The therapeutic use of gene therapy in inflammatory demyelinating diseases of the central nervous system
Roberto Furlan, Stefano Pluchino and Gianvito Martino

Purpose of review
Gene therapy protocols aimed to deliver therapeutic molecules into the central nervous system may represent an alternative therapeutic strategy in patients affected by inflammatory demyelinating diseases of the central nervous system where systemic therapies have shown limited therapeutic efficacy possibly owing to the blood-brain barrier, a major obstacle for the entry of therapeutic molecules into the central nervous system.

Recent findings
Among inflammatory demyelinating diseases of the central nervous system, gene therapy approaches have been so far developed almost exclusively for multiple sclerosis. However, the chronic/relapsing nature of the disease, the restriction to the central nervous system of the pathological process as well as the necessity to inhibit the ongoing inflammatory process but also to foster endogenous remyelinating pathways, have posed several questions which still need to be properly addressed for the development of a successful gene therapy strategy in multiple sclerosis patients.

Summary
The gene therapy approaches for multiple sclerosis have been so far developed and tested only in rodents and monkeys with experimental autoimmune encephalomyelitis, the animal model of multiple sclerosis. The results of these studies clearly indicate that the delivery of therapeutic genes within the central nervous system is superior to the peripheral delivery. In particular, the intracerebral delivery of genes coding for anti-inflammatory and/or neurotrophic molecules, using gene vectors derived from non-replicative viruses, showed to inhibit not only the detrimental function of blood-borne mononuclear effector cells but also to foster proliferation and differentiation of surviving oligodendrocytes within demyelinated areas. Here, we summarize the most recent findings of this novel area of research.

Keywords
gene therapy, inflammation, neuroprotection, demyelination, multiple sclerosis

Introduction
Inflammatory demyelinating diseases of the central nervous system (CNS), such as multiple sclerosis (MS), are characterized by the presence within the CNS of inflammatory infiltrates containing few autoreactive T cells and a multitude of pathogenic nonspecific mononuclear cells in areas of demyelination, axonal loss and severe glial scarring [1,2]. It is currently believed that CNS antigen-specific T cells provide the organ specificity of the pathogenic process and regulate the influx within the CNS of nonantigen-specific mononuclear cells that, in turn, act as effector cells by directly destroying oligodendrocytes and by releasing myelinotoxic substances [3]. In most instances, however, oligodendrocytes or their precursors are morphologically preserved in demyelinating plaques during the early phase of the disease, thus remaining capable of differentiation and remyelination [4]. A successful therapeutic approach in inflammatory demyelinating diseases of the CNS should therefore consider the delivery of ‘therapeutic’ molecules directly into the CNS in order to inhibit blood-borne mononuclear cells acting as ultimate effector cells (antiinflammatory therapies) or to promote migration and differentiation of endogenous myelinating oligodendrocytes into demyelinating areas (neuro-protective/-regenerative therapies).

Among the inflammatory diseases of the CNS, MS has recently been the subject of the most intensive research for the development of new therapeutic strategies, gene therapy included. MS is the most common inflammatory demyelinating disease in young adults. Furthermore, no efficacious therapies are so far available for this disease, the social costs for such a chronic disease are enormous and the majority – if not all – of the novel anti-inflammatory or neuro-protective/neuro-regenerative therapies for MS have been mostly disappointing [5–8].

Abbreviations
BBB blood–brain barrier
CFS cerebrospinal fluid
CNS central nervous system
CTLA cytotoxic T-lymphocyte antigen
EAE experimental autoimmune encephalomyelitis
HSV-1 herpes simplex virus type-1
MBP myelin basic protein
MS multiple sclerosis
PLP proteolipid protein
TGF transforming growth factor
TNF tumor necrosis factor

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One possible explanation for these failures is that the systemic delivery of drugs in patients affected by CNS-confined diseases is therapeutically ineffective due to the presence of the blood–brain barrier (BBB), which forms an inaccessible wall to the majority of CNS targeting molecules [9]. An alternative therapeutic approach in MS based on the delivery of genes coding for therapeutic molecules directly within the CNS is therefore conceivable [10].

