8 Gene and Stem Cell Therapy for Autoimmune Demyelination

S. Pluchino, M. Bacigaluppi, S. Bucello, E. Butti, M. Deleidi, L. Zanotti, G. Martino, R. Furlan

8.1 Introduction

The pathological hallmark of multiple sclerosis (MS) is the presence within the central nervous system (CNS) of inflammatory infiltrates containing few autoreactive T cells and a multitude of pathogenic nonspecific lymphocytes determining patchy demyelination, axonal loss, and severe glial scarring. 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/or by releasing myelinotoxic substances (Kieseier et al. 1999; Martino and Hartung 1999). 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 (Lucchinetti et al. 1996; Martino and Hartung 1999; Prineas et al. 1993). A successful therapeutic approach of MS should therefore be aimed to (a) inhibit the activation of antigen- and nonantigen-specific immune cells, and/or (b) to rescue the surviving oligodendrocytes within demyelinating plaques, or both. CNS gene delivery using nonreplicative viral vectors able to infect postmitotic cells such as those resident in the CNS has being proposed as a useful therapeutic approach to deliver anti-inflammatory cytokine and – possibly – growth factor genes for either inhibiting the activation of antigen- and nonantigen-specific immune cells (anti-inflammatory therapies) or fostering surviving oligodendrocyte progenitors to differentiate into myelin forming cells ("remyelinating" therapies), respectively (Furlan et al. 2003). Experimental cell- based strategies aimed at replacing damaged myelin-forming cells have also been developed in the last few years (Pluchino et al. 2004). Most of these therapeutic approaches have appeared as unfeasible, owing to the mutifocality of the demyelinating process in MS patients and the inability of in vitro growth and of differentiation of large numbers of myelin-forming cells. However, recently proposed adult somatic stem cell-based therapies (Pluchino et al. 2003) have partially overcome these limitations.

Recent advances in the development of gene- and stem cell-based therapies, aimed at promoting multifocal remyelination, are here discussed.

8.2 CNS Delivery of Neuroprotective Genes

8.2.1 Anti-inflammatory Cytokine Genes

Proinflammatory cytokines (i.e., Th1 cytokines such as IFNγ, tumor necrosis factor (TNF)α/β, IL-2) are believed to play a crucial role in MS pathogenic process since they can promote and sustain the development of myelin-specific T cells and the recruitment, within the CNS, of peripheral myelinotoxic effector cells, i.e., monocyte/macrophages (Kieseier et al. 1999). In addition, proinflammatory cytokines such as TNFα can be directly toxic for oligodendrocytes (Selmaj et al. 1998). Proinflammatory cytokines thus represent a suitable therapeutic target in MS.

However, systemic delivery of anti- inflammatory cytokines has shown limited or no efficacy, and considerable toxicity when administered to MS patients. Intramuscular or subcutaneous administration of interferon (IFN)β reduces by only one third the exacerbation rate of the disease, does not change substantially the progression of disability, and induces the production of neutralizing anti-IFNβ antibodies (?????????? TS[a] 1993; ?????????? TS[a] 1996). Systemic administration of TGFβ fails to prevent the appearance of new inflammatory magnetic resonance imaging lesions in the brain of patients and causes reversible nephrotoxicity (Calabresi et al. 1998). Intravenous injection of the recombinant TNF-receptor p55 immunoglobulin fusion protein Lenercept® increases the number of patients experiencing disease relapses and induces the formation of neutralizing antibodies (??????????? TS[a] 1999). These disappointing results are possibly due to the scarce capacity of systemically administered cytokines to cross the blood brain barrier (BBB) and accumulate in the CNS where the MS pathogenic process takes place (Khan et al. 1996). Moreover, cytokines act in an autocrine-paracrine fashion and have a short half-life thus being consumed at the site of production and/or administration. The CNS delivery of anti-inflammatory cytokines (i.e., Th2 cytokines such as IL-4, IL-5, IL-10, IL-13) could partially overcome these limitations. Biological vectors engineered with heterologous genes coding for anti-inflammatory cytokines and injected into the CNS might represent appropriate tools to deliver anti-inflammatory "protective" Th2 cytokines in inflammatory demyelinating diseases of the CNS.

