Neuronal survival following remote adenovirus gene delivery

NICHOLAS M. BOULIS, M.D., DANIELLE E. TURNER, B.S., MICHAEL J. IMPERIALE, PH.D., AND EVA L. FELDMAN, M.D., PH.D.

Section of Neurosurgery and Departments of Neurology, Microbiology, and Immunology, Center for Gene Therapy, University of Michigan, Ann Arbor, Michigan

Object. Virus-mediated central nervous system gene delivery is a promising means of treating traumatized tissue or degenerative diseases. In the present study, the authors examined gene expression and neuronal survival in the spinal cord after sciatic nerve administration of an adenovirus vector expressing a LacZ reporter gene.

Methods. The time course of adenovirus gene expression, DNA fragmentation, and neuronal density were quantified in rat lumbar spinal cord by staining for β-galactosidase (β-Gal), terminal deoxynucleotidyl transferase, and cresyl violet after microinjection of either saline or the reporter virus into rat sciatic nerve. The expression of β-Gal following remote delivery peaked at 7 days and declined thereafter but was not accompanied by neuronal cell death, as measured by DNA fragmentation. No significant difference in spinal motor neuron density was detected between virus-treated and control rats at any time point examined. Although the spinal cords removed from rats treated with cyclosporine prior to adenovirus injection contained substantially more neurons staining for β-Gal at 7 days (67% of total neurons), the decay in the number of stained neurons was not paralleled by a decline in motor neuron density.

Conclusions. The authors conclude that remote gene expression is suppressed by a noncytolytic process.

KEY WORDS • apoptosis • β-galactosidase • central nervous system • gene therapy • spinal cord

Abbreviations used in this paper: Ad5RSWntLacZ = adenovirus containing the LacZ gene under control of the Rous sarcoma virus; ANOVA = analysis of variance; β-Gal = β-galactosidase; CMV = cytomegalovirus; CNS = central nervous system; INFγ = interferon-γ; PBS = phosphate buffered saline; RSV = Rous sarcoma virus; TNFα = tumor necrosis factor-α; TUNEL = terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate nick-end labeling.

Several investigators have demonstrated that retrograde delivery of neural growth factors can protect motor neurons of the spinal cord and brainstem from axotomy-induced cell death. Wilson, et al., demonstrated that retrograde delivery of preproenkephalin complementary DNA to neurons of the dorsal root ganglion with a herpes virus vector reduced hypalgesia without disrupting baseline sensory neurotransmission.

Several viral vector systems are currently in use, including herpes simplex virus, adenovirus, adenoassociated virus, and lentivirus. Lentiviruses, adenoassociated viruses, and manipulation of the adenovirus genome have been explored as means of prolonging gene expression and reducing inflammation associated with first-generation adenovirus vectors. Nonetheless, adenovirus vectors continue to have advantages such as the capacity to generate high titer stocks without the use of helper virus and the ability to transfer genes efficiently over a broad range of host cells or in a targeted fashion. Direct intraparenchymal injection of adenovirus vectors into brain and spinal cord causes a mononuclear inflammatory infiltrate that leads to tissue necrosis and cell death. In contrast, remote delivery of recombinant genes to the CNS through peripheral nervous system injection of adenovirus vectors offers a number of potential advantages over direct injections. A remote injection allows minimally invasive delivery of adenovirus vectors and potential for repeated treatments. In addition, because it has the ability to decrease the number...
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of viral particles at the CNS expression site, it reduces the inflammatory response in this sensitive tissue. Despite these advantages, the time course of viral gene expression following remote delivery parallels that of direct injection. As with direct injection models, immunosuppression amplifies and prolongs remote gene expression, suggesting that the host immune system plays a role in termination of gene expression. Several mechanisms for the termination of expression have been hypothesized. The death of the transfected cells may be caused either by direct viral toxicity or by immune mediators. The immune response to viral vectors is thought to be biphasic with an early response to the viral capsid proteins. This phase of reaction has been shown to involve activation of microglia, CD4+ T cells, and macrophages. These inflammatory cells increase the expression of major histocompatibility complex surface proteins upon activation. In a later phase of the inflammatory response, CD8+ T cells respond to viral proteins presented on major histocompatibility complex Class I molecules in transduced neural cells. Remote delivery is likely to limit the early phase of the inflammatory response in the CNS by reducing the level of viral proteins in this region, but leaky expression of viral proteins, even from replication-defective vectors, may still trigger a late-phase CD8+ response, resulting in neural cell death.