Here we will discuss the most recent attempts to develop new gene therapy strategies for the CNS targeting of therapeutic molecules. This excursus will be based mainly on experimental studies performed in the animal model of MS because these are the only, sufficiently robust data that can be reviewed. The results of these studies are summarized in Table 1. No other inflammatory diseases of the CNS have been the subject of such intense research aimed to develop new gene therapy strategies.

**Immuno-gene therapy for the treatment of central nervous system inflammatory demyelinating diseases: the peripheral approach**

Delivery of immune-modulatory genes into the CNS has never been tested in MS patients, but encouraging results have been obtained in experimental autoimmune encephalomyelitis (EAE). Different approaches have been developed. ‘Protective’ genes have been engineered into viral vectors or plasmids and injected into encephalitogenic T cells, which, in turn, have been used as ‘Trojan horses’ to deliver genes coding for anti-inflammatory cytokines to the CNS of EAE mice. Intramuscular injection of plasmid–DNA (e.g. naked DNA) or DNA–liposome complexes to induce a self-reaction against the heterologous genes engineered into the gene vectors has also been tried. Engineering of myelin antigens into B cells to protect from EAE via a tolerizing B cell-mediated mechanism has recently been reported. The results of a study based on the peripheral delivery of viral vectors engineered with cytokine genes are also available.

**Inhibition of proinflammatory cytokines or delivery of antiinflammatory cytokines**

Retroviral constructs containing the IL-4, IL-10 or tumor necrosis factor (TNF)-α gene have been used to transduce myelin basic protein (MBP)-specific T hybridoma cells that were then transferred into syngeneic (PL/J × SJL/J) F1 mice in which EAE was induced by MBP. IL-4 ameliorated EAE, while TNFα exacerbated the disease and IL-10 was ineffective [14,15]. In a variant of this approach, a MBP-specific BALB/c Th1 clone, transduced with the murine latent transforming growth factor (TGF)-β cDNA subcloned into the pMFG retroviral vector, was injected into the blood stream of (SJL × BALB/c) F1 mice, immunized with the proteolipid protein (PLP) peptide 139–151. The treatment significantly delayed and ameliorated EAE development both when administered at the time of immunization and at EAE onset. The treatment, however, was only partially efficacious when engineered T cells were administered 3 days after EAE onset [33]. Finally, 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 splice 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 × SJL) F1 mice immunized with the PLP139–151 peptide [22].

The therapeutic efficacy of systemic injection of viral vectors engineered with cytokine genes in mice affected by EAE has also been reported [12]. Viral vectors derived from the WR strain of vaccinia virus and engineered to produce IL-6, TNFα, IL-1β, IL-2, IL-10, IL-4 or IFNγ were injected intravenously, at the time of immunization and 6 days later, into SJL/J or (SJL/J × BALB/C) F1 mice affected by EAE induced by syngenic spinal cord homogenate. IL-6, TNFα, IL-1β, IL-2, IL-10, but neither IL-4 nor IFNγ, gene-containing vectors induced amelioration of the disease. The sustained efficacy of this treatment was unclear, because clinical parameters have been evaluated only during a 1-week period from day 10 to day 16 post immunization, and no histo-pathological evidence of a disease-protective effect was provided.

