ELSEVIER

European Journal of Pharmacology 429 (2001) 39–48

ejp

www.elsevier.com/locate/ejphar

Review

Gene therapy of pain: emerging strategies and future directions

Michel Pohl *, Joao Braz

INSERM U288, NeuroPsychoPharmacologie Moléculaire, Cellulaire et Fonctionnelle, C.H.U. Pitié-Salpêtrière, Faculté de Médecine Pitié-Salpêtrière, 91 Boulevard de l'Hôpital, 75634 Paris Cedex 13, France

Accepted 27 July 2001

Abstract

Gene therapy to alleviate pain could appear surprising and perhaps not appropriate when opioids and other active molecules are available. However, the possibility of introducing a therapeutic protein into some targeted structures, where it would be continuously synthesised and exert its biological effect in the near vicinity of, or inside the cells, might avoid some drawbacks of "classical" drugs. Moreover, the gene-transfer techniques might improve present therapies or lead to novel ones. The recent significant and constant advances in vector systems design suggest that these techniques will be available in the near future for safe application in humans. The first experimental protocols attempting the transfer of opioid precursors genes, leading to their overexpression at the spinal level, demonstrated the feasibility and the potential interest of these approaches. Indeed, overproduction of opioid peptides in primary sensory neurones or spinal cord induced antihyperalgesic effects in various animal models of persistent pain. However, numerous other molecules involved in pain processing or associated with chronic pain have been identified and the gene-based techniques might be particularly adapted for the evaluation of the possible therapeutic interest of these new potential targets.

Keywords: Gene therapy; Pain chronic; Herpes simplex virus-derived vector; Opioid peptide

1. Introduction

Although frequently used, the term «gene therapy» remains somewhat confusing. This term was first associated with the idea of treating inherited diseases by replacement or repair of genes directly in the genome of patients. However, this application of gene therapy still seems far away. Indeed, the first experimental data concerning in situ repair of genes with punctual mutations have been only recently published (Kren et al., 1998; Ye et al., 1998; Bandyopadhyay et al., 1999).

On the other hand, gene therapy techniques that consist of the introduction and in situ expression of foreign genes (or part of them) in the organism, and their use as source of therapeutic proteins, represent a rapidly growing field of gene-based therapeutic strategies (Martuza et al., 1991; Boviatis et al., 1994a; Isner et al., 1996; Kessler et al., 1996; During and Ashenden, 1998; Audouy et al., 1999; Snyder et al., 1999). It is noteworthy that these approaches may concern both inherited and acquired diseases in which the therapeutic protein delivery might improve, or represent a new possibility of, treatment.

In spite of strong medical interest and an increasing number of scientific reports published in this field, the results of clinical trials evaluating these strategies have been rather disappointing. Three major problems explain, at least partly, this limited success: (i) whatever the vector system used, gene transfer efficacy was relatively poor; (ii) in general, the expression of transgenes was only transient, due to the extinction of transgene or promoter activity and preferential elimination of transduced cells; (iii) some vector systems had an intrinsic toxicity, inducing limited to violent immune reactions.

Although we are still far removed from an ideal «shuttle» system, enabling targeted transgene transfer, long-term (if possible inducible) expression, and safe use, real progress has been made with the construction of new generations of vectors (Howard et al., 1998; Jacobs et al., 1999; Simonato et al., 2000; Sundaresan et al., 2000). Thus, the fast evolution of gene therapy techniques makes these approaches more «accessible» and «acceptable» not only for «hard» (or otherwise incurable) pathologies but also for some chronic affections such as inflammation and pain. In this review article, we focus on this relatively novel therapeutic concept for the treatment of chronic pain,

* Corresponding author. Tel.: +33-1-40-77-97-09; fax: +33-1-40-77-97-90.

E-mail address: pohl@ext.jussieu.fr (M. Pohl).

0014-2999/01/$ - see front matter
PII: S0014-2999(01)01304-8

the relevant data obtained to date, and some potentially interesting future targets.

2. Vectorisation systems

The experimental evaluation of gene therapy approaches in animal models of various pathophysiological affections rapidly revealed the need for different vector systems, reflecting both the diversity of target tissues or cells and the number of proteins of potential therapeutic interest. The most widely used techniques allowing DNA delivery into the host are either virus-derived vector systems or non-viral transfer methods. The latter approaches use ex vivo transfected (iso- or xeno-genic) cells or various immortalised, in vitro transduced cell lines, which are then transplanted into the target host tissue where they function as biological "pumps" (Dai et al., 1992; Doughty et al., 1997; for review, see Taylor, 1997). Although potentially interesting, the modified-cell grafts present several drawbacks, including the possibility of graft rejection, the risk of tumorigenesis, the unstability of transgene expression, the lack of cell specificity and the need for surgical procedures. Experimental strategies for cell lines desimmortalization (Bergehella et al., 1999) or the use of novel cell lines such as multipotent neural progenitor cells (Taylor, 1997; Liu et al., 1999; Weissman, 2000) will certainly improve the transfer capacity and safety of these methods. Another non-viral system enabling DNA introduction into the host cells is the use of plasmid DNA coated with cationic lipids or complexed with polymers (Abdallah et al., 1995; Hope et al., 1998; Lemkine and Demeneix, 2001). Although these non-viral vectors are safe, versatile and easy to handle, their in vivo efficiency is low (Remy et al., 1995) and their performances are frequently variable, depending on DNA and cationic compounds preparations (Fabre and Martres, personal communication).

In spite of the "a priori" pathogenic character of viruses, the viral-derived vectors take advantage of their natural capacity to penetrate the cells and to deliver their genome (including the transgene) into the host cell nucleus. Thus, these vectors now represent the most effective tools for the introduction of foreign genes, and their efficient expression in various cell types. To date, the most widely used viral vectors derive either from adenoviruses, adeno-associated viruses (AAV), Herpes simplex viruses (HSV) or retroviruses. The biological characteristics of these viruses, summarised in Table 1, suggest that different viral vectors would be particularly adapted for each kind of gene-transfer design, depending on the target cell type, the organ or tissue accessibility, the transgene size, the desired (or undesired) integration of the foreign gene into the host cell genome.... Although current forms of virus-derived vectors are not yet optimal for both highly efficient and truly safe gene introduction and expression in humans, their experimental use in animals provides strong bases for their therapeutic potential. New generations of viral vectors have been engineered in which genes governing viral replication, or responsible for the host immune reaction, have been inactivated (Boviatis et al., 1994b; Howard et al., 1998; Nabel, 1999). AAV vectors appear especially promising for an efficient and safe gene transfer into the central and peripheral nervous systems (Bueler, 1999; Guy et al., 1999; Lein et al., 2000). These vectors, as well as lentivirus vectors, deriving from the human immunodeficiency virus, have also been shown to transduce mouse and human sensory neurones, at least in vitro (Fleming et al., 2001). However, in human dorsal root ganglia cell cultures, both vectors could transfect with similar efficiency neurones, Schwann cells or fibroblast-like cells. Hybrid vectors, such as HSV/AAV amplicon systems, have been developed and their capacity to drive long-term transgene expression in the central and peripheral nervous system has been demonstrated (Jacobs et al., 1999).