Alternatively, the termination of viral gene expression may occur at the level of intracellular gene expression regulation, without the death of the transfected cell. Romero and Smith have noted that immunosuppression with anti-CD4+ and anti-CD4+5 monoclonal antibodies was capable of suppressing T-cell infiltration, although it did not indefinitely stabilize gene expression following direct spinal cord injection. Furthermore, Bromberg, et al., have raised four arguments for the existence of noncytolytic mechanisms for gene expression termination. First, transgene expression declines in several model systems prior to the evolution of a specific immune response. Second, transgene expression in hepatocytes declines 30 to 90% after intravenous administration of adenovirus but fails to show accompanying evidence of significant hepatocyte death. Third, even in the setting of immunosuppression viral gene expression in vivo does not match predicted expression in vitro. Last, viral DNA remains detectable in several systems after transgene expression is suppressed. Indeed, we have previously demonstrated that soluble cytokines turn off most promoters that are commonly used in gene delivery vectors.

Despite the absence of the inflammation and tissue necrosis that accompanies direct spinal cord injection of first-generation adenoviral vectors, gene expression following remote injection is still limited in duration. A determination of whether neurons expressing viral transgenes survive attenuated adenovirus vector infection following remote delivery is critical to future efforts at spinal cord gene therapy. Regardless of the therapeutic transgene that is induced, ultimate glial and neuronal cell death will preclude such strategies from becoming applicable in humans. Some investigators have demonstrated that no neuronal death is detected 7 days after remote delivery of either a control vector or a vector containing the β-Gal transgene. Nonetheless, 7 days is the time point of maximum gene expression. If gene expression is terminated through a cytolytic process, detection of active cell death should occur during the period when gene expression is shutting down.

In the present experiments, DNA fragmentation was examined as an indication of cell death in the spinal cord following sciatic nerve delivery of Ad5RSVntLacZ, an adenovirus carrying the reporter gene, LacZ. The expression of β-Gal peaks 1 week after sciatic nerve injection and declines thereafter, but it is not accompanied by evidence of necrosis or neuronal cell death, as demonstrated by DNA fragmentation. Furthermore, measurements of spinal cord motor neuron density indicate that the number of neurons fails to decline in parallel with transgene expression in either naïve or immunosuppressed animals. Analysis of these data indicates that whereas gene expression declines, neurons survive.

Materials and Methods

Ethical Experimentation

All experiments conducted were reviewed and approved before initiation by the University of Michigan Committee on Use and Care of Animals and the University of Michigan Biological Research Review Committee.

Surgical Procedures

Adult Sprague–Dawley rats weighing 300 to 350 g were anesthetized with sodium pentobarbital (65 mg/kg), and the lateral portion of the right thigh was shaved and sterilized with povidone iodine. A 1.5-cm incision was made posterior to the right femur, and the sciatic nerve was exposed and freed from surrounding tissue. All dissection was performed using a dissecting microscope. Sciatic nerve injections required a small amount of countertraction, accomplished by applying a loosely tied No. 3-0 silk suture around the nerve. The suture tails were cut and the remaining tie left in place post-surgery for direct visualization of the injection site during subsequent tissue preparation. The extent of countertraction and retraction was carefully controlled to minimize nerve damage. The No. 3-0 silk sutures were used to approximate the posterior muscles to the femoral periostium, and the skin was closed using a skin stapler.