8.2.2 The Ephendymal Route

We have developed an alternative approach to CNS gene therapy by using the ependymal route (Martino et al. 2001). Injection into the cerebrospinal fluid (CSF) allows viral vectors to infect only cells lining liquoral spaces, like ependymal and leptomeningeal cells. The large number of viral particles that can be delivered in this relatively small compartment ensures high infection efficiency. If the delivered gene codes for a soluble, secreted molecule, this will be released into the CSF and be able to travel through the ventricular system to reach all brain areas, remaining confined to the brain and unable to induce unwanted

TS[a] Please complete.

Editor's or typesetter's annotations (will be removed before the final TEX run)

side effects in the periphery. This approach has been used in EAE in mice and nonhuman primates (Furlan et al. 2003), and in ischemic stroke (Shimamura et al. 2004), but holds promise for all multifocal brain diseases. Since ependymal and leptomeningeal cells are slow dividing cells that are poorly renewed, their infection allows long-term expression, up to 6 months, of the delivered transgene. Summarizing, the advantages of the ependymal route for CNS gene therapy are:

1. The high concentration of soluble therapeutic proteins that can be achieved in the CSF
2. The possibility of reaching multiple brain areas and thus be useful in multifocal CNS disorders
3. The ability of vectors injected in the CSF to express the transgene long-term, being potentially useful for chronic diseases
4. The absence of unwanted peripheral toxicity and side effects
5. The absence of an intrathecal immune response towards the viral vector which should allow repetitive injections without loss of therapeutic efficacy

Some of these features rely on the nature of the protein encoded by the transferred gene. The therapeutic molecule has to be soluble and secreted, and its ability to travel across the brain–CSF barrier and "soak" the brain parenchyma depends on its physical and chemical properties, and has to be assessed for each molecule. Using an HSV-1-derived vector expressing the cytokine IFNγ in mice, we have been able to show that the biological effect (i.e., induction of MHC-I and -II expression) of the molecule transferred by gene therapy could be detected in the brain parenchyma at least at 1 mm from liquoral spaces (Furlan et al. 2001). With the same type of vector, but delivering the cytokine IL-4, however, we were able to interfere with an inflammatory disease ongoing in the brain of larger mammals such as Rhesus monkeys (Poliani et al. 2001), indicating that data obtained in mice were, most likely, underestimated. HSV-1-derived vectors were used to deliver IL-4 in mice with EAE, either before or after disease onset. I.c. injection of an IL-4-coding HSV-1 vector was able to inhibit chronic-remitting EAE development in Biozzi AB/H mice immunized with the myelin oligodendrocyte glycoprotein (MOG)40–55 peptide (Furlan et al. 1998). Disease prevention was associated to a decreased recruitment within the

CNS of monocyte/macrophages from the peripheral circulation. The i.c., HSV-1- mediated, IL-4 delivery was able also to ameliorate ongoing relapsing-remitting EAE in spinal cord homogenate-immunized Biozzi AB/H mice, determining, in this case, a significant modulation of the local cytokine milieu, leading to downregulation of proinflammatory cytokines and chemokines (Furlan et al. 2001). This latter approach has been tested also in nonhuman primates affected by a very acute, invariably fatal, form of EAE induced by immunization with whole myelin. Sixty percent of monkeys, i.c.-injected at the time of disease onset with an HSV-1 vector engineered with the human IL-4 gene, were completely protected from EAE signs and symptoms (Poliani et al. 2001). The ependymal route using HSV-1-derived vectors has been employed also to deliver the IFNγ gene, which was able to both inhibit or treat MOG35–55-induced chronic EAE in C57BL/6 mice, through the induction of in situ apoptotic death of encephalitogenic T cells (Furlan et al. 2001). Using the same protocol, we used HSV-1 derived vectors to deliver the IL-1 receptor antagonist (IL-1ra) to C57BL/6 EAE mice immunized with MOG35–55 and obtained delay of disease onset and decreased severity only on a prophylactic schedule (R. Furlan, unpublished). HSV-1-derived vectors, however, have a low chance to be employed in a human clinical setting due to: (a) the short term transgene production i.e., up to 4 weeks); (b) their possible immunogenicity; and (c) their derivation from a virus potentially very dangerous for its selective neurovirulence.

