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Original Article

Interstitial tissue-specific gene expression in mouse testis by intra-tunica albuguineal injection of recombinant baculovirus

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Abstract

The purpose of this study is to establish a gene delivery system for interstitial tissue-specific protein expression in mice testes using modified recombinant baculovirus. Green fluorescent protein (GFP)-expressing recombinant baculovirus (GFP-baculovirus), in which the insect cell-specific polyhedron promoter was replaced by the cytomegalovirus (CMV)-IE promoter, was used to transfect testicular cells *in vitro*, and for intra-tunica albuguineal injection of the interstitial tissue of the testis. GFP expression was monitored in frozen testes sections by fluorescence microscopy. Expression of GFP in testicular tissues was also assessed by reverse transcription polymerase chain reaction (RT-PCR), and protein expression was assessed by Western blot. Testicular cells *in vitro* were infected efficiently by modified recombinant GFP-baculovirus. Intra-tunica albuguineal injection of GFP-baculovirus into the mouse testis resulted in a high level of GFP expression in the interstitial tissues. RT-PCR analysis clearly showed GFP gene expression in the testis, particularly interstitial tissues. Intra-tunica albuguineal injection of a modified baculovirus that encoded recombinant rat insulin-like growth factor binding protein (IGFBP)-5 resulted in an increase in IGFBP-5 in testis and semen. In conclusion, we have developed an efficient delivery system for gene expression *in vivo* in testicular cells, particularly cells of the interstitial tissue using intra-tunica albuguineal injection of a modified recombinant baculovirus. This method will be particularly relevant for application that requires gene delivery and protein expression in the testicular cells of the outer seminiferous tubule of the testis.

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1 Introduction

The baculovirus Autographa californica multinuclear polyhedrosis (AcMNPV) has a 130-kilobase (kb) double-stranded DNA genome that is packaged into a rod-shaped (25 nm by 260 nm) enveloped nucleocapsid.

Engineered AcMNPV has been used extensively for the expression of a wide variety of mammalian proteins in insect cells because of the high efficiency of protein expression by recombinant AcMNPV. This system has proven to be particularly useful for the expression of eukaryotic proteins, whose function depends on proper post-translational modifications. Nearly two decades ago, scientists adapted this system to mammalian cells as a therapeutic gene delivery system *in vivo* and *in vitro*. Several groups have shown the possibility of baculovirus-mediated expression of foreign genes under the control of mammalian promoters in hepatic cell lines [1, 2]. Recently, recombinant baculoviruses containing mammalian gene promoters have been used to successfully introduce foreign genes into several other cell types, including those of non-hepatic origins, both *in vivo* and *in vitro* [3–8]. These studies have greatly expanded the potential therapeutic applications of this technology, and baculovirus-mediated gene expression is now a useful and well-accepted viral gene delivery system in mammalian cells [9–11].

The ability to manipulate gene expression by transgenesis and efficiently transfer genes into different cell types *in vivo* is central to the study of protein function and for therapeutic applications that involve gene therapy. Using a lentiviral gene expression system to deliver the gene encoding the c-kit ligand into the efferent ductules, Ikawa *et al*. [12] reported the successful expression of c-kit in Sertoli cells, as well as the restoration of recurring spermatogenesis in an infertile mouse. In a similar study, isolated rat male germ cells that were successfully infected with lentivirus containing a green fluorescent protein (GFP) reporter gene were transplanted into a heterologous recipient. When the transplanted male rat was mated to a female rat, transgenic GFP-expressing offspring were produced [13]. Most recently, it has been shown that recombinant baculovirus containing a modified viral envelope protein can transduce the gene for GFP into mouse testis [14]. A recombinant baculovirus that contained vesicular stomatitis virus envelope G (VSVG) protein viral capsids was directly injected into the efferent ductules of the mouse testis, and GFP expression was subsequently detected in whole testis, including the seminiferous tubules. However, neither GFP-expressing germ cells nor spermatozoa were observed [14].

