VIRAL TRANSFER TECHNOLOGY

REVIEW

Cytokine gene therapy of autoimmune demyelination revisited using herpes simplex virus type-1-derived vectors

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The peripheral delivery of drugs in patients affected by central nervous system (CNS)-confined diseases is therapeutically ineffective due to the presence of the blood–brain barrier which forms an inaccessible wall to the majority of CNS targeting molecules. When molecules with an anti-inflammatory profile have been systemically administered to patients affected by a chronic inflammatory demyelinating disease of the CNS, such as multiple sclerosis (MS), results have been disappointing. A successful therapeutic approach in MS should therefore consider the delivery of anti-inflammatory molecules directly into the CNS in order to inhibit blood-borne CNS-confined mononuclear cells which act as ultimate effector cells directly destroying oligodendrocytes and/or releasing myelinotoxic substances. Biological and physical vectors engineered with heterologous genes coding for immunomodulatory cytokines with an anti-inflammatory

profile might represent the appropriate tool to deliver therapeutic genes into the CNS of patients with MS. So far, cytokine gene therapy has never been attempted in MS, but encouraging results have been obtained in the animal model of MS, experimental autoimmune encephalomyelitis (EAE), using viral vectors or plasmids engineered with cytokine genes and then injected systemically, either in the blood stream or circulating encephalitogenic T cells, or into the CNS. Here, we critically discuss the various attempts made in EAE using gene therapy protocols based on the delivery of immunomodulatory cytokine genes. Special emphasis is put on the use of non-replicative herpes simplex type-1 (HSV)-derived vectors engineered with the gene of the immunomodulatory cytokine interleukin (IL)-4. Gene Therapy (2000) 7, 1087–1093.

Keywords: gene therapy; MS; EAE; cytokines; herpetic vectors; inflammation

The cytokine network in multiple sclerosis

Introductory remarks

Multiple sclerosis (MS) is an immune-mediated chronic inflammatory disease of the central nervous system (CNS) of unknown origin affecting young adults. The pathological hallmark of the disease is the presence in the CNS of patchy inflammatory infiltrates in areas of demyelination and axonal loss, containing few auto-reactive T cells and a multitude of pathogenic non-antigen-specific mononuclear cells.¹ While the origin of MS is still unknown there are solid indications on the pathogenic process underlying the disease that mostly derive from studies performed on the animal model of MS, experimental autoimmune encephalomyelitis (EAE).^{1–3} It is currently believed that myelin antigen-reactive T cells (regulatory cells) provide the organ specificity of the pathogenic process in MS and regulate the re-circulation within the CNS of non-antigen-specific lymphocytes and monocytes (effector cells) releasing myelinotoxic substances.³ T cells that are specific for myelin components,

such as myelin basic protein (MBP), proteolipid protein (PLP), myelin oligodendrocyte glycoprotein (MOG), and mainly display the α/β T cell receptor (TCR) constitute the majority of the CNS antigen-specific T cell population. Blood-borne activated macrophages, B cells that produce antibodies against myelin components (ie MOG) or still unidentified components (ie oligoclonal cerebrospinal fluid bands), and possibly also γ/δ T cells, represent the effector cell population.²

Th1 and Th2 polarized response in MS

As previously mentioned, specific immunity sustained by T cells committed against myelin components plays a key role in the pathogenic process underlying MS since the T cells not only act as regulatory cells but are also crucial to recruit effector cells (ie macrophages) to the CNS. It is therefore advantageous to understand better the immunological mechanisms regulating the ‘anti-myelin’ immune response occurring during MS in order to set the stage for developing new immunomodulatory treatments for this disabling disease.

After antigen presentation, CD4⁺ (and CD8⁺) T cells (in mouse and human) differentiate into two distinct cell subsets differing in their cytokine secretion profile. T helper 1 (Th1) cells secrete interleukin (IL)-2, interferon (IFN) γ and tumor necrosis factor (TNF) α/β whereas T