8.2.3 Neurotrophic Growth Factor Genes

The development of therapies aimed to promote remyelination is a major issue in MS, where repeated episodes of demyelination over time lead to axonal loss and permanent neurological impairment (Lucchinetti et al. 1996; Prineas et al. 1993). Therapies aimed to promote myelin restoration are so far mainly based on transplantation of oligodendrocytes (or oligodendrocyte precursors) or multipotential neural stem cells (NSCs; Franklin 2002), and on the use of neurotrophic growth factors able to promote migration, proliferation, and differentiation of oligodendrocyte precursors (Franklin et al. 2001).

HSV-1-mediated intracisternal delivery of the fibroblast growth factor (FGF)-II gene was able to induce oligodendrocyte precursors proliferation and migration, thus ameliorating ongoing chronic EAE in MOG35–55-immunized C57BL/6 mice (Ruffini et al. 2001). Growth factors have been partially successful in the therapy of EAE (Chen et al. 1998; Croxford et al. 1997; Villoslada et al. 2000), but not in MS patients where their systemic administration caused heavy side effects (Calabresi et al. 1998). However, results in humans are still preliminary and questionable (i.e., natural inactive TGFβ1 was used in EAE while active TGFβ1 was used in MS patients) since there is clear in vitro evidence that growth factors may stimulate the proliferation of glial progenitor cells, and their differentiation into myelinating oligodendrocytes.

8.3 Stem Cell Therapies

Somatic stem cells represent an alternative source of cells that can be used to promote myelin repair (Pluchino et al. 2004). Encouraging preliminary results have been obtained by transplanting adult neural stem cells (aNSCs) (Fig. 1) into rodents affected by CNS demyelination (Ben-Hur et al. 2003b; Pluchino et al. 2003). However, although somatic stem cells may integrate within the CNS and possibly repair the myelin damage, further studies are required to assess the in vivo plasticity of these cells and the safety and efficacy of this therapeutic approach. Furthermore, there are some additional questions we need to confront with before prospecting any potential human application of such therapies: (a) the ideal stem cell source for transplantation; (b) the route of cell administration; (c) the differentiation and persistence of the transplanted cells into the targeted tissue; and, last but not least, (d) functional and long-lasting integration of transplanted cells into the host tissue has to be achieved.

Whatever the organ or tissue necessities, the “gold standard” cell for replacement therapies has to be inherently plastic. Stem cells can meet this criterion since they are intrinsically able either to adapt their terminal cell fate to different environmental needs (differentiation plasticity) or to transdifferentiate (developmental plasticity). Moreover, stem cells represent a potentially unlimited source of myelin-forming cells while

