

NONNEUROTROPIC ADENOVIRUS: A VECTOR FOR GENE TRANSFER TO THE BRAIN AND GENE THERAPY OF NEUROLOGICAL DISORDERS

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I. Introduction: Some History and Other Vectors

For many years it has been virtually impossible to transfer genes into brain cells either to study or manipulate molecular, cellular, or, *in vivo*, behavioral processes. In addition to the physical barriers that protect the brain (bone and three layers of meninges), the earliest gene delivery systems, the retroviral vectors, require cell division to integrate the transgenes into their genomes to express any transgenes. Thus they limited transduction to dividing cells of the nervous system, e.g., astrocytes and oligodendrocytes, neurons in the neonatal brain undergoing cell division, or non-neural cells such as fibroblasts that could then be transplanted to the brain. Because neurons in the adult brain do not divide, retroviruses were of limited use to neurobiologists wanting to manipulate the molecular makeup of neurons *in vivo* (Fisher and Gage, 1993; Suhr and Gage, 1999; Lowenstein and Castro, 2002; Hsich *et al.*, 2002; see Kageyama *et al.*, this volume, for further discussion).

Far greater transduction efficiencies in the nervous system can now be achieved with the recently developed lentiviral vectors. Lentiviruses are a subclass of retroviruses whose genomes contain additional viral proteins. Two of these virion proteins, matrix (MA) and virion protein R (VPR), are used to transport the cDNA/integrase complex (also called preintegration complex) across the

nuclear membrane in the absence of mitosis. This allows lentiviruses not only to infect, but (at least in theory) also to express transgenes in both proliferating, e.g., astrocytes, and nonproliferating cells, such as neurons. *In vivo* experiments with lentiviral vectors, however, have demonstrated that only neurons express transgenes, presumably due to a not yet characterized selectivity of lentiviral vectors to infect neuronal cells (Kay *et al.*, 2001; Consiglio *et al.*, 2001; Brooks *et al.*, 2002).

The first lentiviral vectors were derived from human immunodeficiency virus-1 (HIV-1) but were pseudotyped by using the envelope glycoproteins from other viruses such as the vesicular stomatitis virus G protein (VSV-G), a fusion protein used to improve infection efficiency. The cell line 293 is used to generate these vectors using a three-plasmid cotransfection system. Three separate plasmids encode for the pseudotyped *env* gene, the transgene cassette, and a packaging construct respectively supplying the structural and regulatory genes *in trans* (Naldini *et al.*, 1996). Currently other lentiviruses, e.g., feline immune deficiency virus and equine infectious anemia virus, have also been engineered as gene transfer vectors. An important attraction of these latter systems is the fact that they are derived from animal, rather than human lentiviruses (Brooks *et al.*, 2002; Curran and Nolan, 2002).

Lentivirus vectors have all the desirable properties of the early Moloney murine leukemia virus (Mo-MLV)-based vectors, but, in addition, can infect both dividing and quiescent cells and induce a limited inflammatory response even in the CNS (Blomer *et al.*, 1997; unpublished observations). Lentiviruses have been used very successfully infecting and transducing the central nervous system (CNS), with almost negligible inflammatory responses (Deglon and Aebischer 2002; Hsich *et al.*, 2002; Tate *et al.*, 2002). Lentiviral vectors are currently being evaluated for safety, with a view to removing all nonessential regulatory genes to facilitate and accelerate approval for clinical trials. If the issues of increased production and safety can be overcome, higher titers are achieved, and public fears of some lentiviral vectors being HIV-1 derived are addressed, these vectors hold great promise for clinical applications within the nervous system (Ailles and Naldini, 2002; De Palma and Naldini, 2002; Galimi and Verma, 2002; Huentelman *et al.*, 2002). Novel and/or improved vector systems are constantly being developed to provide a highly effective technology with which to explore the molecular basis of neuronal gene therapy (see Jakobsson *et al.*, for further discussion).

Having briefly reviewed the current status of vectors available for gene transfer into the brain, we will now explore the potential application of replication-deficient adenovirus vectors as vehicles for gene delivery into the CNS (Table I). A significant feature of adenovirus as a potential vector for DNA delivery in human gene therapy protocols has been that a live (replication-competent) vaccine has been safely administered to many million human beings (U.S. military recruits) over several decades to provide protection against natural adenovirus infections (Rubin and Rorke, 1994). More recently, similar replication-competent

NONNEUROTROPIC ADENOVIRUS

TABLE I
A COMPARISON BETWEEN ADENOVIRUS VECTORS AND OTHER GENE TRANSFER METHODS^a

	Vectors								
	Ads	HC-Ad	HSV1/r	HSV1/a	AAV	Retro/ lentivirus	Vaccinia	SFV	Micro injection
Size (kb)	36	36	152	5-30	4.68	7-10	185	11.51	10-30
Cloning capacity (kb)	7.5	~30	10-30	~150	4.5	7-10	30	9-20	10-30
Neuronal transduction									
<i>In vivo?</i>	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
<i>In vitro?</i>	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Long-term gene expression (≥12 months)	Yes	Yes	Yes	Yes	Yes	Yes	No	No	N/A
Gene therapy?	Yes	Yes	Yes	Yes	Yes	Yes	No	No	No
Vaccination	Yes	Yes	Yes	Yes	Yes	No	Yes	Yes	No

^aHSV-1/r, recombinant HSV1-based vectors; HSV-1/a, amplicon HSV1-based vectors; Ad, adenovirus vectors; HC-Ad, high-capacity, helper-dependent adenovirus vectors; SFV, Semiliki Forest virus vectors.

adenovirus recombinants encoding defined immunogens have also been used in human vaccine trials, or as replication-competent vectors with or without additional anticancer genes to induce tumor cell killing. Although most adenovirus vaccine development has been based on exploiting replication-competent systems, replication-deficient adenovirus vectors have also proved to be highly effective agents with which to generate both humoral and cell-mediated immune responses to expressed transgenes. These vectors have definite attributes as agents for immunization. There is therefore considerable experience in using live adenovirus isolates, replication-competent adenovirus recombinants, and replication-deficient recombinant adenovirus as immunizing agents (Top *et al.*, 1971a, b; Schwartz *et al.*, 1974; Morin *et al.*, 1987; Prevec *et al.*, 1989; Eloit *et al.*, 1990; Jacobs *et al.*, 1992; Gallichan *et al.*, 1993; Rubin, 1994; Wilkinson, 1994a,b; Wilkinson and Borysiewicz, 1995; Amalfitano and Parks, 2002; Nicklin and Baker, 2002; Wickham, 2000).

Furthermore, human gene therapy programs have been launched by a number of groups in the United States and Europe, resulting in the application of replication-deficient adenovirus recombinants in the treatment of patients suffering cystic fibrosis, coronary artery restenosis postpercutaneous angioplasty, brain tumors, head and neck carcinomas, ovarian carcinomas, melanoma, and metabolic disorders among others (Rosenfeld *et al.*, 1992; Engelhardt *et al.*, 1993; Mastrangeli *et al.*, 1993; Simon *et al.*, 1993; Boucher *et al.*, 1994; Bout

et al., 1994a,b; Brody *et al.*, 1994a,b; Mittereder *et al.*, 1994; Welsh *et al.*, 1994; Wilson *et al.*, 1994; Yei *et al.*, 1994a,b; Zabner *et al.*, 1994; Kirn *et al.*, 2001; Rutanen *et al.*, 2002; Bauknecht and Meinhold-Heerlein, 2002; Raper *et al.*, 2002). Thus, a wealth of data has accumulated and continues to grow concerning the therapeutic administration of adenovirus and their effectiveness as well as their side effects in human patients.

Interest in exploiting the new capabilities offered by adenoviruses as gene therapy vectors has been very high. The key features that make adenovirus potentially a credible vector for neurological gene therapy specifically are that (1) sufficiently high titers can be easily produced as to allow their administration *in vivo*, (2) they can transduce many different differentiated cell types, including postmitotic neurons, (3) expression in the target cell can be restricted to the transgene only (reviewed in Legrand *et al.*, 2002; Nicklin and Baker, 2002), and (4) they can be scaled up to very high titres, i.e., 10^{12} – 10^{13} IU/ml. So far adenovirus recombinants have been used to transduce cells of the lung (see above for CFTR transfer; Gilardi *et al.*, 1990; Rosenfeld *et al.*, 1991; Yei *et al.*, 1994a,b), liver (Herz and Gerard, 1993; Ishibashi *et al.*, 1993; Engelhardt *et al.*, 1994; Hayashi *et al.*, 1994; Kozarsky *et al.*, 1994), arteries and other blood vessels (Lemarchand *et al.*, 1992; Roessler *et al.*, 1993; Kingston *et al.*, 2001), joints, bone marrow cells, and various differentiated circulating cells of the immune system (Haddada *et al.*, 1993), heart (Stratford-Perricaudet *et al.*, 1992; Kass-Eisler *et al.*, 1993), skeletal muscle (Quantin *et al.*, 1992; Stratford-Perricaudet *et al.*, 1990, 1992; Acsadi *et al.*, 1994a,b), brain (Akli *et al.*, 1993; Bajocchi *et al.*, 1993; Davidson *et al.*, 1993; Le Gal La Salle *et al.*, 1993; Ambar *et al.*, 1999; Dewey *et al.*, 1999; Thomas *et al.*, 2000a,b, 2001a,b), and spinal cord (Lisovoski *et al.*, 1994), and to release factor IX into the bloodstream (Smith *et al.*, 1993). The widespread potential applications of adenovirus systems has promoted further developments of the adenovirus vectors, in particular, efficient strategies have been devised for the insertion of transgenes into recombinant adenovirus. Also, the further developments have concentrated on two main limitations of the adenoviral vectors, namely, the limited transgene capacity of first-generation adenoviral vectors and the inflammatory and immune responses caused by the adenoviral virions and its genome. Inserts of up to 8 kbp are feasible in replication-deficient recombinants, which can be grown without helper virus on *trans*-complementing 293 cells (Graham *et al.*, 1977; Berkner, 1988; Wilkinson, 1994a,b), gutless vectors, and the new helper-dependent high-capacity vectors have a theoretical capacity of up to 30–36 kbp (Lowenstein *et al.*, 2002; Hartigan-O'Connor *et al.*, 2002; Zhou *et al.*, 2002).

Recent reviews have provided detailed protocols and methodologies involved in constructing and utilizing adenovirus vectors for gene transfer to cells of the brain both *in vitro* and *in vivo* (Lowenstein, 1995; Southgate *et al.*, 2001; Thomas *et al.*, 2001a,b; see Phillips, 2002). In this chapter, we will consequently concentrate on progress that has been made in the application of such vectors within

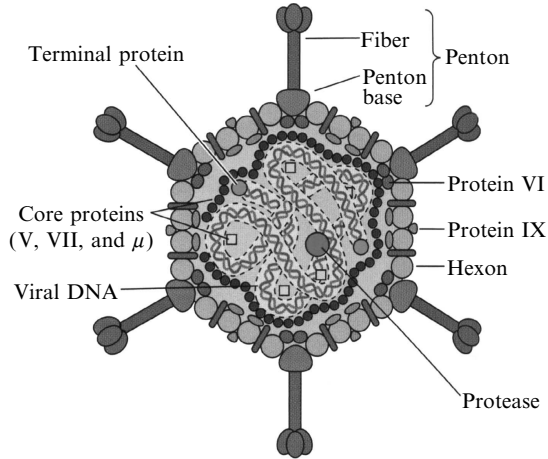
the context for their exploitation for neurobiology and discuss potential future developments in this field.

II. The Vector System

Adenoviruses have the ability to infect a wide range of different tissues and cell types. It has been shown convincingly that adenovirus type 5 serotype initiates infection by binding to the coxsackievirus and adenovirus receptor (CAR) through the knob domain located at the distal extreme of the fiber protein (Bergelson *et al.*, 1997; Roelvink *et al.*, 1999). CAR is a 46-kDa integral membrane protein with a typical transmembrane region and an extracellular region composed of two immunoglobulin (Ig)-like domains (Tomko *et al.*, 1997). The C-terminal knob of the fiber protein confers the specificity of the cellular receptor recognition. Following its binding to CAR, adenovirus is subsequently internalized through an interaction with integrins on the cell surface of the target cells, especially $\alpha_v\beta_5$ (Wickham *et al.*, 1993). Entry into the cell is by receptor-mediated endocytosis (Wickham *et al.*, 1994), after which the viral nucleocapsid is released from endosomes into the cytoplasm, and transported to the nucleus (Greber *et al.*, 1993; Trotman *et al.*, 2001). The double-stranded DNA genome (~ 36 kb; Fig. 1) is then released into the nucleus of the infected cell (Trotman *et al.*, 2001). Upon infection of a permissive cell, the replication cycle can be divided into three phases of gene expression: immediate early (E1 genes), early (E1–E4 genes), and finally the late events, which follow the onset of viral DNA replication. The very first region of the genome to become active is the left-hand region of the left strand, E1, which encodes a series of transcriptional activators, which will promote progression into the early phase of gene expression. These phases of virus replication are associated with host cell cycle progression to S1, onset of viral DNA replication, as well as the production of gene products associated with evasion of host antiviral defenses. Five late transcriptionally active regions are found at the right of the left strand, and are produced from a single major late promoter by alternative splicing (L1, L2, L3, L4, and L5). Expression of late viral genes becomes activated after the start of viral DNA replication and will eventually lead to the assembly of progeny virions, in excess of 10^5 new virions per infected cell (Shenk, 1995).