helper 2 (Th2) produce IL-4, IL-5, IL-10, IL-13 and, to some extent, transforming growth factor (TGF) β .^{4,5} Their precursor cells, termed Th0 cells, can produce IL-2, IFN γ and IL-4 simultaneously. Th1 cytokines, whose production is mostly due to IL-12 stimulation, play a role in activation of macrophages (proinflammatory function), in delayed hypersensitivity (DTH) and in IgG2a (mouse)/IgG1 (human) but not IgE synthesis. Conversely, Th2 cytokines which are considered growth/differentiation factors for B cells, eosinophils and mast cells, play a key role in immediate-type hypersensitivity (IL-4 is in fact the critical stimulus inducing a switch to IgE antibody production). The *in vitro* immunological characteristics of these two cell subsets and the *in vivo* results collected from experimental animal models has led to the assumption that Th1 cytokines are pivotal in the induction of human organ-specific autoimmune disease (ie Hashimoto's thyroiditis, Graves' disease, rheumatoid arthritis, insulin-dependent diabetes mellitus and MS) while Th2 cytokines are important in allergic diseases and helminth infections.⁴ From a therapeutic point of view it is important to stress that cytokines elaborated by each Th-cell subset are inhibitory for the opposite subset and that Th2 cytokines inhibit several macrophage functions.⁵

Although there is no clear cut evidence that T cell lines and/or clones obtained from MS patients and reacting against major myelin proteins (PLP, MBP) have a Th1-polarized cytokine secretion profile, there are several *in vivo* indications that MS can be considered a 'bona fide' Th1 disease.¹⁻³ (1) MS patients experience clinical exacerbations when treated with IFN γ .^{6,7} (2) TNF α and IFN γ are present in demyelinating plaques⁸ and increase in the circulation during active phases of MS.⁹ (3) TNF α and IFN γ gene expression is routinely seen in cerebrospinal fluid (CSF) cells from MS patients where IL-4 gene expression is virtually absent.¹⁰ (4) Th1 cytokines (ie TNF α , IL-6, IFN γ) along with IL-2 can activate circulating memory CD4⁺ T cells in MS patients.¹¹ (5) PLP-specific T cell clones retrieved during acute attacks of MS display a Th1-like profile while T cell clones raised from the same patient during remission exhibit a Th0/Th2 pattern of cytokine secretion.¹² (6) The chemokine receptors CXCR3 and CCR5, which are consistently expressed on Th1-polarized cells, are found on mononuclear cells in active MS lesions and are significantly enriched in the CSF cells from MS patients.¹³ Finally, evidence in humans is paralleled by those obtained in EAE: (1) myelin-specific Th1 cell lines and clones transfer EAE to syngenic recipients;^{3,14} (2) mRNA and protein levels of Th1 cytokines increase before the onset of clinical EAE signs and then parallel EAE progression;^{14,15} and (3) MBP-specific T cells from non-human primates producing Th1 cytokines are able to induce EAE upon transfer into irradiated autologous recipients.¹⁶

Th1 cytokines as a therapeutic target in MS

Considering the detrimental effect of Th1 cytokines in EAE/MS it is conceivable to propose the therapeutic use in MS of Th1 antagonizing molecules, such as Th2 cytokines, to stop or at least partially ameliorate the neurological impairment due to demyelination and axonal loss. Moreover, the macrophage de-activating effect of Th2 cytokines could be of further help in the treatment of a disease, such as MS, in which ultimate effector cells are macrophages.¹⁻³

The therapeutic use of Th2 cytokines (IL-10, TGF β) or of cytokines with an anti-inflammatory profile (IFN β , TNF α receptor p55-Ig) in MS patients has so far been mostly disappointing. Subcutaneous as well as intramuscular administration of IFN β in patients with relapsing remitting MS is only partially effective since it reduces by only one third the exacerbation rate and does not substantially change the progression of disability.^{17,18} Systemic administration of TGF β does not induce any change in the disability status of patients or in the appearance of inflammatory magnetic resonance imaging lesions in the brain.¹⁹ The intravenous injection of the recombinant TNF receptor p55-Ig fusion protein Lenercept (Hoffmann-La Roche, Basel, Switzerland) in MS patients induces an increase in the number of patients experiencing exacerbations.²⁰ The disappointing results obtained using systemically administered cytokines to patients with MS might be due to the limited crossing of these molecules through the blood-brain barrier thus scarcely accumulating in the CNS where the MS pathogenic process takes place. The blood-brain barrier, which is made by close interacting endothelial cells, glial cells (mainly astrocytes) and pericytes, is physiologically crossed only by molecules using: (1) a lipid-mediated transport which is restricted to small molecules with a molecular weight less than a threshold of approximately 700 Da and is generally, but not always, proportional to the lipid solubility of the molecule; or (2) a catalyzed transport due to carrier-mediated (few milliseconds) or receptor-mediated (few minutes) processes.²¹ Moreover, cytokines have a short half-life, act in an auto/paracrine fashion thus being rapidly consumed in the periphery; and cause unpredictable and undesirable systemic side-effects (ie reversible nephrotoxicity = TGF β , binding and neutralizing anti-cytokine antibodies = IFN β and Lenercept).^{19,20,22,23}

A delivery system able to provide an *in situ* (ie CNS) cytokine production could overcome some of the limitations of the systemic delivery although practical considerations (ie injection schedule and technique) as well as potential CNS toxicity should be taken into consideration.

