Long-Term Transgene Expression within the Anterior Pituitary Gland in Situ: Impact on Circulating Hormone Levels, Cellular and Antibody-Mediated Immune Responses*

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ABSTRACT

Adenoviral vectors have been identified as useful tools for gene transfer to the pituitary gland with the aim of providing therapeutic treatments for pituitary diseases. Although successful adenovirus-mediated gene transfer to the pituitary has been shown, the duration of transgene expression, local immune responses and consequences on circulating pituitary hormone levels have not been investigated. These are critical not only for the successful implementation of these gene transfer techniques both for physiological and/or therapeutic applications but also for assessing the safety of these approaches. We have therefore assessed duration and levels of transgene expression 3 days, 14 days, 1, 2, and 3 months after delivery of adenoviruses expressing herpes simplex virus type 1 thymidine kinase (HSV1-TK), under the control of the major immediate early human cytomegalovirus (RAd-hCMV/TK) or human PRL (RAd-hPrl/TK) promoters, to the anterior pituitary (AP) gland in situ. The presence of vector genome and cellular immune infiltrates within the AP gland were also studied along with the levels of circulating anti-adenovirus neutralizing antibodies and AP hormones in sera. Ubiquitous or cell-type specific expression of HSV1-TK within the AP gland was seen from RAd-hCMV/TK and RAd-hPrl/TK respectively at all time points, although a reduction in expression was seen over time. PCR amplification of HSV1-TK specific sequences showed the persistence of adenoviral genomes for up to 3 months. Analysis of the AP showed the presence of a virus-induced inflammation that peaked around day 14 and was resolved between 2–3 months. ED1-positive macrophages, CD8-positive T-cells and CD161-positive NK cells were identified up to 1 month after virus administration. A virus-induced humoral immune response was also present as anti-adenovirus neutralizing antibodies were detected from 14 days after virus administration. Levels of circulating pituitary hormones were unaffected by virus administration with the exception of the stress hormone ACTH which was increased at 3 days but normalized by 14 days. In conclusion, our data indicates that adenovirus-mediated delivery to the AP gland in situ may be a useful tool for the treatment of pituitary diseases as no major cytotoxicity or disruption of AP hormonal functions are seen. Despite of this, further developments to this approach still need to be made to combat the reduced transgene expression seen over time and the induction of virus-induced immune responses. (Endocrinology 142: 464–476, 2001)

THE ANTERIOR pituitary (AP) gland is a very attractive target to develop gene therapy strategies because it controls critical physiological parameters, and there are numerous diseases that involve the pituitary that are currently poorly treated. These include macroadenomas, especially nonfunctional adenomas and some cases of macroprolactinomas, and GH secretory adenomas. Also, some diseases of a chronic nature, i.e. GH deficiency, would benefit from more efficient and safer treatments.

Adenoviral vectors (RAds) have been used successfully to transfer genes into the anterior pituitary gland both in vitro (1, 2) and in vivo (3, 4, 5). Work from our laboratory has shown efficient reversion of an in situ lactotroph hyperplasia induced by estrogen/sulpiride implants after treatment with a RAd encoding herpes simplex virus type 1-thymidine kinase (HSV1-TK) driven by the human cytomegalovirus (hCMV) promoter (3). On the other hand, when a different RAd, in which the expression of HSV1-TK was driven by the human PRL (hPrl) promoter, was used in the same experimental paradigm, it was not effective in either reducing the hyperplasia or circulating PRL levels (4). Using RAd-hPrl/TK transgene expression was almost exclusively restricted to lactotrophs within the AP gland (4), but since transgene expression was weaker when compared with the hCMV promoter, this could account for the lack of beneficial therapeutic outcome. Lee et al. (6) have shown increased survival in nude mice bearing GH3 transplanted tumors when they were treated with a RAd expressing HSV1-TK driven by the human GH promoter. The discrepancy between the Southgate et al. (4) and Lee et al. (6) results could be explained by the different animal model used to test these therapeutic strategies. Southgate et al. (4) used a slow growing adenoma in situ, while Lee et al. (6), used a transplantable fast growing tumor, and since HSV1-TK in combination with ganciclovir only kills actively proliferating cells, the rapidly growing

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transplantable tumor would be more readily killed. The previously discussed results provide a compelling argument for the need to test these therapies in relevant preclinical animal models before they are transferred into the clinic. Also, in the light of the recent death of a patient undergoing gene therapy for ornithine transcarbamylase (OTC) deficiency after adenoviral gene transfer to the liver (http://www.med.upenn.edu/~ihgt/findings.html), it is imperative that the toxicity and other potential side effects of the gene transfer vehicles are very carefully investigated.

