Rapid Publication

Baculovirus-Mediated Gene Transfer Into Pancreatic Islet Cells

Li Ma, Natalia Tamarina, Yong Wang, Andrey Kuznetsov, Nipam Patel, Cory Kending, Bernhard J. Hering, and Louis H. Philipson

Baculovirus transduction is a gene transfer method that uses a moth cell virus for mammalian cells in culture, which results in a high-level prolonged expression. Here we demonstrate that recombinant baculoviruses can serve as efficient gene transfer vehicles for delivering foreign genes driven by mammalian promoters into human and mouse pancreatic islet cells. Existing methods, such as various transfection and electroporation techniques, either suffer from low efficiency or cause extensive membrane damage. Viral vectors have emerged as an important tool for gene delivery and expression in mammalian cells but suffer from several drawbacks, such as lengthy construction time and expression of viral genes. The baculovirus *Autographa californica* multiple nuclear polyhedrosis virus is widely used as a vector for expression of foreign genes in insect cells and, more recently, in some mammalian cells. Using several green fluorescent protein– and LacZ-expressing constructs in a cytomegalovirus promoter cassette, we obtained efficient gene expression in primary human and mouse islet cells. There was no impairment of glucose-stimulated intracellular free calcium responses after baculovirus infection. The safety and the relative ease of construction and propagation of the virus makes the baculovirus system a useful tool for facilitating the transfer of foreign genes. *Diabetes* 49:1986–1991, 2000

Delivering foreign genes into mammalian cells is an important tool to study the expression and functions of those genes in a particular cellular environment. Many approaches have been used for gene transfer, including transfection, electroporation, direct injection, and viral vectors. The efficiency of gene transfer varies dramatically depending on the target cells, culture conditions, vectors, and methods. The extent of cell damage also varies greatly for each method. Development of methods for efficient and stable gene transfer to primary pancreatic islet cells is very important for understanding the function of pancreatic β-cell genes and for misexpression analysis, promoter analysis, and gene therapy. Nonviral techniques including lipofection, electroporation, and biolistic particles lack either sufficient gene transfer or stable gene expression, or result in substantial damage (1). The most commonly used viral vectors for β-cell and islet expression are derived from adenovirus, adenoassociated virus, or HIV-1 lentiviruses (2–5). Newgard and colleagues (2,5) have convincingly demonstrated the utility of recombinant adenovirus for gene delivery systems for rat islets, either in vitro or via the perfused pancreas. Although recent improvements using replication-deficient virus have reduced some of the difficulties, additional methods for gentle and efficient gene transfer in the absence of the expression of viral genes are desirable.

The baculovirus *Autographa californica* multiple nuclear polyhedrosis virus has widely been used for recombinant gene expression in insect cells (6–9). Recently, it was shown that baculovirus can be transported into some primary cells and into many cell lines derived from mouse, rat, porcine, and human tissues (10–17). Using mammalian promoters, baculovirus enabled efficient transient expression of reporter genes in mammalian cells (10–17). Condreay et al. (17) also reported stable baculovirus transduction of several mammalian cell types with expression of a reporter gene for multiple passages.

In this study, we examined the ability of a baculovirus construct to deliver a reporter gene to pancreatic β-cells and islets in culture. We used recombinant baculovirus vectors comprising the cytomegalovirus (CMV) immediate early promoter and green fluorescent protein (GFP) or the lacZ reporter gene. We determined that the efficiency of gene delivery into primary mouse and human islet cells using this viral vector was, on average, 85%, with persistent expression lasting at least 7 days. We also examined the effects of the baculovirus transduction on glucose-induced changes in intracellular free Ca^{2+} ($[Ca^{2+}]_i$) in mouse pancreatic β-cells and found no significant effect on oscillatory responses in $[Ca^{2+}]_i$.

RESEARCH DESIGN AND METHODS

Recombinant baculovirus construction. One of the baculoviruses and its transfer vector (pFastBacMam1 GFP and pFastBac GFP, respectively) were provided by P. Condreay (GlaxoWelcome) and are described in the article by Condreay et al. (17). BacCMV-GFP and BacCMV-*LacZ* were also used in this study. BacCMV-GFP cassette was derived from pEGFP-C3 (Clontech) as an

From the Department of Medicine (L.M., N.T., Y.W., A.K., L.H.P.) and the Department of Organismal Biology and Anatomy, Howard Hughes Medical Institute (N.P., C.K.), the University of Chicago, Chicago, Illinois; and the Department of Surgery (B.J.H.), the University of Minnesota, Minneapolis, Minnesota.

Address correspondence and reprint requests to Louis H. Philipson, MD, PhD, Dept. of Medicine, MC 1027, 5841 S. Maryland Ave., Chicago, IL 60637. E-mail: l-philipson@uchicago.edu.

