neuronal cell turnover, may also be responsible for loss of transgene signal. Infection of neuroepithelial stem cells[17] rather than terminally differentiated cells would result in diminution of signal over time. However, we do not believe that brain cell turnover fully explains the decline in the percentage of *lacZ* expressing cells.

Because adenoviruses are ubiquitous amongst mammals including mice[18,19] it is possible that cellular factors from endogenous wild type murine adenovirus (MAV-1) may have transcomplemented the E1A gene product, allowing for productive intracranial infection and eventual loss of transgene expression[20]. To address this possibility, we assayed by western blot whole brain lysates from mice 4 and 7 days post infection with AdCMVlacZ or wild type sub360 for the presence of adenoviral specific proteins, but none were evident (data not shown). Another characteristic of productive adenoviral infection would be the development of viremia and/or infection of other organ systems. To determine if extracranial spread of the adenoviral vectors had occurred, we performed X-gal staining on sections of liver and spleen obtained from mice infected with AdCMVlacZ or E3 deleted wild type sub360 adenovirus. No *lacZ* expressing cells were seen at 4 or 7 days post infection. To detect evidence for viremia emanating from the right cerebral hemisphere, blood was obtained via the right retro-orbital sinus on days 4 and 7 post infection from animals infected with both AdCMVlacZ or wild type sub360 adenovirus. When aliquots of these peripheral blood cell lysates or heat inactivated plasma were applied to permissive LE293 cells no viable plaque forming units appeared (data not shown). These results suggest that in our system delivery and uptake of the adenoviral vectors was confined to parenchymal brain cells, with no evidence of a concomitant productive adenoviral infection.

Other factors that may be responsible for loss of *lacZ* expression include alterations in the level of transcription associated with the CMV promoter, host immune response to infected cells, or direct toxicity of transgene product. Strong promoters may prevent normal host cell functions by overloading the transcriptional/translational machinery. This may be particularly important in those cell types which are highly metabolically active, such as neurons. Further studies to specifically address the issues of stability and possible pathogenicity of adenovirally expressed transgenes are underway.

Methodology

Animals. Animal experiments were performed in accordance with institutional guidelines and approved by the University Committee on Use and Care of Animals. Mice were anaesthetized with a single dose of intramuscular ketamine/xylazine (80 mg per kg mouse; 30 mg ketamine to 7 mg xylazine) and secured on a murine stereotactic platform (David Kopf Instruments). Using sterile technique, the skull was exposed and a 1 mm burr hole was made at a point 0.3 mm caudal and 3.0 mm lateral to the zero coordinate using a high speed drill. (The zero coordinate is defined as the intersection between the sagittal and coronal sutures.) 5 µl of adenoviral suspension containing 1×10^{10} particles ml^{-1} in 20% sucrose/PBS were injected into the right caudate putamen via a 25 µl Hamilton syringe and 30 gauge needle over the course of 10 min (infusion rate = 0.5 µl min^{-1}). The needle was then withdrawn over the course of 2 min and the wounds were closed using cyanoacrylate.

Histochemical staining. 2×10^9 pfu ml^{-1} of AdCMVlacZ was brought to 20% sucrose in PBS, and delivered stereotactically into the right cerebral hemisphere in the region of the caudate putamen of 7 week old C3H/HeJ mice. At one week post-infection mice were perfused with 2% paraformaldehyde in 0.1 M PIPES pH6.9. Whole brains were excised, and post fixed in the same solution. The brain was then blocked, rinsed in PBS/2 mM $MgCl_2$, and reacted in 5 mM $K_3Fe(CN)_6$, 5 mM $K_4Fe(CN)_6$, 2 mM $MgCl_2$, 0.01% sodium deoxycholate, 0.02% NP-40 and 1 mg/ml 5-bromo-4-chloro-3-indolyl-β-D-galactoside[7] (X-gal) in PBS for 2 h, and photographed (Fig.1).

For brain sections shown in Fig. 2, 10 or 40 µm cryostat sections from brains fresh frozen in O.C.T. (Sigma), or perfused brains, respectively, were placed onto poly-L-lysine coated slides, fixed in 0.5% glutaraldehyde for 10 min, rinsed in PBS/2 mM $MgCl_2$ and stained in X-gal staining solution followed by counter-staining in neutral red.

Electron microscopy. After anaesthetization and perfusion of animals in 2% paraformaldehyde, mice brains were removed, post-fixed in the same solution, and processed for examination by EM. Briefly, tissues were post-fixed in 2% glutaraldehyde, 1.5% paraformaldehyde, and 0.0015% $CaCl_2$ in 0.1M Na cacodylate, followed by 1% osmium tetroxide in 0.1M Na cacodylate containing 5% sucrose. Samples were then dehydrated in ethanol and embedded in Spurr's embedding medium. Spurr's was chosen so that tissues could be processed from 100% ethanol without the need for propylene oxide, which causes dissolution of the X-gal precipitate from tissues. Thin sections were lightly stained with 2% uranyl acetate and photographed in a Phillips CM10 electron microscope. Use of a 15 µm objective aperture gave sufficient contrast between the electron-dense X-gal reaction product and tissues to permit viewing and photography.

Immunohistochemistry. At 4 weeks post infection mice were sacrificed, brains removed immediately and fresh frozen in O.C.T. (Sigma). 10 µm cryostat sections were placed onto poly-L-lysine coated slides, fixed in methanol at -20 °C, and blocked by incubation with 1% BSA in PBS. Slides were then incubated for 1 h with rabbit anti-neurofilament (Sigma) and biotin conjugated mouse anti-b-galactosidase (Sigma). Following several rinses in PBS sections were incubated with FITC labeled goat-anti rabbit (Sigma) and avidin-AMCA (Vector Labs) for 1 h. Slides were mounted under an anti-fadent and examined using fluorescent and light microscopy.

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