Most available recombinant adenoviruses for use as vectors for gene transfer are rendered replication defective through deletions in the E1, E2, E3, or E4 regions, or through combinations thereof. These vectors, have been termed “first (and sometimes second and third)-generation” adenovirus vectors, depending on the authors, and the particular deletions to increase the cloning capacity (Gilardi *et al.*, 1990; Davidson *et al.*, 1993; Brody *et al.*, 1994b; Wilkinson, 1994a,b; see

A



B

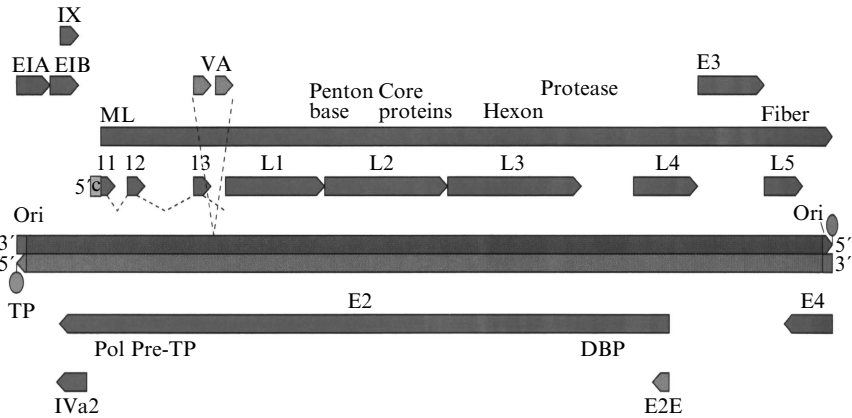


FIG. 1. (A) Schematic representation of an adenovirus particle based on the current understanding of its polypeptide constituents and genome. (B) Genome is 100 map units (mu) in length (1 mu = 360 bp). Early primary mRNA transcripts are designated in bold. Certain polypeptides are identified by conventional numbering system (roman numerals). Modified from Flint *et al.* (2000). (See Color Insert.)

Philips, 2002). The regions deleted comprise some genes that are necessary for replication (e.g., those located in the E2 region), to others that do not appear to be necessary for replication (e.g., the E3 and E4 region) and that encode genes associated with modulating the host immune response and transcriptional control. No deletions are neutral, and they will have varying effects on the production, replication, expression, and toxicity of the particular viral vectors.

Replication-deficient adenovirus recombinants deleted in various genes or genomic regions are commonly grown on *trans*-complementing 293 cells that express an integrated copy of the Ad5 E1 gene, complemented by other regions in the case of deletions in the E2 region (Graham *et al.*, 1977). Transcomplementation with the E3 or E4 region has usually not been necessary.

Efficient systems to construct adenovirus recombinants are now available both from a number of different laboratories, and more recently also from various commercial sources (e.g., Microbix Biosystems Inc., Toronto, Canada; QBio Gene, Montreal, Canada). These allow the construction of both replication-competent and replication-deficient recombinant adenoviruses for gene transfer (Prevec *et al.*, 1989), using conventional and well-established DNA cloning and transfection procedures (Sambrook, 1989). Most published reports utilize adenovirus (Ad) recombinant derived from either serotypes 5 or 2 (see Table II), although there are some 47 human serotypes identified and many more animal isolates, some of which have also been used within the context of gene transfer (Cotten *et al.*, 1993). The exploration of other adenoviral serotypes has substantially increased since 1995 as some of their distinct properties concerning the receptors used for entry, the consequent cell types transduced, have been discovered to differ in important ways from adenovirus type 2 and 5 (Zabner *et al.*, 1999; Segerman *et al.*, 2000; Shayakhmetov *et al.*, 2000). In Ad E1-deleted vectors, transgenes are usually inserted into the E1 region under the control of exogenous viral promoters (e.g., RSV-LTR, IE-hCMV). Cell specific alone, or combined with inducible promoters, or other elements that increase expression levels can also be used effectively to target expression to specific cell types, to regulate its expression, and to increase expression levels (Babiss *et al.*, 1986; Bessereau *et al.*, 1994; Grubb *et al.*, 1994; Smith-Arica *et al.*, 2000, 2001; Southgate *et al.*, 2001; Gerdes *et al.*, 2000; Harding *et al.*, 1998; Ralph *et al.*, 2000; Glover *et al.*, 2002). Most recently the use of replication-competent adenoviruses for the treatment of tumors has also begun to be explored (Nanda *et al.*, 2001; Doronin *et al.*, 2000, 2001).

III. Developments in the Use of Adenovirus Recombinants as Vectors

The deletion of the Ad E1 gene region results in a failure of the replication-deficient Ad vector to activate both early and late phase transcription from the viral genome. Consequently expression of only a transgene under the control of a constitutive or cell-type-specific or inducible promoter is achieved in the infected target cell. Although this genetic barrier to adenovirus gene expression is efficient, it is not absolute. Even limited breakthrough of Ad gene expression does occur in many if not most tissues, and can thus be problematic,

TABLE II
REVIEW OF PUBLICATIONS USING RECOMBINANT ADENOVIRUS VECTORS FOR GENE TRANSFER INTO THE BRAIN OR CONSTITUENT CELLS

References ^a	Recombinant adenovirus E1 vector	Target <i>in vitro</i> and <i>in vivo</i>	Therapeutic efficacy/type of experiment
(1) "Early" Adenoviral Gene Transfer into the Brain (1993–1994)			
Akli <i>et al.</i> (1993)	Ad-RSVlacZ, Ad type 5	Brain <i>in vivo</i> ; rats	Gene transfer into the rodent brain <i>in vivo</i>
Bajocchi <i>et al.</i> (1993)	Ad-RSVlacZ, Ad- α 1AT, Ad type 5	Intraventricular or intrastriatal administration; 5×10^8 – 10^9 pfu/animal	Gene transfer of marker enzymes and transgenes into the rodent ventricles and brain <i>in vivo</i>
Bennett <i>et al.</i> (1994)	Ad-CMVlacZ; Ad5	Adult mouse mammalian retina; subretinal injection of 10^2 – 10^9 pfu/retina in 1–10 μ l	Gene transfer into retina <i>in vivo</i>
Caillaud <i>et al.</i> (1993)	Ad-RSVlacZ; Ad5	Primary cultures of neurons and glial cells	Gene transfer into neural cells <i>in vitro</i>
Davidson <i>et al.</i> (1993)	Ad-CMVlacZ Ad type 5 E1; E3	<i>In vivo</i> : intrastriatal, mice	Gene transfer into rodent brain <i>in vivo</i>
Jomary <i>et al.</i> (1994)	Ad2/CMV lacZ	Retinal cells	Gene transfer of marker cells into retina
Le Gal La Salle <i>et al.</i> (1993)	Ad-RSVlacZ, Ad type 5	SCG and astrocytes in culture. Hippocampus and substantia nigra (SN) <i>in vivo</i> : 3 – 5×10^7 pfu/injection	Gene transfer into brain cells <i>in vivo</i> and <i>in vitro</i>
Li <i>et al.</i> (1994)	Ad-CMV β -Actin-nls lacZ; Ad5	Subretinal injection in normal and mutant mice of 4×10^4 – 4×10^7 pfu in 0.3–0.5 μ l/retina	Gene transfer into retina <i>in vivo</i>
Ridoux <i>et al.</i> (1994b)	Ad-RSVlacZ	Astrocytes <i>in vitro</i> ; 2×10^7 pfu/confluent dish	Gene transfer into astrocytes prior to intracranial transplantation
(2) <i>Ex Vivo</i> Gene Transfer and Transplantation of Transduced Cells			
Hughes <i>et al.</i> (2002)	Ad-lacZ or eGFP	<i>In vitro</i> : embryonic murine neural progenitor cells	FIV-transduced progenitors retain potential for differentiation; Ad induces differentiation into astrocytes

Corti <i>et al.</i> (1999)	Ad-tet-TH	<i>In vitro</i> : human neural progenitor cells	Ad encoding TH under negative control of the tetracycline regulatory system produces high levels of TH in neural progenitor cells
Boer <i>et al.</i> (1997)	Ad-lacZ	<i>In vivo</i> : third ventricle of rats	<i>Ex vivo</i> Ad delivered transgene expression of neurografts implanted in the third ventricle is compared to explant cultures
Yoon <i>et al.</i> (1996)	Ad-lacZ or p75	<i>In vivo</i> : intraventricular injection in mice	Ad-mediated reporter genes are introduced into progenitor cells to monitor their migration and assess stability of transgene expression
(3) Recent Vector Developments			
Umana <i>et al.</i> (2001)	HD-Ad-lacZ	<i>In vivo</i> : rats	Use of FLPe recombinase to improve quality and quantity of HD Ad production
Xia <i>et al.</i> (2000)	Ad-GFP, Ad5GFPBxHI, where "x" is the epitope number	<i>In vitro</i> : engineered cell lines and human brain microcapillary endothelia	The Ad HI loop is modified to achieve binding to a specific receptor; this is used to achieve a disseminated pattern of transduction
Cregan <i>et al.</i> (2000)	HD-Ad-lacZ	<i>In vitro</i> : primary cerebellar neuronal cultures	Efficacy and cytotoxicity of an HD Ad is compared to a first-generation Ad
Zheng <i>et al.</i> (2000)	Chimaeric Ad-retroviral vector	<i>In vitro</i> ; <i>in vivo</i> : submandibular gland, cortex, and caudate nucleus	Examination of the characteristics of an Ad construct containing LTR sequences of a retroviral vector
Beer <i>et al.</i> (1998)	Encapsulated RAdS	<i>In vitro</i> ; <i>in vivo</i> : mice	Administration of PLGA encapsulated Ad to diminish immunogenicity of the viral vectors

(Continued)

TABLE II (Continued)

References ^a	Recombinant adenovirus E1 vector	Target <i>in vitro</i> and <i>in vivo</i>	Therapeutic efficacy/type of experiment
Sato <i>et al.</i> (1998)	Ad-bc12; regulatable by <i>cre</i>	<i>In vitro</i> : PC12, a hybrid motoneuronal cell line and primary chicken motoneurons	A new strategy for delivery of Bcl-2 mediated by Cre/loxP recombination using Ad vectors
Peltekiean <i>et al.</i> (2002)	CAV2	<i>In vivo</i> : rat	Selective transduction of neuronal cells by CAV2 vectors introduced as a new tool for targeting key structures of neurodegeneration
Soudais <i>et al.</i> (2001)	CAV2	Rodent CNS neurons <i>in vitro</i> , and <i>in vivo</i>	Neurotropism of CAV2 and its efficient retrograde transport are demonstrated
(4) Transcriptional Targeting			
15 Kugler <i>et al.</i> (2001)	Ad-NSE, tubulin α 1, synapsin promoters-lacZ or Bcl-X(L)	<i>In vitro</i> : primary neuronal cultures; <i>in vivo</i> : rats	Different cellular promoters are investigated in an adenoviral context to determine their capability of neuron-restrictive transgene expression
Smith <i>et al.</i> (2000)	Ad-CMV, RSV, E1A promoters-lacZ or eGFP	<i>In vitro</i> : hippocampal brain slices; <i>in vivo</i> : rat hippocampus	Cytotoxicity and transgene expression from three viral promoters in the same Ad backbone are compared
Ralph <i>et al.</i> (2000)	Ad-synapsin 1, GFAP-tet-eGFP	<i>In vitro</i> : primary hippocampal cultures; <i>in vivo</i> : rat hippocampus	Ad system employing regulatable, cell-specific transgene expression
Navarro <i>et al.</i> (1999); Nillecamps (1999)	Ad-NSE, RSV promoters-LacZ	<i>In vivo</i> : rat	NSE and RSV promoters driving LacZ in an Ad are compared
	Ad-xNRSE-PK-luc, x denotes ≤ 12 NRSE elements	<i>In vitro</i> : different nonneuronal and neuronal cell lines and primary SCG neurons; <i>in vivo</i> : mouse tongue injection, rats	More specific neuronal transgene expression is achieved with an Ad vector construct encoding luciferase with increasing numbers of NRSEs upstream of the phosphoglycerate promoter