Received for publication 24 July 2000 and accepted 14 September 2000. Posted on the World Wide Web at www.diabetes.org/diabetes on 7 November 2000.

AM, acetoxymethyl; BSA, bovine serum albumin; $[Ca^{2+}]_i$, intracellular free Ca^{2+}; CMV, cytomegalovirus; EGFP, enhanced GFP; GFP, green fluorescent protein; KRB, Krebs-Ringer buffer; MOI, multiplicity of infection; PBS, phosphate-buffered saline.

*Nsi*I-*Mlu*I fragment. The ends were polished with Klenow fragment and cassette inserted into the EcoRV site of transfer vector 3272 (18) (Fig. 1). To construct the BacCMV-*LacZ* cassette, hp 70/nuclacZ plasmid (18) was digested with *Hind*III and *Xba*I to remove the hp 70 promoter, and the pCMV–β-gal (Stratagene) *Nsi*I-*Nhe*I fragment containing CMV promoter was inserted in its place upstream of the *LacZ* gene. The resulting cassette was excised with *Sal*I and *Not*I, blunted, and inserted into the EcoRV site of transfer vector 3272. Two transfer vector constructs with the opposite orientation of the CMV-*LacZ* cassette relative to the baculoviral genes were named BacCMV-*LacZ*for and BacCMV-*LacZ*rev (Fig.1). Baculoviruses were constructed via homologous recombination using BacPak (Clontech) as the parental virus and the transfer vector containing the expression cassettes. Recombinant viruses were purified by three rounds of the end-point dilution method.

Recombinant baculovirus amplification. The transfer vector containing the expression cassettes and the parental virus were cotransfected into Sf9 (*Spodoptera frugiperda*) cells by lipofection. The recombinant virus was further amplified by propagation in the Sf9 cell line in Grace-supplemented insect media containing 10% fetal bovine serum (HyClone) with 0.1% (vol/vol) pluronic F-68 and was incubated at 28°C for 2 days. The culture medium was then harvested and used to infect Sf9 cells in a standard plaque assay. The inserted *LacZ* gene was detected using β-galactosidase expression with X-gal substrate. The plaques were then isolated and purified by a third round of plaque isolation. The virus was concentrated by centrifugation at 80,000*g* in sucrose cushion solution (25% sucrose [wt/wt] in 5 mmol/l NaCl and 10 mmol/l EDTA) for 75 min at 4°C and was resuspended in Dulbecco's phosphate-buffered saline (PBS)-supplemented medium containing 2% (vol/vol) fetal bovine serum. The viral titers were determined by an end-point dilution method (19).

Cell culture. The islet and dispersed islet cells were isolated from pancreata of 8- to 10-week-old C57BL/6J mice (The Jackson Laboratory, Bar Harbor, ME) using collagenase digestion followed by discontinuous Ficoll gradient centrifugation (20). The islet cells were dissociated using 0.25 mg/ml trypsin. The cells were then plated on the glass cover slips and incubated in RPMI-1640–supplemented medium containing 11 mmol/l glucose, 10% fetal calf serum, 100 U/ml penicillin, and 100 μg/ml streptomycin at 37°C for 2–3 days before the transduction.

Transduction of islet cells with recombinant baculovirus. The islet cells were cultured for 2–3 days. The culture medium was removed and replaced with the medium containing 10% (vol/vol) heat-inactivated fetal calf serum and the recombinant baculovirus and was incubated at 37°C for 1 h. The cells were then washed with PBS three times and were further incubated at 37°C for 2–10 days. GFP expression was determined using fluorescence microscopy.

Detection of β-galactosidase expression. Islet cells were washed twice in PBS and fixed in 2% paraformaldehyde/0.2% glutaraldehyde in PBS (5 min). β-Galactosidase expression was detected using 1 mg/ml 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside (X-Gal) in a medium containing (in mmol/l) 5 $K_3Fe(CN)_6$, 5 $K_4Fe(CN)_6$, and 2 $MgCl_2$.

Immunofluorescence detection of GFP and insulin coexpression. After baculovirus transduction, the islet cells on coverslips were fixed in 1% paraformaldehyde (room temperature, 3 h). The cells were then washed three times with PBS and incubated in 2% bovine serum albumin (BSA)/PBS (30 min). The cells were double-labeled, and the following primary antibodies were used: a polyclonal rabbit GFP antibody (1:200; Clontech) and guinea pig anti–bovine insulin antibody (1:150; Linco Research) incubated in 2% BSA/PBS (room temperature, 60 min). The cells were then washed and incubated in 2% donkey serum/PBS for 30 min. The conjugated second fluorescent antibodies, fluorescein isothiocyanate–conjugated anti-rabbit (1:100; Jackson Research Laboratories), and tetramethyl rhodamine–conjugated anti– guinea pig (1:100; Jackson ImmunoResearch Laboratories) were then added and incubated in the mixture for 60 min. The insulin and GFP expressions were visualized using a fluorescent microscope and specific filters.