IL-4, TGFβ, IFNβ, p55TNF receptor-Ig, p75TNF receptor-Ig, or IL-10 cytokine plasmids injected either into resting or proliferating muscle cells failed to ameliorate EAE in Biozzi AB/H mice immunized with spinal cord homogenate [11,25,35]. Conversely, clinical and histo-pathological signs of MBP-induced EAE have been significantly reduced by intramuscular injection of plasmid DNA expression vectors encoding for TGFβ1 or IL-4-IgG chimeric protein. This approach induced a pronounced downregulation of MBP-specific T cell proliferation as well as IFNγ and TNFα production [16]. Repeated intramuscular injections of IL-4 naked DNA have also been performed in SJL and C57BL/6 mice affected by EAE as a preventive or therapeutic treatment. While the preventive approach in SJL mice was partially successful in ameliorating the disease course, the therapeutic approach in C57BL/6 mice did not show any consistent result [17**].

**Blocking proinflammatory chemokines**

IFNγ-inducible protein (IP)-10, a CXC chemokine that stimulates the directional migration of activated pro-
<table>
<thead>
<tr>
<th>Therapeutic gene</th>
<th>Delivery route</th>
<th>Gene vector</th>
<th>Animal strain</th>
<th>Antigen used for immunization</th>
<th>Administration schedule(a)</th>
<th>Clinical efficacy(b)</th>
<th>Refs</th>
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<td>IFN(\beta)</td>
<td>Intracerebral</td>
<td>Liposomes</td>
<td>Biozzi AB/H mice</td>
<td>SCH</td>
<td>Therapeutic</td>
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<td>[11]</td>
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<td>IFN(\gamma)</td>
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<td>Vaccinia virus</td>
<td>SJL mice</td>
<td>SCH</td>
<td>Preventive</td>
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<td>Ependymal route</td>
<td>HSV-1</td>
<td>C57BL/6 mice</td>
<td>MOG35–55</td>
<td>Therapeutic</td>
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<td>+</td>
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<td></td>
<td>HSV-1</td>
<td>C57BL/6 mice</td>
<td>MOG35–55</td>
<td>Therapeutic</td>
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<td><strong>IL-1(\beta)</strong></td>
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<td>SJL mice</td>
<td>SCH</td>
<td>Preventive</td>
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<td>Vaccinia virus</td>
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<td>Retrovirus (PL/J x SJL)F1 mice</td>
<td>MBP</td>
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<td>Therapeutic</td>
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<td>MBP-specific T cells</td>
<td>Retrovirus (PL/J x SJL)F1 mice</td>
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<td>Therapeutic</td>
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<td>Biozzi AB/H mice</td>
<td>SCH</td>
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<td>+</td>
<td>[14]</td>
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<td></td>
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<td>Biozzi AB/H mice</td>
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<td>Therapeutic</td>
<td>+</td>
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<td>–/+</td>
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<td>PLP-specific T cells</td>
<td>Retrovirus SWXJ mice</td>
<td>PLP139–151</td>
<td>Therapeutic</td>
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<td>HSV-1</td>
<td>Biozzi AB/H mice</td>
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<td>HSV-1</td>
<td>Biozzi AB/H mice</td>
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<td>Therapeutic</td>
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<td>SJL mice</td>
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<td>Therapeutic</td>
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<td>[12]</td>
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<td>Therapeutic</td>
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<td>[14]</td>
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<td>Biozzi AB/H mice</td>
<td>SCH</td>
<td>Therapeutic</td>
<td>+</td>
<td>[12]</td>
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<td>Therapeutic worsening</td>
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<td>[12]</td>
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<td>p55 TNFR-lg</td>
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<td>Liposomes</td>
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<td>Therapeutic</td>
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<td>[11]</td>
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<td>Intramuscular</td>
<td>Liposomes</td>
<td>Biozzi AB/H mice</td>
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<td>Therapeutic</td>
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<td>[11]</td>
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<td>Lewis rats</td>
<td>MBP68–86</td>
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<td>CTLA4-IgG</td>
<td>Intrapertonal</td>
<td>Adenovirus</td>
<td>NA</td>
<td>NA</td>
<td>Preventive</td>
<td>+</td>
<td>[27]</td>
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<tr>
<td><strong>Myelin antigens</strong></td>
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<td>PLP139–151</td>
<td>Intramuscular</td>
<td>Naked DNA</td>
<td>SJL mice</td>
<td>PLP139–151</td>
<td>Preventive</td>
<td>+</td>
<td>[29*]</td>
</tr>
<tr>
<td>PLP139–151+IL-4</td>
<td>Intramuscular</td>
<td>Naked DNA</td>
<td>SJL mice</td>
<td>PLP139–151</td>
<td>Preventive</td>
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<td>MOG35–55</td>
<td>Intramuscular</td>
<td>Naked DNA</td>
<td>C57BL/6 mice</td>
<td>MOG35–55</td>
<td>Therapeutic</td>
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<td>[17**]</td>
</tr>
<tr>
<td>MOG35–55+IL-4</td>
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<td>Naked DNA</td>
<td>C57BL/6 mice</td>
<td>MOG35–55</td>
<td>Therapeutic</td>
<td>+</td>
<td>[17**]</td>
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<td>PLP100–154</td>
<td>B cells</td>
<td>Retrovirus (SJL x BALB/c)F1 mice</td>
<td>bMBP</td>
<td></td>
<td>Therapeutic</td>
<td>+</td>
<td>[29**]</td>
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<td><strong>MBP–IgG1</strong></td>
<td>B cells</td>
<td>Retrovirus (PL/J x SJL)F1 mice</td>
<td>bMBP</td>
<td></td>
<td>Therapeutic</td>
<td>+</td>
<td>[30**]</td>
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(continued overleaf)
inflammatory T cells (i.e. Th1 cells), is the only chemokine so far tested in a gene therapy protocol in EAE mice. Administration of plasmid DNA encoding IP-10 was capable of inducing the generation of self-specific immunity to the gene product of the vaccine (including specific Abs) that, in turn, suppressed full-blown ongoing EAE in Lewis rats and C57BL/6 mice [26].