Cytokine gene therapy in experimental autoimmune encephalomyelitis

Gene therapy in MS patients has not been attempted to date. However, some experiments of gene therapy based on the delivery of Th2 'protective' cytokine genes able to counteract *in vivo* the 'detrimental' activity of Th1 cytokines have been performed in animals affected by EAE. Generally speaking, cytokine gene therapy approaches so far attempted in EAE can be divided into two distinct categories: (1) cytokine genes have been incorporated into viral vectors or plasmids and injected directly into the blood stream or into circulating encephalitogenic T cells; and (2) cytokine genes have been directly transferred into the CNS, either in the parenchyma or into CSF spaces, using viral vectors, plasmid-DNA or DNA-liposome complexes.

Attempts made by transferring cytokine genes into encephalitogenic T cells are based on the idea of generating 'suppressor' cells that will home to the sites of inflammation/demyelination in the CNS as a result of their specificity for myelin antigens. After crossing the

blood-brain barrier these cells are supposed to be reactivated to produce Th1-antagonizing molecules *in situ* thereby inhibiting neighboring Th1 encephalitogenic T cells via a bystander effect. These are the results obtained so far. (1) Retroviral constructs containing the IL-4, IL-10 or TNF α genes were used to transduce an MPB-specific, H-2s restricted T cell hybridoma which was, in turn, adoptively transferred into syngeneic (PL/J $\times$ SJL/J)F1 mice in which EAE was induced using MBP. IL-4 ameliorated EAE, while TNF α exacerbated the disease and IL-10 was ineffective.^{24,25} (2) An MBP-specific BALB/c Th1 clone, transduced with the murine latent TGF β cDNA subcloned into the pMFG retroviral vector, was injected into the blood stream of (SJL $\times$ BALB/c) F1 mice, immunized with PLP₁₃₉₋₁₅₁. The treatment significantly delayed and ameliorated EAE development either when administered before (at the time of immunization) or at EAE onset. Only partial efficacy of this treatment was observed when modified T cells were administered 3 days after EAE onset.²⁶ (3) PLP₁₃₉₋₁₅₁-activated T cells transfected with a transgenic construct containing the IL-10 mouse cDNA driven by the IL-2 promoter (along with intron splices sites and the SV40 polyadenylation signal region to ensure high levels of IL-10 expression) were effective in ameliorating clinico-pathological signs of EAE in (SWR $\times$ SJL)F1 mice immunized with the PLP₁₃₉₋₁₅₁ peptide.²⁷ All these efforts based on the genetic modification of encephalitogenic T cells, although successful in EAE, are challenged by the following considerations: (1) no clear cut evidence is provided that encephalitogenic T cells, however modified, cross the blood-brain barrier and release *in situ* the protective cytokine; (2) constitutive cytokine gene expression using hybridoma cells lines can eventually kill mice due to the overgrowth of the tumor cells; (3) T cells transduced with IL-2 promoter IL-10 cDNA construct, and showing high levels of endogenous IL-10 production, were not examined for production of other cytokines, thus questioning the protective role of IL-10 in that experiment; and (4) *in vitro* pre-activation of encephalitogenic T cell lines engineered with the TGF β gene may induce cytokine release into the blood stream thus causing serious side-effects (ie TGF β administration in humans causes reversible nephrotoxicity).¹⁹

There is only one report dealing with the therapeutic efficacy of systemic injection of viral vectors engineered with cytokine genes in mice affected by EAE.²⁸ Viral vectors derived from the WR strain of vaccinia virus (VV) and engineered to produce IL-6, TNF α , IL-1 β , IL-2, IL-10, IL-4 or IFN γ were intravenously injected, at the time of immunization and 6 days later, in SJL/J or (SJL/J $\times$ BALB/C)F1 hybrid mice affected by syngeneic spinal cord homogenate-induced EAE. A disease ameliorating effect was observed in EAE mice when the IL-6, TNF α , IL-1 β , IL-2, IL-10 but not IL-4 or IFN γ gene-containing vectors were used. It is unclear whether or not this therapy relies on consistent data since clinical parameters have only been calculated during a 1 week period spanning day 10 to day 16 after immunization and no pathological evidence of a disease protective effect was provided. Apart from the inconsistency of the experimental results dealing with the use of VV-derived vectors in EAE, it has to be clearly stated that VV-derived vectors are still hampered by their intrinsic toxicity and immuno-

genicity that renders such technology unsuitable for transfer to humans.