$[Ca^{2+}]_i$ measurements. Between 2 and 7 days after transduction with the recombinant baculovirus, the islet cells were incubated in Krebs-Ringer buffer (KRB) medium containing 5 μmol/l fura 2-AM (acetoxymethyl ester) (Molecular Probes, Eugene, OR) for 30 min at 37°C, and $[Ca^{2+}]_i$ was estimated using a dual-wavelength fluorescence video microscopy imaging system as previously described (21). The positive infected cells were visualized by GFP detection.

Electron microscopy. The mouse pancreatic β-cells were incubated with BacCMV-GFP virus for 1.5 h at 37°C. The cells were washed, fixed with 1.25% glutaraldehyde and 4% paraformaldehyde in 0.1 mol/l sodium cacodylate buffer (pH 7.3), and embedded in Spurr resin. Ultrathin sections were cut, placed on copper grids, and stained with uranyl acetate at the electron microscopy core facility at the University of Chicago. Grids were examined in a Philips CM-120 transmission electron microscope.

Data presentation and statistical analysis. Results are expressed as means ± SE unless otherwise stated.

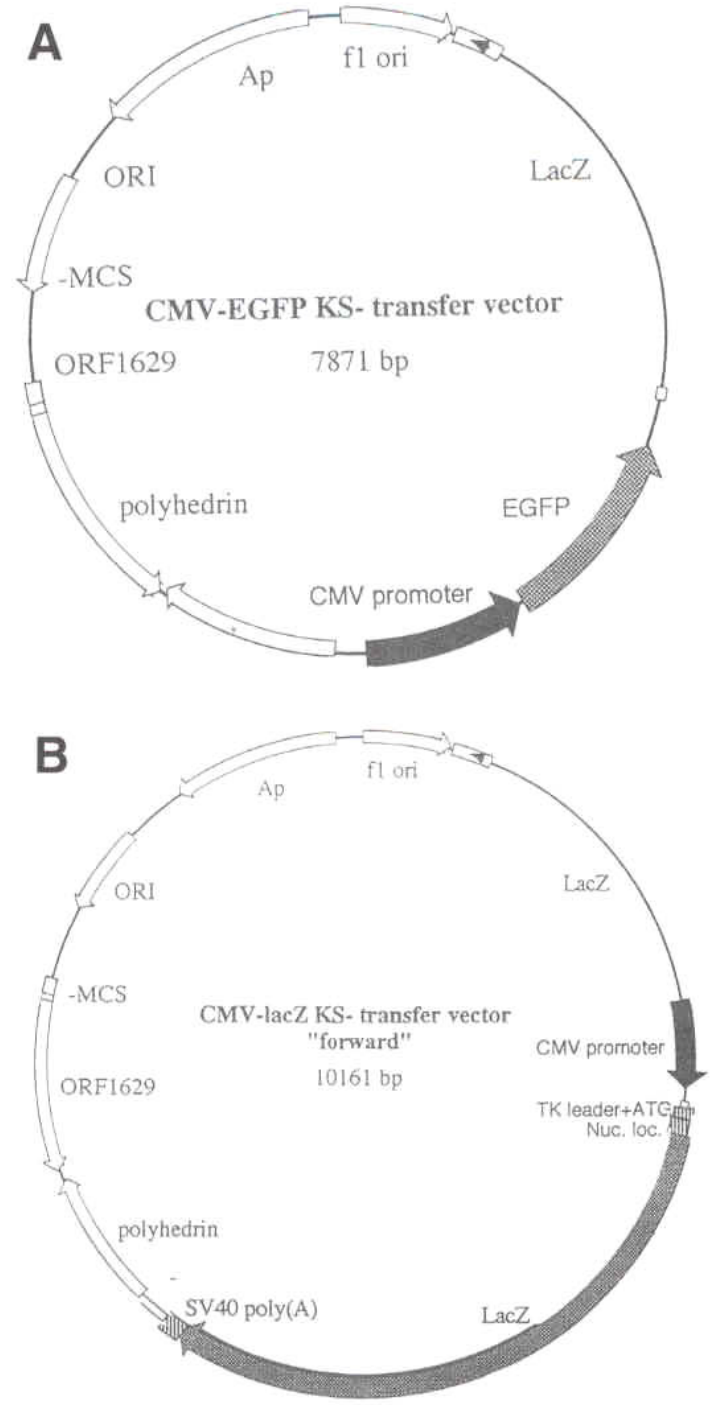

FIG. 1. Transfer vectors used to generate recombinant baculovirus. *A*: BacCMV-GFP. *B*: BacCMV-lacZ-forward. BacCMV-lacZ-reverse transfer vector sequences between nucleotides 2216 and 6144 were present in reverse orientation.