Numerous biological characteristics of Herpes simplex type 1 (HSV-1) viruses make HSV-1-derived vectors particularly suitable for the introduction and long-term expression of genes into neurones. These highly neurotropic viruses infect efficiently post-mitotic nerve cells, in which they can establish a latent state of infection. Latency is characterised by the long-term persistence of viral genome in an episomal chromatin structure, which does not disturb host cell function. During latency, viral protein synthesis is not detected and a unique transcriptionally active unit leads to the synthesis of two stable nuclear RNAs, called

Table 1
Main characteristics of viruses used for the construction of viral-derived vectors

	Adenovirus	Adeno-associated virus	Retrovirus		Herpes virus
			MLV[a]	Lentivirus	
Viral genome	double strand DNA	simple strand DNA	diploid RNA + strand		double strand DNA
Integration into the host genome	no	yes	yes	yes	no
Possible insert size	~ 7.5 kb	~ 4.5 kb	~ 5 kb	~ 5 kb	~ 35 kb
Non-dividing cell infection	yes	yes	no	yes	yes
Viral protein expression	yes	no	no	yes/no	latency–no
Transgene expression	transient (days–weeks)	persistent (month)	persistent (month–years)		transient (days–weeks)

[a] MLV = murine leukemia virus.

"latency associated transcripts" (LATs). The LATs, which are not translated, are synthesised under the control of a LAT promoter, particularly active in neuronal cells. The HSV genome, that has been completely sequenced, is relatively large (~152 kb), and consists of at least 72 genes. Nearly half of them are non-essential genes that can be deleted and replaced by exogenous DNA sequences, thus allowing the introduction of inserts of about 30–40 kb. After penetration into the host cell, HSV can be transported both retro- and anterogradely. Numerous HSV-derived vectors have been developed (Levatte et al., 1998; Simonato et al., 2000; Sundaresan et al., 2000) both for solving some problems including lytic viral replication, immune response induction, and for ensuring high-rate and long-term transgene expression. These vectors have been extensively studied for the transfer of potentially therapeutic genes into the central (Kaplitt et al., 1994; Carlezon et al., 1997; Yamada et al., 1999) and peripheral nervous systems (Antunes Bras et al., 1998; Levatte et al., 1998), or into different types of non-neuronal tissues (Fraefel et al., 1997; Akkaraju et al., 1999; D'Angelica et al., 1999) and also in suicide-gene approaches in cancer therapy (Boviatis et al., 1994a,b). Beside these applications, HSV derived vectors are particularly adapted for gene introduction into primary sensory nerves. Indeed, these neurones are the natural targets of HSV infection and, after several cycles of replication in epithelial cells, viral particles penetrate into the nerve terminals and are then retrogradely transported, sometimes over long distances, to the cell bodies of sensory neurones. Peripheral inoculation of HSV-derived vectors, either on abraded skin (Lokensgard et al., 1994; Antunes Bras et al., 1998) or scarified cornea (Shimeld et al., 1995), allows the introduction of the transgene and its expression in sensory ganglia neurones without the need for direct (sometimes not easily achievable) delivery of vectors.

Together, these characteristics have led several laboratories, including ours, to develop recombinant HSV vectors for the experimental study of the modulation of gene expression in primary sensory neurones, associated with chronic pain, inflammation or nerve plasticity.

3. First experimental data in gene therapy of pain

At a first glance, the idea of gene therapy for pain may appear somewhat surprising. Why introduce foreign genes or some part of them into sensory neurones or other tissues when opioids and other active molecules are available? However, the notion of protein as a therapeutic drug is really tempting. The transgene-derived protein that would be synthesised (either continuously or preferably in an inducible manner) in a closely targeted structure (or organ), would act in the near vicinity of, or inside, the cells, circumventing some problems related to the half-life of classical drugs. Moreover, the need for high doses of some drugs to achieve biological activity, possibly resulting in undesirable side effects, would be eliminated.

There have been numerous attempts to alleviate pain using cellular therapy approaches in both experimental animal models and clinical trials. An antinociceptive agent "cocktail" were thus supplied through autologous (Hama and Sagen, 1994; Pappas et al., 1997), heterologous (Buchser et al., 1996), or immortalized chromaffin cells grafts (Eaton, 2000). Various engineered clonal cell lines that synthesise and secrete potentially antinociceptive molecules have also been experimented with (for review, see Eaton, 2000). This technique of exogenous protein delivery, however, remains relatively unspecific and does not allow real targeting of host tissue or cells.

More recently, two different strategies have been used for direct gene transfer of opioid peptide precursors into dorsal root ganglia neurones or the spinal cord.

In our laboratory, we chose to introduce the rat proenkephalin A (pEnkA) gene into rat dorsal root ganglia sensory neurones (Antunes Bras et al., 1998). The pEnkA coding sequence was placed under the control of a modified HSV latency promoter, consisting of a fusion between the region upstream from the HSV Lat core promoter and elements of Moloney murine leukemia virus long terminal repeat (LTR). This binary promoter has been previously shown to drive long-term expression of the ß-galactosidase gene in mouse dorsal root ganglia (Lokensgard et al., 1994). The Lat-LTR-pEnkA transcriptional unit was introduced into the glycoprotein C locus of the HSV genome using homologous recombination. The recombinant vectors synthesising pEnkA derived peptide (HSVLatEnk1) were inoculated on the slightly scarified footpad surface of rat hind limbs. Three weeks after their infection, i.e. during the latent phase of infection, HSVLatEnk1-inoculated animals presented with: i) a ~7-fold increased number of pEnkA mRNA expressing neurones and a ~24-fold higher levels of pEnkA mRNA in L4-6 dorsal root ganglia; ii) a ~160% and ~40% increase of [Met^5]enkephalin-like material concentrations in dorsal root ganglia and the dorsal part of the lumbar spinal cord, respectively, as compared with the values in both sham- and ß-galactosidase encoding recombinant vector (HSVLatß-gal)-infected rats (Fig. 1). Overexpressed pEnkA was apparently completely processed, as shown by both reverse phase high performance liquid chromatography analysis and immunohistochemical detection of [Met^5]enkephalin-like material in cell bodies and processes of sensory neurones using anti-[Met^5]enkephalin monoclonal antibodies (Antunes Bras et al., 2001). The transgene-derived peptides (as well as endogenous [Met^5]enkephalin-like material) were preferentially, but not exclusively, transported into the peripheral processes of primary afferent neurones. The [Met^5]enkephalin-like material accumulated in vesicular profiles and reached a releasable compartment in nerve terminals on both the central and peripheral sides, as the electrical stimulation of either the dorsal roots or the