In this paper we have compared the longevity of HSV1-TK expression driven by either the hCMV or hPrl promoter. We have also assessed the cellular and antibody-mediated immune responses elicited after adenovirus delivery into the AP gland in situ. Effect of viral infection on AP physiology was also assessed by monitoring circulating AP hormone levels up to three months after viral delivery in vivo.

Our results demonstrate that transgene expression can be sustained up to 3 months post viral delivery within the AP gland in vivo, although there is a gradual decline in transgene expression during this period. Also, both cellular and antibody mediated immune responses were elicited in the RAd treated groups. No significant changes were observed in circulating hormone levels at all time points tested post viral delivery into the AP in situ, except for ACTH which was significantly higher only at 3 days post viral infection.

**Materials and Methods**

**Recombinant adenoviruses**

RAd-hCMV/TK (7, 8), RAd-hPrl/TK (4) and RAd35 (9, 10) have been described in detail previously. Production of high titer stocks, purification by double cesium chloride density gradient separation and titration of viruses was carried out as previously described (11, 12). After cesium chloride purification virus stocks were assayed for the presence of replication competent adenovirus using a supernatant rescue assay (13), which can detect the presence of a single replication competent virus within 10⁷ recombinant viruses, and were ascertained to be RCA free. Viruses were also assayed for the presence of endotoxin (lipopolysaccharide [LPS]) using the E-TOXASE assay (Sigma, Dorset, UK) according to the manufacturer’s instructions. Viruses were deemed negative, as defined by Cotten et al. (14), when having LPS levels less than 6 × 10⁻⁸ endotoxin units per dose of adenovirus injected. The particle number to infectious unit (iu) ratios for RAd-hCMV/TK and RAd-hPrl/TK were determined according to Mittereder et al. (15) and found to be 8.85:1 and 26.16:1 respectively.

**In vivo gene delivery to the anterior pituitary gland**

Male 8-week old Buffalo rats were house bred at the University of Manchester Biological Safety Unit. All animals had free access to food and water, a 12-h light, 12-h dark cycle, and constant housing temperature and humidity. Experiments were conducted according to the United Kingdom Animal (Scientific Procedures) Act of 1986. Animals were anesthetized with halothane, placed in a stereotaxic frame and injected in the anterior pituitary using a 26 gauge Hamilton syringe as previously described (4). Briefly, animals received 6 injections per pituitary gland (3 sites per globe) of 1 μl recombinant virus (total of 1 × 10⁶ iu per pituitary) or saline, delivered over 1 min per injection site. Animals were then given 10 ml of saline sc and allowed to recover. At time points of 3 days, 14 days, and 1, 2, and 3 months, blood was collected for extraction of serum for anterior pituitary hormone measurements. Animals were then perfused transcardially with tyrode solution (132 mM NaCl, 1.9 mM CaCl₂, 0.32 mM NaH₂PO₄, 5.56 mM glucose, 11.6 mM NaHCO₃ and 2.68 mM KCl), and pituitary glands were removed and placed in 4% paraformaldehyde solution in 0.1 M PBS for 3 h. Tissue was then paraffin embedded, sectioned using a Leica Corp. (Nussloch, Germany) RM2145 microtome (5 μm) and mounted onto 3-aminopropyltriethoxysilane (APES)-coated slides.

**Immunohistochemical detection of transgene, hormones and immune markers within the anterior pituitary gland in vivo using fluorescence microscopy and histological analysis**

Immunohistochemistry was carried out as described previously (3, 4). HSV1-TK was detected using a rabbit anti-HSV1-TK antibody (1/1000), which was kindly provided by M. Janicot, Rhone-Poulenc-Rorer, France. The different hormone producing cell types within the anterior pituitary were identified using polyclonal antibodies to the following hormones: guinea pig anti-rat β-TSH (1/100), guinea pig anti-rat Prl (1/500), guinea pig anti-rat α-LH (1/100), guinea pig anti-human GH (1/500), guinea pig anti-human β-FSH (1/100), and rabbit or sheep anti-human ACTH (1/500) (provided by Dr. A. F. Parlow at NIDDK National Hormone and Pituitary Program, Bethesda, MD). Inflammatory cell types within the anterior pituitary were identified using mouse monoclonal antibodies to the following markers: anti-rat ED1 (1/500) (activated macrophages, Serotec, Oxford, UK), anti-rat CD161 (1/1000) (NK cells and T cell subset, Serotec) and anti-rat CD86 (1/1000) (cytotoxic T lymphocytes and NK cells, PharMingen, San Diego, CA).