Leydig cells reside in the interstitium of the testis, and destruction of these cells results in a decrease of more than 75% of total testosterone in the adult males [15–17], suggesting that these cells play a key role in spermatogenesis and in the maintenance of a masculine phenotype. The development of techniques for the isolation and purification of Leydig cells [18–20] and the establishment of Leydig cell lines [21, 22] have been instrumental in the study of the various functions of these cells during testis development. However, the role of Leydig cells in steroidogenesis and spermatogenesis is intimately linked to the function of other testicular cells, such as Sertoli and germ cells. Thus, an understanding of the molecular mechanisms of Leydig cell functions requires the development of *in vivo* gene delivery systems for the expression of specific genes in the interstitial tissue area of the testis.

In this study, we examined the feasibility of using intra-tunica albuguineal injection of recombinant baculovirus into the interstitial tissue of the mouse testis to express foreign genes in the mouse testis. The results of this study expand the utility of baculovirus as an *in vivo* gene delivery system for the functional analysis of gene expression.

2 Materials and methods

2.1 Construction and production of recombinant baculovirus

The baculovirus-mammalian expression vector was constructed using shuttle vectors derived from pFastBac1 and pCDNA3, as described earlier by Condreay *et al.* [8]. The vector carried the cytomegalovirus (CMV)-IE promoter and the G418 resistance gene. The genes for GFP and rat IGFBP-5 were subcloned using *Eco*R1 and *Xba*1 restriction sites. Recombinant baculoviruses were produced and titrated using the method of Song *et al*. [23].

2.2 Cell culture

Hela, Cos-7, GC-SPG1, TM3 and TM4 cells were maintained at 37ºC in Dulbecco's modified Eagle's medium containing 10% fetal bovine serum (Gibco, Carlsbad, CA, USA) and 1% penicillin/streptomycin (Gibco). Confluent cells were washed with phosphate buffered saline (PBS), and then detached from the plastic tissue culture dish by incubation at 37ºC in the presence of 5 mL of Trypsin–EDTA solution (Gibco) for several minutes. PBS was added to the cells, and they were subjected to centrifugation at 200 × *g* for 5 min. The supernatant was then discarded and the cell pellets were resuspended in culture medium.

2.3 Infection of mammalian cells in vitro

Before inoculation with GFP-baculovirus, cells were seeded in a six-well plate (1×10^5 cells per well) and then incubated for 16 h at 37ºC. Under serum-free conditions, GFP-baculovirus was added to each well at a multiplicity of infection (MOI) of 5. Cells were incubated for 24 h at 37ºC, and GFP expression was confirmed using fluorescence microscopy (Olympus, Hamburg, Germany).

2.4 Infection of the mouse testis

For gene delivery to the mouse testis, 10-week-old Balb/c male mice were anesthetized and the testes were exposed by laparotomy. The tunica albuginea of each testis was partially pierced with a 28-gauge needle, and 50 μL of 1×10^{11} plaque-forming units (pfu) of GFP-baculovirus was slowly injected just under the tunica albuginea using a 31-gauge injection needle. As a negative control, an equal volume of PBS containing 5 mg of pBacMamGFP vector only (empty vector) was injected. GFP expression was confirmed using fluorescence microscopy (Olympus) at 48 h after injection.

2.5 Histological analysis

Testes were collected and washed in PBS and then frozen in optimal cutting temperature compound (Sakura Finetek, Tokyo, Japan) at −70ºC for 72 h. The frozen tissue was then cut into 20-μm slices using a cryo-sample microtome (Leica Microsystems, Mussloch, Germany), and the slices were mounted on a glass slide. GFP expression was detected using fluorescence microscopy (Nicon , Tokyo, Japan).

2.6 Analysis of GFP gene expression using reverse transcription-polymerase chain reaction (RT-PCR)

Testes were removed 7 days after injection, and the tunica albuginea was separated from the testis body. Whole testis, tunica albuginea, interstitial tissue, seminiferous tubules, epididymis and sperm from the epididymis were isolated, and total RNA was extracted using an RNeasy mini kit (QIAGEN, Venlo, Netherlands), according to the manufacturer's instructions. For cDNA synthesis, reaction mixtures containing total RNA, oligo dT primer and reverse transcriptase were incubated at 25ºC for 10 min, 50ºC for 50 min, 85ºC for 5 min and 4ºC for 10 min. GFP expression was analyzed by PCR using cDNA as the template, and the following primers: 5′-CATGGTGAGCAAGGGCGAG (forward) and GTACAGCTCGTCCATGCCG-3′ (reverse). Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) amplification was used as a loading control for the samples. The following primers were used to amplify the GAPDH gene: 5′-CAATGAATACGGCTACAGCAAC (forward) and AGGGAGATGCTCAGTGTTGG-3′ (reverse). PCR conditions were as follows: 95ºC for 10 s, 57ºC for 30 s and 72ºC for 1 min.