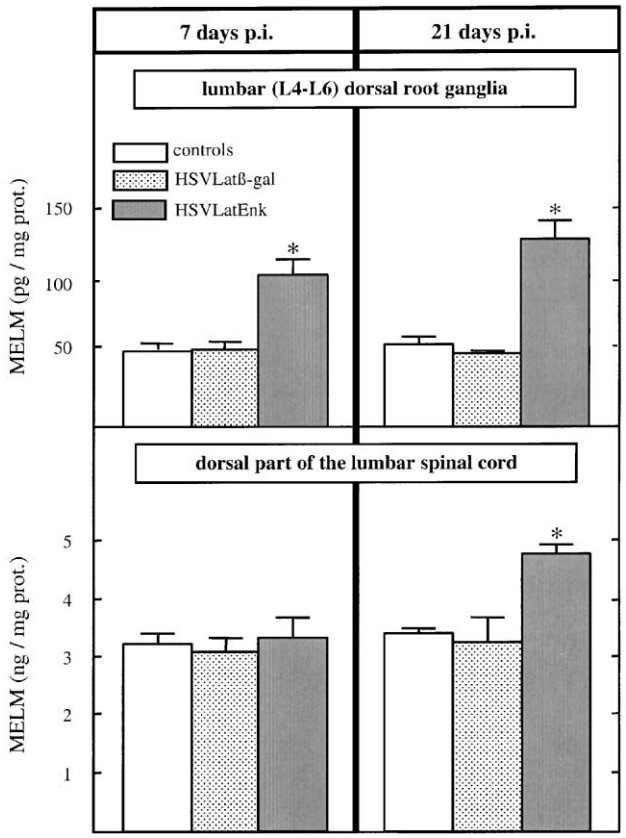

Fig. 1. Concentrations of [Met^5]enkephalin-like material in lumbar (L4–L6) dorsal root ganglia and in the dorsal half of the lumbar spinal cord of control rats and HSVLatß-gal- or HSVLatEnk1-infected animals. [Met^5]enkephalin-like material levels (in pg or ng/mg protein) are the means ± S.E.M. of five independent determinations. $^*P < 0.05$ as compared with respective values from control rats or HSVLatß-gal-infected animals.

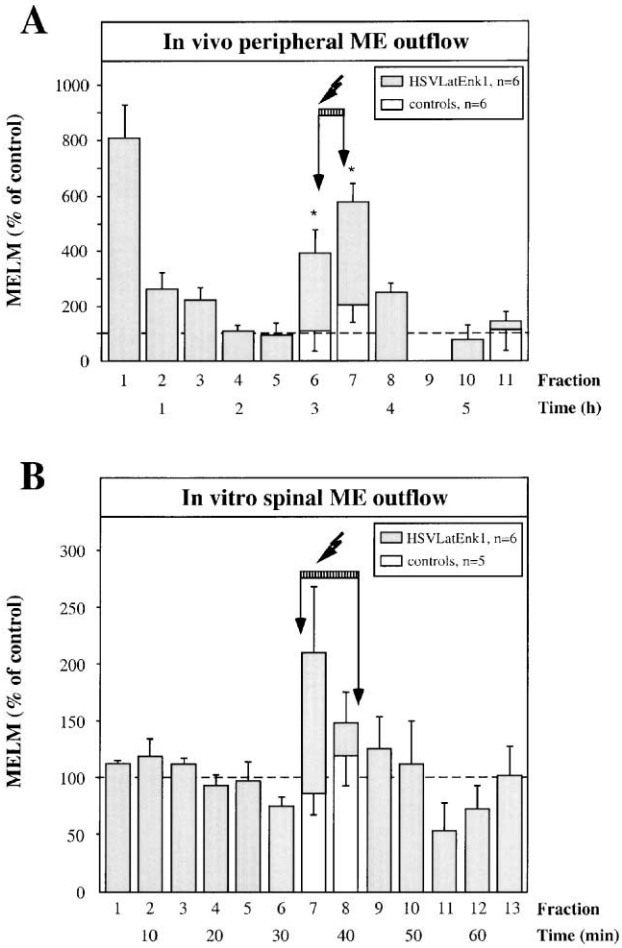

Fig. 2. Effects of electrical stimulation of the sciatic nerve (A) or L4–L5 dorsal roots (B) on of [Met^5]enkephalin-like material outflow in peripheral or spinal cord tissues. (**A**) A microdialysis probe was inserted into the interstitial space of hind paw plantar tissues in anaesthetised control and HSVLatEnk1-infected rats, and continuously perfused with a Ringer's solution. After a 90-min washout period (fractions 1–3), two fractions (4, 5), corresponding to the basal of [Met^5]enkephalin-like material outflow, were collected and the ipsilateral sciatic nerve was electrically stimulated (5 mA, 1 ms, 0.7 Hz—15 min) during the collection of fractions 6–7. [Met^5]enkephalin-like material content of each collected fraction (mean ± S.E.M.) is expressed as percentage of the mean content of fractions 4–5 corresponding to the basal of [Met^5]enkephalin-like material outflow in HSVLatEnk1-infected rats (100%, dashed line). In control rats (open bars), [Met^5]enkephalin-like material was detected only during the collection of fractions 6–7 and 11. In HSVLatEnk1-infected rats (grey bars), microdialysate-[Met^5]enkephalin-like material was measurable under resting conditions, and stimulation of the sciatic nerve resulted in a ~3-fold increase in [Met^5]enkephalin-like material levels ($P < 0.05$, $n = 6$) compared to control rats. (**B**) At the spinal level, spontaneous in vitro outflow of [Met^5]enkephalin-like material was measurable in HSVLatEnk1-infected rats (grey bars) but not in control animals (open bars). In the latter animals, electrical stimulation of L4–L5 dorsal roots (5 mA, 2 ms, 1 Hz—10 min) rendered of [Met^5]enkephalin-like material levels measurable in fractions 7–8 collected during stimulation. In HSVLatEnk1-infected rats stimulation of dorsal roots resulted in a ~70% increase in [Met^5]enkephalin-like material concentrations in fractions 7–8, over baseline in fractions 1–6 (100%, dashed line) of [Met^5]enkephalin-like material content of each fraction (mean ± S.E.M.) is expressed as percentage of this baseline value in HSVLatEnk1-infected rats.

sciatic nerve led to [Met^5]enkephalin-like material release (Fig. 2). Interestingly, although [Met^5]enkephalin-like material could be co-localised with substance P-like material or calcitonin gene-related peptide-like material in sensory neurones, the [Met^5]enkephalin-like material-containing vesicular profiles seen all along the sciatic nerve sensory fibres were distinct from those endowed with substance P-like material or calcitonin gene-related peptide-like material. These results suggest that the release of overproduced [Met^5]enkephalin-like material might occur independently of that of other neuropeptides. In spite of this apparently functional enkephalinergic supply, the basal nociceptive response evoked by noxious thermal stimulation did not differ in pEnkA-overexpressing rats compared to their controls.