Miyaguchi <i>et al.</i> (1999)	Ad-SCG10-NRSE-lacZ	<i>In vitro</i> : rat hippocampal slice cultures	NRSE in an Ad-encoding lacZ to achieve selective neuronal expression
Smith-Arica <i>et al.</i> (2000)	Ad-tet-GFAP, NSE, β -Actin-CMV promoters	<i>In vitro</i> : different cell lines and primary neocortical cultures; <i>in vitro</i> : rats	A dual Ad system encoding for cell-type-specific and regulatable transcription units is tested
Morelli <i>et al.</i> (1999)	Ad-NSE, GFAP, hCMV promoters—FasL	<i>In vitro</i> : primary, neuronal, glial, fibroblastic, and epithelial cell lines; <i>in vivo</i> : systemic administration in mice	Ads encoding FasL controlled by non-specific and cell-type specific promoters are tested in different cell types and <i>in vivo</i>
Shering <i>et al.</i> (1997)	Ad, HSV-1, HSV-1 amplicon vectors—hCMV	<i>In vitro</i> : rat nervous system cell cultures	Cell-specific expression from a short HCMV major IE promoter enhancer is assessed in Ad and HSV-1 viral vectors
(5) Targeting through the Blood–Brain Barrier			
Nilaver <i>et al.</i> (1995)	Ad-RSVlacZ, HSV-1-lacZ	<i>In vivo</i> : intracerebral xenografts of human LX-1 small cell lung carcinoma in nude rats	Distribution of LacZ delivered by Ad and HSV-1 vectors after intratumor or intracarotid administration +/- osmotic disruption of the BBB
Muldoon <i>et al.</i> (1995)	Ad, HSV	<i>In vivo</i> : rat and cat	Delivery of Ad, HSV, and MION into normal brain or intraarterially is compared in animals with intact or disrupted BBB
Doran <i>et al.</i> (1995)	Ad-lacZ	<i>In vivo</i> : rats	Short-term gene expression is evaluated after intracarotid administration of an Ad encoding lacZ after or without osmotic BBB disruption
(6) Brain Immunity/Inflammation			
Zermansky <i>et al.</i> (2001)	Ad-HSV1-TK	<i>In vivo</i> : rats	12 months persisting high-level and widespread expression of Ad delivered HSV1-TK is transgene dependent

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TABLE II (Continued)

References ^a	Recombinant adenovirus E1 vector	Target <i>in vitro</i> and <i>in vivo</i>	Therapeutic efficacy/type of experiment
Thomas <i>et al.</i> (2001a)	Ad-hCMV-lacZ or HPRT	<i>In vivo</i> : rats	Mechanisms contributing to the decline in transgene expression delivered by first generation Ad vectors are examined
Thomas <i>et al.</i> (2002)	Ad-hCMV-lacZ, luc with different capsid modifications	<i>In vivo</i> : rats	Engineered viral capsids ablated for CAR and/or integrin and their inflammatory potential are examined
Thomas <i>et al.</i> (2001b)	Ad-hCMV-lacZ or HPRT, HD-Ad-hCMV-lacZ	<i>In vivo</i> : rats	Effects of preexisting antiadenoviral immunity on transduction of brain cells with HD Ad and first-generation Ad are compared
†1 Zou <i>et al.</i> (2001)	Ad-lacZ, HD-Ad-lacZ	<i>In vivo</i> : intraventricular and intrahippocampal administration in rats	Toxicity and immunogenicity of HD and first-generation Ad vectors and their effect on transgene expression are compared
Cua <i>et al.</i> (2001)	Ad-IL10	<i>In vivo</i> : animal model of autoimmune encephalomyelitis	Therapeutic effects of intracranial and systemic administration of an Ad encoding an antiinflammatory cytokine are compared
Gerdes <i>et al.</i> (2000)	Ad-hCMV, mCMV promoters-lacZ	<i>In vivo</i> : rats	Ad-delivered MIE _m CMV promoter performance is characterized
Driesse <i>et al.</i> (2000)	Ad-TK or lacZ	<i>In vivo</i> : rats and nonhuman primates	Analysis of immune response to CSF administration of Ad encoding TK with subsequent GCV or lacZ
Zou <i>et al.</i> (2000)	Ad and HD-Ad- β -Geo	<i>In vivo</i> : hippocampus of rats	Immunogenicity and its effects on transgene expression and stability are compared in HD and first-generation Ad

Kajiwara <i>et al.</i> (2000)	Ad-lacZ	<i>In vivo</i> : mice	Investigation of the humoral immune response to a first-generation Ad and its transgene delivered into the brain
Dewey <i>et al.</i> (1999)	Ad-TK	<i>In vivo</i> : rat glioma model	Characterization of long-term effects of Ad-mediated conditional cytotoxic gene therapy for glioma
Ohmoto <i>et al.</i> (1999)	Ad-lacZ	<i>In vivo</i> : different mouse strains	Influence of environmental conditions and animal priming with Ad on quality and duration of immune response to Ad gene delivery is examined
Cartmell <i>et al.</i> (1999)	Ad-hCMV-lacZ, Ad-0, Ad-TK	<i>In vivo</i> : intrastriatal and intraventricular administration in rats	Role of TNF- α and IL- β in mediating an early inflammatory response to Ad administered to different CNS targets is delineated
Proescholdt <i>et al.</i> (1999)	Ad-CMV-VEGF, Ad-CMV-lacZ	<i>In vivo</i>	Inflammatory response to Ad-mediated VEGF versus pump-injected protein and its effects on the integrity of the BBB are studied
Shine <i>et al.</i> (1997)	Ad-RSV-TK	<i>In vivo</i> : cotton rat	Toxicity of Ad-mediated TK +/- GCV at different doses in naive and pre-immunized rats is tested
Kajiwara <i>et al.</i> (1997)	Ad-lacZ	<i>In vivo</i> : mice	Quantitative analysis of the immune response to an Ad-encoding lacZ and transgene longevity
Byrnes <i>et al.</i> (1996)	Ad-lacZ	<i>In vivo</i> : mice	Immune response to Ad-delivered lacZ is examined
Byrnes <i>et al.</i> (1996a)	Ad-RSV-lacZ, HSV-1 RSV-lacZ, HSV-1 tsK	<i>In vivo</i> : caudate nucleus of rats	Duration of Ad-mediated transgene expression and related inflammation in immune-naive animals and subsequent peripheral exposure to the vector are compared

(Continued)

TABLE II (Continued)

References ^a	Recombinant adenovirus E1 vector	Target <i>in vitro</i> and <i>in vivo</i>	Therapeutic efficacy/type of experiment
Byrnes <i>et al.</i> (1995)	AdR1, Ad0, Ad5	Brain, <i>in vivo</i> ; 3×10^6 pfu/0.5 μ l, injection site	Study of the immune response to the direct administration of adenovirus recombinants into the brain.
Davidson <i>et al.</i> (1994)	Ad5-RSV-lacZ and RSV-rHPRT	Striatum and cortex <i>in vivo</i>	Gene therapy into primate brain; immune response to adenovirus vectors and encoded transgenes
Wang <i>et al.</i> (2001)	Ad-WldS	(7) Neuroprotection <i>In vitro</i> : rat DRG neurons	Ad-mediated WldS protein utilized against axonal degeneration
Tominaga-Yoshino <i>et al.</i> (2001)	Ad-APP, Ad-lacZ	<i>In vitro</i> : cultured hippocampal neurons	Adenoviral introduction of APP cDNA to enhance neurotoxic and neuroprotective effects of glutamate
Yuan <i>et al.</i> (1999)	Ad-CMV-APP or lacZ	<i>In vitro</i> : central neuron cultures	Ad is used as a vector for protein trafficking and processing studies to elucidate pathogenesis of AD
Horellou <i>et al.</i> (1994)	Ad-RSV-hTH, Ad-RSVlacZ	Brain <i>in vivo</i>	Gene therapy of Parkinson's disease; significant reduction in abnormal behavior of intrastriatal AdRSVhTH vs AdRSVlacZ
Apoptosis Zhu <i>et al.</i> (2002)	Ad-TGF- β 1	<i>In vivo</i> : mouse brains	Transfer of an antiapoptotic gene to the CNS for neuroprotection
Araki <i>et al.</i> (2001)	Ad-(wt or mutant) PS2, Ad-lac	<i>In vitro</i> : rat primary hippocampal cells <i>In vitro</i> : rat primary cortical neurons	The proapoptotic effect of Ad-mediated PS2 overexpression is examined <i>in vitro</i>

Xia <i>et al.</i> (2001)	Ad-EGFP, Ad-JBD	<i>In vitro</i> : human neuroblastoma cells; <i>in vivo</i> : mouse MPTP model of PD	Ad-induced inhibition of the JNK pathway protects MPTP lesioned DA neurons
Neurotrophic factors Dorsey <i>et al.</i> (2002)	Ad-trkB	<i>In vitro</i> : cultured primary hippocampal neurons	Correction of dysregulated receptor expression with an adenoviral vector relating to an animal model of Down's syndrome
Benraiss <i>et al.</i> (2001)	AdCMV-BDNF-IRES-hGFP	<i>In vivo</i> : intraventricular administration in rats	Ad delivered BDNF to induce neuronal recruitment from endogenous progenitor cells in the adult forebrain
Connor <i>et al.</i> (2001)	Ad-lacZ, Ad-GDNF	<i>In vivo</i> : intrastriatal and intra-SN administration in a 6-OHDA rat model of PD	Neuroprotective mechanisms of Ad-mediated GDNF are elucidated
Kozlowski <i>et al.</i> (2001)	Ad-hGDNF	<i>In vivo</i> : monkey caudate nucleus	Quantitative assessment of Ad-mediated hGDNF expression, using ELISA and (RT-)PCR techniques
Kozlowski <i>et al.</i> (2000)	Ad-lacZ, Ad-GDNF	<i>In vivo</i> : intrastriatal and SN injection in a 6-OHDA rat model	Behavioral testing of Ad-mediated GDNF therapy into the striatum or SN is compared
Connor <i>et al.</i> (1999)	Ad-LacZ, Ad-GDNF, Ad-muGDNF	<i>In vivo</i> : 6-OHDA PD model in aged rats	Effects of Ad-mediated GDNF delivery to the striatum and SN are compared
Kitagawa <i>et al.</i> (1999)	Ad-LacZ, Ad-GDNF	<i>In vivo</i> : rats	Effects of Ad-mediated GDNF on ischemic brain injury are studied
Bemelmans <i>et al.</i> (1999)	Ad-LacZ, Ad-BDNF	<i>In vivo</i> : rat model of Huntington's disease	Therapeutic value of an intrastriatal injection of Ad-mediated BDNF in Huntington's disease is assessed
Choi-Lundberg <i>et al.</i> (1998)	Ad-lacZ, Ad-mGDNF, Ad-GDNF	<i>In vivo</i> : intrastriatal injection in 6-OHDA rat model of PD	Effects of intrastriatal administration of an Ad encoding GDNF on dopaminergic cell survival are examined

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TABLE II (Continued)

References ^a	Recombinant adenovirus E1 vector	Target <i>in vitro</i> and <i>in vivo</i>	Therapeutic efficacy/type of experiment
Choi-Lundberg <i>et al.</i> (1997)	Ad-lacZ, Ad-mGDNF, Ad-GDNF	<i>In vivo</i> : 6-OHDA rat model of PD	Neuroprotective effect of Ad-mediated GDNF injected near the SN is examined
		(8) Traumatic or Ischemic Brain Injury/Excitotoxicity	
Qiu <i>et al.</i> (2002)	Ad-FasL	<i>In vivo</i> : mouse parietal cortex and contused human cortical tissue samples	Role of Fas receptor in traumatic brain injury is addressed with an Ad encoding FasL
Kim <i>et al.</i> (2002)	Ad-Cu/Zn SOD	<i>In vitro</i> : embryonal cortical neurons <i>In vivo</i> : rats	Intracisternal administration of an Ad encoding human Cu/Zn SOD to restore CBF autoregulation after fluid percussion injury
Matsuoka <i>et al.</i> (2002)	Ad-bFGF, Ad-Bcl-xL	<i>In vitro</i> : rat neuronal cells	Synergistic neuroprotective effect through Ad-mediated bFGF and Bcl-xl against EAA toxicity
Aoki <i>et al.</i> (2001)	Ad-GRP78	<i>In vitro</i> : rat hippocampal neurons; <i>in vivo</i> : hippocampus of gerbils	Ad-mediated overexpression of GRP78 preserves rat neurons; hypothermic treatment is neuroprotective by restoring GRP78 expression in ischemic brain
Masada <i>et al.</i> (2001)	Ad.RSVlacZ, Ad-IL-1ra	<i>In vivo</i> : cerebral intraventricular injection in rats	Ad-mediated overexpression of IL-1ra reduces ischemic brain injury
Yang <i>et al.</i> (2001)	Ad-CMV-lacZ, Ad-CMV-mSAG, Ad-CMV-SAG	<i>In vivo</i> : mouse	Overexpression of SAG, an antioxidant protein, via an Ad vector to prevent ischemic injury of brain cells