Immunosuppression Treatment

Pilot experiments involving cyclosporine to amplify motor neuron gene delivery demonstrated the efficacy of immunosuppression for 14 days followed by a brief delay in immunosuppression prior to surgery. The goal of this delay was to provide immunosuppression during the initial period of viral exposure but not during the period of gene expression, which begins 3 days after injection. For immunosuppression, rats received cyclosporine pretreatment for a period of 14 days prior to sciatic nerve injections. Subcutaneous injections were performed twice daily (5 mg/kg), with injections terminating 4 days prior to surgery.

Tissue Handling and Counting Criteria

The rats were killed by pentobarbital overdose and perfused intracardially with 0.9% saline and buffered 2% paraformaldehyde. A 1-cm segment of the lumbar spinal cord and the site of sciatic nerve injection were removed. The spinal cord segment was generated by transecting the cord once at the insertion of the lumbar nerve roots and again at a point 1 cm rostral to the insertion. On tissue removal, a 2% paraformaldehyde postfixation was performed at 0°C for 1 hour. The lumbar segment was further divided into eight smaller 1.25-mm segments to allow for consistent staining. Tissue was dehydrated in 20% sucrose in PBS for at least 12 hours until sections lost buoyancy. Tissue sections were frozen, two per block, in optimal cutting temperature gel over dry ice and then cryosectioned at 10 μm. Ten sections were obtained from each block. Sections were taken at random from each block for β-Gal, TUNEL, and cresyl violet staining. Tissue sections torn during cryosectioning were discarded.
Viral Injections

The E1/E3-deleted adenovirus Ad5RSVntLacZ was used in all experiments. This vector contains the LacZ gene under the control of the RSV promoter and a nuclear localization sequence. Glass micropipettes, tapered to a 100-μm tip were placed on a Nanject that delivers 50-nl bursts of virus. The micropipette tip was advanced through perineurium along the axis of the sciatic nerve by using a micromanipulator. Gentle countertraction was applied to the nerve as 0.5-μl boluses were injected (10 bursts of 50 nl) at the puncture site over a 1-minute period, and the process was repeated four times to administer the desired dose of 1.8 × 10^7 pfu. This dose was selected as the titer associated with optimum spinal cord gene expression following remote intrasciatic nerve injection, as previously documented in an examination of the dose–response characteristics of this phenomenon. Four separate nerve punctures were conducted to improve absorption and prevent viral solution from refluxing out of the nerve.

Staining and Quantification

Beta-Galactosidase Gene Expression. Beta-galactosidase staining was performed on whole tissue segments (1.25 mm) as well as on 10-μm sections to ensure complete and accurate staining. Neuronal counts of β-Gal were conducted in four sections under ×400 magnification, one from each block, and a mean count was calculated for each group. Each section was washed three times in Laci-Z rinse (2 mM MgCl₂, 0.1 mg/ml sodium deoxycholate, 1/5000 × Triton-X-100 in PBS) at 0°C for 5 minutes each and then incubated in X-Gal stain for 15 hours at 37°C.

Terminal Deoxynucleotidyl Transferase–Mediated Deoxyuridine Triphosphate Nick-End Labeling. Serial sections adjacent to those stained for β-Gal were stained for DNA fragmentation as an indication of cell death. After quenching endogenous peroxidase activity in 3% H₂O₂ in PBS, the sections were incubated with terminal deoxynucleotidyl transferase and digoxigenin–deoxyuridine triphosphate for 1 hour at 37°C. The sections were washed three times in PBS and then incubated with anti–digoxigenin-peroxidase. The sections were developed using diaminobenzidine and counterstained with eosin. All TUNEL-stained cells were quantified under magnification (×400), and the mean for four sections was reported for each animal.