Wilson et al. (1999) achieved human pEnkA transfer into the mouse dorsal root ganglia using a similar approach. These authors introduced the pEnkA encoding sequence under the control of the human cytomegalovirus promoter into the viral thymidine kinase gene, thereby preventing replication of the recombinant vectors in ner-

vous tissue. The baseline latencies of foot withdrawal responses evoked by noxious heat were similar in both infected pEnkA-overproducing animals and the controls. Only previous sensitisation of sensory afferents by topical application of dimethyl sulfoxide or capsaicin revealed the antihyperalgesic effect of pEnkA overexpression in primary sensory neurones. Although intrathecal naloxone (1 mg/kg) administration was effective to reverse the antihyperalgesia associated with pEnkA overexpression, very high doses (50 mg/kg) were necessary to partially reverse this effect when the opioid receptor antagonist was delivered systemically. The authors concluded that viral introduction of pEnkA into nociceptive primary afferents altered the responsiveness of both C and Aδ fibres to stimuli which would normally produce hyperalgesia by acting, particularly, at the spinal level. They also suggested that the basal release of the transgene-derived peptide(s) should be minimal and that only high-level or persistent stimulation of sensory nerves should evoke peptide release. In line with this hypothesis, we used subcutaneous microdialysis in the hind paw plantar area of pEnkA-overexpressing rats, and we showed that only very low levels of [Met^5]enkephalin-like material could be detected under resting conditions but that electrical stimulation of the sciatic nerve resulted in a ~5-fold increase of microdialysate [Met^5]enkephalin-like material levels (Fig. 2).

Another strategy to introduce and overexpress an opioid peptide was explored by Finegold et al. (1999). An artificial fusion gene, previously used by Beutler et al. (1995) to drive ß-endorphin production and secretion in primary fibroblasts, was introduced into an adenovirus-derived vector under the cytomegalovirus promoter. Recombinant vectors were delivered in rats by intrathecal infusion. Under these conditions, recombinant vectors did not penetrate and infect the spinal cord neurones but remained restricted to pia mater cells. One week after recombinant vector infusion, ß-endorphin concentrations in cerebrospinal fluid (CSF) increased about 4-fold as compared with control animals. However, probably because of the promoter used, ß-endorphin secretion into the CSF was found to fall rapidly (i.e. 15 days later) to control levels. As in the other studies, the ß-endorphin overproduction and secretion into the CSF did not alter the baseline nociceptive sensitivity of animals to radiant thermal stimulus. However, inflammation of the hind paw induced by subcutaneous carrageenan injection revealed the antihyperalgesic potency of overproduced ß-endorphin. The increases in paw withdrawal latencies observed in recombinant vector-injected rats, compared to latencies in control animals, were attenuated after i.p. administration of naloxone, suggesting a specific, opioid receptor-mediated effect.

Together these data clearly demonstrate the feasibility of such approaches and suggest that exogenous supplying of various opioid peptides at the spinal level (via primary sensory neurones or using the "paracrine" route) has no antinociceptive effect but an antihyperalgesic effect only seen after the induction of persistent pain. This observation is interesting because the therapeutic approaches based on gene transfer would be particularly adaptable to chronic pain states. In addition, plastic changes in the neuronal systems involved in the transmission of pain or its control, particularly at the spinal level, associated with chronic inflammatory and neuropathic pain, are well documented (for a review, see Millan, 1998). There is now accumulating data concerning the alteration of the expression of genes encoding neuropeptides, receptors or ion channels.

In this context, it would be particularly interesting, using targeted gene transfer, to attempt the "normalisation" of some gene expression and to study its potential effects. Among animal models of chronic inflammatory pain, rats with adjuvant-induced polyarthritis have been studied extensively. We previously showed that, in these animals, pEnkA mRNA were almost undetectable in lumbar dorsal root ganglia (which contain cell bodies of sensory neurones innervating the hind limbs, which are particularly affected by the disease), whereas about 2–3% of dorsal root ganglia neurones synthesise pEnkA mRNA in healthy rats (Pohl et al., 1997). In addition, it has also been reported that [Met^5]enkephalin-like materiel levels were markedly reduced in nerve terminals innervating the synovial tissues of hind paw joints in polyarthritic rats (El Hassan et al., 1998). Using an approach similar to that of our previous studies (see above), we tried to introduce and overproduce rat pEnkA gene in dorsal root ganglia sensory neurones in these animals. The resulting biochemical data were comparable to those obtained following recombinant vector infection of normal rats, but HSVLatEnk inoculation of polyarthritic rats resulted in an antihyperalgesic effect against thermal noxious stimulus, which lasted for at least 8 weeks after infection. However, an acute painful stimulus does not actually reflect the dimension of persistent, spontaneous pain. This is particularly true in polyarthritic rats in which the most prominent behavioural change is a marked reduction of locomotion, due to intense inflammatory pain and alterations of joints. We thus explored in an open field the spontaneous horizontal and vertical mobility of these animals in order to assess as accurately as possible their "functional disability" (Larsen and Arnt, 1985; Cain et al., 1997; Lindner et al., 1999). Compared to untreated polyarthritic rats, there was an impressive improvement of the locomotor activity of HSVLatEnk-infected polyarthritic rats, who recovered up to nearly 70% of the activity of healthy rats (Fig. 3). Thus, our results confirmed the data obtained with normal animals and, more importantly, suggest the actual therapeutic potency of overproduced pEnkA-derived opioid peptides in sensory neurones of chronically suffering rats.

The gene transfer techniques that we have mentioned so far consist of the transfer and synthesis of precursors of biologically active peptides. However, another possible application of the viral vector-mediated transfer of DNA sequences is, on the contrary, the introduction into the cell

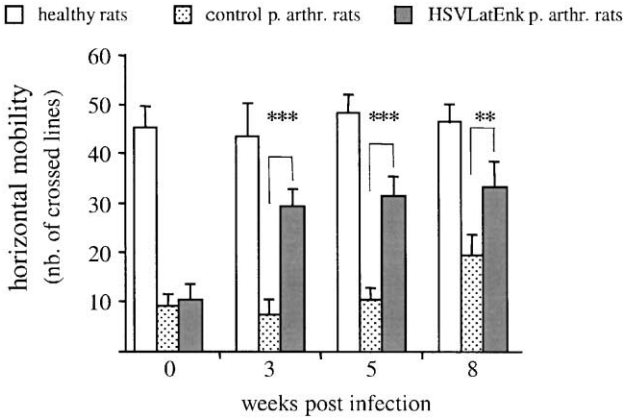

Fig. 3. HSVLatEnk-infected polyarthritic rats exhibited improved spontaneous locomotor activity. Horizontal locomotor activity was measured in a red-lighted open-field, video-monitored and assessed every minute during a 7-min period. Long-term improvement of functional ability in HSVLatEnk-infected polyarthritic rats was evaluated by comparing the locomotor activity of normal healthy rats ($n = 5$) and both control ($n = 8$) and HSVLatEnk-infected ($n = 10$) polyarthritic rats 3, 5 and 8 weeks after infection. $^{**}P < 0.01$; $^{***}P < 0.001$ for control versus HSVLatEnk-infected ($n = 10$) rats: two-tailed unpaired *t*-test.

body of a DNA sequence in antisense orientation in order to reduce the synthesis of the targeted gene. Compared with the widely used techniques of antisense oligonucleotides injection, the viral vector delivery of an antisense molecule has the advantage of continuous and perhaps better targeted production. The possible limitation of the delivery of "naturally" synthesised antisense RNA is their increased sensitivity to enzymatic degradation compared to that of oligonucleotides modified by the introduction of different chemical groups (phosphotriesters, phopshorothiates, methylphosphonates, ...) that have been shown to improve their stability. However, abundant data support the efficacy of the in situ synthesis of antisense RNA whose biological effect probably takes place mainly in the nucleus, affecting gene expression prior to translation (for a review, see Hélène and Toulmé, 1991). These techniques might be relevant for the "correction" of the increased synthesis of numerous proteins whose genes are overexpressed in chronic pain states.