Gupta <i>et al.</i> (2001)	Ad-RSV-LacZ, Ad-RSV-GT	<i>In vitro</i> : hippocampal cultures	Ad-induced overexpression of the Glut-1 glucose transporter <i>in vitro</i> enhances neuronal survival after exposure to the excitotoxin KA
Ralph <i>et al.</i> (2001)	Ad-TRE-pep2m, Ad-TRE-pep4c, Ad-TRE-EGFP, Ad-Syn-tTA	<i>In vitro</i> : primary hippocampal neurons	AMPA receptors convey postischemic neurotoxicity; Ad-mediated reduction of AMPA receptor expression provides neuroprotection
Ooboshi <i>et al.</i> (2001)	Ad-lacZ	<i>In vivo</i> : spontaneously hypertensive rats	Effects of brain ischemia on expression of an Ad-delivered marker gene are examined
Yang <i>et al.</i> (1999)	Ad-RSV-lacZ, AdRSV-IL-1ra	<i>In vivo</i> : CD-1 mouse	Ad-mediated overexpression of IL-1ra is used to assess the role of interleukin-1 in cerebral ischemia
Yang <i>et al.</i> (1999)	Ad-RSV-lacZ, AdRSV-IL-1ra	<i>In vivo</i> : intraventricular administration in CD-1 mice	Effects of Ad-delivered IL-1ra on ICAM-1 protein in cerebral ischemia
Yang <i>et al.</i> (1997)	Ad-RSV-lacZ, AdRSV-IL-1ra	<i>In vivo</i> : intraventricular administration in mice	Effect of Ad-mediated IL-1ra on cerebral ischemia is assessed
Hagan <i>et al.</i> (1996)	Ad-lacZ, Ad-IL-1ra	<i>In vivo</i> : perinatal rats	Impact of Ad-mediated overexpression of IL-1ra is assessed in a model of excitotoxicity
Betz <i>et al.</i> (1995)	Ad-RSV-lacZ, AdRSV-IL-1ra	<i>In vivo</i> : intraventricular injection in rats	Suitability of Ad-mediated IL-1ra in reducing ischemic brain damage is tested
Abe <i>et al.</i> (2002)	Ad-lacZ, Ad-p53	(9) Brain Tumors <i>In vivo</i> : athymic rat brains	Intraarterial infusion of p53-containing Ad
Shinoura <i>et al.</i> (2002)	Ad-APAF1, Ad-Casp9, Ad-Bax, Ad-Fas, Ad-p53	<i>In vivo</i> : glioma cells harboring a mutated p53	Cotransduction of Apaf-1 and caspase-9 via Ad to increase p53-mediated apoptosis in glioma cells

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TABLE II (Continued)

References ^a	Recombinant adenovirus E1 vector	Target <i>in vitro</i> and <i>in vivo</i>	Therapeutic efficacy/type of experiment
Miller <i>et al.</i> (2002)	Ad-CMV-CD	<i>In vitro</i> : different human tumor cell lines; <i>in vivo</i> : immunodeficient mouse model of glioma	Glioma cell lines can be as sensitive as GI tumor cells to the antineoplastic effects of 5-FU; efficacy and specificity of Ad-mediated CD and subsequent systemically delivered 5-FC are tested in a mouse model of human glioma
Adachi <i>et al.</i> (2002)	Ad-uPAR/p16	<i>Ex vivo</i> intracerebral tumor model and <i>in vivo</i> subcutaneous tumor model	Ad-mediated delivery of a bicistronic construct containing antisense uPAR and sense p16 genes to suppress glioma invasion and growth
Nanda <i>et al.</i> (2001)	IG.Ad5E1(+); E3Luc, HSV1-TK gene [IG.Ad5E1(+).E3TK]	<i>In vitro</i> : human glioma cell lines, rat gliosarcoma cell line, other tumor cell lines; <i>in vivo</i> : a mouse s.c. glioma xenograft model	Combination of replication-competent Ad and the HSV1-TK/GCV suicide system to improve glioma treatment outcome
Maleniak <i>et al.</i> (2001)	AdhCMV-mFasL, Ad-CMV-TK	<i>In vitro</i> : primary human glioma-derived cell cultures	Ad delivered FasL or HSV1-TK/GCV therapy to induce cell death in some glioma cell lines resistant to the chemotherapeutic agent CCNU
Okada <i>et al.</i> (2001)	Ad-TK	<i>In vivo</i> : rat glioma model	s.c. tumor injection of Ad expressing HSV1-TK/GCV shows vaccination effect by inducing a CTL response
Galanis <i>et al.</i> (2001)	Ad-MV-F/H	<i>In vitro</i> : cultured glioma cell lines; <i>in vivo</i> : glioma xenografts in nude mice	Assessment of two fusogenic membrane glycoproteins as adenovirally delivered transgenes for glioma therapy
Lammering <i>et al.</i> (2001)	Ad-EGFR-CD533	<i>In vitro</i> : human glioma cell lines; <i>in vivo</i> : glioma xenografts	Ad-mediated overexpression of a dominant-negative epidermal growth factor receptor to achieve radiosensitization of glioma cells

Glaser <i>et al.</i> (2001)	Ad-TK	Human malignant glioma cells	Molecular pathways mediating TK/GCV-induced cell death are elucidated
Eck <i>et al.</i> (2001)	Ad-CMV-hIFN- β	Phase I trial in humans	Assessment of toxicity and efficacy of hIFN- β intralesional gene therapy for patients with recurrent or progressive malignant glioma with tumor resection
Sandmair <i>et al.</i> (2000)	Ad-CMV-TK	Phase I trial in humans	Evaluation of safety and efficacy of TK gene therapy by using retrovirus packaging cells and Ad
Trask <i>et al.</i> (2000)	Ad-RSV-TK	Phase I clinical study	Patients with recurrent malignant brain tumors are treated with HSV TK/GCV
Lawinger <i>et al.</i> (2000)	Ad-REST-VP16	<i>In vitro</i> : three types of human medulloblastoma cells	Effects of expression of REST-VP16 mediated by an Ad vector are studied in medulloblastoma cells
Brust <i>et al.</i> (2000)	Ad-TK	<i>In vitro</i> : RT2 rat glioma cells; <i>in vivo</i> : soft tissue RT2 cell tumors in rats	Combination of Ad-delivered TK/GCV therapy and subsequent radiosensitization with BrdC
Shinoura <i>et al.</i> (2000)	Ad-FasL, Ad-p53	<i>In vitro</i> : A-172 and U251 glioma cell lines	Coinfection with Ad-mediated p53 and FasL to enhance apoptosis in glioma cells
Kurihara <i>et al.</i> (2000)	AxCALNLNZK, Ax2iNPNCre	<i>In vitro</i> : seven human glioma cell lines, HeLa and COS-7 cells	Glioma-cell specific Ad-mediated expression is compared in different glioma and nonglioma cell lines
Cowsill <i>et al.</i> (2000)	Ad-TK, Ad Δ TK	<i>In vitro</i> : primary neuronal and glial cultures; <i>in vitro</i> : rat striatum	Ad encoding TK or its truncated form +/- GCV is compared
Adachi <i>et al.</i> (2000)	Ad-lacZ, Ad-CAG-UPRT, Ad-CD	<i>In vivo</i> : rat brain tumor model	Ad-mediated combined CD/5-FC and UPRT therapy is evaluated

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TABLE II (Continued)

References ^a	Recombinant adenovirus E1 vector	Target <i>in vitro</i> and <i>in vivo</i>	Therapeutic efficacy/type of experiment
Fathallah-Shaykh <i>et al.</i> (2000)	Ad-lacZ, AdIFN γ	<i>In vivo</i> : mouse model of metastatic brain tumor	Therapeutic effect of Ad-mediated IFN- γ is examined
Biroccio <i>et al.</i> (1999)	Ad-p53, Ad-0	<i>In vitro</i> : different glioblastoma cell lines	wt-p53 gene transfer enhances BCNU sensitivity in glioblastoma cells depending on the administration sequence
Harada <i>et al.</i> (1999)	Ad-p16		Evaluation of the effect of restoring Ad-mediated p16 on tumor angiogenesis in p16-deleted glioma cells
Shinoura <i>et al.</i> (1999)	Adv-lacZ-F/K20 and Adv-lacZ-F/wt	<i>In vitro</i> U-373 MG glioma cells; <i>in vivo</i>	Therapeutic effect of replication-competent Ad with a fiber mutation is compared to its corresponding Ad with wt fiber
Wagenknecht <i>et al.</i> (1999)	Ad-XIAP	<i>In vitro</i> : different human glioma cell lines	Expression and biological activity of XIAP in human malignant glioma are examined using Ad as a delivery tool
Driesse <i>et al.</i> (1998)	Ad-TK	<i>In vivo</i> : nonhuman primates	Toxicity study of Ad-mediated TK with GCV therapy
Puumalainen <i>et al.</i> (1998)	Ad-lacZ, BAG retrovirus-lacZ	<i>In vivo</i> : humans	Safety and efficiency of retrovirus and Ad-mediated β -galactosidase in human malignant glioma are examined
Gomez-Manzano <i>et al.</i> (1997)	Ad-p53/p21	<i>In vitro</i> : human glioma cell lines	Ad-mediated p53 and p21 is examined to characterize their role and timing in p53-induced apoptosis

Smith <i>et al.</i> (1997)	Ad-GFP, Ad-GFP/5-HT3	<i>In vivo</i> : rats and monkeys	Toxicity and dose profiles are presented for Ad-mediated TK and systemic GCV administration in healthy animals
Tanaka <i>et al.</i> (1997)	Ad-sPF4	RT2 glioma cells <i>in vitro</i> and <i>in vivo</i> , injected into the subrenal capsule or the caudate nucleus of nude mice	Analysis of the activity of retroviral and Ad vectors expressing a secretable form of PF4, an antiangiogenic protein
Eck <i>et al.</i> (1996)	Ad-TK	<i>In vivo</i> : human phase I trial in patients with recurrent glioma	Evaluation of safety and efficiency of Ad-mediated TK followed by systemic GCV therapy
Vincent <i>et al.</i> (1996a)	Ad-MLP-luc, Ad-MLP-TK	<i>In vitro</i> : several human cell lines; <i>in vivo</i> : rats	Efficiency of Ad-mediated TK and systemic GCV administration is assessed in a rat model of leptomeningeal metastases
Goodman <i>et al.</i> (1996)	Ad-RSV-TK	<i>In vivo</i> : baboons	Toxicity studies of Ad-mediated TK and systemic GCV in nonhuman primates
Vincent <i>et al.</i> (1996b)	Ad-TK	<i>In vivo</i> : 9L tumors in rats	Ad-mediated TK transfer is compared to retroviral mediation followed by systemic GCV in a rat gliosarcoma model
Viola <i>et al.</i> (1995)	Ad-lacZ	<i>In vitro</i> : human glioma cell cultures; <i>in vivo</i> : rat models of solid brain tumors and meningeal cancer	Transduction profiles of Ad encoding β -galactosidase in solid and leptomeningeal tumor models are assessed
Badie <i>et al.</i> (1994)	AdLacZ	Rat C6 glioma cells	Delivery of marker genes to the CNS and brain tumors
Boviatsis <i>et al.</i> (1994)	Ad5MLP lacZ	Rat 9L gliosarcoma cells	β -Galactosidase expression in tumors and neurons; tumor necrosis
Chen <i>et al.</i> (1994)	Ad5, Ad/RSV-TK	C6 glioma	Tumor treatment/significant tumor decrease/long-term tumor regrowth
Perez-Cruet <i>et al.</i> (1994)	Adv-tl	<i>In vivo</i> : 9L in Fischer rats	Tumor treatment effective for >120 days

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TABLE II (Continued)

References ^a	Recombinant adenovirus E1 vector	Target <i>in vitro</i> and <i>in vivo</i>	Therapeutic efficacy/type of experiment
		(10) Metabolic Brain Disease	
Kosuga <i>et al.</i> , 2001a	Ad-lacZ, Ad-GUSB	<i>In vivo</i> : monkey	<i>In vitro</i> enzyme deficiency of MPSVII reduced by transduction with an Ad-expressing GUSB; adenovirally transduced mAEC transplanted into monkey brain to determine their migration and distribution
Shen <i>et al.</i> , 2001	Ad-GALC	<i>In vivo</i> : intraventricular administration in a murine model of human globoid cell leukodystrophy	Effects of Ad-mediated replacement of GALC in Krabbe disease
Kosuga <i>et al.</i> , 2001b	Ad-GUSB	<i>In vivo</i> : mice	Adenovirally transduced mAEC overexpressing GUSB transplanted into mice with MPSVII
Ghodsi <i>et al.</i> , 1998	Ad-GUSB	<i>In vivo</i> : intraventricular or intrastriatal injection in MPSVII or wt mice	Activity of Ad-delivered GUSB is examined up to 84 days postadministration
Peltola <i>et al.</i> , 1998	Ad-AGA, Ad-AGU(Fin)	<i>In vitro</i> and <i>in vivo</i> : primary neuronal cultures from/and mouse model of AGU	Effects of systemic and intraventricular administration of AD encoding for the deficient enzyme AGA
Plumb <i>et al.</i> , 1994	Ad-RSV-rHPRT	<i>In vivo</i> : mice	Utility of Ad encoding HPRT in HPRT-deficient mice is examined