Cresyl Violet Density Measurements. Sections used for motor neuron density measurements were adjoining those used for both TUNEL and β-Gal counting. These sections were briefly rehydrated and then stained with a 2% cresyl violet solution for 12 hours. They were then dehydrated in graded alcohols (70–100%), cleared in Hemo-D, and placed on cover slips. Cells were considered motor neurons if a nucleus and at least one distinct process were apparent. Motor neuron quantification was performed on the four sections adjacent to the TUNEL-stained sections. The area of the anterior horns was measured from the axis of the central canal and below to the anterior horn. Area measurements were performed using the Image 1 at ×4 magnification and neuronal counts were performed under ×400 magnification. Density was calculated as the number of motor neurons divided by the area of the anterior horns. A density measurement was calculated for each of the four sections, and a mean density was determined for each animal.

Data Analysis

A blinded scorer conducted all tissue quantifications and assessments of inclusion criteria. All data are depicted in histograms as the mean of a given value for the group of animals (five rats in each group; total 40 specimens) examined with the standard error of the mean included as an error bar. In the first experiment involving multiple time points and different treatments (virus compared with saline), ANOVA was used to detect group and time effects as well as group-by-time interactions. In the second experiment, to generate the percentage of anterior horn motor neuron staining, β-Gal–staining motor neurons were counted in four sections evenly distributed throughout the lumbar spinal cord. The total number of motor neurons was counted using cresyl violet in the adjoining tissue sections. The number of β-Gal–positive neurons was then divided by the total number of neurons. A mean percentage of anterior horn motor neuron staining was generated for each animal. The comparison of groups (15 rats in each group; total 30 rats) was conducted using unpaired two-tailed t-tests to analyze the difference in motor neuron density and percentage of anterior horn motor neuron staining.

Sources of Supplies and Equipment

We purchased the dissecting microscope (Sterozoom 6) from Leica (Buffalo, NY). The injector (Nanject) was obtained from Drummond (Broomall, PA). The skin stapler (Reflex TL) was manufactured by Richard Allen (Richland, MI). Cresyl violet density measurements were acquired using the Image 1 from Universal Imaging Co. (West Chester, PA). Both micropipette (model PP-83) and the micromanipulator (model N-152) were purchased from Narishige (Tokyo, Japan).

We acquired the E1/E3-deleted adenovirus Ad5RSVntLacZ from Vector Core (Iowa State University, Ames). The β-Gal staining kit was obtained from Invitrogen (Carlsbad, CA) and the Apotag staining kit from Intergen (Purchase, NY). Sakura Finetek, Inc. (Torrance, CA), produced the optimum cutting temperature gel.

Results

Spinal cord sections were histologically examined at various time points after injection of Ad5RSVntLacZ into the right sciatic nerve of adult rats, and expression of the LacZ reporter gene was quantified. Cells expressing β-Gal were seen predominately on the ipsilateral side, with the most staining seen in ipsilateral anterior horn motor neurons (Fig. 1). Gene expression increased from 3 to 7 days and deteriorated over the next 11 days (Fig. 2). An ANOVA comparing virus- and saline-treated rats revealed a significant group-by-time interaction at 3, 7, 13, and 18 days (p < 0.0001). To determine whether the reduction in number of β-Gal staining cells results in part from the death of neurons expressing β-Gal, DNA fragmentation was assayed throughout this time course as an indication of cell death. An ANOVA comparing virus- and saline-treated rats showed no significant difference in the number of cells with fragmented DNA (p = 0.989; 40 samples) (Fig. 3). The cell death detected by TUNEL staining in both groups occurred universally in nonneuronal cells, and no evidence of motor neuron cell death was detected. In addition to an absence of DNA fragmentation, which was often associated with apoptotic cell death, microscopic histological analysis revealed no evidence of the cellular swelling associated with necrotic cell death. Evidence of TUNEL staining was present in all positive control spinal cord tissue treated with DNase prior to the assay.

