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, adeno-associated virus, or HIV-1 lentiviruses (2–5). Newgard and colleagues (2,5) have convincingly demonstrated the utility of recombinant adeno virus for gene delivery systems for rat islets, either in vitro or via the perfused pancreas. Although recent improvements using replication-deficient viruses 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). Condrea 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 consisting of 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 Ca²⁺ ([Ca²⁺]) in mouse pancreatic β-cells and found no significant effect on oscillatory responses in [Ca²⁺].

**RESEARCH DESIGN AND METHODS**

**Recombinant baculovirus construction.** One of the baculoviruses and its transfer vector (pFastBacMan1 GFP and pFastBac GFP, respectively) were provided by P. Condrea (Gliox&Welcome) and are described in the article by Condrea 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
Electron microscopy. The mouse pancreatic islets described (21). The positive infected cells were visualized by GFP detection using a transmission electron microscope.

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

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...
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 transduced 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
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-transduced 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

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**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.

**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).
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 pCMVcat than from pRSVcat 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 [Ca2+]i transients, and [Ca2+]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 [Ca2+]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 Ca2+ 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.

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