**Suppression of co-stimulation using gene therapy**

A gene therapy protocol consisting of intravenous injections of an adenovirus vector (Adex1CACTLA4IgG) coding for a chimeric protein consisting of an extracellular portion of cytotoxic T lymphocyte-associated antigen (CTLA4) and an Fc portion of human IgG1(CTLA4IgG) were used to prevent the development of EAE. This approach inhibited the development of EAE up to 8 months after a single injection of intravenous Adex1CACTLA4IgG [27].

Lewis rats vaccinated with naked DNA coding for the membrane-bound form of the Fas ligand were protected from EAE. The gene therapy protocol induced Fas ligand-specific Abs, which, in turn, were capable of downregulating the production of TNFα by T cells. Protection was observed only in rats treated at the onset of EAE and in rats treated after the peak of the acute phase of disease [28].

**Inducing tolerance to myelin-antigens**

A novel and interesting gene therapy-based model has recently been proposed. Mice susceptible to EAE have been transformed into resistant mice by specifically downregulating an autoreactive T-cell population. This was accomplished by using a retroviral gene transfer protocol. Normal B cells were genetically modified to constitutively express the SJL-specific PLP encephalitogenic determinant (amino acids 139–151) and then adoptively transferred into syngeneic hosts. To ensure appropriate presentation of the exogenous encephalitogenic peptide in association with MHC class II, the encephalitogenic sequence was fused to a lysosomal targeting sequence. Adoptive transfer of syngeneic B cells expressing the PLP139–151 into normal, naive, genetically susceptible mice induced PLP-specific unresponsiveness and completely protected the majority of mice (from 62 to 83%) from EAE induction. The remaining mice displayed delayed disease onset and lower disease severity [29].

Another B cell-mediated gene therapy approach has recently been proposed. A retroviral construct, expressing a chimeric IgG–MBP construct into B cells, was intravenously injected into syngeneic EAE mice. This protocol lead to the amelioration of ongoing EAE induced by the transfer of primed cells from (PL × SJL) F₁ mice. The treatment was effective even when administered after the appearance of EAE symptoms. The effect was specific and did not involve bystander suppression because treatment with MBP–IgG did not affect the disease induced by immunization with the PLP immunodominant peptide plus MBP [30].