Motor neuron densities in the anterior horn of the spinal cords obtained in virus- and saline-treated rats were calculated by dividing the number of cresyl violet–stained neurons (Fig. 4 upper) in the spinal cord anterior horn by the area of this region. An ANOVA comparing motor neuron densities in control and infected rats at all time points revealed no significant group effect (p = 0.913, 40 samples) (Fig. 4 lower), indicating that virus infection did not reduce motor neuron density.

Examination of the percentage of motor neurons expressing LacZ in the previous experiment revealed that only 13.6 ± 1.9% of motor neurons showed gene expression at the optimum dose (1.8 × 10⁷ pfu) and time (7 days) postinjection. Because of the relatively small percentage of motor neurons that are transduced upon sciatic nerve vec-
tor injection, cyclosporine was used to amplify and prolong gene expression. Cyclosporine pretreatment results in viral gene expression in 67.1 ± 4.0% of the spinal motor neurons 7 days after sciatic nerve injection (Fig. 1B). Forty-five days after viral injection in cyclosporine-pretreated rats, the reporter gene is detected in only 12.8 ± 1.2% of the motor neurons. Comparison of these values with an unpaired two-tailed t-test revealed a markedly significant reduction in neuronal staining (Fig. 5 left: p = 0.0001; 30 samples). Despite the loss of expression in roughly half of the spinal cord’s total motor neurons, neuronal density remained constant (p = 0.7; 30 samples) (Fig. 5 right). These data suggest that no neuronal death accompanies the reduction in β-Gal expression.

Discussion

Remote delivery of transgenes using adenovirus as a vector represents a potential strategy for the treatment of the spinal cord and brainstem trauma and neurodegenerative diseases. Both regions of the CNS are exquisitely sensitive to surgical manipulation, limiting the potential for direct injection of viral vectors. These limitations are particularly important in the context of an already compromised CNS. In addition to creating a mechanical insult, direct-injection procedures expose eloquent neural tissue to high concentrations of immunogenic viral capsid proteins. Because remote vector delivery of adenovirus likely occurs through axonal transportation of the virus, antigen-presenting cells in the CNS are not directly exposed to virus, resulting in a dramatic reduction in the local inflammatory response. Nonetheless, the decrease in viral gene expression occurs over the same time course observed in direct spinal cord injection. Such termination of gene expression may represent neuronal cell death or a noncytolytic process.

Neuronal death could result from a late-phase CD8+-mediated cell death of the transduced neurons or direct viral toxicity. In the present experiment we demonstrated that no DNA fragmentation or histological markers of cell death accompany the termination of gene expression in the spinal cord. Furthermore, no reduction in spinal cord neuronal density can be detected in animals following remote injection of Ad5RSVntLacZ into the peripheral nervous system. Likewise, no neuronal loss was detected in rats injected after pretreatment with cyclosporine despite the conversion of 54% of anterior horn neurons from β-Gal positive to negative. Because animals were examined 49 days after termination of cyclosporine pretreatment, the absence of cell death in this experiment was unlikely to result from persistent immunosuppression.