Using this approach, we attempted to reduce the enhanced synthesis of calcitonin gene-related peptide (CGRP) induced in dorsal root ganglia neurones by peripheral inflammation (Collin et al., 1993). In the same viral backbone as used in studies on pEnkA overexpression, we introduced, in antisense orientation, the complete sequence of the exon 5 of the rat α-calcitonin-CGRP gene (Amara et al., 1982). The recombinant vectors expressing antisense CGRP RNA (HSVLatCGRP$_{AS}$) were inoculated into rats on the right hind-paw plantar surface as described above. Profound inflammation was induced 2 weeks later by subcutaneous injection of complete Freund's adjuvant into the right hind paw plantar surface. As expected, 5 days later, the concentrations of CGRP-like material in the ipsilateral lumbar dorsal root ganglia of both sham-and HSVLatß-gal-infected rats were significantly higher (+ ~ 40%) than in control animals. In contrast, in HSVLat-CGRP$_{AS}$-infected rats, the CGRP-like material levels were increased by only about 20% as compared to non-inflammed animals. However, this effect was transient because the reduction of the increased CGRP-like material concentrations was no longer significant 12 days after induction of inflammation. The hind paw volumes of control and HSVLatCGRP$_{AS}$-infected rats were measured, before and after inflammation, using a plethysmometer. As shown in Fig. 4, the paw volumes of infected and uninfected rats were similar. Two days after the induction of inflammation, the paw volumes increased parallely in both HSVLatCGRP$_{AS}$-infected rats and control animals. However, whereas the hind paw volume of control rats was still increasing until days 4–7 after the beginning of inflammation, in CGRP antisense RNA expressing rats, the paw volume remained almost stable during the whole 16 days observation period. The discrepancy between the biochemical marker of antisense efficacy, i.e. decreased dorsal root ganglia CGRP-like material levels, and biological efficacy suggests that limited variations in endogenous neuropeptides levels might be sufficient to sustain a functional effect. In fact, other authors using antisense RNA or antisense oligonucleotides delivery reported similar data (see Hélène and Toulmé, 1991). Nevertheless, our data support the idea that down-regulation of gene expression in sensory neurones might also be achieved using these approaches and we are currently trying out different anti-

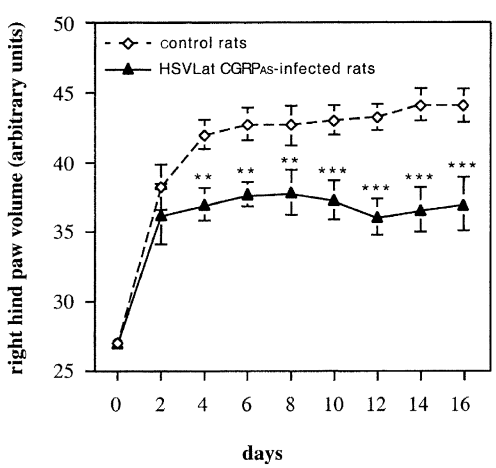

Fig. 4. Infection of rats with HSVLatCGRP$_{AS}$ limited the hind paw volume increase induced by local Freund's adjuvant injection. Inflammation was induced in right hind paws of control rats (sham- and HSVLatß-gal-infected rats) or HSVLatCGRP$_{AS}$-infected animals by injection of 150 μl of complete Freund's adjuvant. Paw volumes were then measured every 2 days using a Ugo Basile plethysmometer in both control (sham- and HSVLatß-gal-infected animals; $n = 10$) and HSVLatEnk-infected ($n = 10$). $^{**}P < 0.01$; $^{***}P < 0.001$ for control versus HSVLat-CGRP$_{AS}$-infected ($n = 10$) rats: two-tailed unpaired *t*-test.

sense constructions with the aim of modulating the expression of some relevant genes.

4. Future directions and new targets

The first experimental assays of gene therapy of pain, which have (logically) targeted the endogenous opioid system by inducing the overexpression of opioid precursors, seem promising. However, numerous other targets which may be more relevant and more specific, have to be explored. Several of these have been identified (Na^+ channels, Baker and Wood, 2001; cyclooxygenase Dannhardt and Kiefer, 2001; NMDA receptors, Gordh et al., 1995; ...), and drugs (available or in development) allow already or will allow their use in "classical" therapeutic protocols. Other molecules have been identified recently but their real therapeutic potential remains unclear, mainly because of the lack of pharmacological tools (ion channels, Waldman et al., 1997; Fosset et al., 1999; isoforms of protein kinase C, Malberg et al., 1997; Dina et al., 2000; proteinase activated receptor 2, Steinhoff et al., 2000; ...). Finally, some molecules, whose involvement in pain was demonstrated only very recently (osteoprotegerin, Honoré et al., 2000, for instance), will certainly be tried out in gene-based therapeutic procedures in the near future.

Although chronic pain should be considered as a real disease state of the nervous system, the starting point of persistent pain is, in most cases, related to specific pathophysiological conditions. Thus, associated with gene therapy of some of these affections, gene therapy of pain might take place. Apart from the different gene-based strategies for cancer treatment, representing almost 60% of current clinical trials (Kahn, 2000), these therapeutic approaches are also being actively evaluated for the treatment of inflammatory diseases (for review, see Hedley, 2000), particularly rheumatoid arthritis (for review, see Junker and Böhnlein, 1999). Given the major role that cytokines such as tumor necrosis factor-α and interleukin-1 are thought to play in local inflammation and joint destruction, inhibition of their biological activity using tumor necrosis factor-α "decoy" receptor or interleukin-1 natural receptor antagonist might be of real benefit in the therapy of arthritic diseases. The efficacy of these approaches has been demonstrated in several clinical trials in patients with advanced rheumatoid arthritis. Another possibility of cytokine-based treatments is the delivery of anti-inflammatory cytokines, including transforming growth factor-ß, interleukin-4, interleukin-10 and interleukin-13.

However, due to the relatively short half-life of this kind of drug and their possible harmful side-effects, experiments were undertaken for the targeted and long-lasting delivery of cytokines or their antagonists using gene transfer techniques. Experimental data from animal models of inflammation and arthritis clearly demonstrate the therapeutic potency of the introduction and overproduction of local cytokine or cytokine-related proteins (Ghivizzani et al., 1998; Lubberts et al., 2000; Woods et al., 2000). Interestingly, in mouse, Lubberts et al. (2000) have shown that local interleukin-4 overexpression in knee reduces the levels of synovial interleukin-17 and osteoprotegerin ligand and prevents bone erosion. On the other hand, osteoprotegerin, the secreted soluble tumor necrosis factor receptor-related molecule that sequestres osteoprotegerin ligand thereby blocking its biological activity, has recently been shown to prevent bone destruction and pain behaviour in an animal model of bone cancer (Honoré et al., 2000). These data suggest that targeted expression of the osteoprotegerin gene would be particularly relevant for the treatment of both bone erosion and pain in rheumatoid arthritis and bone cancer. The potential therapeutic interest of various other targets has been evaluated using viral vector-mediated local expression. For instance, the transfer of cDNAs encoding the cyclin-dependent kinase inhibitor (Nasu et al., 2000), the natural inhibitors of metalloproteinases (enzymes involved in cartilage destruction), the angiogenesis inhibitors or the proteins involved in apoptosis (FasL ligand...) (for review, see Junker and Böhnlein, 1999) has been experimented with. Finally, numerous genes or proteins, associated with inflammatory diseases, have been identified in the last 5 years. Although the biological functions of these molecules are not completely understood, several patents have already been filled for their potential use in novel, gene-based therapeutic strategies (Junker and Böhnlein, 1999).