RESULTS

Baculovirus-mediated gene transfer to human and mouse pancreatic islet dissociated cells. Using the recombinant baculovirus BacCMV-GFP, enhanced GFP (EGFP) expression was observed in both primary mouse and human pancreatic dissociated islet cells (Figs. 2 and 3) at a multiplicity of infection (MOI) of 100 plaque-forming units per cell. Expression of EGFP could be detected as early as 10–12 h after virus treatment. Expression continued to increase from 24 to 36 h (data not shown); no statistically significant differences in expression

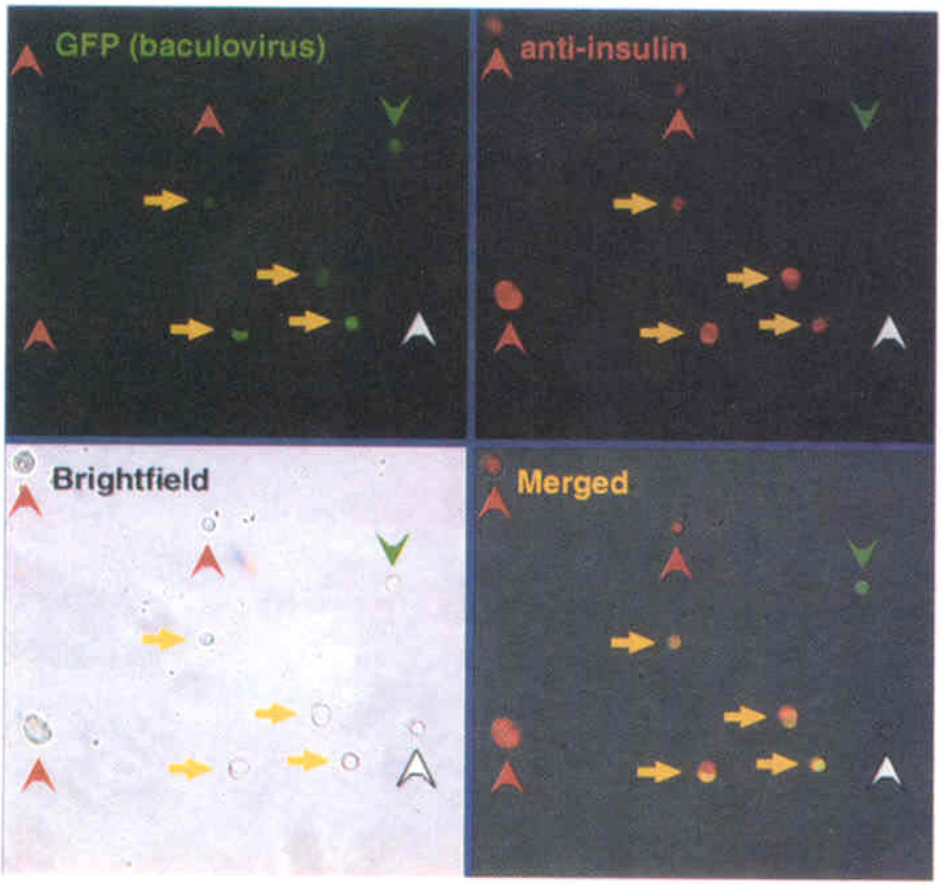

FIG. 2. Immunofluorescence images of mouse pancreatic dissociated islet cells infected with BacCMV-GFP. Cells were visualized after immunofluorescence double-labeling combining antiserum to GFP (green color and green arrows), antiserum to insulin (red color and red arrows), and uninfected cells (white arrows). Cells positive for both GFP and insulin are indicated by the yellow arrows in each panel.

level between dissociated islet cells cultured for 2 or 7 days after infection were noted. There was no significant background fluorescence expression in uninfected human or mouse pancreatic dissociated islets cells.

Pancreatic β-cells can be transducted efficiently by baculovirus. BacCMV-GFP infected mouse (Fig. 2) and human (Fig. 3) pancreatic dissociated islet cells with similar efficiency. The expression rate shows a dose-dependent