Viruses have the capacity to trigger neural injury either directly or through the induction of host immunity. Easton, et al.,12 have begun the study of the innate neurotoxicity of first-generation adenovirus vectors. These investigators noted that cultured sympathetic neurons underwent morphological changes including shrunken cell bodies and neurite fragmentation 5 to 7 days after infection of an adenovirus vector expressing the CMV promoter–controlled LacZ gene, leading ultimately to neuronal cell death. These overt changes were preceded by a reduction in β-Gal protein expression beginning at Day 5. Although this effect was dependent on the titer of virus, it plateaued at a multiplicity of infection of 200 pfu/cell. Reduction in protein synthesis occurred in a similar fashion after exposure to Ad5RSVntLacZ, the same virus used in the present experiment. Seventy-two hours postinfection, these cells demonstrated an increase in c-jun phosphorylation, indicative of a stress response. Despite the deletion of the E1 region of the vector, the viral E3 and E4 genes were expressed within 16 hours of infection. One of the E4 gene products has been found to interact with the transcription factor E2F, which is known to drive tran-
The expression of viral gene products may trigger both a stress response and the activation of cellular genes, ultimately culminating in toxic effects. Viral toxicity is likely to trigger an apoptotic pathway of neuronal cell death. Induction of c-myb and cyclin D1 has previously been associated with apoptosis in sympathetic neurons. An apoptotic pathway for neuronal cell death following viral toxicity is also supported by the observation of chromatin condensation in infected sympathetic neurons. Further, other investigators have documented signs of apoptosis following in vitro adenovirus infection. Because of these observations, TUNEL staining was used in the present experiment to screen for neuronal apoptosis in vivo following remote adenovirus gene delivery. No evidence of neuronal TUNEL staining was detected in our experiments. Although TUNEL staining is not specific to apoptosis, all apoptosis involves DNA fragmentation. Therefore, the absence of variance in the limited TUNEL staining detected in spinal cords of virus- and saline-injected rats at multiple time points suggests that remote adenovirus vector delivery does not trigger neuronal apoptosis in the CNS.

The direct neuronal toxicity of first-generation adenovirus vectors detected in vitro might not be observed in vivo for several reasons. The in vitro findings were only documented in sympathetic neurons, not the CNS motor neurons evaluated in the present experiment. Additionally, neurons in vivo may be more capable of tolerating the stress alteration of cellular protein synthesis induced by adenovirus gene expression. Thus, the interaction between the neuron and its environment may affect its tolerance of viral infection.

Neuronal death after exposure to adenovirus vector has been noted in vivo in direct-injection paradigms. Romero and Smith have noted that spinal cord tissue sections show “cell balling” and characteristic cytopathological features following direct adenovirus vector delivery; such degenerative morphologies correlated with immunohistochemical localization of T cells and macrophages. Other investigators have shown both a B- and T-cell response against viral vector components limiting gene expression. Peltekian, et al., have reviewed several mechanisms by which cell death might occur. They found that a late-phase CD8+ response to adenovirus vectors depended on the presentation of viral genes by major histocompatibility complex Class I molecules. Although deletion of
the E1 gene should prevent replication and subsequent activation of the viral late promoter. Leakage of this promoter may result in late expression of the viral gene and the production of viral antigens. These authors have also described alternative mechanisms for direct vector toxicity in vivo. They proposed that, because there is no limitation to the number of viral particles that can transduce a given cell, exposure to high numbers of viral particles might create both extracellular debris as well as a high number of empty cytoplasmic nucleocapsids in target neurons.28 Alternatively, Breakefield4 has proposed that the viral envelope might act in a neurotoxic fashion in some viruses. These aforementioned mechanisms are dependent on exposure of the neuronal cell body to viral capsids. Because the remote delivery paradigm removes the target neurons from the region of maximum capsid concentration, it has the potential to avoid such mechanisms. The absence of neuronal cell death in the present experiment argues against a late-phase CΔ8+-mediated mechanism of cell death.

The absence of evidence for neuronal apoptosis or reduced neuronal cell densities in the present experiment suggests a noncytolytic mechanism for termination of vector gene expression. The observation that cyclosporine prolongs gene expression correlates the immune system to gene termination. Bromberg, et al.,5 have cited several points of evidence for noncytolytic mechanisms of termination. First, viral transgene expression has been shown to decline in several systems within 2 to 5 days. Such a time course is inadequate to allow for adenovirus-specific cytotoxic T lymphocytes to eliminate these transduced cells. In addition, no necrosis or apoptosis was evident in the livers of animals treated with intravenously administered adenovirus vectors following transient transgene expression. Transient immunosuppression induced by drugs such as tacrolimus (FK506) and cyclosporine in other model systems has been shown to prolong adenovirus gene expression significantly.22 More novel approaches, such as blocking specific lymphocytic receptors, have been tested for improving transgene persistence. In this paradigm, gene expression endured for up to 60 days, but it declined thereafter, again suggesting that noncytotoxic mechanisms play an important role in reducing gene expression.31 Lastly, viral DNA has been demonstrated to persist in several model systems despite the elimination of viral transgene expression.20,43