2.7 Western blot analysis

Testes were injected with either a control empty vector or a recombinant baculovirus that encoded IGFBP-5 (IGFBP-5-baculovirus). Baculovirus-injected testes were extracted and separated into the testicle and epididymis. The testicle was decapsulated and sliced to isolate the testicular cells. Cells were incubated in lysis buffer (25 mmol L^{-1} Hepes, 150 mmol L^{-1} NaCl, 5 mmol L^{-1} $MgCl_2$, pH 7.5) containing 1% triton X-100 for 1 h. Semen was extracted from the epididymis and subjected to centrifugation at 6 000 × *g* for 15 min. The supernatants were then collected, and total protein was quantified using the DC protein assay kit (Bio-Rad, Chester, UK). Samples containing equal amounts of total testis protein were subjected to 11% polyacrylamide gel electrophoresis. As a positive control, we analyzed 1 μg of purified IGFBP-5 that was expressed in Sf9 insect cells. The membrane was incubated in TBS-T (20 mmol L^{-1} Tris-HCl, 137 mmol L^{-1} NaCl, pH 7.6 supplemented with 0.1% Tween 20) containing 3% bovine serum albumin for 1 h at room temperature. After rinsing in TBS-T, the membrane was incubated with an anti-rat IGFBP-5 antibody (UpstateBiotechnology, Lake Placid, NY, USA) for 1 h at room temperature and then washed thrice in TBS-T. The membrane was incubated with HRP-conjugated goat anti-rabbit IgG antibody (Santa Cruz Biotechnology, Santa Cruz, CA, USA) in 50 mL of TBS-T containing 5% skimmed milk for 1 h at room temperature, and immunoreactive proteins were detected using enhanced chemiluminescence (Amersham, Buckinghamshire, UK) and HyperfilmTM ECLTM (Amersham). Films were processed in developer and fixer solutions to visualize the protein bands.

3 Results

3.1 Infection of cells by GFP-baculovirus in vitro

To determine the efficiency of gene delivery into

mammalian cells using recombinant baculovirus, Hela and Cos-7 cells were infected with GFP-baculovirus at an MOI of 5. As shown in Figure 1A, more than 70% of the cells expressed GFP 18 h after infection. For the infection of testicular cells *in vitro*, GC-SPG1 (a spermatogonial cell line), TM3 (a Leydig cell line) and TM4 (a Sertoli cell line) cells were infected with GFP-baculovirus. GFP was expressed in all three testicular cell lines; however, the level of expression did not exceed that seen in Hela and Cos-7 cells (Figure 1B). The highest level of GFP expression was observed in TM3 cells, whereas TM4 cells exhibited minimal GFP expression (Figure 1B).

3.2 Interstitial tissue-specific gene delivery by intra-tunica albuguineal injection

We first determined the localization and distribution over time of an indicator dye, trypan blue, which was injected into the mouse testis by intra-tunica albuguineal injection. Immediately following injection, the whole testis turned blue. By day 3 after injection, most of the trypan blue had moved to the caput epididymis. At 1 week after injection, trypan blue was eliminated from the epididymis, but a trace of blue color remained in the main testis body (Figure 2A). These results indicated that the injectant was able to flow through the testis body and eventually move to the epididymis. We next analyzed this method for the delivery of recombinant baculovirus into the mouse testis.

Equal volumes of PBS, pBacMamGFP vector (control, 5 mg), or GFP-baculovirus were administered by intra-tunica albuguineal injection to male mice. As shown in Figure 2, GFP expression in the testis was observed only in mice that received GFP-baculovirus (Figure 2D). Mice that received PBS or the control vector did not exhibit green fluorescence (Figure 2B and C). In GFP-baculovirus-injected testes, GFP appeared to form a line along the long seminiferous tubule (Figure 2D). To examine the localization of GFP more closely, seminiferous tubules were carefully isolated and examined by fluorescence microscopy. As shown in Figure 3, strong GFP expression was detected in the outer seminiferous tubule cells.