Taking into account the important number of identified or possible future targets, gene therapy of pain might take a place as a complementary and parallel form of treatment. Indeed, as previously mentioned, DNA sequences of relatively important size could be inserted in some viral genomes (namely in HSV-derived vectors), allowing concomitant transfer and expression of several genes. Thus, association of antihyperalgesic molecules (such as opioid peptide precursors) with a "therapeutic" transgene should be explored in gene therapy clinical trials relevant to various chronic painful pathologies.

5. Conclusion

In spite of their apparent complexity, gene therapy approaches have, in some applications, numerous advantages as compared with the "classical" treatments. However, it must be pointed out that, to date, most of the available data have been obtained only in experimental animal models or in very preliminary clinical trials. Gene therapy of pain will be, or at least will long remain, a complementary treatment. Nevertheless, the development of safe vector systems as well as their application in well selected pathophysiologies might provide real therapeutic

benefit, lead to novel therapies and contribute to the amelioration of life of patients suffering chronic pain.

Acknowledgements

The research performed in our laboratory has been supported by grants from INSERM, Bristol-Myers Squibb Foundation (unrestricted biomedical research grant) and Institut UPSA de la Douleur.

References

Abdallah, B., Sachs, L., Demeneix, B.A., 1995. Non-viral gene transfer: applications in developmental biology and gene therapy. Biol. Cell. 85, 1–7.

Akkaraju, G.R., Huard, J., Hoffman, E.P., Goins, W.F., Pruchnic, R., Watkins, S.C., Cohen, J.B., Glorioso, J.C., 1999. Herpes simplex virus vector-mediated dystrophin gene transfer and expression in MDX mouse skeletal muscle. J. Gene Med. 1, 280–289.

Amara, S.G., Jonas, V., Rosenfeld, M.G., Ong, E.S., Evans, R.M., 1982. Alternative RNA processing in calcitonin gene expression generates mRNAs encoding different polypeptide products. Nature 298, 240–244.

Antunes Bras, J.M., Epstein, A.L., Bourgoin, S., Hamon, M., Cesselin, F., Pohl, M., 1998. Herpes simplex virus-1 mediated transfer of preproenkephalin A in rat dorsal root ganglia. J. Neurochem. 70, 1299–1303.

Antunes Bras, J.M., Becker, C., Bourgoin, S., Lombard, M.C., Cesselin, F., Hamon, M., Pohl, M., 2001. Met-enkephalin is preferentially transported into the peripheral processes of primary afferent fibres in both control and HSV1-driven proenkephalin A overexpressing rats. Neuroscience 103, 1073–1083.

Audouy, S., Mallet, J., Privat, A., Gimenez y Ribotta, M., 1999. Adenovirus-mediated suicide gene therapy in an in vitro model of reactive gliosis. Glia 25, 293–303.

Baker, M.D., Wood, J.N., 2001. Involvement of Na^+ channels in pain pathways. TIPS 22, 27–31.

Bandyopadhyay, P., Ma, X., Linehan-Stieer, C., Kren, B.T., Steer, C.J., 1999. Nucleotide exchange in genomic DNA of rat hepatocytes using RNA/DNA oligonucleotides. Targeted delivery of liposomes and polyethyleneimine to the asialoglycoprotein receptor. J. Biol. Chem. 274, 10163–10172.

Bergehella, L., De Angelis, L., Coletta, M., Berarducci, B., Sonnino, C., Salvatori, G., Anthonissen, C., Cooper, R., Butler-Browne, G.S., Mouly, V., Ferrari, G., Mavilio, F., Cossu, G., 1999. Reversible immortalization of human myogenic cells by site-specific excision of a retrovirally transferred oncogene. Hum. Gene Ther. 10, 1607–1617.

Beutler, A.S., Banck, M.S., Bach, F.W., Gage, F.H., Porreca, F., Bilsky, E.J., Yaksh, T.L., 1995. Retrovirus-mediated expression of an artificial ß-endorphin precursor in primary fibroblast. J. Neurochem. 64, 475–481.

Boviatis, E., Chase, M., Wei, M., Tamiya, T., Hurford, R., Kowall, N., Tepper, R., Breakefield, X., Chiocca, A., 1994a. Gene transfer into experimental brain tumors mediated by adenovirus, herpes simplex virus and retrovirus vectors. Hum. Gene Ther. 5, 183–191.

Boviatis, E.J., Scharf, J.M., Chase, M., Harrington, K., Kowall, N.W., Breakefield, X.O., Chiocca, E.A., 1994b. Antitumor activity and reporter gene transfer into rat brain neoplasms inoculated with herpes simplex virus vectors defective in thymidine kinase or ribonucletotide reductase. Gene Ther. 1, 323–331.

Buchser, E., Goddard, M., Heyd, B., Joseph, J.M., Favre, J., de Tribolet, N., Lysaght, M., Aebischer, P., 1996. Immunoisolated xenogenic chromaffin cell therapy for chronic pain. Initial clinical experience. Anesthesiology 85, 1005–1012.

Bueler, H., 1999. Adeno-associated viral vectors for gene transfer and gene therapy. Biol. Chem. 380, 613–622.

Cain, C.K., Francis, J.M., Plone, M.A., Emerich, D.F., Lindner, M.D., 1997. Pain-related disability and effects of chronic morphine in the adjuvant-induced arthritis model of chronic pain. Physiol. Behav. 62, 199–205.

Carlezon Jr., W.A., Boundy, V.A., Haile, C.N., Lane, S.B., Kalb, R.G., Neve, R.L., Nestler, E.J., 1997. Sensitization to morphine induced by viral-mediated gene transfer. Science 277, 812–814.

Collin, E., Mantelet, S., Frechilla, D., Pohl, M., Bourgoin, S., Hamon, M., Cesselin, F., 1993. Increased in vivo release of calcitonin gene-related peptide-like material from the spinal cord in arthritic rats. Pain 54, 203–211.

Dai, Y., Roman, M., Naviaux, R.K., Verma, I.M., 1992. Gene therapy via primary myoblasts: long-term expression of factor IX protein following transplantation in vivo. Proc. Natl. Acad. Sci. U. S. A. 89, 10892–10895.