(11) Other Applications: Brain Imaging, Pathway Tracing, Substance Abuse

Umegaki <i>et al.</i> (2002)	Ad-CMV-DopD(2)R	<i>In vivo</i> : rats	PET is shown to be useful for longitudinal <i>in vivo</i> assessment of D(2)R expression mediated by an Ad vector in rat brain
Ross <i>et al.</i> (1995)	Ad-TK	<i>In vivo</i> : 9L gliosarcoma rat model	Ad-mediated TK and GCV therapy is evaluated with MRI and localized H magnetic resonance spectroscopy
Mackler <i>et al.</i> (2000)	Ad-NAC-1	<i>In vivo</i> : rats	Ad-mediated overexpression of NAC-1 protein affects long-term behavior in psychostimulant abuse
Lisovoski <i>et al.</i> (1994)	Ad-RSV-LacZ, Ad type 5	Spinal cord	Neuroanatomy: cell labeling for morphological studies
Kuo <i>et al.</i> (1995)	Ad-RSVlacZ	<i>In vivo</i> ; injection into the laterodorsal striatum	Suitability of an Ad vector encoding β -galactosidase for neuronal pathways tracing is tested
Ridoux <i>et al.</i> (1994a)	Ad-RSVLacZ	CNS, basal ganglia	Pathway tracing in the CNS

Abbreviations

Ad	Adenovirus
AD	Alzheimer's disease
AGA	Aspartylglucosaminidase
AGU	Aspartylglucosaminuria
APP	Amyloid precursor protein
α_1 -AT	α_1 -Antitrypsin
BBB	Blood-brain barrier
BCNU	Carmustine
BDNF	Brain-derived neurotrophic factor
bFGF	Basic fibroblastic growth factor
BrdC	5-Bromo-2'-deoxycytidine

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TABLE II (Abbreviations Continued)

CAR	Coxsackie and adenovirus receptor
CAV2	Canine adenovirus type 2
CBF	Cerebral blood flow
CCNU	1-(2-Chloroethyl)-3-cyclohexyl-1-nitrosourea
CD	Cytosine deaminase
CMV	Human cytomegalovirus
CTL	Cytotoxic T-lymphocyte
DA	Dopaminergic
DRG	Dorsal root ganglion
D(2)R	Dopamine D(2) receptors
EAA	Excitatory amino acid
FasL	Fas ligand
5-FC	5-Fluorocytosine
FIV	Feline immunodeficiency virus
FPI	Fluid percussion injury
5-FU	5-Fluorouracil
GALC	Galactocerebrosidase
GI	Gastrointestinal
GRP78	Glucose-regulated protein 78
hGDNF	Human glial cell-line-derived neurotrophic factor
GUSB	β -Glucuronidase
HCMV major IE	Human cytomegalovirus major immediate early
HD	Helper dependent
HPRT	Hypoxanthine-guanine-phosphoribosyltransferase
HSV1	Herpes simplex virus type 1
ICAM-1	Intercellular adhesion molecule-1
IL-1 β	Interleukin-1 β
IL-1ra	Interleukin-1 receptor antagonist
IRES	Internal ribosomal entry site
JBD	JNK-binding domain

JNK	c-Jun N-terminal kinase
KA	Kainic acid
LTR	Long terminal repeat
Luc	Luciferase
MIE _m CMV	Major immediate early murine CMV
MION	Monocrystalline iron oxide nanoparticles
MLP	Major late promoter
MPSVII	Mucopolysaccharidosis type VII
MPTP	1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine/1-methyl-4-phenylpyridinium
MRI	Magnetic resonance imaging
Nls	Nuclear localization signal
NSE	Neuron-specific enolase
NRSE	Neuron-restrictive silencer element
6-OHDA	6-Hydroxydopamine
PD	Parkinson's disease
PET	Positron emission tomography
PF4	Platelet factor 4
PK	Phosphoglycerate kinase
PLGA	Polylactic glycolic acid
PS2	Presenilin 2
RAd	Recombinant adenovirus
REST	Repressor element 1 silencing transcription factor
RPE	Retinal pigment epithelium
RSV-LTR	Rous sarcoma virus-long terminal repeat
SCG	Superior cervical ganglia
SN	Substantia nigra
TGF- β 1	Transforming growth factor- β 1
TH	Tyrosine hydroxylase
TK	Thymidine kinase
TNF- α	Tumor necrosis factor- α
trkB	Tyrosine receptor kinase B

(Continued)

TABLE II (Abbreviations Continued)

UPAR	Urokinase-type plasminogen activator receptor
UPRT	5-Fluorouridine 5'-monophosphate
VEGF	Vascular endothelial growth factor
VP16	Activation domain of a viral protein
WldS protein	Unique protein derived from a spontaneous mutant mouse, characterized by slow Wallerian degeneration
Wt	Wild type

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it may either by itself affect the physiology of the target cell, induce cytotoxic responses, a cellular immune response, as well as provide antigenic epitopes recognized by the adaptive arm of the immune response (Engelhardt *et al.*, 1994; Yang *et al.*, 1994a,b; Thomas *et al.*, 2000b, 2001b; Lowenstein and Castro, 2002; Mahr and Gooding, 1999; Wold *et al.*, 1994, 1999; Hackett *et al.*, 2000; Wood *et al.*, 1996a). Although infections at low multiplicity with E1-deleted vectors are unlikely to lead to high level adenoviral gene expression, the same is not true at high multiplicity of infections such as used in gene therapy applications. This is likely to remain the case, even if deletion of E1a, E1b, and other regions of the adenovirus genomes provides a better block than simply the E1a deletion (Imperiale *et al.*, 1984; Spergel and Chen-Kiang, 1991). Nevertheless extremely high input multiplicities of infection (in excess of 100 or even higher infectious units per cell regularly achieved in *in vivo* experimental designs) can result in breakthrough of low level expression of the Ad genome (Engelhardt *et al.*, 1994; Kass-Eisler *et al.*, 1994; Yang *et al.*, 1994a,b). A further modification to current vectors is therefore of interest, i.e., the supplementation of deletions in E1, E3, with an additional conditional mutation in E2a, the so-called "second-generation vectors" (Engelhardt *et al.*, 1994; Yang *et al.*, 1994a,b). The E2a region plays a role in activating late phase gene expression. Recombinants carrying this additional mutation have proved to be less reactogenic and permit prolonged expression of the transgene (Engelhardt *et al.*, 1994; Yang *et al.*, 1994a,b). Expression of late genes from the adenoviral genome that are normally expressed only following adenoviral DNA replication suggests that limited replication of the adenoviral genome may occur in some cells. This remains to be proven. In view of the fact that the liver contains proteins that can substitute for E1a function, this topic ought to be investigated carefully.

Further, forskolin can increase the breakthrough to late gene expression. Forskolin results in enhanced levels of intracellular calcium and activation of the transcription factor cAMP responsive element binding protein (CREB or ATF), a factor that is known to bind and stimulate transcription from the Ad early promoter (Muchardt *et al.*, 1990). It is therefore not surprising that forskolin treatment of cells infected with a replication-deficient Ad recombinant can enhance breakthrough to late phase gene expression. Both a high input multiplicity of infection (around the site of virus inoculation or instillation) and elevated calcium levels are likely to occur in transduced cells following *in vivo* gene transfer. Both these phenomena are very likely to coincide in neurons surrounding the site of adenovirus direct injection into the brain. Physiological conditions at the site of gene transfer could thus further regulate transgene expression from viral vectors.

The induction of an immune response to elements of the Ad vector is now perceived as a significant limitation of the vector in long-term gene therapy protocols, especially when systemic administration is needed. Several groups have characterized the effects of the virions on brain inflammation, and the

effects of peripheral injections on the stimulation of adaptive immune responses (Byrnes *et al.*, 1995, 1996a,b; Matyszak and Perry, 1996; Wood *et al.*, 1996a; Wood, 1996; Morral *et al.*, 1997; Matyszak 1998; Perry, 1998; Gerdes *et al.*, 2000; Thomas *et al.*, 2000b, 2001a,b, 2002). The effects of each of these on transgene expression are reviewed in detail elsewhere (Lowenstein and Castro, 2002). Although gene transduction remains possible in seropositive animal models and thus possibly in human clinical trials, repeated exposure to viral antigens will be expected to reduce the efficiency of gene transfer. The lack of need to utilize adjuvant when immunizing against adenovirus indicates that even small amounts of vectors leaking into the peripheral immune compartments could activate such immune responses. In the nervous system elements of the adaptive immune response, but not innate inflammatory processes and cells, are able to completely shut-off transgene expression, apparently in the absence of cytotoxicity (unpublished observations). Whether similar phenomena occur in other organs remains to be determined.

Utilizing different Ad serotypes as vectors in sequential treatment administrations could to some extent circumvent this problem. Breakthrough to early phase transcription was demonstrated by the identification of a humoral immune response to early phase proteins. In a mouse model breakthrough has been strongly correlated with the induction of CD8⁺ cytotoxic T-lymphocyte (CTL) response to adenovirus proteins, which is responsible both for an inflammatory response and an elimination of target cells expressing a transgene (Yang *et al.*, 1994a,b). It has, however, been difficult to conclusively demonstrate that such CTLs are effectively cytotoxic *in vivo*, and thus some of these results and their implications for immune-mediated killing of target cells have been challenged (Wadsworth *et al.*, 1997). Many experiments have been performed using extremely high doses of input virus. If lower doses of adenovirus recombinant are inoculated into a mouse, major histocompatibility complex-I (MHC-I) responses specific for a viral transgene in the absence of a detectable response to the vector can be induced (Schadeck *et al.*, 1999). However, high input doses of recombinant may be essential for efficient *in vivo* gene transfer.

It is also important to consider that many experiments are performed in non-permissive or semipermissive species (Ginsberg *et al.*, 1991; Ross and Ziff, 1992), e.g., mice, rats, and nonhuman primates. In such systems, Ad E1 recombinants are unable to replicate due to a "species block." It would thus be useful to test recombinants in a more relevant species, such as "permissive" cotton rat (*Sigmodon hispidus*), to determine whether cell-specific factors in addition to species-specific factors regulate the capacity of adenovirus to replicate in particular target organs (Oualikene *et al.*, 1994).

Many researchers see the current cloning capacity of ~7–8 kb as a limitation toward the more general applicability of adenoviral vectors. There is a stringent limitation exerted by the adenovirus particle, which will package only an additional 3% (~1 kb) in addition to the full-length viral genome (Shenk, 1995).

Thus, a new generation of high-capacity helper-dependent adenoviral vectors (also known as “gutless” or “guttled” vectors; HD-Ad) has been developed that is devoid of all viral coding sequences (Fig. 2) (Kochanek *et al.*, 1996; Mitani *et al.*, 1995; Chen *et al.*, 1997, 1999; Morral *et al.*, 1998; Morsy *et al.*, 1998; Schiedner *et al.*, 1998; Burcin *et al.*, 1999; Sandig *et al.*, 2000; Akagi *et al.*, 1997; Maione *et al.*, 2001; Parks *et al.*, 1996, 1999a,b; Cregan *et al.*, 2000; Ng *et al.*, 2001, 2002; Umana *et al.*, 2001; Lowenstein *et al.*, 2002). These vectors have a minimum requirement for the extreme termini of the linear adenovirus genome, containing only those *cis*-acting elements for viral DNA replication and packaging, mainly the inverted terminal repeat (ITR) sequences and packaging signal. Because these elements are contained ~500 bp from the ends of the genome

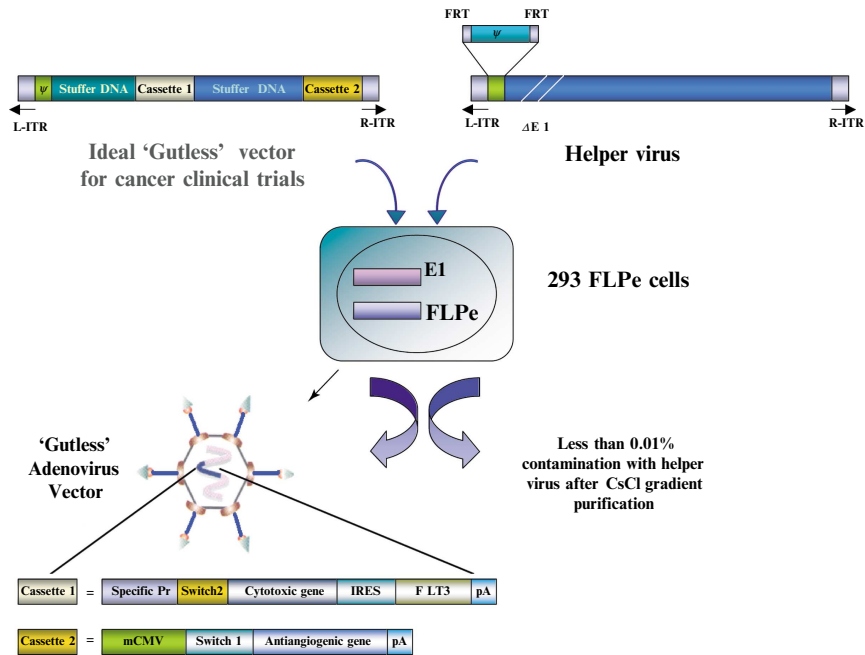


FIG. 2. An E1-, E3-deleted helper adenovirus (FL helper) with a packaging signal sensitive to FLP3-mediated excision. (A) FL helper was generated by homologous recombination in 293 cells after cotransfection with plasmid p Δ E1sp1A-2xFRT and pBHG10-CMVluc-I. The bottom half of the figure indicates the left-end region of the helper virus genome, before and after FLP3-mediated recombination; the figure also indicates the location of the PCR primers used to determine excision of the packaging signal, ϕ (flanked by black arrows). (B) Production of gutless adenoviral vectors. Diagram of an ideal gene delivery vector for neurological gene therapy using the “gutless” adenoviral vector. The packaging signal of the helper virus, flanked by FRT sites, is excised after growth in 293 cells expressing FLPe recombinase. The cloning capacity of this system (N30 kbp) will enable the simultaneous use of two or more therapeutic genes and also includes inducible promoter systems. (See Color Insert.)