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**Table 1. (continued)**

<table>
<thead>
<tr>
<th>Therapeutic gene</th>
<th>Delivery route</th>
<th>Gene vector</th>
<th>Animal strain</th>
<th>Antigen used for immunization</th>
<th>Administration schedule</th>
<th>Clinical efficacy</th>
<th>Refs</th>
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</thead>
<tbody>
<tr>
<td>B cells</td>
<td>Retrovirus</td>
<td>(PL/J × SJL)F₁ mice</td>
<td>bMBP + MBP1-17</td>
<td>Therapeutic</td>
<td>+</td>
<td>[30]</td>
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<tr>
<td>B cells</td>
<td>Retrovirus</td>
<td>(PL/J × SJL)F₁ mice</td>
<td>PLP139–151</td>
<td>Therapeutic</td>
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<td>[30]</td>
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<tr>
<td>B cells</td>
<td>Retrovirus</td>
<td>(PL/J × SJL)F₁ mice</td>
<td>bMBP + PLP139–151</td>
<td>Therapeutic</td>
<td>–</td>
<td>[30]**</td>
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</table>

**Growth factors**

- **PDGFα**
  - PLP-specific T cells
    - Retrovirus
    - SWXJ mice
    - PLP139–151
    - Therapeutic
    - +
    - [31]
  - FGF-II
    - Ependymal route
    - HSV-1
    - C57BL/6 mice
    - MOG35–55
    - Preventive
    - +
    - [32]
    - Ependymal route
    - HSV-1
    - C57BL/6 mice
    - MOG35–55
    - Therapeutic
    - +
    - [32]
- **TGFβ**
  - MBP-specific T cells
    - Retrovirus
    - (SJL × BALB/c)F₁ mice
    - PLP139–151
    - Therapeutic
    - +
    - [33]
  - Intramuscular
    - Liposomes
    - Biozzi AB/H mice
    - SCH
    - Preventive
    - –
    - [11]
  - Intramuscular
    - Naked DNA
    - SJL mice
    - SCH
    - MBP
    - Preventive
    - –
    - [16]
  - Intracerebral
    - Liposomes
    - Biozzi AB/H mice
    - SCH
    - Therapeutic
    - +
    - [11]
  - NGF
    - MBP-specific T cells
    - Retrovirus
    - Lewis rats
    - MBP
    - Preventive
    - –
    - [34]

*SCH, spinal cord homogenate; HSV-1, herpes simplex virus type-1; MOG, myelin oligodendrocyte glycoprotein; MBP, myelin basic protein; PLP, proteolipid protein; TNF, tumor necrosis factor; p55 TNFR-Ig, p55 TNF receptor-Ig fusion protein; p75 TNF dimeric receptor; IP-10, IFNγ-inducible protein 10; CTLA4, cytotoxic T-lymphocyte antigen 4; NA, not available; FasL, Fas ligand; bMBP, bovine myelin basic protein; PDGF, platelet-derived growth factor; FGF-II, fibroblast growth factor II; TGF, transforming growth factor; NGF, nerve growth factor. *Preventive, at the time of immunization or shortly after; therapeutic, close to or after disease onset. +, Disease amelioration; -, no effect.
Finally, it has been shown that the co-delivery of the IL-4 gene with a DNA vaccine encoding the self-peptide PLP139–151 provided protective immunity against EAE. The mechanism was due to secretion of IL-4 expressed from the naked DNA acting locally on autoreactive T cells. The same approach also worked to reverse established EAE when co-vaccination with IL-4 and the myelin oligodendrocyte glycoprotein genes was performed [17**].