Secondary antibodies used for either single or double labeling were: swine anti-rabbit conjugated to fluorescein isothiocyanate (FITC) or donkey anti-rabbit R-phycocrythrin from DAKO Corp. Ltd., High Wycombe, UK, and Jackson ImmunoResearch Laboratories, Inc., West Grove, PA respectively; goat anti-guinea-pig conjugated to FITC or Texas red from Jackson ImmunoResearch Laboratories, Inc.; donkey anti-mouse conjugated to FITC from Jackson ImmunoResearch Laboratories, Inc. Hematoxylin and eosin staining was carried out as described previously (8).

**Determination of hormone levels in peripheral blood**

Serum was extracted from blood taken at 3 days, 14 days, 1, 2, and 3 months post virus delivery into the AP gland in situ. Serum levels of PRL, GH, LH, FSH, and TSH β were determined using specific RIA kits provided by Dr. A. F. Parlow and the National Hormone and Pituitary Program, NIH. Serum ACTH was measured using a specific immunoradiometric assay that has been described previously (16).

**Anti-adenovirus neutralizing antibody assay**

Serum was extracted from blood taken from 3 days, 14 days, 1, 2, and 3 months post virus delivery into the AP gland in situ. The presence of circulating anti-adenovirus neutralizing antibodies was assessed as described previously (17). Briefly, blood serum samples were heat inactivated at 56 C for 30 min before serially diluting 2-fold in MEM containing 10% FCS. Each serum dilution (100 μl) was then incubated in duplicate with 10⁶ iu of RAd35 in 10 μl for 90 min before 50 μl of each dilution was placed on 4 × 10⁴ low passage 293 cells in a 96-well plate at 37 C for 1 h. 50 μl of MEM containing 10% FCS was then added to each well, cells were left at 37 C for 20 h, fixed in 4% paraformaldehyde and 5-bromo-4-chlororo-indoyl-b-D-galactoside (X-gal) stained. Titters were taken as the reciprocal serum dilution factor that caused 50% inhibition of histochemical staining to detect β-galactosidase enzyme, compared with controls.

**Detection of transgene sequences in pituitary sections using PCR**

HSV1-TK transgene sequence was detected in 50 μm paraffin imbedded micrometre-cut pituitary sections using PCR. Sections were deparaffinized twice by mixing with 1 ml of xylene for 30 min at room temperature, left in 100% ethanol for 10 min and then air-dried. Sections were then digested for 3 h at 55 C in 200 μl of 50 μM Tris HCl (pH 7.8), 1 mM EDTA, 0.5% Tween 20, and 1 mg/ml proteinase K. The proteinase K was then heat-inactivated at 95 C for 10 min. Sequences of HSV1-TK and β-actin were detected using two different primer pairs. Primers a and b (see Fig. 5) are specific to HSV1-TK and produce a PCR product of 364 bp. Primers c and d are specific to exon 4 of rat cytoplasmic β-actin and produce a PCR product of 340 base pairs. In a 100 μl PCR reaction, 50 μl of genomic DNA was used in a solution
containing 1x PCR buffer (Promega Corp., Southampton, UK), 200 μM dATP, 200 μM dTTP, 200 μM dCTP, 200 μM dGTP, 2 mM MgCl₂, 2 ng/μl each primer and 3U Taq polymerase (Promega Corp., Southampton, UK). PCR conditions comprised 35 cycles of 30 sec of denaturing, 30 sec of annealing and a 1 min extension followed by another 5 min of extension. An annealing temperature of 63°C was used for both primer pairs. Primer sequences were a, 5'-AAAACCACCACCACGCAACT-3'; b, 5'-GTCATGCTGCCATAAAGTA-3'; c, 5'-CCAGCCATGCTAGCCATCC-3'; d, 5'-G CAGCTCATAGCTCTTCTCCAGG-3'.