(Grable and Hearing, 1992), helper-dependent vectors have the potential encode range of few hundred base pairs to up to ~30 kb of foreign DNA, which is close to the size of the native genome.

HD-Ad are copropagated with an E1-deleted helper virus, which provides in *trans* all of the proteins required for packaging the vector. Up to now, several systems have been developed to prevent packaging of the helper virus. The Cre/loxP-based system for the generation of HD-Ad involves the use of a first-generation helper virus, where the packaging signal is flanked by loxP recognition sites (Hardy *et al.*, 1997). Infection of Cre-expressing 293 cells with the helper virus results in excision of the viral packaging signal, rendering the helper virus DNA unpackageable but still able to replicate and provide helper functions for HD-Ad vector propagation (Chen *et al.*, 1996). Purification by cesium chloride centrifugation is necessary to reduce the titer of the helper virus to negligible levels, typically ranging from 0.1 to 0.01% of the HD-Ad titer (Morsy *et al.*, 1998). Recently, another Flp/rtt-base system has been developed. The Flp recombinase was used in place of Cre, and shown to excise the rtt-flanked packaging signal in helper virus efficiently (Lowenstein and Castro, 2001; Ng *et al.*, 2001; Umana *et al.*, 2001). The most recent improvement to this system is the development a new Cre-expressing cell line based on E2T, an E1 and E2a complementary cell line (Zhou *et al.*, 2001). Thus an E1 and E2a double-deleted helper virus can be used with the new cell line to produce HD-Ad vector with low helper contamination, further improving HD vector safety (Zhou *et al.*, 2001). Compared with first-generation adenovirus vectors, the HD-Ad vector can efficiently transduce a wide variety of cell types from numerous species in a cell cycle-independent manner as first generation, but HD-Ad vector has the added advantage of increased cloning capacity, reduced toxicity and immune responses, and prolonged stable transgene expression *in vivo* (Schiedner *et al.*, 1998; Thomas *et al.*, 2000a,b; Thomas *et al.*, 2001a,b). This system is essentially analogous to herpes simplex virus (HSV1) amplicons and shares some of their limitations (Lowenstein, 1994, 1995), e.g., low titers and the presence of variable amounts of helper virus in viral stocks, although novel systems that produce vectors in a helper-free fashion are now available (Wade-Martins *et al.*, 2001; Logvinoff and Epstein, 2001; Wang *et al.*, 2002). Eventually, it should be possible to develop this system so that the recombinants can be produced in the absence of helper virus to generate a safer vector with markedly reduced potential to be reactogenic.

IV. Adenoviral Recombinant Vectors: Applications to Basic Neuroscience

The technology for gene transfer to the brain has progressed substantially during the past 5 years, and new improved systems are being rapidly developed.

Table I compares the major systems for gene transfer into neurons *in vitro* and into the brain *in vivo*. Vectors vary dramatically in their efficiency of gene transfer, longevity of expression, associate toxicity, and size of the transgene they can harbor. Some vectors mainly direct short-term expression of transgenes, either because of their intrinsic toxicity [e.g., Semliki Forest virus (SFV), vaccinia], or promoter shut-off, a poorly understood phenomenon by which promoters within viral vectors eventually stop being active in spite of the vector genomes being present. Adenoviruses mediate long-term transgene expression in the central nervous system (up to over 1 year) in naive (nonimmunized) animals. Immunization completely eliminates expression from first-generation vectors but not high-capacity helper-dependent adenoviral vectors, whereas in preimmunized animals, transgene expression mediated by first-generation adenoviruses declines within 2–4 weeks postinjection, but expression from the high-capacity helper-dependent adenoviral vectors is reduced only to approximately 50% and stays stable thereafter (Thomas *et al.*, 2000a,b; Thomas *et al.*, 2001b).

In 1993 four groups independently reported for the first time *in vivo* gene transfer into several types of brain cells using adenovirus recombinant encoding β -galactosidase (Akli *et al.*, 1993; Bajocchi *et al.*, 1993; Davidson *et al.*, 1993; Le Gal La Salle *et al.*, 1993). Since these initial breakthroughs, there has been a logarithmic increase of original papers using adenovirus recombinants to transduce brain cells (see Table II). Although there is some specificity in the capacity of different adenovirus serotypes to infect/express in different target cells (Acsadi *et al.*, 1994a,b; Bessereau *et al.*, 1994; Grubb *et al.*, 1994; Millecamps *et al.*, 1999) a recombinant adenovirus-derived vector can indeed express transgenes in all brain cells, e.g., neurons, astrocytes, oligodendrocytes, ependymal cells, fibroblasts, macrophages, endothelial blood vessel cells, retinal pigment epithelium, photoreceptors, as well as retinal neurons proper, and peripheral nerve Schwann cells either *in vivo* or *in vitro* (Table II; Akli *et al.*, 1993; Bajocchi *et al.*, 1993; Caillaud *et al.*, 1993; Davidson *et al.*, 1993; Le Gal La Salle *et al.*, 1993; Bain *et al.*, 1994; Bennett *et al.*, 1994; Jomary *et al.*, 1994; Li *et al.*, 1994; Byrnes *et al.*, 1995; Lowenstein, 1995; Shering *et al.*, 1997; Morelli *et al.*, 1999; Smith-Arica *et al.*, 2000; Thomas *et al.*, 2000a,b; Umana *et al.*, 2001; Thomas *et al.*, 2000b, 2001a,b, 2002).

While initial experiments were performed using recombinant adenovirus to transfer marker proteins into the brains of both rodents and primates, recombinant vectors encoding therapeutic genes for treatment of neurological diseases have now been generated (Table III) (Chen *et al.*, 1994; Davidson *et al.*, 1994; Perez-Cruet *et al.*, 1994; Shewach *et al.*, 1994; Lowenstein, 1995; Geddes *et al.*, 1997; Ambar *et al.*, 1999; Dewey *et al.*, 1999; Gupta *et al.*, 2001; Bohn *et al.*, 1999; Lawrence *et al.*, 1999; Bohn, 2000; Connor *et al.*, 1999, 2001; Kozlowski *et al.*, 2000, 2001; Amalfitano and Parks, 2002). Expression of transgenes after *in vivo* administration of vectors to the brain was detected up to 5–6 months

TABLE III
NEUROLOGICAL GENE THERAPY: WHAT CAN BE ACHIEVED WITH ADENOVIRUS VECTORS?^{a,d}

Disease	Noninherited		Inherited				
	Tumors	PD ^e	HD	CMT	Leukodystrophies	Lesch-Nyhan	Frax-A
Outcome	Fatal	Fatal	Fatal	Nonfatal	Fatal	Fatal	Nonfatal
Distribution	Focal	Focal	Diffuse	Diffuse	Diffuse	Diffuse	Diffuse
Age at manifestation	Variable	Late	Variable	Variable	Early	Early	Prenatal
Presymptomatic detection ^b	No	No	Yes	Yes ^d	Yes ^d	Yes ^d	Yes ^d
Genotype predicts disease severity	ND	ND	Yes	Yes	Yes/no ^e	Yes	No
Gene cloned	Yes ^f	Yes ^g	Yes	Yes	Yes	Yes	Yes
Expression needed	Short term	Long term	Long term	Long term	Long term	Long term	Long term
Experimental	Tumor	Dopamine	Reduce	Normalize	BMT	Express	Express
Gene therapy	Cell killing	Replacement, GDNF, AADC	Expression of expanded allele	PMP22 expression	Expressing missing genes	Brain	Brain
Clinically relevant therapeutic protocols available	Yes	Yes	No	No	Yes	No	No
Available adenoviral vectors	Yes	Yes	No	No	No	Yes	No

^aHD, Huntington's disease; CMT, Charcot-Marie-Tooth disease (the commonest genetic alteration is duplication of the region of chromosome 17p11.2 containing the PMP 22 gene); Frax-A, fragile X syndrome type A; PD, Parkinson's disease (idiopathic); BMT, bone marrow transplant. This table is modified from MacMillan and Lowenstein (1996).

^bEven if presymptomatic detection is available, it is not done routinely during pregnancies; it is performed only if there is any suspicion due to a positive family history.

^cThe primary pathology is loss of dopaminergic neurons from the pars compacta of the substantia nigra, but other lesions within PD are distributed throughout the brain.

^dMolecular genetic diagnosis is possible in the fetus, but it is arguable whether this would be prior to the occurrence of pathological changes.

^eGenotype predicts disease severity in metachromatic and Krabbe's leukodystrophy, but not yet in adrenoleukodystrophy.

^fThe genes mutated in inherited brain tumors have been cloned. However, therapeutic gene therapy is more likely to utilize the transduction of cytotoxic genes to eliminate tumor cells.

^gThe genes for tyrosine hydroxylase and dopa decarboxylase have been cloned. These are not, however, mutated in either familial or sporadic PD.

and even after 18 months postinoculation (Geddes *et al.*, 1997; Thomas *et al.*, 2000a,b; Thomas *et al.*, 2000b, 2001a,b; Zermansky *et al.*, 2001).

Other examples of adenoviruses encoding therapeutic transgenes for preclinical applications include recombinant adenoviral vectors encoding hypoxanthine phosphoribosyltransferase (HPRT), which could be utilized in the treatment of the Lesch–Nyhan syndrome. Although these vectors have mainly been utilized to transduce HPRT in primate (Davidson *et al.*, 1994) and rodent brains (Davidson *et al.*, 1994; Lowenstein, 1995; Southgate *et al.*, 2001), there is yet no published account of their capacity to complement an HPRT deficiency in animal models for the Lesch–Nyhan syndrome. Adenovirus vectors expressing tyrosine hydroxylase, and their therapeutic efficacy in a rat model of Parkinson's disease, have been reported (Horellou *et al.*, 1994; Corti *et al.*, 1999a,b; Hida *et al.*, 1999). Recombinant adenoviruses encoding neuroprotective factors such as glial cell line-derived neurotrophic factor (GDNF), aimed to prevent dopaminergic neuron degeneration in a rat model of Parkinson disease, have also been successfully developed (Bilang-Bleuel *et al.*, 1997; Choi-Lundberg *et al.*, 1997, 1998; Bohn *et al.*, 1999; Lawrence *et al.*, 1999; Bohn, 2000; Connor *et al.*, 1999, 2001; Kozlowski *et al.*, 2000, 2001).

Because adenovirus recombinants can be used to transduce essentially all brain cells, they have also been postulated to be applicable to gene therapy protocols for the treatment of brain tumors, by utilizing them to deliver cytotoxic genes directly into the tumors. Thus, a number of groups have utilized several adenovirus recombinants, e.g., expressing HSV1 thymidine kinase or Fas ligand, Fas receptor, or p53, under the control of viral promoters. In experimental paradigms the use of these viruses appears to be promising (Badie *et al.*, 1994; Boviatsis *et al.*, 1994; Brody *et al.*, 1994a; Chen *et al.*, 1994; Perez-Cruet *et al.*, 1994; Shewach *et al.*, 1994; Ambar *et al.*, 1999; Dewey *et al.*, 1999; Morelli *et al.*, 1999; Shinoura *et al.*, 2000a,b; Shono *et al.*, 2002; see George *et al.*, this volume).

Recombinant retroviruses have been used in the treatment of brain tumors (Ram *et al.*, 1993; Palu *et al.*, 1999; Tamura *et al.*, 2001), and replication-competent and conditional HSV1 vectors as well as HSV1 amplicon vectors have also been tried (Martuza *et al.*, 1991; Markert *et al.*, 1993; Kramm *et al.*, 1997; Pechan *et al.*, 1999; Herrlinger *et al.*, 2000; Papanastassiou *et al.*, 2002; see Hu and Coffin, this volume). In a recent phase I clinical trial the efficacy and safety profiles of retrovirus and adenovirus expressing HSVI TK have been compared (Sandmair *et al.*, 2000). Adenoviruses showed very promising results, both in terms of their safety and also their efficacy in glioma growth control (Sandmair *et al.*, 2000). However, the only vector system that has progressed to a phase III clinical trial has been a retroviral vector expressing HSV-1 thymidine kinase. The trial was halted because of the absence of positive effects in the gene therapy arm of patients treated (Rainov, 2000; Rainov and Kramm, 2001).