Plasmid DNA constructs (naked DNA or DNA-liposome complexes) engineered with cytokine genes and injected intramuscularly or intracerebrally have been also used as alternative therapeutic treatment in EAE mice. (1) IL-4, TGF β , IFN β , p55TNF receptor-Ig, p75TNF receptor-Ig, or IL-10 cytokine plasmids were injected both into resting or proliferating muscle cells (systemic) or into the right frontal cortex (intracerebral) of Biozzi AB/H mice affected by spinal cord homogenate-induced EAE.^{29,30} Systemic or intracerebral administration of naked cytokine DNAs was not successful in ameliorating EAE. In contrast, with IL-4, TGF β , IFN β , p75TNF receptor-Ig, p55TNF receptor-Ig but not of IL-10, DNAs complexed with lipofectin (DNA-liposome complexes) ameliorated EAE when intracerebrally injected 3 days before disease onset. (2) Naked plasmid DNA expression vectors encoding for TGF β 1 (pVR-TGF β 1) or for IL-4-IgG chimeric protein (pVR-IL-4-IgG1) were injected intramuscularly into mice affected by MBP-induced EAE. Both these therapeutic protocols significantly reduced clinical and histopathological signs of EAE by inducing a pronounced down-regulation of proliferating activity of MBP-specific T cells which have been found to produce less IFN γ and TNF α . Moreover, enhanced secretion of endogenous IL-4 was observed when the IL-4-IgG1 vector was administered. In the CNS, treatment with either vectors suppressed IL-12 and IFN γ mRNA expression, while IL-4 and TGF β 1 mRNA levels were increased compared with control mice.³¹ Although to some extent contradictory, the results of using naked DNA and/or plasmid DNA-cationic liposome complexes in preventing EAE onset or ameliorating the ongoing disease has some practical and theoretic drawbacks. Intracerebral injection of these constructs leads not only to a short-term *in situ* transgene expression, thus limiting their therapeutic potential in a chronic disease such as MS, but might also be ineffective since MS is a multifocal disease and plasmid DNA constructs have a short range of CNS diffusion when intraparenchymally injected. On the other hand, recently it has been shown that regulatory sequences (eg CG repeats) used in plasmids for DNA vaccination protocols can modulate *per se* cytokine production *in vivo* when intramuscularly injected thus being able to suppress or worsen clinico-pathological signs and symptoms of EAE.^{32,33}

Cytokine gene therapy in EAE using HSV-1-derived vectors

We have been working on a different viral vector-mediated CNS delivery system for cytokine genes. Our main goal was to have a delivery system: (1) able to reach different CNS areas (MS is a multifocal disease with a preferential periventricular distribution of the affected areas); (2) not toxic at all for CNS resident cells; (3) unable to induce peripheral side-effects; and (4) able to persist sufficiently within the CNS in order to avoid repeated intrathecal injections.

The novel system we established to deliver cytokine genes into the CNS is based on the use of non-replicative herpes simplex virus (HSV) type-1-derived vectors. These vectors are considered as an alternative to classical retroviral and adenoviral vectors, mainly because HSV is able

to accommodate multiple foreign genes and to infect post-mitotic cells, such as neurons.^{34–37} We used the non-replicative HSV-1-derived vector named d120^{37,38} (a gift from J Glorioso, Department of Molecular Genetics and Biochemistry, School of Medicine, University of Pittsburgh, PA, USA) which is an HSV type-1 virus (Kos) strain containing a deletion in both copies of the immediate-early (IE) *ICP4* gene. *In vivo* preliminary experiments using the d120 vector containing the *LacZ* gene (d120:LacZ) inserted into the thymidine kinase (TK) locus under the *ICP4* promoter indicated that the vector can be easily transferred within the mouse CNS through an intracisternal (i.c.) injection (Figure 1a and b). In the CNS the vector diffuses consistently in all CSF spaces and efficiently infects the ependymal cell layer surrounding the ventricles as well as choroidal and leptomeningeal cells (Figure 1c and d). When mice were i.c. injected with a d120 vector containing the *IFN* γ gene (d120:LacZ: *IFN* γ), we found that CNS-infected cells started to produce discrete amounts of the cytokine into the mouse CSF from a few hours until 4 weeks after vector injection. *IFN* γ levels, measured using a home-made sandwich ELISA¹⁵ in CSF samples obtained before death from the cisterna magna using a 25G needle, were high 2 days after i.c. injection (4.5 ng/ml) and decreased thereafter, but were still measurable 28 days after i.c. injection (0.7 ng/ml). *IFN* γ was also measurable (0.3 ng/ml) in CSF samples obtained from a control mouse injected with the d120:LacZ vector and killed 2 days after injection but not in the sample from another control mouse killed at 14 days after injection; no detectable levels of *IFN* γ were present in CSF samples from naive mice. These latter