### Statistical analysis

The in vivo experimental results were analyzed using ANOVA, followed by the Students-Newman-Keuls multiple comparisons test.
FIG. 2. Expression of HSV1-TK within immunocytochemically identified hormone producing cells of the AP 14 days after delivery of 1 × 10⁸ IU of RAd-hCMV/TK. Fourteen days after virus infection the AP glands were removed, embedded in paraffin wax, sectioned, and double-labeled for HSV1-TK and individual hormones using immunofluorescence techniques. White arrows indicate double-labeled cells with hormones (indicated along the left margin) visualized in the green immunofluorescent channel and HSV1-TK labeled in the red immunofluorescent channel. Expression of HSV1-TK is present within all hormone producing cell types at all time points post viral delivery into the AP gland in vivo (data not shown). Scale bar (bottom right panel), 10 μm.

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FIG. 3. Expression of HSV1-TK within PRL producing cells of the AP in vivo, after delivery of $1 \times 10^6$ iu of RAd-hPrl/TK. After 3 days, 14 days, 1, 2, or 3 months, AP glands were removed, embedded in paraffin wax, sectioned, and double-labeled for HSV1-TK and PRL using immunofluorescence techniques. White arrows indicate cells double labeled for PRL (green) and HSV1-TK (red). Expression of HSV1-TK was evident in PRL positive cells at all time points although fewer cells were seen at 2 and 3 months. Note that most cells double labeled with antibodies against HSV1-TK and PRL are not seen as yellow cells because HSV1-TK is mostly nuclear, while PRL is mostly cytoplasmic. Scale bar (bottom right panel), 10 μm.
Results

Persistent HSV1-TK transgene expression within the anterior pituitary gland in vivo

To assess the longevity of transgene expression encoded from recombinant adenoviruses, RAds encoding HSV1-TK under the control of either the hCMV, or the hPRL promoter were injected into the AP gland of adult Buffalo rats. Expression of the transgene was evaluated at various time points after virus delivery. With either virus, the highest levels of HSV1-TK expression were seen at 3 days after intrapituitary injection. The areas of transduction achieved using the hCMV promoter were larger than the areas transduced using the hPRL promoter. Transgene expression driven by both promoters decreased to lower levels at later time points after virus delivery.
points, but immunoreactive cells could still be detected for up to 3 months postviral vector delivery (Fig. 1).

The hCMV promoter furthermore provided transgene expression in all different pituitary cell types examined (Fig. 2), as expected from a non cell-type specific promoter element, and as shown by us earlier (3, 4). Expression in all endocrine cell types was observed at all time points examined, up to 3 months. This explains the higher levels of expression observed when transgene expression is analyzed on its own. The PRL promoter, however, restricted transgene expression almost exclusively to lactotrophic cells. This specificity was also maintained at all time points examined, up to 3 months (Fig. 3). As expected from the specificity known for this promoter, expression was observed mainly in lactotrophic cells, and a subpopulation of GH expressing cells (illustrated in Fig. 4). This cell-type specificity explains the lower number of cells within the areas transduced by RAd-hPrl/TK when compared with RAd-hCMV/TK, and is expected because lactotrophic cells only compose approximately 15–20% of all endocrine AP cell types.

Long-term presence of HSV1-TK immunoreactive cells could have been due to the continued presence of HSV1-TK messenger RNA or protein, or to the persistence of actively expressing adenoviral genomes within AP cells. There has been some controversial data in the literature suggesting that adenoviral genomes were eliminated rather rapidly postdelivery to various tissues, such as the liver (18). However, some authors have demonstrated that even first generation adenovirus genomes can persist in liver and muscle for several months (19, 20). To determine if adenoviral genomes could be stably maintained in the AP gland, we examined the presence of the viral genome, after the administration of either vector in vivo. We demonstrated the presence of viral genomes within the AP gland, for at least 3 months (Fig. 5). We thus conclude that both viral vector genomes, and HSV1-TK protein can persist in AP tissue for up to 3 months post adenoviral delivery in situ.
Cellular immune infiltrates in response to the delivery of adenoviruses into the anterior pituitary gland