To determine the location of the GFP-expressing cells in the testis, we carried out a histological analysis. Although not all interstitial tissues expressed GFP, there was a marked GFP expression in the cells between the seminiferous tubules, including Leydig cells (Figure 4B). GFP expression was not observed in the inner seminiferous tubule, even under high magnification (Figure 4D).

We also examined the expression of GFP in different parts of the testis 7 days after intra-tunica albuguinea injection of GFP-baculovirus. Testes were removed and separated from the epididymis into whole testes, tunica albuguinea, interstitial tissue, seminiferous tubules and sperm, and the expression of GFP mRNA was analyzed by RT-PCR. GFP mRNA was present in whole testes and interstitial tissue, but not in the seminiferous tubules and sperm (Figure 5).

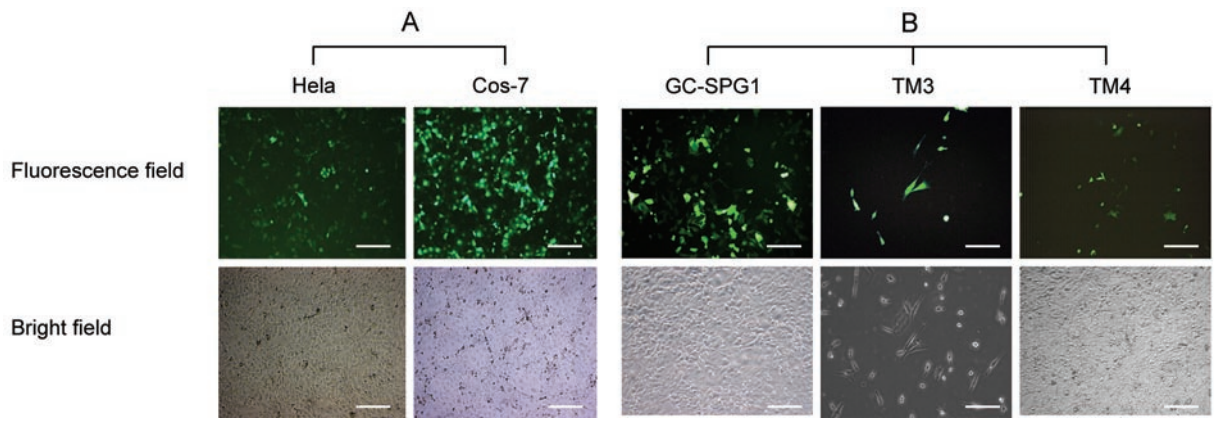

Figure 1. Transfection of mammalian cell lines with pBacMam-green fluorescent protein (GFP), a recombinant baculovirus containing the GFP reporter gene. GFP expression was detected 18 h after viral infection. Cells were observed through bright field and fluorescence microscopy. (A): Non-testicular HeLa and Cos-7 cells. (B): GC-SPG1 (spermatogonial cells), TM3 (Leydig cells) and TM4 (Sertoli cells) cells. The level of GFP expression was assessed by calculating the number of cells exhibiting fluorescence divided by the number of cells transfected. Bars = 50 μm.