D'Angelica, M., Tung, C., Allen, P., Halterman, M., Delman, K., Delohery, T., Klimstra, D., Brownlee, M., Federoff, H., Fong, Y., 1999. Herpes simplex virus (HSV)-mediated ICAM-1 gene transfer abrogates tumorigenicity and induces anti-tumor immunity. Mol. Med. 5, 606–616.

Dannhardt, G., Kiefer, X., 2001. Cyclooxygenase inhibitors—current status and future prospects. Eur. J. Med. Chem. 36, 109–126.

Dina, O.A., Barletta, J., Chen, X., Mutero, A., Martin, A., Messig, R.O., Levine, J.D., 2000. Key role for the epsilon isoform of protein kinase C in painful alcoholic neuropathy in the rat. J. Neurosci. 20, 8614–8619.

Doughty, L., Patrene, K.D., Evans, C.H., Boggs, S.S., Robbins, P.D., 1997. Constitutive systemic expression of IL-1Ra or soluble TNF receptor by genetically modified hematopoietic cells supresses LPS induction of IL-6 and IL-10. Gene Ther. 4, 252–257.

During, M.J., Ashenden, L.M., 1998. Towards gene therapy for the central nervous system. Mol. Med. Today 4, 485–493.

Eaton, M.J., 2000. Emerging cell and molecular strategies for the study and treatment of painful peripheral neuropathies. J. Peripher. Nerv. Syst. 5, 59–74.

El Hassan, A.M., Lindgren, J.U., Hultenby, K., Bergstrom, J., Adem, A., 1998. Methionine-enkephalin in bone and joint tissues. J. Bone Miner. Res. 13, 88–95.

Finegold, A.A., Mannes, A.J., Iadarola, M.I., 1999. A paracrine paradigm for in vivo gene therapy in the central nervous system: treatment of chronic pain. Hum. Gene Ther. 10, 1251–1257.

Fleming, J., Ginn, S.L., Weinberger, R.P., Trahair, T.N., Smythe, J.A., Alexander, I.E., 2001. Adeno-associated virus and lentivirus vectors mediate efficient and sustained transduction of cultured mouse and human dorsal root ganglia sensory neurons. Hum. Gene Ther. 12, 77–86.

Fosset, M., Lesage, F., Lazdunski, M., Honoré, E., 1999. TRAAK is a mammalian neuronal mechano-gated K^+ gated ion channel. J. Biol. Chem. 274, 1381–1387.

Fraefel, C., Jacoby, D.R., Lage, C., Hilderbrand, H., Chou, J.Y., Alt, F.W., Breakefield, X.O., Majzoub, J.A., 1997. Gene transfer into hepatocytes mediated by helper virus-free HSV/AAV hybrid vectors. Mol. Med. 3, 13–25.

Ghivizzani, S.C., Lechman, E.R., Kang, R., Tio, C., Kolls, J., Evans, C.H., Robbins, P.D., 1998. Direct adenovirus-mediated gene transfer of interleukin 1 and tumor necrosis factor alpha soluble receptors to rabbit knees with experimental arthritis has local and distal anti-arthritic effects. Proc. Natl. Acad. Sci. U. S. A. 95, 4613–4618.

Gordh, T., Karlsten, R., Kristensen, J., 1995. Intervention with spinal NMDA, adenosine, and NO systems for pain modulation. Ann. Med. 27, 229–234.

Guy, J., Qi, X., Muzyczka, N., Hauswirth, W.W., 1999. Reporter expression persists 1 year after adeno-associated virus-mediated gene transfer to the optic nerve. Arch. Ophthalmol. 117, 929–937.

Hama, A.T., Sagen, J., 1994. Reduced pain-related behavior by adrenal medullary transplants in rats with experimental painful peripheral neuropathy. Pain 52, 223–231.

Hedley, M.L., 2000. Gene therapy of chronic inflammatory disease. Adv. Drug Delivery Rew. 44, 195–207.

Hélène, C., Toulmé, J.J., 1991. Specific regulation of gene expression by antisens, sense and antigene nucleic acids. Biochim. Biophys. Acta 1049, 99–125.

Honoré, P., Luger, N.M., Sabino, M.C., Schwei, M.J., Rogers, S.D., Match, D.B., O'Keefe, P., Ramnaraine, M.L., Clohisy, D.R., Mantyh, P.W., 2000. Osteoprotegerin blocks bone cancer-induced skeletal destruction, skeletal pain and pain related neurochemical reorganisation of the spinal cord. Nat. Med. 5, 521–528.

Hope, M.J., Mui, B., Ansell, S., Ahkong, Q.F., 1998. Cationic lipids, phosphatidylethanolamine and the intracellular delivery of polymeric, nucleic acid-based drugs. Mol. Membr. Biol. 15, 1–14.

Howard, M.K., Kershaw, T., Gibb, B., Storey, N., MacLean, A.R., Zeng, B.Y., Tel, B.C., Jenner, P., Brown, S.M., Wollf, C.J., Andersen, P.N., Coffin, P.N., Latchman, D.S., 1998. High efficacy gene transfer to the central nervous system of rodents and primates using herpes virus vectors lacking functional ICP27 and ICP34.5. Gene Ther. 5, 1137–1147.

Isner, J.M., Walsh, K., Symes, J., Pieczek, A., Takeshita, S., Lowry, J., Rosenfield, K., Weir, L., Brogi, E., Jurayj, D., 1996. Arterial gene transfer for therapeutic angiogenesis in patients with peripheral artery disease. Hum. Gene Ther. 7, 959–988.

Jacobs, A., Breakefield, X.O., Fraefel, C., 1999. HSV-1-based vectors for gene therapy of neurological diseases and brain tumors: Part II. Vector systems and applications. Neoplasia 1, 402–416.

Junker, U., Böhnlein, E., 1999. Gene therapy for arthritis 1996–1999. Exp. Opin. Ther. Patents 9, 1491–1498.

Kahn, A., 2000. Dix ans de thérapie génique: déceptions et espoirs. Biofutur 202, 16–20.

Kaplitt, M.G., Kwong, A.D., Kleopoulos, S.P., Mobbs, C.V., Rabkin, S.D., Pfaff, D.W., 1994. Preproenkephalin promoter yields region-specific and long-term expression in adult brain after direct in vivo gene transfer via a defective herpes simplex viral vector. Proc. Natl. Acad. Sci. U. S. A. 91, 8979–8983.

Kessler, P.D., Podsakoff, G.M., Chen, X., McQuiston, S.A., Colosi, P.C., Matelis, L.A., Kurtzman, G.J., Byrne, B.J., 1996. Gene delivery to skeletal muscle results in sustained expression and systemic delivery of a therapeutic protein. Proc. Natl. Acad. Sci. U. S. A. 93, 14082–14087.

Kren, B.T., Bandyopadhyay, P., Steer, C.J., 1998. In vivo site-directed mutagenesis of the factor IX gene by chimeric RNA/DNA oligonucleotides. Nat. Med. 4, 285–290.

Larsen, J.J., Arnt, J., 1985. Reduction in locomotor activity of arthritic rats as parameter for chronic pain: effect of morphine, acetylsalicylic acid and citalopram. Acta Pharmacol. Toxicol. 57, 345–351.