Immune mediated responses to adenoviral vectors can determine both the longevity of transgene expression and also adverse side effects to therapy. We therefore assessed the immune consequences of delivering RAds into the AP gland in vivo at the same doses as were effective in eliciting a beneficial therapeutic outcome in an animal model of lactotroph hyperplasia (3, 4). A nonspecific inflammatory response was observed in pituitary tissue postvirus injections. Virus induced inflammation was significantly higher in virus injected pituitaries, compared with saline controls, but was indistinguishable between glands injected with either virus i.e. RAd-hCMV/TK or RAd-hPrl/TK. Because equal titers were injected, but different levels of transgene obtained, we conclude that most of the early innate inflammatory response is due to inflammatory effects of the viral capsid itself, rather than the amount of transgene expressed, or the cell-types expressing the transgene (21). The influx of inflammatory cells peaked around 14 days and could still be detected at 1 month post infection (Fig. 6). Inflammation resolved between 2-3 months postviral delivery to the pituitary. Examination of specific cell populations indicated that the influx of ED1-positive macrophages lasted 14 days in saline injected pituitaries, but up to 1 month in adenovirus injected pituitaries. Again, we could not detect any significant difference in the amount of monocyte influx in pituitaries injected with either virus (Fig. 7). The same pattern of influx was detected when we examined the distribution of CD8β-positive T-cells (Fig. 8), or CD161-positive natural killer cells (Fig. 9).

Circulating antiadenovirus neutralizing antibody responses

Anti-adenovirus neutralizing antibody titers could not be detected in any of the animals injected with saline as expected but were detected in animals injected with both adenoviral vectors (Table 1). Higher antibody titers were obtained in animals injected with RAd-hPrl/TK, compared with those injected with RAd-hCMV/TK. In animals injected with the lactotrophic specific promoter, significant antibody titers were detected from 14 days post injection, whereas antibody titers were only detected after 1 month in those animals injected with the adenovirus encoding the promiscuous hCMV promoter driving expression of the transgene. In both groups titers remained above background values for up to 3 months. It is likely that the higher antibody titers against RAd-hPrl/TK are due to the higher particle:iu ratio of this virus (26.16:1 vs. 8.85:1), indicating that higher total numbers of virions were injected into these animals.

Impact of intrapituitary adenoviral injection on circulating hormone levels

Levels of circulating AP hormones are key indicators of AP physiology. We therefore assessed the impact of RAd delivery into the AP gland in vivo on the levels of circulating ACTH, PRL, FSH, TSH, GH, and LH at all time points post viral infection, up to 3 months. Our results indicate that there were no major significant changes in the levels of circulating AP hormones (Fig. 10). Only at 3 days post injection, circulating ACTH levels were significantly higher than at later time points (P < 0.0276, n = 4). This is most likely due to the post surgical stress involved in the intrapituitary injection of RAds. The level of all other hormones remained within normal values for up to 3 months postviral delivery.

Discussion

Successful virus mediated gene delivery to the AP gland can be achieved through several methods although most studies have looked at short-term gene transfer. The need for extended treatment of pituitary diseases has led us to investigate the longevity of transgene expression obtainable...
within the AP gland in situ and the immune and inflammatory consequences of this invasive form of gene delivery in situ.

In this study we have explored the distribution of transgene expression seen within the AP up to 3 months after adenovirus delivery when transgene expression is driven by constitutive (hCMV) or cell-type specific (hPrl) promoters. Expression of HSV1-TK transgene is seen in all hormone producing cell types encountered within the AP gland with the hCMV promoter but remains restricted to PRL producing cells with the hPrl promoter. Also, transgene expression from both the hCMV and hPrl promoters can be seen at 3 months post injection although a decrease in expression is seen over time. The exact cause of the decline in transgene expression seen in the AP over time is unclear, although activation of virus induced immune responses or decreased levels of adenoviral genome may play a part. PCR analysis of pituitary sections at 3 months revealed the presence of HSV1-TK transgene and as the PCR primers used in this study were designed to be specific for the HSV1-TK transgene present in RAd-hCMV/TK and RAd-hPrl/TK, its presence is indicative of the presence of adenoviral genomes. The importance of the presence and levels of vector genome in maintaining transgene expression is still unclear, as similarly, liver, muscle and brain, which are known to have different transgene expression profiles, all show persistence of first generation adenoviral genomes at 3 months (7, 19, 20). To assess the role of vector genomes in loss of transgene expression over time quantitative analysis would have to be carried out to determine whether a reduced level of genome is present at 3 months in the pituitary gland.