With Phase I and II being essentially toxicity trials, Phase III efficacy clinical trials are crucial in order to determine whether any of the new therapies are indeed effective, because such results can be obtained only rarely from the much smaller Phase I and II trials.

Two groups have also made interesting observations on the use of adenovirus recombinants that should be of interest to neuroanatomists, namely the use of adenovirus for pathway tracing and cell labeling. Ridoux and colleagues (1994a) observed retrograde labeling of substantia nigra neurons after injections of recombinants expressing β -galactosidase into the striatum; similar results were also observed by Byrnes *et al.* (1995). Thus, replication-defective adenovirus could be used for retrograde labeling of neuronal pathways. Interestingly, HSV1-derived recombinants can also be used to trace neuronal pathways (Ugolini *et al.*, 1989), with some recombinants being specific for anterograde transport and others for retrograde transport (Zemanick *et al.*, 1991), while certain pseudorabies recombinants have been shown to be specific markers for individual neuronal circuits and capable of retrograde axonal transport (Card *et al.*, 1991, 1992; Mazarakis *et al.*, 2001; Enquist *et al.*, 1998; DeFalco *et al.*, 2001). Lisovoski *et al.* (1994) utilized adenovirus recombinants to label neurons of the spinal cord at different stages of development to study their morphological development. The staining they obtained after *in vivo* administration filled the dendritic arbors of neurons, thus allowing for their morphological and morphometric examination during spinal cord development.

An effective way of delivering gene products to the brain, rather than by introducing it into constituent cells, is by transplanting genetically engineered cells directly into the CNS. So far, most genetically engineered cells expressing transgenes for transplantation have been transduced with recombinant retroviral vectors. Ridoux *et al.*, (1994b) have used adenovirus to transduce primary cultures of rat astrocytes *in vitro* and then transplanted these into the CNS of host rats. Expression of transgene was detected for at least 5 months. Thus, adenovirus might constitute an alternative to retrovirus for transducing cells in preparation for transplantation into the brain. In many cases expression from retroviral vectors ceases after a few days to a few weeks; thus, it will be interesting to compare both systems vis-à-vis length of transgene expression.

Expression of transgenes after the administration of adenovirus recombinants into adult brains has been seen by some groups to last from 6 to 18 months. It also was reported that injection of recombinants into neonates has allowed expression to be sustained over periods up to a year. Long-term modulation of the immune response and the elucidation of its exact role in the regulation of long-term expression from adenoviral recombinants administered into the CNS will be crucial to achieve stable expression over a long period of time, which will be needed to implement gene therapy for chronic neurological disorders in humans. (Stratford-Perricaudet *et al.*, 1990; Kass-Eisler *et al.*, 1994; Geddes

et al., 1997; Thomas *et al.*, 2000a,b; Thomas *et al.*, 2000b, 2001a,b; Zermansky *et al.*, 2001).

Scant information is available on the interactions of adenovirus with the brain. Thus it will be of great importance to explore this field, e.g., adenovirus entry into brain cells, transport to the nucleus, and viral replication in neurons of permissive species. In addition, the effect of adenovirus recombinants on the electrophysiology of specific neuronal populations will have to be investigated. It also will be critical to assess the effects of adenoviral delivery into different brain regions on animal behavior. It is surprising that not much work on these topics has been published in the past 5 years.

V. Adenoviral Recombinant Vectors: Applications to Neurological Gene Therapy

Essentially there are two types of applications for which vectors for gene transfer into the brain could be used. Short-term expression could be used, for example, to express cytotoxic products to kill tumor cells, or to provide drugs to block ischemia-induced neurotoxicity. Ideally, the area of brain tissue to be targeted should be focal, and therapeutic benefit should be predicted after short-term transgene expression (Table III). Long term expression would be needed to treat chronic neurodegenerative disorders, such as Parkinson's disease, Alzheimer's disease, or amyotrophic lateral dystrophy. In this case, the area of brain tissue will be larger, and the results of the therapeutic intervention may not be seen until after several years.

The availability of viral vectors that express short term at high levels of expression could lead to important new treatments for brain diseases. A case in point are brain tumors, which are now being treated experimentally with adenovirus recombinants expressing conditional cytotoxic gene products (Badie *et al.*, 1994; Boviatsis *et al.*, 1994; Brody *et al.*, 1994a,b; Chen *et al.*, 1994; Perez-Cruet *et al.*, 1994; Shewach *et al.*, 1994; Takamiya *et al.*, 1992; Barba *et al.*, 1994; Benedetti *et al.*, 1997; Ram *et al.*, 1997; Bansal and Engelhard, 2000; Ikeda *et al.*, 2000; Trask *et al.*, 2000; Jacobs *et al.*, 2001; Rainov and Kramm, 2001). Brain tumors constitute a good target disease for gene therapy for the following reasons: (1) the disease is focal; (2) the therapeutic objective—the destruction of tumor cells—needs to be achieved within the short term, and this can be done using cytotoxic or conditionally cytotoxic gene products; (3) the disease is life threatening within 6–12 months of diagnosis; (4) no effective treatments are available; and (5) adenovirus-particle-induced inflammation could be a beneficial adjunct to tumor cell elimination. Similarly, replication-deficient and replication-competent HSV1-derived vectors have also been also developed (Martuza *et al.*, 1991; Markert *et al.*, 1993) and these vectors have already been tested in Phase I–III clinical trials

(Martuza *et al.*, 1991; Markert *et al.*, 1993, 2000; Rampling *et al.*, 1998, 2000; Rainov, 2000).

Achieving long-term expression in brain following the administration of recombinant vectors will open up the development of gene therapy clinical trials for human neurological diseases where long-term expression is paramount, such as Alzheimer's disease, Parkinson's disease, Huntington's disease, and the metabolic and degenerative brain disorders (Amalfitano and Parks, 2002; Hsich *et al.*, 2002; Lowenstein, 2002; Lowenstein and Castro, 2002). What makes it difficult to treat such diseases is that they affect large areas of the brain, many extending throughout large anatomical regions of the human CNS, and they progress relatively slowly over years or decades. For Parkinson's diseases there are as yet no presymptomatic diagnostic methods available, so treatment can be instituted only when symptoms appear. In families suffering from Huntington's disease, presymptomatic diagnosis is now possible (Holloway *et al.*, 1994) and this might also soon become possible in Alzheimer's disease (Saunders *et al.*, 1993; Nalbantoglu *et al.*, 1994; Scinto *et al.*, 1994). The diffuse nature of these diseases has led to the development of other therapeutic interventions that could target genes and their products to different areas located throughout the brain, such as transplantation of genetically engineered cells (Fisher, 1993), the direct intracranial administration of neuronal growth factors (NGF), (Seiger *et al.*, 1993), or more recently the development of embryonic and or neural stem cells. HSV1 (Lokensgard *et al.*, 1994; Burton *et al.*, 2001; Kay *et al.*, 2001; Latchman and Coffin, 2001; Lilley *et al.*, 2001) and adeno-associated virus vectors (Kaplitt *et al.*, 1994; Sanlioglu *et al.*, 2001; Fu *et al.*, 2002; Muramatsu *et al.*, 2002) might also provide alternative vectors to achieve long-term transgene expression in brain for clinical applications in humans.

Although human brain infections caused by adenovirus are extremely rare, it has been reported that adenovirus can cause central nervous system disease, especially in children and immunocompromised individuals, although rare cases have also been described in nonimmunocompromised patients (Ginsberg and Prince, 1994; Engel, 1995; Chirmule *et al.*, 1999; Ginsberg, 1999). Meningoencephalitis, encephalitis, and cerebellar ataxia have been reported in children in association with adenovirus infection of the CNS, mostly adenovirus type 7, 1, 2, or 32 (Chou *et al.*, 1973; Kelsey, 1978; Davis *et al.*, 1988; Osamura *et al.*, 1993). Direct inoculation of adenovirus into the brain of rhesus monkeys has demonstrated that several adenovirus serotypes can cause neuropathology, although adenovirus did not appear to replicate to a great extent in the primate brain (Ginsberg and Prince, 1994; Engel, 1995; Chirmule *et al.*, 1999; Ginsberg, 1999). Also, neuropathology due to an anamnestic response of the immune system following the injection of the adenovirus type 5 into the primate brain was recently described (Davidson *et al.*, 1994).

The possibility that latent adenovirus infection might be localized to the human brain, and that adenovirus could enter the brain through infected macrophages from the periphery, has not yet been examined in enough detail, although widespread neuronal inclusions diagnostic of adenovirus particles have been seen in some fatal encephalitis cases (Chou *et al.*, 1973). However, all these data taken together with our observations that wild-type adenovirus can express endogenous proteins in neurons, and brain glial cells, strongly suggest that adenovirus could replicate, or at least express many of its genes after infecting human neurons or glial cells and that expression might proceed for a longer time than predicted, even in the absence of symptoms of encephalitis. If there is a risk of adenoviral encephalitis it will have to be carefully considered at a time adenovirus vector is directly administered into the brain. It is clear that many more studies will be required to clarify the origins of neurotoxicity after direct injections of adenovirus virions into the brain. The use of the completely deleted high-capacity helper-dependent adenoviral vectors will completely avoid these potential complications.

VI. Immune Responses to Recombinant Adenoviral Vectors Delivered into the Brain

As stated above, recombinant adenoviruses are very attractive vectors for gene transfer and therapy within the CNS, however, they still cause acute inflammation, and any of their expressed genes can be the target of adaptive immune responses. Local inflammation is elicited in the brain in response to the injection of either a first-generation, or a high-capacity helper-dependent adenoviral vector. This acute inflammation is dose dependent, self-limited (disappears within 30 days), adenoviral-capsid dependent, and does not affect long-term adenoviral-mediated transgene expression (Byrnes *et al.*, 1995, 1996a; Kajiwara *et al.*, 1997; Gerdes *et al.*, 2000; Thomas *et al.*, 2000b, 2001a,b, 2002). Injection of adenoviral vectors into the brain leads to persistent expression (e.g., up to 18 months). This is thought to be due to the failure to stimulate an effective antiadenoviral T cell response following the careful injection of these vectors into the brain parenchyma (Byrnes *et al.*, 1996b; Wood *et al.*, 1996a; Kajiwara *et al.*, 1997; Gerdes *et al.*, 2000; Thomas *et al.* 2000b, 2001a,b, 2002). Activation of antiadenoviral immune response by peripheral immunization with adenovirus leads to massive lymphocyte infiltration of the brain parenchyma, macrophage/microglial activation, up-regulation of MHC I and II, and loss of vector-mediated transgene expression (Byrnes *et al.*, 1996a; Gerdes *et al.*, 2000; Thomas *et al.*, 2000b, 2001a,b, 2002). Mechanisms of loss of transgene expression have not been completely elucidated. Either cytotoxicity of transduced cells or downregulation of mRNA expression could explain it. Thus, loss of transgene expression from first-generation adenoviral vectors in the

brain following systemic immunization against adenovirus limits their utility for long-term neurological gene therapy applications (Lowenstein and Castro, 2002).

Recent work from our laboratory indicates that high doses of E1/E3-deleted viral vectors ($>10^8$ IU) cause direct acute cytotoxicity, and chronic inflammation, which lead to reduced transgene expression and substantial tissue damage (Thomas *et al.*, 2001a,b). We thus reasoned that an absolute reduction in the dose of vectors would be necessary to eliminate both the acute cytotoxicity and chronic inflammation. The use of the powerful mCMV promoter in adenoviral vectors has recently uncovered the capacity to transfer transgenes into the brain, and achieving similar results in the levels of transgene expression while using 2–3 logs lower doses of vectors when compared to vectors employing the hCMV promoter. The important reduction in total viral dose needed thus allows transduction in the absence of acute cytotoxicity or chronic brain inflammation (Gerdes *et al.*, 2000).

Additionally, E1/E3-deleted adenoviruses do express adenoviral proteins encoded within their genomes (Yang *et al.*, 1994a,b). These can either generate specific immune responses or provide target epitopes recognized by the activated adaptive immune system. We thus studied transgene expression and inflammatory responses elicited by the intrastriatal injection of E1–E3-deleted or HC-Adv vectors in four different paradigms: (1) acutely in naive rats, (2) long term, in naive animals, (3) long term, in naive animals, followed by a peripheral immunization against adenovirus type 5, and (4) medium term, in animals immunized against adenovirus type 5 preceding intracranial vector injection (Thomas *et al.*, 2000b, 2001a,b, 2002).