results indicate that the i.c. injection procedure is safe and induces only a moderate, transient and self-limited inflammatory CNS reaction. After secretion into the CSF, *IFN* γ was shown to be biologically active since it was able to up-regulate MHC class I molecules on CNS resident cells.^{39,40}

Due to the *in vivo* results indicating that the intrathecal delivery of HSV-1-derived vectors containing cytokine genes induces the release into the CSF spaces of bioactive cytokines thus allowing the continuous travelling of these potentially 'therapeutic' molecules across the whole CNS, we exploited the therapeutic potential of our gene delivery system in EAE mice using the same d120:LacZ vector but engineered with IL-4 gene under the HCMV promoter (d120:IL-4:LacZ). We preliminary tested whether IL-4 was produced by vector-infected CNS resident cells using either immunocytochemistry on CNS tissue sections or a home-made ELISA¹⁵ on CSF samples (obtained at different time-points after vector i.c. injection). IL-4 was consistently found by immunohistochemistry in the cell layers surrounding ventricular and subarachnoid spaces. In the same areas the presence of the vector was detected using an anti-HSV-1 antibody thus indicating that vector-infected CNS resident cells were able *in vivo* efficiently to transcribe and translate the IL-4 gene engineered into the vector.⁴⁰ IL-4 was never found in CSF samples thus indicating its rapid *in vivo* consumption. We then tested the therapeutic potential on EAE of the IL-4-containing vector i.c. administered. Either preventive or therapeutic experiments were performed. The preventive treatment was based on the i.c. delivery of the d120:IL-4:LacZ vector twice (days 0 and 7 after immunization) to

Figure 1 Macroscopic (panels a and b) and microscopic (panels c and d) distribution of the HSV-1-derived d120:LacZ vector after intracisternal injection. In panel a blue spots indicate cells infected by the vector which are visible around the injection site (arrow). No blue staining is visible in a brain from a control mice (panel b). In panels c and d staining for β Gal (panel c) and HSV-1 (panel d) co-localize in two adjacent cerebellar tissue sections thus indicating that the intracisternal delivery of the d120:LacZ vector induces infection of leptomeningeal cells within CSF spaces which in turn transcribe and translate the heterologous gene (ie *LacZ*) contained into the vector.

Biozzi AB/H mice immunized with 200 μ g per mouse of MOG_{40–55}.⁴⁰ The therapeutic protocol was based on one i.c. injection of the d120:IL-4:LacZ vector to Biozzi AB/H mice, immunized with 2 mg per mouse of spinal cord homogenate, the same day as the first appearance of EAE signs and symptoms.⁴¹ Neither protocol induced toxic or immune reactions up to 3 months after vector injection but did confirm that the gene delivery technique employed led to IL-4 secretion in the CSF spaces by ependymal cells (including choroidal cells) infected by the vectors. A significant amelioration of clinical and pathological (inflammatory infiltrates, demyelination and axonal loss) CNS features characteristic of EAE was observed in IL-4-treated compared with control mice both when the vector was administered before or after EAE onset (Figure 2). The protective effect of the preventive therapy (cumulative EAE score: 36.6 ± 9.5 in IL-4-treated mice *versus* 64.1 ± 11.3 in control mice treated with the empty vector) was mostly due to the ability of IL-4 to inhibit recruitment and trafficking of macrophages within the CNS (number of macrophages per mm² of spinal cord: 34.4 ± 2.3 in IL-4-treated mice *versus* 51.4 ± 4.6 in control mice). On the other hand, the protective effect of the therapeutic protocol (47% reduction of EAE-related deaths in IL-4-treated mice *versus* control mice) was due to the *in situ* inhibition of proinflammatory cytokines (TNF α , IL-1 β) and monocyte chemoattractant chemokines (MCP-1, Rantes). Finally, we found that lymph node cells from mice preventively treated with the IL-4-containing vector proliferated in response to the encephalitogenic antigen (ie MOG_{40–55}) and differentiated into IFN γ -producing Th1 cells (9% of lymph node cells were anti-MOG_{40–55} CD4⁺ IFN γ -producing cells in IL-4-treated mice *versus* 7% in control mice) as well as lymph node cells from control mice, thus indicating that CNS delivery of IL-4 does not affect the peripheral immune system.⁴⁰