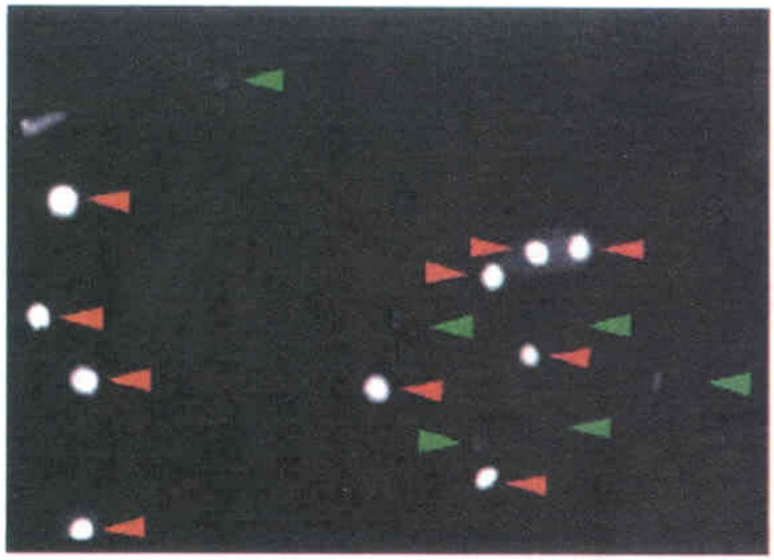

FIG. 3. Transduction of human pancreatic dissociated islet cells with BacCMV-GFP baculovirus. The cells expressing GFP showed bright fluorescence and were clearly distinguished from nontransfected cells by laser scanning confocal microscopy. The green arrowheads indicate cells that were visible but not expressing GFP, whereas the GFP$^+$ cells are indicated by the red arrowheads. This is a typical field with 10 of 16 cells positive for GFP.

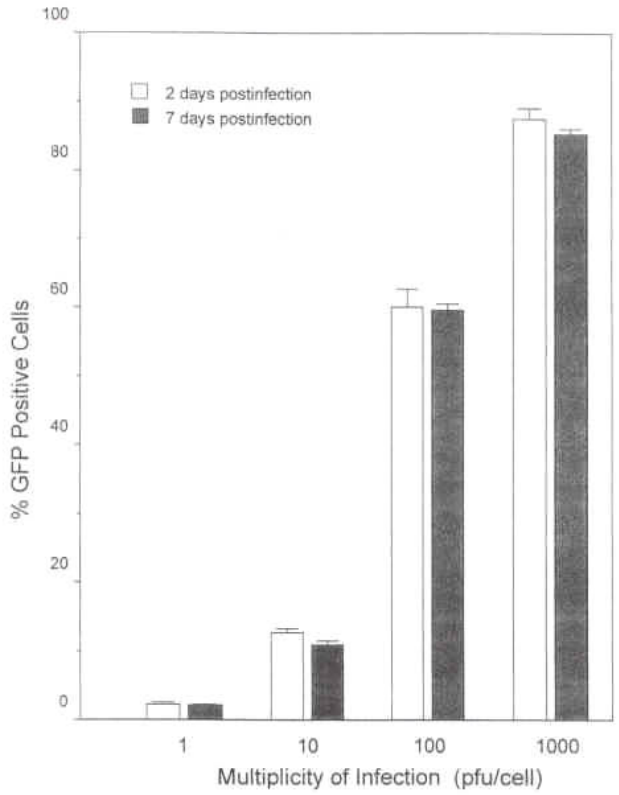

FIG. 4. Transfer and expression of mouse pancreatic β-cells with BacCMV-GFP baculovirus. Results are averages of three independent transductions, and the errors are SEs. Data represent means ± SE.

relationship to the viral dose (MOI) during 48 h of coculture, equal plaque-forming units in the SF9 cell plaque assay (Fig. 4). The GFP signal could be detected at an MOI of 1:0.1, and the highest expression was achieved with coculture at an MOI of 1:1,000.

A critical issue for both gene therapy and basic science applications of gene transfer technology is the ability of the various vectors to infect pancreatic β-cells. After direct double-labeling combining antiserum to GFP with antiserum to insulin, most insulin-positive cells were transducted with BacCMV-GFP viruses, as indicated by strong positive staining for GFP (Fig. 2). Electron microscopy demonstrated pancreatic β-cells identified by the presence of typical granules that were taking up and uncoating baculovirus (Fig. 5). This result is similar to the findings of Condreay et al. (17) in CHO cells and Hofmann et al. (10) in HuH-7 cells incubated with a baculovirus vector.

Pancreatic β-cell function after baculovirus transduction. One of the characteristics of islet β-cells is their ability to increase $[Ca^{2+}]_i$ in response to an increase in glucose concentration. Pancreatic islet cells (as well as the control cells) transducted with BacCMV-GFP at an MOI of 1:1,000, 75.2% of the GFP$^+$ BacCMV–GFP-transducted cells demonstrated an increase in $[Ca^{2+}]_i$ upon stimulation with 14 mmol/l glucose, and 100% did so after exposure to 30 mmol/l KCl (Fig. 6). The number of glucose-responsive and KCl-responsive cells in the control sample was similar (77.7 and 100%). Culturing the transducted islet cells for up to 7 days did not change their normal response to glucose and KCl.

Baculovirus transduction of intact mouse islets. Mouse islets (freshly isolated or 2 days old) were cocultured for 1 h at 37°C with BacCMV-GFP at an MOI of 1:1,000. Unlike dissociated mouse islet cells (85% at an MOI of 1:1,000), only a few cells per islet would typically express GFP after 1–7 days incubation (data not shown).