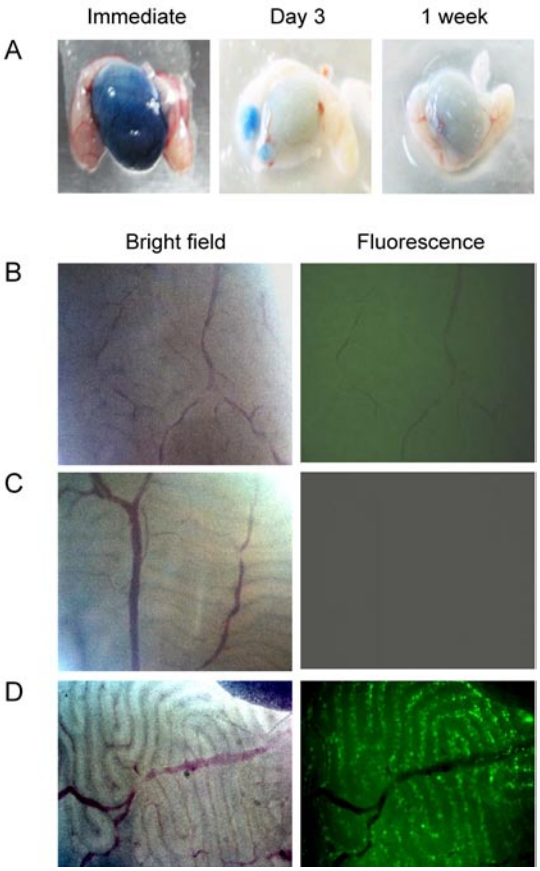

Figure 2. Intra-tunica albuguineal injection of trypan blue and green fluorescent protein (GFP)-baculovirus into the testis. Testes were isolated 48 h after injection and observed by bright field and fluorescence microscopy. (A): Testes that received a solution of trypan blue as a marker for the movement of injectant. Testes were examined at the indicated time points after injection. To determine the efficiency of gene delivery by intra-tunica albuguineal injection, mice were injected with PBS (B), 5 mg of pBacMamGFP vector only (empty vector) (C), or 1×10^{11} plaque forming unit (pfu) of GFP-baculovirus (D) between the tunica albuginea and the seminiferous tubules.

3.3 Expression of IGFBP-5 in testes by intra-tunica albuguineal injection

To determine whether the baculovirus system could be used to express a foreign gene of interest in the testis, we injected recombinant-baculovirus-encoding rat IGFBP-5 into the interstitial tissue of the testis and examined protein expression in whole testis through Western blot analysis. We also examined the level of IGFBP-5 secretion into semen (Figure 6). The protein expression level of rIGFBP-5 in whole testis extract was increased following injection of the IGFBP-5-baculovirus, as compared with the control baculovirus. We also detected strong rIGFBP-5 immunoreactivity in

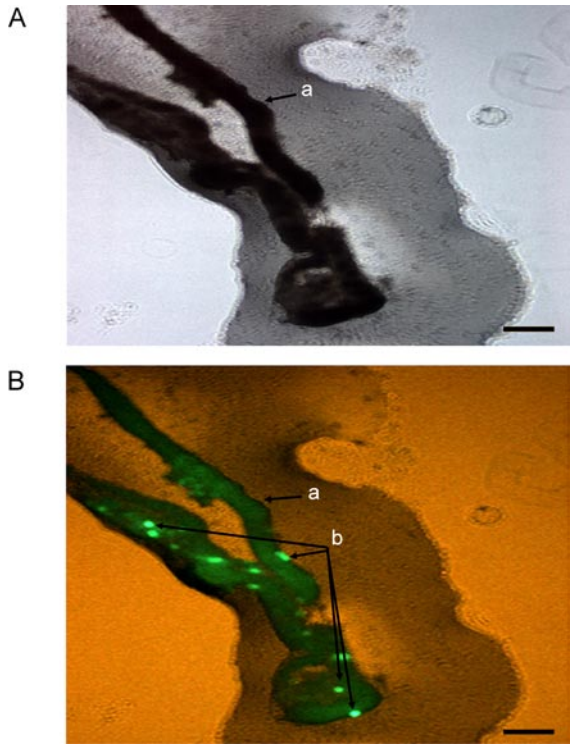

Figure 3. Analysis of green fluorescent protein (GFP) expression in seminiferous tubules. Mouse testes that were injected with GFP-baculovirus were decapsulated, and the seminiferous tubules were separated by mild physical force without enzyme treatment. Cells were observed by bright field (A) and fluorescence microscopy (B). Arrows indicate (a) isolated seminiferous tubule with gentle agitation in Trypsin–EDTA solution, and (b) that interstitial cells remained on the seminiferous tubule after enzyme treatment. Scale bar = 100 μm.

semen, which indicated that rIGFBP-5 was produced in the interstitial tissue and then migrated to the epididymis (Figure 6). These data were consistent with those in Figure 2A, which show that intra-tunica albuguineal injection of trypan blue resulted in the migration of dye into the epididymis.

