes the use of AcMNPV as a vector for expression of an exogenous gene in mammalian cells. A RSV-*lacZ* expression cassette was inserted into the 134-kb double-stranded circular DNA comprising the viral genome. The baculoviral virion then served as a shell for delivery of this large plasmid to mammalian cells. The human liver tumor line HepG2 was able to internalize, uncoat, and express the RSV-*lacZ* gene packaged within AcMNPV virions. Primary cultures of rat hepatocytes were also highly susceptible to infection, with >70% of cells expressing β-galactosidase after exposure to the virus. This efficiency of transfection of primary liver cells exceeds that obtained with conventional retroviral vectors (17), VSV-G pseudotyped retrovirus (18), or herpes simplex virus vector (19). Importantly, the virus does not appear to replicate or cause cytopathology in HepG2 cells, and the viral polyhedrin gene transcription unit was inactive. Further experiments will be required to determine whether any of the viral genes are expressed after gene transfer into mammalian cells.

Previous studies have described uptake of AcMNPV by mammalian cell lines without expression of viral genes or viral replication (9, 20, 21). The human lung carcinoma line A549 was found to be more susceptible to AcMNPV entry than were other cell lines (22), but when a baculovirus containing a RSV-CAT cassette was used to infect A549 cells, no evidence of *de novo* expression of CAT was observed (9). Our studies confirm that AcMNPV virions can be internalized within mammalian cells and that viral genes are not expressed after infection of mammalian cells. However, using a *lacZ* reporter gene under the control of the RSV promoter, we have detected *de novo* expression of β-galactosidase in mammalian cells. The capability to express a reporter gene varied widely between cell types, with the human liver cell line HepG2 and primary cultures of rat hepatocytes expressing β-galactosidase 10- to 1000-fold more frequently than other cell types. This does not appear to be due to increased internalization of the virus since both high (HepG2) and low (Sk-Hep-1) expressing cell lines internalized similar amounts of virus. Rather, the block to expression appears to occur during uncoating of the virus and transport to the cellular transcription apparatus. Although our experiments suggest that AcMNPV enters HepG2 cells via an endosomal pathway, further experiments will be needed to define the events by which AcMNPV is expressed in liver cells.

Current gene transfer vectors are based on animal viruses that must be engineered to remove functions involved in expression of viral genes and viral replication. In contrast, the strategy used in this study utilized a virus that was previously known not to replicate or express viral genes after exposure to mammalian cells. Through the addition of vertebrate transcriptional control sequences, we were able to achieve transient expression of an exogenous gene in mammalian cells. Thus, our strategy is based on the addition of useful elements rather than on the elimination of unwanted ones. It is not difficult to envision engineering of additional features into the virus, such as elements that will allow autonomous replication or integration of the virus to give persistence of gene expression. Because recombinant baculovirus are simple to manipulate, are able to accommodate large inserts, and can be concentrated to titers of $>10^{10}$-plaque-forming units/ml, they are an attractive platform for further innovation.

Much more work will be necessary to evaluate the safety and efficacy of AcMNPV as a tool for human gene therapy. However, our studies clearly demonstrate that a nonanimal virus may be used as a shell for delivery of nucleic acids to animal cells. This raises the possibility that other viruses with unique properties may also be engineered for use as "xenovectors" for human gene therapy.

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