DISCUSSION

Our results demonstrate that recombinant baculovirus can be used to deliver functional genes into human and mouse pancreatic insulin-expressing cells. The percentage of GFP$^+$ cells after transduction with baculovirus was similar to that reported after adenovirus and lentivirus expression; all three viral vectors can induce gene expression in dissociated islet cells at an efficiency >85% (1–5). However, both adenoviral and lentiviral vectors are based on animal viruses that must be engineered to remove functions involved in the expression of viral genes and viral replication. Baculovirus infection does not lead to expression of viral genes or viral

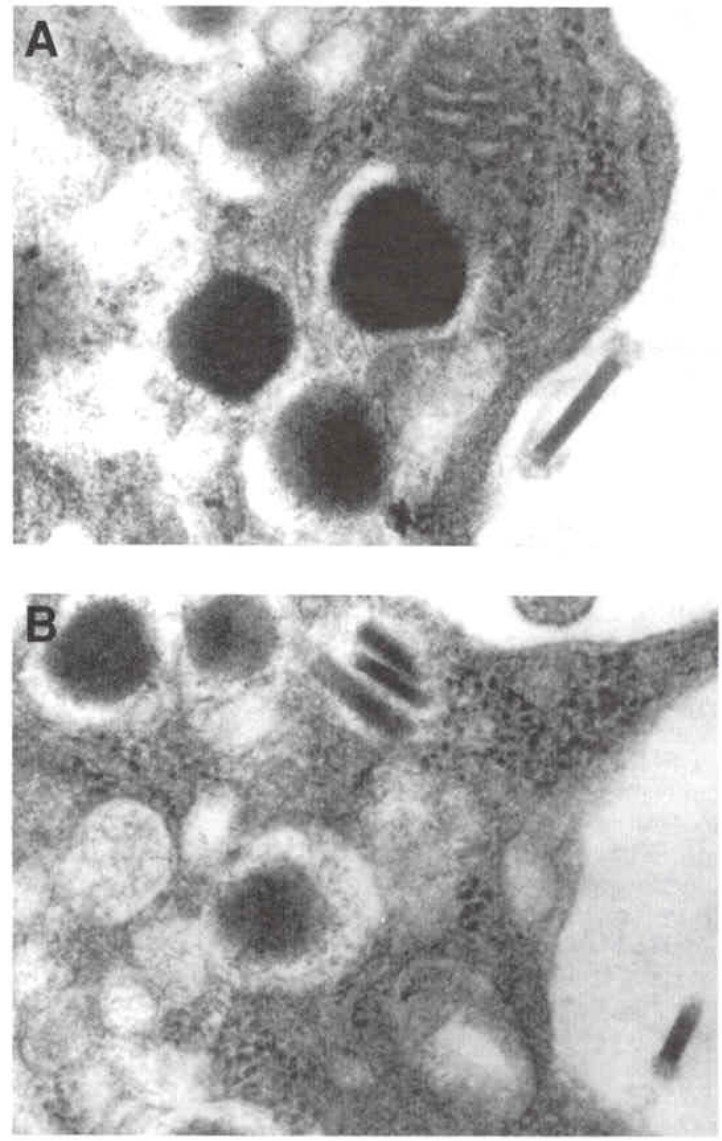

FIG. 5. Detection of baculovirus particles in mouse pancreatic β-cells by electron microscopy. The pancreatic β-cells were transducted with the recombinant baculovirus BacCMV-GFP at a MOI of 1,000 for 1.5 h. *A*: Virus particle at the surface of cell (magnification ×90,000). *B*: Virus particles enclosed on membrane vesicle (magnification ×90,000).