4 Discussion

Baculovirus vectors have many advantages over other gene transduction methods for gene delivery. Baculoviruses can incorporate large pieces of DNA and efficiently infect not only insect cells but also a variety of mammalian cells, with no apparent cytotoxic effects or evidence of viral replication [6, 11, 24]. The safety of baculovirus has been examined, and based on clinical chemistry analyses, it has been shown that the injection

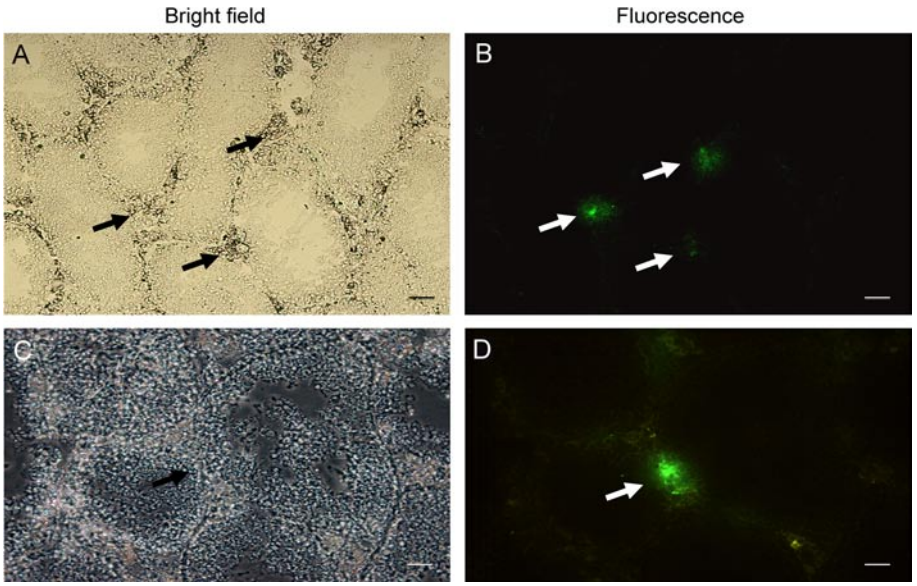

Figure 4. Histological analysis of green fluorescent protein (GFP)-baculovirus-injected testis. Testes were injected with GFP-baculovirus and then analyzed 48 h after injection. Frozen sections were viewed through bright field and fluorescence microscopy. (A) and (B) are lower magnification images than (C) and (D). Arrows indicate the localization of GFP-positive cells. Scale bar = 100 μm.

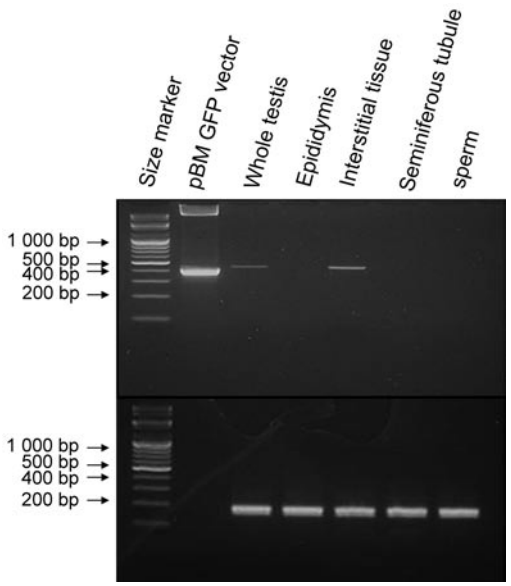

Figure 5. Reverse transcription-polymerase chain reaction (RT-PCR) analysis of green fluorescent protein (GFP) expression in baculovirus-injected testes. RT-PCR analysis of whole testes, interstitial tissue, epididymis, seminiferous tubules and sperm from the epididymis (upper panel). RT-PCR using pBacManGFP as the template was used as a positive control (band at approximately 450 bp). Lower panel, analysis of Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) as a loading control.