**Immuno-gene therapy for the treatment of inflammatory demyelinating diseases: the central nervous system approach**

Genes have been incorporated into viral vectors, plasmid–DNA, DNA–liposome complexes, or cells (e.g. fibroblasts) and then directly injected into the CNS. So far, two different approaches have been developed and tested in EAE. In the intraparenchymal approach, ‘protective’ genes have been engineered into viral vectors or plasmids and injected directly into brain areas. In the intrathecal approach, genes have been incorporated into viral vectors and then injected into the cerebrospinal fluid (CSF) circulation.

**Cytokine gene therapy**

IL-4, TGFβ, IFNβ, p55TNF receptor-Ig, p75TNF receptor-Ig or IL-10 containing plasmids were injected intracerebrally into the right frontal cortex of Biozzi AB/H mice affected by spinal cord homogenate-induced EAE, but no amelioration of the disease was observed. In contrast, DNAs complexed with lipfectin (DNA–liposome complexes) containing IL-4, TGFβ, IFNβ, p75TNF receptor-Ig, or p55TNF receptor-Ig ameliorated EAE when injected intracerebrally 3 days before the disease onset. IL-10 DNA–liposome complexes were, however, ineffective [11,25,35]. An adenoviral vector coding for mouse IL-10 failed to inhibit EAE when injected into the CNS parenchyma, despite producing high amounts of IL-10 protein. In contrast, intracranial injection of fibroblasts transduced with a retroviral vector expressing IL-10 inhibited mouse EAE. The disease ameliorating effect was associated with increased numbers of CD8+ T and B cells in CNS infiltrates rather than a decreased cell recruitment [24*]. Finally, partially replicating herpes simplex virus type-1 (HSV-1) γ(1)34.5 deletion viruses coding for IL-4 and IL-10 have been used in gene therapy of EAE in Balb/c mice [18]. Intracranial injection of the IL-4– but not of IL-10-producing virus showed protection from EAE symptoms and signs.

A novel gene therapy approach based on the CSF injection of HSV-1-derived nonreplicative vectors engineered with cytokine genes has recently been established [19,36*]. HSV-1-derived vectors were easily transferable into the CNS through the CSF route and diffused in the CSF space leading to efficient infection of ependymal and leptomeningeal cells surrounding cerebral ventricles and leptomeningeal spaces [13*,19,20,21**,36*]. Within the CNS, viral vectors redirected the machinery of the infected cells to produce discrete levels of the cytokines in the CSF for at least 4 weeks [13*,19,20,21**,36*]. This approach was used to deliver IL-4 and IFNγ in mice with EAE either before or after disease onset [13*,19,20]. Both protocols showed ameliorating EAE – either in rodents or in nonhuman primates – by inhibiting CNS lymphocyte trafficking and proliferation or by inducing encephalitogenic cell death by apoptosis. The ependymal approach has recently been further validated in EAE by the demonstration that the injection into the CSF of a replication-defective E1/E3/PIX-deleted adenoviral vector engineered with IL-10 could ameliorate EAE in mice [23]. These results are also consistent with previous data showing that intraventricular injection in naive mice and rats of first-generation replication defective recombinant adenoviral vectors (Ad.Svbgal, Ada1AT, and AxCAHBG) leads to the expression of reporter genes in ependymal cells [37,38] and to CSF production of proteins coded by the transgenes, such as β-glucuronidase and z1-antitrypsin [38,39]. Moreover, a recent report confirmed that the injection into the CSF space, either sub-occipitally (within the cisterna magna) or more caudally by lumbar puncture of an adenoviral vector containing the β-gal reporter gene determined a widespread infection of ependymal cells surrounding the CSF space in non-human primates [40].