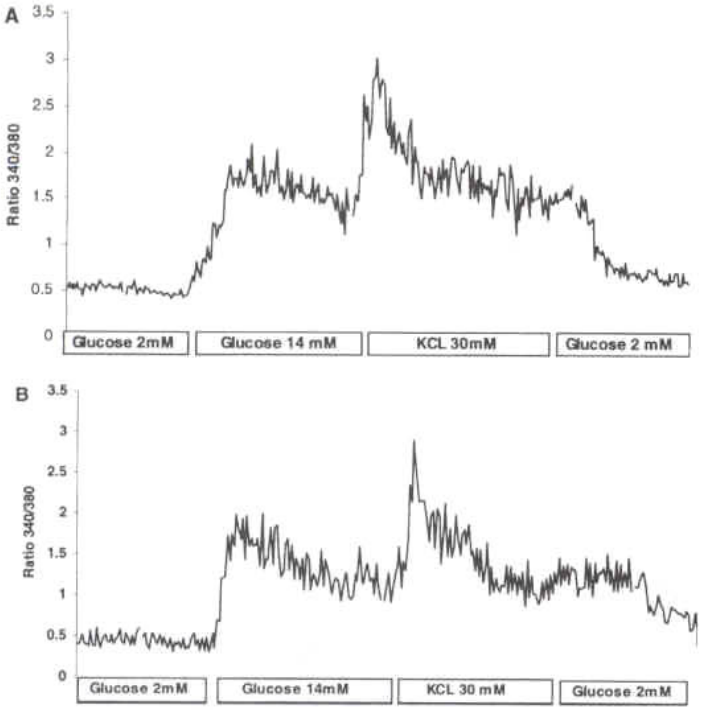

FIG. 6. $[Ca^{2+}]_i$ responses to glucose and KCl in GFP$^+$ cells after 3 days of infection with baculovirus. GFP$^+$ (*A*) and control islet cells (*B*) were loaded with 5 μmol/l fura 2-AM with 2 mmol/l glucose 30 min in KRB and were then perfused with KRB with 14 mmol/l glucose and 30 mmol/l KCl. Changes in $[Ca^{2+}]_i$, as reflected by the ratio A340:380, were monitored with a computerized digital imaging system. Percentage of cells showing increases in $[Ca^{2+}]_i$ as a response to 14 mmol/l glucose and 30 mmol/l KCl were 75.2 and 100% for GFP$^+$ cells (n = 109) and 77.7 and 100% for control cells (n = 121) (n is the total islet cell number in each category).

replication after uptake by mammalian cells (8,9). Whereas most replication-deficient viral gene delivery systems for mammalian cells require the use of a helper virus for propagation, the baculovirus is propagated in insect cells, thus avoiding any risk of a replication-competent virus.

Previous studies have described the ability of baculovirus to enter mammalian cells and express genes from a mammalian-active promoter (Rous sarcoma virus–long-terminal repeat), but the efficiency was <0.05% in mammalian cells at an equivalent MOI at room temperature (8). The most successful use of baculovirus for delivery and expression of genes in mammalian cells used a CMV promoter and transduction at 37°C (10–17). We found that transduction of primary pancreatic dissociated islet cells and other cell lines using baculovirus at 37°C was four- to fivefold higher than that at room temperature (L.M., L.H.P., unpublished data). Additionally, the level of expression of a foreign gene from a recombinant vector is determined in part by the strength of both the enhancer and promoter that drive its transcription. Expression of chloramphenicol acetyl transferase activity was >12-fold stronger from *p*CMV*cat* than from *p*RSV*cat* in the same mammalian cell lines (not endocrine cells) (22).

Multiple gene–expression vectors offer an alternative to cotransduction. Multiple gene–expression vectors enable reproducible ratios of products to be provided in each transduced cell. We constructed different orientations of CMV-*LacZ* cassette relative to the baculovirus genes and found that they gave the same level of expression in mammalian cells (data not shown). Thus, we can use a single recombinant baculovirus for delivery and expression of multiple genes.

Glucose-stimulated insulin secretion by β-cells is initiated via induction of $[Ca^{2+}]_i$ transients, and $[Ca^{2+}]_i$ plays a critical permissive role in the secretory process (23). We showed that recombinant baculovirus transduction does not alter islet β-cell function as assessed by the $[Ca^{2+}]_i$ responses to glucose and KCl.

For whole-islet transductions, the baculovirus, like adenovirus, only affects cells at the periphery of the islets (3–5). It is likely that the geometry of the islet and the existence of tight junctions between islet cells does not allow cells in the inner core of the islet to be exposed to viral particles.

In summary, we found that transduction of mouse and human pancreatic islet cells using recombinant baculovirus can provide efficient gene transfer without impairing β-cell Ca^{2+} responses. The safety and the relative ease of construction and propagation of the virus makes the baculovirus system a useful tool for the study of gene expression and the function of gene products by facilitating transfer of foreign genes.

ACKNOWLEDGMENTS

This work was supported by National Institute of Diabetes and Digestive Disease and Kidney Diseases grants DK-48494 (L.H.P.) and DK-44840, the Diabetes Research and Training Center at the University of Chicago, and the Blum-Kovler Foundation. This work was also supported by the Juvenile Diabetes Foundation International cooperative agreement 598002 (B.J.H.). N.H.P. is an Investigator with the Howard Hughes Medical Institute.

We would like to thank Patrick Condreay, Vytas Bindokas, and Daniel Oppenheimer for helpful discussions. We would also like to thank Jeff Ansite, Thomas Gilmore, Jeremy Oberbroeckling, and Hui-Jian Zhang, MD, for isolating islets used in this study.