Lein, R., Mandel, R.J., Muzyczka, N., 2000. Adeno-associated virus vector-mediated gene transfer to somatic cells in the central nervous system. Adv. Virus Res. 55, 507–528.

Lemkine, G.F., Demeneix, B.A., 2001. Polyethylenimines for in vivo gene delivery. Curr. Opin. Mol. Ther. 3, 178–182.

Levatte, M.A., Cassam, A.K., Dekaban, G.A., Weaver, L.C., 1998. Analysis of a multi-mutant herpes simplex virus type 1 for gene transfer into sympathetic preganglionic neurons and a comparison to adenovirus vectors. Neuroscience 86, 1321–1336.

Lindner, M.D., Plone, M.A., Francis, J.M., Cain, C.K., 1999. Chronic morphine reduces pain-related disability in a rodent model of chronic, inflammatory pain. Exp. Clin. Psychopharmacol. 7, 187–197.

Liu, Y., Himes, B.T., Solowska, J., Moul, J., Chow, S.Y., Park, K.I., Tessler, A., Murray, M., Snyder, E.Y., Fischer, I., 1999. Intraspinal delivery of neurotrophin-3 using neural stem cells genetically modified by recombinant retrovirus. Exp. Neurol. 158, 9–26.

Lokensgard, J., Bloom, D., Dobson, A., Feldman, L., 1994. Long-term promoter activity during herpes simplex virus latency. J. Virol. 68, 7148–7158.

Lubberts, E., Joosten, L.A.B., Chabaud, M., Van den Bersselaar, L., Oppers, B., Coenen-de Roo, C.J.J., Richards, C.D., Miossec, P., Van den Berg, W.B., 2000. IL-4 gene therapy for collagen arthritis suppresses synovial IL-17 and osteoprotegerin ligand and prevents bone erosion. J. Clin. Invest. 105, 1697–1710.

Malberg, A.B., Brandon, E.P., Idzerda, R.L., Liu, H., McKnight, G.S., Basbaum, A.I., 1997. Diminished inflammation and nociceptive pain with preservation of neuropathic pain in mice with a targeted mutation of the type I regulatory subunit of cAMP-dependent protein kinase. J. Neurosci. 17, 7462–7470.

Martuza, R.L., Malick, A., Markert, A.M., Ruffner, K.L., Coen, D.M., 1991. Experimental therapy of human glioma by means of genetically engineered virus mutant. Science 252, 854–856.

Millan, M.J., 1998. The induction of pain: an integrative review. Prog. Neurobiol. 57, 1–164.

Nabel, G.J., 1999. Development of optimized vectors for gene therapy (Commentary). Proc. Natl. Acad. Sci. U. S. A. 96, 324–326.

Nasu, K., Kohsaka, H., Nonomura, Y., Terada, Y., Ito, H., Hirokawa, K., Miyasaka, N., 2000. Adenoviral transfer of cyclin-dependent kinase inhibitor genes suppresses collagen-induced arthritis in mice. J. Immunol. 165, 7246–7252.

Pappas, G.D., Lazorthes, Y., Bes, J.C., Tafani, M., Winnie, A.P., 1997. Relief of intractable cancer pain by human chromaffin cell transplants; experience at two medical centers. Neurol. Res. 19, 71–77.

Pohl, M., Ballet, S., Collin, E., Mauborgne, A., Bourgoin, S., Benoliel, J.J., Hamon, M., Cesselin, F., 1997. Enkephalinergic and dynorphinergic neurons in the spinal cord and dorsal root ganglia of the polyarthritic rat-in vivo release and cDNA hybridization studies. Brain Res. 749, 18–28.

Remy, J.S., Kichler, A., Mordvinov, V., Schuber, F., Behr, J.P., 1995. Targeted gene transfer into hepatoma cells with lipopolyamine-condensed DNA particles presenting galactose ligands: a stage toward artificial viruses. Proc. Natl. Acad. Sci. U. S. A. 92, 1744–1748.

Shimeld, C., Whiteland, J.L., Nicholls, S.M., Grinfeld, E., Easty, D.L., Gao, H., Hill, T., 1995. Immune cell infiltration and persistence in the mouse trigeminal ganglion after infection of the cornea with herpes simplex virus type 1. J. Neuroimmunol. 61, 7–16.

Simonato, M., Manservigi, R., Marconi, P., Glorioso, J., 2000. Gene transfer into neurones for the molecular analysis of behaviour: focus on herpes simplex vectors. TINS 23, 183–190.

Snyder, R.O., Miao, C., Meuse, L., Tubb, J., Donahue, B.A., Lin, H.F., Stafford, D.W., Patel, S., Thompson, A.R., Read, M.S., Bellinger, D.A., Brinkhous, K.M., Kay, M.A., 1999. Correction of hemophilia B in canine and murine models using recombinant adeno-associated viral vectors. Nat. Med. 5, 64–70.

Steinhoff, M., Vergnolle, N., Young, S.H., Tognetto, M., Amadesi, S., Ennes, H.S., Trevisani, M., Hollenberg, M.D., Wallace, J.L., Caughey, G.H., Mitchell, S.E., Williams, L.M., Geppetti, P., Mayer, E.A., Bunnett, N.W., 2000. Agonists of proteinase-activated receptor 2 induce inflammation by a neurogenic mechanism. Nat. Med. 6, 151–158.

Sundaresan, P., Hunter, W.D., Martuza, R.L., Rabkin, S.D., 2000. Attenuated, replication-competent herpes simplex virus type 1 mutant G207: safety evaluation in mice. J. Virol. 74, 3832–3841.

Taylor, R., 1997. Cell vehicles for gene transfer to the brain. Neuromuscular Disord. 7, 343–351.

Waldman, R., Champigny, G., Bassilana, F., Heurteaux, C., Lazdunski, M., 1997. A proton gated cation channel involved in acid sensing. Nature 386, 173–177.

Weissman, I.L., 2000. Translating stem and progenitor cell biology to the clinic: barriers and opportunities. Science 28, 1442–1446.

Wilson, S.P., Yeomans, D.C., Bender, M.A., Lu, Y., Goins, W., Glorioso, J.C., 1999. Antihyperalgesic effects of infection with a preproenkephalin-encoding herpes virus. Proc. Natl. Acad. Sci. U. S. A. 96, 3211–3216.

Woods, J.M., Katchke, K.J., Tokuhira, M., Kurata, H., Arai, K.I., Campbell, P.L., Koch, A.E., 2000. Reduction of inflammatory cytokines and prostaglandin E2 by IL-13 gene therapy in rheumatoid arthritis synovium. J. Immunol. 165, 2755–2763.

Yamada, M., Oligino, T., Mata, M., Goss, J.R., Glorioso, J.C., Fink, D.J., 1999. Herpes simplex virus vector-mediated expression of Bcl-2 prevents 6-hydroxydopamine-induced degeneration of neurons in the substantia nigra in vivo. Proc. Natl. Acad. Sci. U. S. A. 96, 4078–4083.

Ye, S., Cole-Strauss, A.C., Franck, B., Kmiec, E.B., 1998. Targeted gene correction: a new strategy for molecular medicine. Mol. Med. Today 4, 431–438.