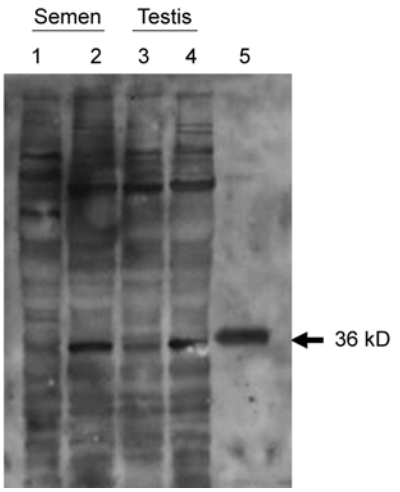

Figure 6. Western blot analysis of testis and semen after injection of IGFBP-5-baculovirus. The control (empty vector) and insulin-like growth factor binding protein (IGFBP)-5-baculovirus vector were injected into mice testes using the intra-tunica albuguineal injection method. The testis was separated into the testicle and epididymis, and the testicle was sliced to extract testicular cells. Total protein was isolated from semen by centrifugation. Lanes 1 and 3 represent protein isolated from control baculovirus-injected testes; lanes 2 and 4 represent IGFBP-5-baculovirus-injected testes; lane 5 represents purified rat IGFBP-5 expressed in Sf9 insect cells. Proteins were separated by 11% polyacrylamide gel electrophoresis, and analyzed by Western blot using polyclonal anti-rat IGFBP-5 antiserum.

of baculovirus into the rat brain is not associated with any major adverse event [7]. Furthermore, there is no evidence of viral replication or transcription of viral DNA following the introduction of baculovirus into mammalian cells, although viral genomes have been detected [25]. In this study, we generated a modified recombinant baculovirus vector, in which the insect cell-specific polyhedrin promoter was replaced by

the CMV promoter to drive gene expression. It was shown earlier that this type of modified baculovirus vector is highly efficient in infecting nearly all types of mammalian cells, with the exception of some immune cells and primary cells [8]. The results of this study confirm these earlier observations, and expand the spectrum of cellular targets of modified recombinant baculovirus to include testicular cells (GC-SPG1, TM3 and TM4 cells), which are difficult to transfect using chemical gene-transfer methods.

Recombinant baculoviruses that contain a modified viral envelope can efficiently transfer foreign genes into the human liver *ex vivo* [9], and into rat hepatocytes [26], the rabbit artery [4], mouse skeletal muscle [6], rodent glial cells [5] and mouse retinal cells *in vivo* [27]. Although baculovirus-mediated gene transfer *in vivo* has many technical problems that need to be resolved, this system has been widely applied to the transfer of genes *in vivo*. Gene transfer into testicular cells has been accomplished using non-viral delivery systems, such as liposomes [28], polyethylenimine [29] and electroporation [30, 31], as well as viral vectors, such as adenovirus [32]. Recently, it was reported that the injection of baculovirus containing a VSVG-modified envelope into the seminiferous tubule area resulted in strong GFP expression in seminiferous tubular cells, but not in germ cells, in the testis [14]. In this study, intra-tunica albuguineal injection of recombinant baculovirus resulted in the delivery and expression of foreign genes in the outer seminiferous tubule of the adult mouse testis. These results strengthen and expand the potential applications of recombinant baculovirus as a vector for DNA transfer, particularly into the testis.

Following intra-tunica albuguineal injection of GFP-baculovirus, histological analysis showed that not all interstitial areas expressed GFP, and that some cells were non-fluorescent. Cells that are located under the tunica albuguinea lie between the seminiferous tubules and contain tight junctions. Therefore, some of the interstitial cells located proximal to the tunica albuguinea are easily exposed to baculovirus, whereas cells located farther from the tunica albuguinea are less easily exposed to the baculovirus in the intra-tunica albuguinea injection. This failure to distribute the baculovirus evenly may be linked to the limited volume of solution that can be injected. The maximum volume of baculovirus used in this study was 50 μL. When injection volumes greater than this were used, either leakage of the solution into the outside of the testis or a breakdown of the tunica albuguinea was observed. Thus, because 50 μL is insufficient to distribute the baculovirus over the entire intertubular compartment, new methods that allow foreign DNA to be delivered to the entire intertubular areas are required to improve the efficiency of gene delivery into the testis.