REFERENCES

1. Saldeen J, Curiel DT, Eizirik DL, Andersson A, Strandell E, Buschard K, Welsh N: Efficient gene transfer to dispersed human pancreatic islet cells in vitro using adenovirus-polylysine/DNA complexes or polycationic liposomes. *Diabetes* 45:1197–1203, 1996
2. Becker TC, BeltrandelRio H, Noel RJ, Johnson JH, Newgard CB: Overexpression of hexokinase I in isolated islets of Langerhans via recombinant adenovirus. *J Biol Chem* 269:21234–21238, 1994
3. Sigalla J, David A, Anegon I, Fiche M, Huvelin JM, Boeffard F, Cassard A, Soulillou JP, Le Mauff B: Adenovirus-mediated gene transfer into isolated mouse adult pancreatic islets: normal β-cell function despite induction of an anti-adenovirus immune response. *Hum Gene Ther* 8:1625–1634, 1997
4. Leibowitz G, Beattie GM, Kafri T, Cirulli V, Lopez AD, Hayek A, Levine F: Gene transfer to human pancreatic endocrine cells using viral vectors. *Diabetes* 48:745–753, 1999
5. Wang MY, Shimabukuro M, Lee Y, Trinh KY, Chen JL, Newgard CB, Unger RH: Adenovirus-mediated overexpression of uncoupling protein-2 in pancreatic islets of Zucker diabetic rats increases oxidative activity and improves β-cell function. *Diabetes* 48:1020–1025, 1999
6. Himeno M, Sakai F, Onodera K, Nakai H, Fukada T, Kawade Y: Formation of nuclear polyhedral bodies and nuclear polyhedrosis virus of silk worm in mammalian cells infected with viral DNA. *Virology* 33:507–512, 1967
7. McIntosh AH, Shamy R: Biological studies of a baculovirus in a mammalian cell line. *Intervirology* 13:331–341, 1980
8. Corbonell LF, Klowden MJ, Miller LK: Baculovirus-mediated expression of bacterial genes in dipteran and mammalian cells. *J Virol* 56:153–160, 1985
9. Corbonell LF, Miller LK: *Appl Environ Microbiol* 53:1412–1417, 1987
10. Hofmann C, Sandig V, Jennings G, Rudolph M, Schlag P, Strauss, M: Efficient gene transfer into human hepatocytes by baculovirus vectors. *Proc Natl Acad Sci U S A* 92:10099–10103, 1995
11. Boyce FM, Bucher NLR: Baculovirus-mediated gene transfer into mammalian

cells. *Proc Natl Acad Sci U S A* 93:2348–2352, 1996
12. Sabdug V, Hofmann C, Steinert T, Jennings G, Schlag P, Strauss M: Gene transfer into hepatocytes and human liver tissue by baculovirus vectors. *Hum Gene Ther* 7:1937–1945, 1996
13. Murges D, Kremer A, Knebel-Mörsdorf D: Baculovirus transactivator IE1 is functional in mammalian cells. *J Gen Virol* 78:1507–1510, 1997
14. Shoji I, Aizaki H, Tani H, Ishii K, Chiba T, Saito I, Miyamura T, Matsuura Y: Efficient gene transfer into various mammalian cells, including non-hepatic cells, by baculovirus vectors. *J Gen Virol* 78:2657–2664, 1997
15. Palombo F, Monciotti A, Recchia A, Cortese R, Ciliberto G, Moneca NL: Site-specific integration in mammalian cells mediated by a new hybrid baculovirus-adeno-associated virus vector. *J Virol* 72:5025–5034, 1998
16. Fipaldini C, Bellei B, Monica NL: Expression of hepatitis C virus cDNA in human hepatoma cell line mediated by a hybrid baculovirus-HCV vector. *Virology* 255:302–311, 1999
17. Condreay JP, Witherspoon SM, Clay WC, Kost TA: Transient and stable gene expression in mammalian cells transduced with a recombinant baculovirus vector. *Proc Natl Acad Sci U S A* 96:127–132, 1999
18. Oppenheimer DR, MacNicol AM, Patel N: Functional conservation of the wingless engrailed interaction as shown by a widely applicable baculovirus misexpression system. *Curr Biol* 9:1288–1296, 1999
19. O'Reilly DR, Miller LK, Luckkow VA: *Baculovirus Expression Vectors: A Laboratory Manual*. Oxford UP, New York, 1992
20. Lacy PE, Mostianovaky M: Method for isolation of intact islets of Langerhans from the rat pancreas. *Diabetes* 16:35–39, 1967
21. Roe MW, Mertz RJ, Lancaster ME, Lancaster Worley III, JF, Dukes ID: Thapsigargin inhibits the glucose-induced decrease of intracellular Ca^{2+} in mouse islets of Langerhans. *Am J Physiol* 266:E852–E862, 1994
22. Foecking MK, Hofstetter H: Powerful and versatile enhancer-promoter unit for mammalian expression vectors. *Gene* 45:101–105, 1986
23. Philipson LH: β-cells ion channels: key to endodermal excitability. *Horm Metab Rec* 31:455–461, 1999