Whether the decline in transgene expression was as a consequence of a virus induced immune response is also unclear although increased levels of ED1 (activated macrophages), CD8\(^+\) (T cells) and CD161 (NK cells) positive cells were seen following adenovirus delivery into the AP gland in vivo. The adenoviral capsid alone is able to induce an inflammatory response independent of viral gene expression (22) and this may be responsible for the inflammatory infiltrate witnessed. The pituitary gland lies outside of the blood brain barrier and upon infection we have shown that, unlike infection of the brain parenchyma (23), an adenovirus specific neutralizing humoral immune response can be mounted (Table 1). The ability of the host to mount a specific cellular adaptive immune response following delivery to the AP was not determined in this study so the role of the cellular immune response, known to play a role in the reduction of transgene expression following adenovirus-mediated deliv-

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**Fig. 7.** Expression of ED1 within the AP after delivery of saline, \(1 \times 10^8\) iu of RAd-hCMV/TK or \(1 \times 10^8\) iu of RAd-hPrl/TK. After 3 days, 14 days, 1, 2, or 3 months, the AP glands were removed, embedded in paraffin wax, sectioned and stained for ED1 using immunofluorescence techniques. Note that ED1 expression is maximal at 3 days and decreases with time thereafter, with little or no staining seen at 2 and 3 months post virus delivery. The ED1 staining was more pronounced in the virus-injected pituitaries than those injected with saline, which showed no ED1 staining after 1 month. Scale bar (bottom right panel), 100 \(\mu\)m.
ery to the periphery (24, 25), in the reduction of transgene expression in the AP remains unclear.

In addition to loss of vector genome and adenovirus-specific cellular immune responses, another cause of reduced transgene expression could be promoter shut off. The shut off of transgene expression from viral promoters has been shown previously when using viral vector-mediated gene transfer approaches (26, 27). Our results, however, demonstrate that should loss of transgene expression within the AP be due to promoter shut off it is independent of the nature of the promoter (i.e. viral vs. AP cell-type specific). Alternatively transcriptional shut down could occur via promoter methylation, cytokine mediated destabilization of transgene RNAs or cytokine-mediated down-regulation of promoter transactivators (28–30).

For effective treatment of pituitary diseases using an adenovirus-mediated approach the levels of circulating hormones following direct injection into the AP gland must remain unaffected so that the regulatory functions of the pituitary gland remain intact. Following direct injection into the AP gland we monitored circulating LH, FSH, GH, TSH, PRL, and ACTH levels at all time points and found no significant differences in hormone levels with the exception of ACTH. An increase in the stress hormone ACTH was seen at 3 days following the invasive surgery but levels were normalized by 14 days. The increase in ACTH levels was expected as a stress response would be expected following pituitary injection. An alternative reason for this increase could be due to a local increase in cytokine levels. Following adenovirus infection levels of cytokines IL-1, IL-6, and TNF-α are known to increase (21), and these cytokines in turn are able to induce the production of ACTH (31). These data are encouraging as it suggests that adenovirus-mediated transgene delivery will be useful for the treatment of pituitary diseases as it does not appear to overtly affect regulatory hormone production within the AP gland.

To further develop the adenovirus-mediated gene transfer approach so that longer gene transfer can be attained within the AP gland the use of engineered vector systems will have to be investigated. The level of transgene expression currently achievable with first generation vectors is adequate for short-term treatments such as the elimination of pituitary tumors (3, 5), but the decline in expression seen over time will limit the use of first generation adenoviruses for long-term

![Figure 8](image-url)
Therapies such as the treatment of GH deficiency. Although an inflammatory response to all adenoviral vectors is inevitable due to the inflammatory nature of the adenoviral capsid, the newer high capacity adenoviral vectors, which are devoid of all viral genes, may be of use for long-term gene transfer to the AP gland as they are able to prolong transgene expression in liver, muscle, and brain, even in the presence of a peripheral anti-adenovirus immune response as may be present in humans (17, 32, 33).

Although adenovirus-mediated gene transfer has been identified as a potential tool for the treatment of pituitary disorders, before any clinical studies can be done the extent of any virus-mediated side effects needs to assessed in a relevant animal model. We have attempted to assess some of the possible pre-clinical side effects that could be deleterious to both the success of this technique and more importantly the safety of the patients. With the recent death of a patient in a clinical trial in the USA following adenoviral gene transfer into the liver (http://www.med.upenn.edu/ihgt/findings.html), the importance of gaining good preclinical data...
has become more critical. Our results have shown that adenovirus-mediated gene transfer can be a safe and effective tool in a relevant pre-clinical model of AP gene transfer in vivo.

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