The study of testis biology is often hampered by the lack of gene delivery systems for the expression of proteins in specific cells of the testis, including cells of the intertubular compartment elements, as well as by difficulties encountered in functionally separating specific cells. Our system represents a useful and efficient method for the delivery of foreign genes to intertubular compartments. In the intertubular compartment, Leydig cells and macrophages interact functionally and structurally, and testicular macrophages are potential sources of several growth and differentiation factors of Leydig cells [33–39]. Furthermore, the close physical association of macrophages with Leydig cells would allow them to provide some of the regulatory factors that control Leydig cell proliferation and differentiation. It therefore seems worthwhile to pursue approaches that would elucidate Leydig cell–macrophage interactions and steroidogenesis. This could best be achieved by performing intra-tunica albuguinea transfection with baculovirus that expresses specific genes for regulating the macrophage–Leydig cell interaction.

In addition, this system may be particularly relevant for the gene therapy of human male infertility associated with Leydig cell and macrophage dysfunction. It would be interesting to determine whether a deficiency in proteins related to spermatogenesis and testis development from Leydig cells and macrophages causes human male infertility, and whether gene therapy using recombinant baculovirus offers a therapeutic benefit. In this context, our results are significant in that the system we have developed will (1) facilitate the study of outer seminiferous tubule tissue-mediated testis biology, (2) facilitate the functional analysis of Leydig cell- and macrophage-specific gene expression in spermatogenesis and testis development and (3) enable functional studies of target genes from non-Leydig cells in all aspects of testis biology, including spermatogenesis, testis abnormalities and testis toxicity.

Intra-tunica albuguineal injection of trypan blue showed that dye remained in the epididymis up to 3 days after injection, after which it was completely removed. Several earlier testis-mediated gene transfer

studies showed that the introduced solutions penetrated to the epididymal areas, and that the injected foreign DNAs were bound to epididymal spermatozoa [40]. All these findings suggest that materials introduced in this way are moved to the epididymal lumen. Moreover, when injected into the testis, trypan blue dye is dispersed to the interstitial compartments, where it frequently migrates to the seminiferous tubule and the testicular capsule, but rarely to the seminiferous tubule; it eventually ends up in the epididymis [40]. This may suggest the presence of two 'gates' in the rete testis: one leading to the testicular sperm cells and the other to the interstitial fluid leading to the rete testis. The one leading to the sperm cells would be connected to the seminiferous tubules, and through this pathway testicular spermatozoa would be transferred out of the seminiferous tubules. The one leading to the interstitial fluid would be connected to intertubular compartments and would accommodate the transport of the interstitial fluid between the seminiferous tubules and the testicular capsule to the rete testis.

It seems more likely that the two pathways are involved in the transport of the interstitial compartment elements to the epididymis. This would involve the active transport of elements from the interstitial compartment to the lumen of the epididymis through the seminiferous tubules and the rete testis, or the direct delivery (leakage) of elements into the epididymal lumen through the rete testis. In this study, the injected dye and the rIGFBP-5 produced in interstitial cells by the baculovirus infections might have been directly moved to the epididymal areas (through leakage); however, we cannot rule out the possibility that the dye or rIGFBP-5 entered the seminiferous tubules and was transported to the lumen of the epididymis. It is therefore important to investigate the transportation and movement of secreted proteins to the seminiferous tubules from the interstitial compartments and through the seminiferous tunica propria. Transportation or movement of the IGFBP-5 through the seminiferous tunica propria would be possible because IGFBP-5 is a 30-kDa protein, and some secreted proteins, such as androgen-binding protein, move freely through the seminiferous tunica propria.

The observation that GFP was expressed in the interstitial tissue, but not in the epididymis, implies that baculovirus has a relatively limited amount of time to infect cells. Thus, the risk of non-specific side effects due to long-term exposure to baculovirus appears to be relatively low in this system, as compared with other viral gene delivery systems that have a longer half-life.

In summary, we have shown the feasibility of gene transfer to testicular cells *in vitro* and *in vivo* using a modified recombinant baculovirus. High-efficiency expression was achieved in the outer seminiferous tubule cells through intra-tunica albuguineal injection of the interstitial tissue of the testis. This method will be useful for the study of genes that are expressed in the outer seminiferous tubule and that are involved in the regulation of spermatogenesis and other functions of the testis. Future studies that examine the replication and transcription of baculovirus *in vivo* will aid in determining the safe use of this gene delivery system for therapeutic applications. Finally, the construction of recombinant baculovirus vectors that contain cell-type- or tissue-specific promoters will greatly enhance our understanding of the mechanisms of spermatogenesis and other testis functions.

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