**Central nervous system delivery of neurotrophic growth factor genes to foster remyelination in inflammatory demyelinating diseases**

The development of therapies aimed to promote remyelination is a major issue in MS, as repeated episodes of demyelination over time lead to axonal loss and permanent neurological impairment [4**]. Therapies aimed to promote myelin restoration are so far mainly based on transplantation of oligodendrocytes or oligodendrocyte precursors and on the use of neurotrophic growth factors able to promote migration, proliferation and differentiation of endogenous oligodendrocyte precursors. While transplantation experiments have been successful in repairing myelin sheet and restoring axonal conduction only in defined demyelinating areas of experimental animals [4**], thus being inadequate, at present, in multifocal CNS demyelinating diseases, growth factor therapy has been partially successful in EAE [11,33], but not in MS patients in which their systemic administration caused heavy side effects [6,41]. An alternative approach for delivering growth factors into the CNS has thus been attempted in EAE mice using gene therapy.
Delivery of growth factor genes into the central nervous system via the blood stream

Encephalitogenic mouse T cells transfected with an antigen-inducible transgene for platelet-derived growth factor (PDGF)-z, a growth factor important in regulating the development of oligodendrocytes, migrated to the CNS where they released PDGF-z that, in turn, ameliorated ongoing EAE [39]. MBP-specific CD4+ T cells transduced with a recombinant retrovirus encoding nerve growth factor (NGF) have also been used in EAE. These modified T cells, which secreted high levels of NGF, were unable to mediate clinical EAE, when transferred alone, but efficiently suppressed induction of clinical EAE by nontransduced MBP-specific T cells. Suppression of clinical EAE was associated with a general reduction of inflammatory CNS infiltrates, with a most pronounced decrease of the monocyte/macrophage component, thus suggesting that the gene therapy protocol might have interfered with monocyte migration through the activated BBB endothelium [34].

Intrathecal delivery of growth factor genes into the central nervous system

Injection into the CSF of HSV-1-derived vectors coding for fibroblast growth factor (FGF)-II, a growth factor inducing differentiation and proliferation of oligodendrocyte progenitors, was shown to be able to ameliorate ongoing EAE without toxic reaction. The protective mechanism was exerted via the induction of oligodendrocyte precursor proliferation and migration into demyelinating areas [32*]. EAE, however, was ameliorated when FGF-II gene therapy was administered for up to 4 weeks but not when it was administered for longer periods of time (i.e. months), since chronic administration induced diffuse astrocitic proliferation thus blocking oligodendrocyte progenitor cell migration to demyelinated areas.

Conclusion

Gene therapy can be bona fide considered as an alternative option for the treatment of MS owing to the promising results so far obtained in EAE. Gene therapy in MS, however, has a long way to go because several challenging questions have to be solved before considering its translation into the clinic.

The ‘peripheral’ gene therapy approach shows some disadvantages. When the targets of the gene transfer are encephalitogenic T lymphocytes, no clear cut evidence is available to demonstrate that these cells, however modified, do cross the BBB and release in situ the protective cytokine. Moreover, T cells have to be in vitro pre-activated before in vivo transfer, thus it is possible that they start releasing the transgene’s product into the blood stream. This can cause serious side effects as it has been shown in MS patients after systemic TGFβ administration (e.g. nephrotoxicity) [6]. When B cells are used as tolerizing agent, one should take into consideration that this approach, although promising, is limited by the fact that the putative autoantigen to tolerate is clearly identifiable in EAE mice but not in MS. The recent failure of the therapeutic use of altered peptide ligands in MS highlights the difficulty to clearly identify an autoantigen to tolerate in MS [42,43]. Systemic delivery of vaccinia virus-derived vectors is hampered by the intrinsic toxicity and immunogenicity of these vectors, thus preventing their applicability to clinical situations. Naked DNA vaccination and liposome-mediated gene transfer also have some problems. It has been shown that regulatory sequences (e.g. CG repeats) used in plasmids for DNA vaccination protocols can per se modulate cytokine production in vivo when injected intramuscularly, thus inhibiting or worsening clinicopathological signs and symptoms of EAE [44,45]. These effects are independent of the transgene and reflect an immune response against the plasmid DNA.