These studies have shown the following. (1) Injection of 1×10^7 IU of either E1/E3-deleted vectors or HC-Adv into the brain causes an acute, transitory inflammatory response, with both cellular (e.g., activation of microglia) and molecular components (e.g., upregulation of MHC I-I). This acute inflammation does not affect long-term transgene expression (see Figs. 3–6). (2) Within 12 months the expression of a marker transgene from first generation vectors decreases. This decrease is less when expression is directed by an HC-Adv. (3) Following peripheral immunization against adenovirus type 5, transgene expression from an E1/E3-deleted adenovirus vector is completely abolished within 45 days, whereas expression from a HC-Adv remains unaffected (Fig. 4). (4) Down-regulation of transgene expression appears to be mediated at the transcriptional level (specific reduction of transgene mRNA levels), rather than cytotoxicity (unpublished data). (5) Expression from an E1/E3-deleted adenovirus in brains of animals previously immunized against adenovirus type 5 is almost completely eliminated by 14 days postinjection, whereas expression from an HC-Adv is reduced to only 50%, and remains stable for at least 2 months (Thomas *et al.*, 2000b, 2001a,b, 2002; Lowenstein and Castro, 2002). The complexities, causes, and consequences of using viral vectors as gene transfer vectors in the brain have been explored elsewhere (Lowenstein, 2002).

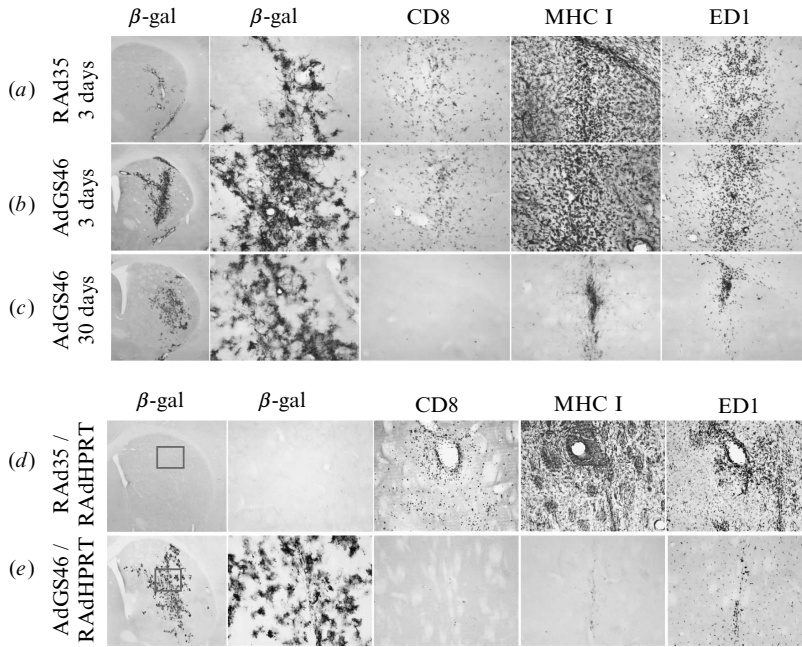


FIG. 3. Transgene expression (β -galactosidase) and inflammation in the brains of animals injected with either the E1/E3-deleted vector RAd35 or the HC-Adv AdGS46. Rows *a* and *b* show β -galactosidase expression (columns 1 and 2), CD8⁺ cell infiltration (column 3), MHC class I upregulation (column 4), and microglial cell activation (ED1, column 5), 3 days after injection of 1×10^7 IU of RAd35 (row *a*) or AdGS46 (row *b*) into the brains of naive animals. Levels of transgene expression and inflammation mediated by the E1/E3-deleted, or the HC-Adv vector, were indistinguishable in naive animals. Transgene expression from 1×10^7 IU of both RAd35 and AdGS46 is stable for at least 30 days in naive animals and inflammation resolves within this time frame (shown in row *c* only for AdGS46). However, transgene expression from E1/E3-deleted vectors in the CNS is rapidly eliminated and accompanied by severe brain inflammation if animals receive a subsequent *peripheral* infection with adenovirus [row *d*; RAd35 injected in the brain at Day 0, RAdHPRT (Southgate *et al.*, 1999) injected in the skin at Day 60. See also the quantitative analysis of all markers, in panel *f*]. In contrast, transgene expression from HC-Adv remains stable, and no brain inflammation is elicited when animals are subsequently injected with RAdHPRT in the skin (row *e*; AdGS46 injected in the brain at Day 0, RAdHPRT injected in the skin at Day 60).

VII. Summary: Targeting the Brain with Adenovirus-Derived Vectors

The early generations of replication-deficient adenovirus vectors, as well as the most recently developed high-capacity helper-dependent adenovirus vectors are extremely versatile vehicles for gene transfer into the brain, and have thus

NONNEUROTROPIC ADENOVIRUS

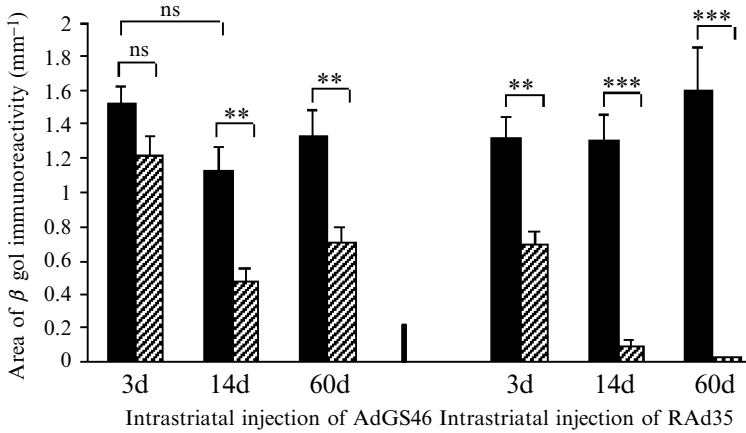


FIG. 4. Preimmunization against adenovirus eliminates rapidly transgene expression from E1/E3-deleted vectors, but not from high-capacity vectors. Quantification of the area within 40- μ m-thick brain sections occupied by β -galactosidase and ED1. Error bars show the SEM value from the five animals in each experimental group. Student's *t* test was used to calculate the degree of significance of differences between levels of transgene expression and inflammation in the brains of nonimmunized animals (black bars) and immunized animals (hatched bars) after intrastriatal injection of RAD35 (right) or AdGS46 (left). The x-axis indicates the days after vector injection; vector was injected on day 0, and animals were immunized 14 days before the brain injection.

become invaluable reagents to neuroscientists. Current applications cover wide areas from helping to address basic neurobiological problems to clinical trials for the treatment of human diseases. The main advantages of adenovirus-derived vectors are as follows:

1. the existence of well-characterized methods to produce high titer GMP grade replication-defective vectors for human clinical trials, as well as methods to produce high titers of high-capacity helper-dependent vectors with contamination levels of helper virus <0.01%. Although these last ones have yet to be used in clinical trials, the production of high levels of such vectors is being pursued by several groups;
2. a capacity to infect, in the adult brain, both terminally differentiated neurons and dividing brain astrocytes, both *in vivo* and *in vitro*;
3. a capacity to transduce both immature neonatal and mature neurons;
4. the potential to achieve cell type-specific and regulated transgene expression using appropriate promoters and transcriptional regulatory elements;
5. an ability to achieve multiple infections of a single target cell with separate vector virions;
6. their use to elucidate neuronal morphology and connectivity;
7. long-term high-level expression *in vivo* in naive nonimmunized animals;

8. low incidence of adenovirus-induced brain infections in humans; and
9. large experience in the use of adenovirus to immunize humans against natural infections.

Although the results so far have been highly encouraging (Tables II and III), adenovirus vector systems continue to be optimized for application to somatic cell gene therapy and specifically for neurological gene transfer. Currently, progress in clinical neurological gene therapy is both dependent on and limited by the technology for gene transfer. Selection of the most appropriate vector system for each application is crucial. Adenoviral vectors clearly have distinct advantages for efficient gene delivery into nervous system both *in vitro* and *in vivo*. Vector development will undoubtedly further enhance the utility of these vectors and allow their implementation for gene therapy applications to treat neurological diseases.

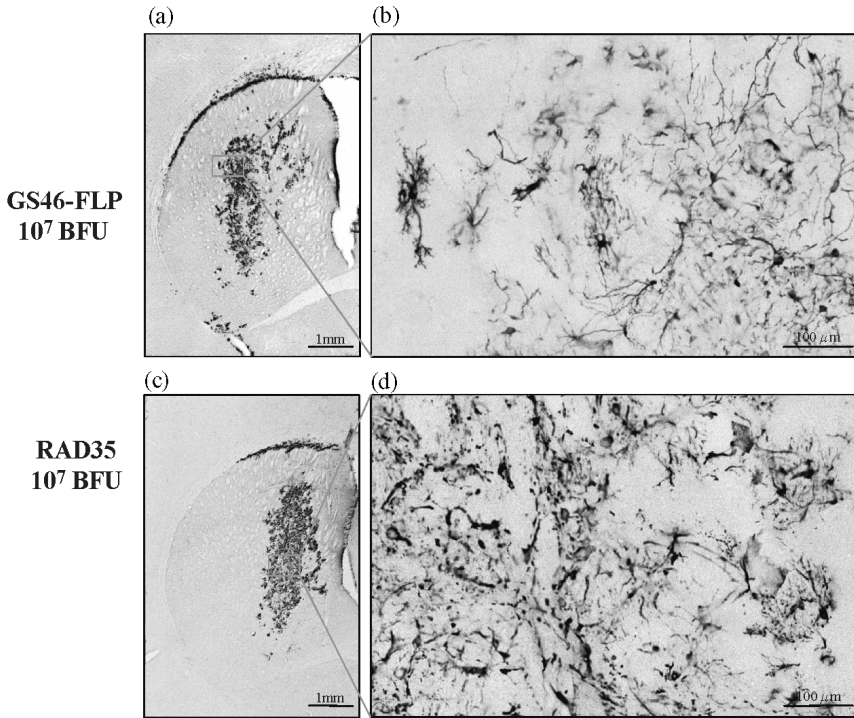


FIG. 5. *In vivo* β-galactosidase expression in rat brain infected with either GS46-FLP HC-Adv vector or E1/E3-deleted adenovirus (RAd35). 7×10^7 BFU of either virus (in $3 \mu\text{l}$) was injected into the striatum of male Sprague–Dawley rats. Six days after virus inoculation, β-galactosidase expression was analyzed by immunohistochemistry. (a, b) GS46-FLP HD virus. (c, d) RAd35 virus. β-Galactosidase is expressed under the control of the hCMV promoter in both viruses. From Umana *et al.* (2001b).

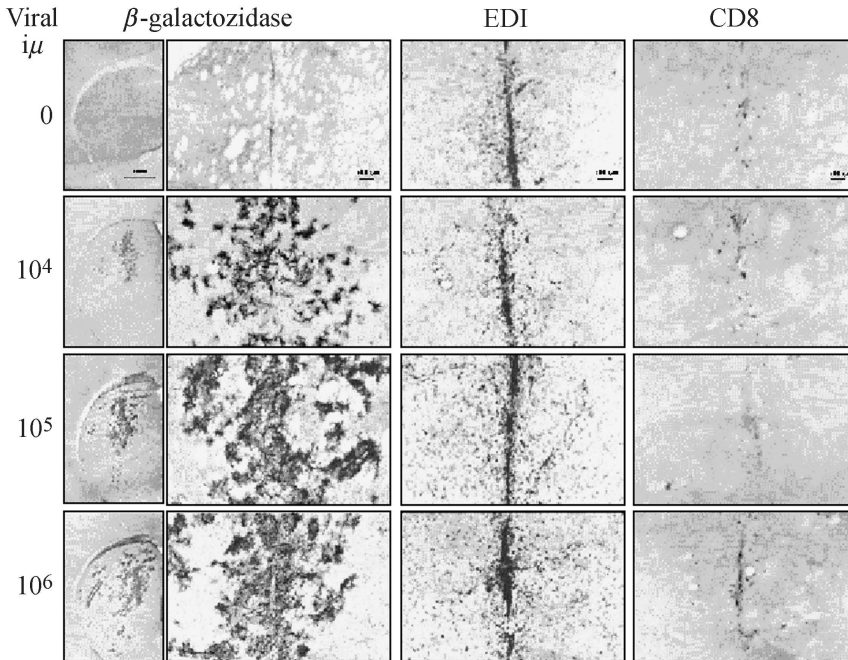


FIG. 6. The mCMV promoter allows high-level expression at low doses of vector, without inflammation. Increasing doses of RAd36 (mCMV- β gal) (10^4 – 10^6) were injected into the striatum of adult Sprague–Dawley rats in a total volume of 3 μ l, and animals were perfused 5 days later. Brains were processed for immunohistochemistry to detect β -galactosidase (transgene expression; first two left hand side columns), ED1 (macrophages-microglial cells), and CD8 (infiltrating lymphocytes and NK cells). Low-power view of the striatum is shown in the left-hand side column, and a higher power view is shown in the column next to it. Scale bar shown in the upper left = 1 mm; all others = 100 μ m. RAd36 allows strong transduction of the striatum, in the absence of recruitment of inflammatory cells, compared to animals injected with saline controls. This image illustrates three doses of animals injected with RAd36. The quantification of this experiment is shown in Fig. 4, above.

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