Final remarks

Although the oversimplistic Th1 *versus* Th2 therapeutic view of human organ-specific autoimmune disease has not yet led to identify the 'golden standard' Th2 cytokine able consistently to halt Th1 diseases such as MS, the results so far obtained in EAE support the continuous research and development of gene delivery techniques aimed at transferring heterologous 'therapeutic' cytokine genes into the CNS. However, gene therapy techniques used so far in EAE models might have major limitations when transferred to humans: (1) autoreactive T cells can be easily isolated in the animal model of MS but not in humans where the MS antigen is still unknown; (2) DNA-liposomes as well as VV have limited transfection efficiency when introduced into post-mitotic cells such as those resident in the CNS; (3) regulatory sequences used in plasmids for naked DNA vaccination can *per se* modulate *in vivo* cytokine production leading to EAE suppression/worsening; (4) VV as well as adenoviral vectors still have residual toxicity and immunogenicity; (5) CG repeated motifs contained in bacterial plasmids used to transfer heterologous genes into EAE animals may be *per se* either 'detrimental' or 'protective'; (6) non-replicative viral vectors infecting post-mitotic cells have short transgene expression; (7) vectors using regulatable promoters can be scarcely accessible to (de)activating molecules when homed into CNS resident cells; and (8) intracerebral delivery of viral vectors able to infect post-mitotic cells determines a short-range of diffusion of the vectors from the injection site thus preventing their efficacy in multifocal CNS diseases.

Some of the limitations of the gene vector technology so far employed in EAE/MS has been overcome by the use of HSV-1-derived vectors that, in our hands, showed to be easily transferred within the CNS through the CSF circulation with no major reactions of resident cells

Figure 2 Clinical features of EAE in mice i.c. injected with the HSV-1-derived d120:LacZ:IL-4 vector (open circles) or the d120:LacZ control vector (closed circles). Panel a shows mean EAE score per day in each of the Biozzi AB/H mice affected by a chronic-relapsing form of the disease induced using 200 μ g of myelin MOG_{40–55} peptide emulsified in complete Freund's adjuvant (CFA); delivery of d120:LacZ:IL-4 and d120:LacZ vectors was performed on days 0 and 7 after immunization (preventive protocol). Panel b shows mean EAE score per day in each of the Biozzi AB/H mice affected by a relapsing-remitting form of the disease induced using 2 mg of spinal cord homogenate emulsified in CFA; i.c. delivery of d120:LacZ:IL-4 and d120:LacZ vectors was performed on the day of EAE onset (therapeutic protocol). IL-4 gene delivery was shown to be effective in ameliorating EAE both in the preventive and in the therapeutic protocol. EAE clinical score is as follows: 0 = healthy; 1 = flaccid tail; 2 = ataxia and/or paresis of hindlimbs; 3 = paralysis of hindlimbs and/or paresis of forelimbs; 4 = tetraparesis; 5 = moribund or death.

whose intracellular machinery was used to produce the heterologous gene (eg cytokine genes). The main advantages of this system are: (1) the availability of high cytokine levels in different areas of the CNS; (2) the persistent therapeutic effect (ie 4 weeks) after a single vector administration; and (3) the lack of interference of this procedure with the proper functioning of the peripheral immune system. Moreover, the possibility of accommodating multiple genes within HSV-1-derived vectors suggests that this therapeutic device might be useful when different cytokines with synergistic activity have to be used at the same time. On the other hand, the still short-term effect of this technology (no more than 4 weeks after *in vivo* delivery) as well as the impossibility of regulating gene transcription are the major limiting factors of a cytokine-based therapy developed for chronic diseases such as MS. These results, however, suggest that research into applicability of HSV-1-derived vectors as an alternative therapeutic approach to CNS-confined diseases is worthwhile.

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