The CNS approach definitely seems more promising and superior to the peripheral approach. Some disadvantages have to be considered, however, and more work is needed to solve these problems. Injection into discrete CNS areas of naked DNA, plasmid DNA–cationic liposome complexes, or first generation adenoviral vectors determines a short-term transgene expression and a restricted CNS diffusion of the vectors to the injection site. Thus, this approach is limited in a multifocal chronic disease such as MS. The intrathecal viral vector-mediated CNS delivery of cytokine and growth factor genes shows some major advantages: (1) availability of high levels of the ‘therapeutic molecule’ in all areas of the CNS, provided that the proteins coded by the transgenes inserted into the vectors are secreted into the CSF circulation; (2) persistent therapeutic effect after a single vector administration, irrespective of the injection site into the CSF circulation; (3) lack of interference with the peripheral immune system; and (4) absence of CNS and peripheral side effects. However, the intrathecal approach, based on the use of HSV-1-derived or first generation adenoviral vectors, although more effective than direct intracranial injection, still has some disadvantages that have to be solved to permit its application in chronic diseases such as MS. In particular, the relatively short-lasting cytokine production (no more than 4 weeks) as well as the inability to regulate gene transcription, may represent important limitations of the therapeutic use of HSV-1-derived vectors. Some concrete hopes were raised from a recent study showing that the CNS delivery of the new generation of helper-dependent adenoviral vectors does not determine an immune response to the vectors and make the vectors persist in the CNS for months [36*,46,47]. Another major advantage is the possibility
of accommodating into these new vectors, due to their size, multiple genes under regulatable promoters.

Gene therapy in MS still has a long way to go before it reaches the clinic. Great hope has been raised, however, for the cure of a disease for which conventional treatments often fail to prevent disease progression. A futuristic but realistic gene therapy scenario can therefore be envisaged in MS. It might include different ‘human-grade’ vectors which could be used to deliver different antiinflammatory cytokines as well as neuroprotective/neuroregenerative agents in a flexible, timed, safe and effective way to control the different phases of the disease.

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References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

• of special interest

•• of outstanding interest

A comprehensive review dealing with the apparently contradictory role played by inflammation in MS.
A complete review dealing with one of the most difficult challenges in multiple sclerosis. The topic, which is discussed and analysed in detail, provides realistic explanations for the failure of remyelination in MS and suggests strategies for successful remyelination.
The unexpected and challenging demonstration that a proinflammatory cytokine, such as IFNγ, may be beneficial in EAE when delivered in the CNS using a gene therapy protocol.
The paper clearly demonstrates that the co-delivery of the IL-4 gene and of a DNA vaccine encoding the self-peptide PLP139–151 provides protective immunity against EAE. This is a very interesting work suggesting a possible, although futuristic, combination therapy for multiple sclerosis.
The first successful gene therapy protocol in a non-human primate model of MS based on the intrathecal delivery of the gene coding for the prototypical antiinflammatory cytokine IL-4 using HSV-1-derived viral vectors.
An interesting work aimed to compare different gene therapy approaches to deliver the antiinflammatory IL-10 gene within the CNS of EAE mice.
The first gene therapy protocol in EAE aimed to therapeutically target a potential detrimental chemokine.

The first approach in EAE based on the use of engineered B cells as tolerogenic agents. Retrovirally transduced B cells deliver an anergic signal to encephalitogenic T cell thus preventing EAE onset.


One of the first attempts to use genetically modified B cells as tolerogenic agents in autoimmunity. This protocol determined the amelioration of ongoing EAE.


The first gene therapy protocol based on the CNS delivery of a growth factor showing efficacy to both dampen inflammation and foster remyelination in rodent EAE.


A point of view describing in detail the ‘ependymal route’ as an alternative and feasible way to approach cytokine-gene therapy in EAE/MS.