Cytokine-mediated regulation of gene expression is one possible noncytolytic immune mechanism for controlling viral transgene expression. The CD8+ lymphocytes have been demonstrated to attenuate the amount of cellular hepatitis B virus messenger RNA found in transgenic mice designed to replicate the virus through an IFNγ and TNFα mediated mechanism. Both cytoplasmic and nuclear hepatitis B virus messenger RNA are thought to be reduced through accelerated clearance. Promoter elements located between nucleotides 3157 and 1239 are thought to be critical to this response to IFNγ and TNFα.18,36 Cytokine sensitivity may be specific to the individual viral promoter in question. The expression of viral transgenes in vectors controlled by the SV40 or CMV promoters is inhibited in vitro by IFNγ. Similarly, Qin, et al.,29 have demonstrated that IFNγ and TNFα can act independently or synergistically to suppress gene expression driven by the CMV or RSV promoters but not the β-actin promoter in adenovirus vectors, as well as expression driven by Maloney murine leukemic virus–long-terminal repeat promoter in a retroviral vector. Importantly, gene expression resumes after cytokine withdrawal, suggesting the absence of cell death. Interferon-gamma has also been noted to suppress the CMV immediate early genes by inhibiting nuclear factor–κB activity.35 It is possible that the inhibitory effect of inflammatory mediators on viral promoters represents a mechanism that evolved to decrease viral load during infections by reducing the expression of viral early genes required for replication.3

The conclusion that termination of adenoviral gene ex-

![Figure 5](https://example.com/figure5.png)

**FIG. 5.** Neuronal survival following transient β-Gal expression in the spinal cords of cyclosporine-pretreated rats. Left: Percentage of total anterior horn neurons with staining indicative of decline in viral gene expression over 45 days (p = 0.0001). Right: Anterior horn motor neuron density remains constant (p = 0.7), despite conversion of 53% of neurons from β-Gal positive to negative.
pression after remote delivery results from the termination of gene expression rather than cell death has implications for the engineering of long-acting adenoviral vectors. It is possible that alternative promoters possessing less inherent sensitivity to inflammatory mediators exist. Analysis of preliminary data suggests that the substitution of the herpes latency promoter for the RSV promoter may prolong gene expression. The herpes latency promoter drives gene expression in a small group of genes in the herpes genome throughout the life of the host, regardless of the host. This promoter may not be susceptible to inflammatory cytokines. Other future directions for investigation of the remote-delivery paradigm include quantification of the variance in gene expression that is apparent between motor neurons. This variance has implications for the types of transgenes that may be capable of a clinical impact in this setting. Given the relatively low level of gene expression that occurs in some motor neurons, transgenes for potent intracellular proteins may perform better than those aimed at increasing the secretory proteins.

Conclusions

The termination of transgene expression in vivo where viral vector components are present at the expression site has previously been linked to both cytolytic and noncytolytic mechanisms. In the present experiment, cell death accompanying the suppression of viral gene expression remote from the site of injection could not be demonstrated. We hypothesized that the separation of gene expression from virion particles reduced CNS inflammation and prevented cell death. Nonetheless, the immune system still played a role in suppression of gene expression, as demonstrated by cyclosporine administration. We hypothesize that the direct promoter effects of circulating cytokines may explain this phenomenon. The absence of neuronal death following remote vector delivery renders this model feasible for spinal cord gene therapy.

References

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Address reprint requests to: Nicholas M. Boulis, M.D., Kresge III, Room 4424, Zina Pitcher Place, Ann Arbor, Michigan 48109. email: nboulis@umich.edu.