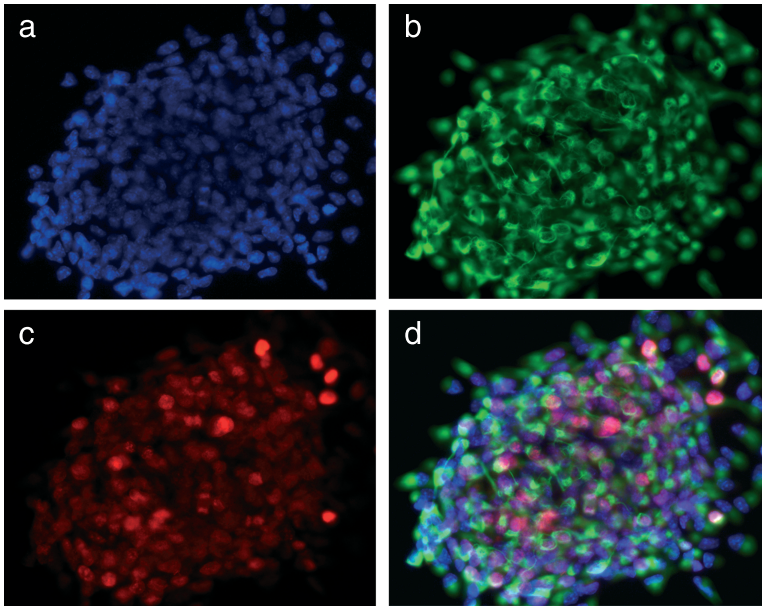

Fig. 1A–D. Adult neural stem cells (aNSC) for transplantation in CSN demyelination. Double immunofluorescence of mouse subventricular zone (SVZ)- derived aNSCs grown as neurospheres in vitro and labeled with antibodies against nestin (**B**, *green*), and the proliferation marker Ki67 (**C**, *red*). Nuclei have been counterstained with dapi (**A**, *blue*). Merged image of **A**–**C** is shown in **D**. ×40

either more mature or even postmitotic myelin-forming cells are difficult to manipulate and can be expanded in vitro only scarcely (Franklin 2002). Both embryonic stem cells (ES) and aNSCs might represent the ideal cell source for cell replacement-based therapies in myelin CNS disorders. aNSCs showed the potential to repair demyelinating lesions by acquiring a preferential glial cell-fate once transplanted in vivo into rodents suffering from either acute or chronic autoimmune inflammatory demyelination (Ben-Hur et al. 2003b; Bulte et al. 2003; Einstein et al. 2003; Pluchino et al. 2003). ES cells have been able to differentiate into glial cells and re-ensheath in vivo demyelinated axons when transplanted in animal models of either genetically determined or

chemically-induced demyelination (Brustle et al. 1999; Chu et al. 2003; Liu et al. 2000; McDonald et al. 1999;Reubinoff et al. 2001; Zhang et al. 2001). However, transplantation of ES cells has been complicated by the formation of heterologous tissues and teratomas within the organ of transplantation (Brustle et al. 1997, 1999; Deacon et al. 1998; Yanai et al. 1995).

Other somatic stem cells of nonneuronal origin have been recently used to repair the myelin sheath in vivo. Rats with an acute demyelinated lesion of the spinal cord showed varying degrees of remyelination – which was proportional to the number of injected cells – after systemic or intralesional injection of acutely isolated mononuclear bone marrow-derived stem cells BMSCs; (Akiyama et al. 2002a; Inoue et al. 2003). Moreover, bone marrow-derived stromal cells induced remyelination and improvement of axonal conduction velocity once transplanted by direct microinjection into the demyelinated spinal cord of immunosuppressed rat (Akiyama et al. 2002b). These results, although encouraging, are still too preliminary to draw any meaningful conclusion about the therapeutic use of BMSCs in demyelinating disorders.

The route of cell administration represents another key issue for stem cell transplantation in multifocal CNS diseases. While direct intralesional cell transplantation can be instrumental in focal CNS disorders (e.g., Parkinson's disease or spinal cord injury), alternative approaches have to be established in multifocal CNS disorders (e.g., MS), where multiple CNS injections would be impractical. Interestingly enough, some recent experiments have shown that stem cells e.g., bone marrow cells, mesenchymal cells, (aNSCs) may reach multiple areas of the CNS once injected into the blood stream (i.v.) or into the cerebrospinal fluid circulation (i.c.) of rodents with multifocal demyelinating disorders of inflammatory origin (Ben-Hur et al. 2003b; Einstein et al. 2003; Pluchino et al. 2003). This specific homing has been explained, at least in part, by the constitutive expression by transplanted stem cells of a wide array of inflammatory molecules such as adhesion molecules (i.e., integrins, selectins, immunoglobulins, etc.), cytokines, chemokines, and chemokine receptors (Ben- Hur et al. 2003a; Coulombel et al. 1997; Klassen et al. 2003; Luo et al. 2002; Papayannopoulou 2003; Pluchino et al. 2003; Schmid and Anton 2003).

Ideally, once in the target organ, transplanted stem cells should differentiate into the appropriate daughter cells and persist as long as needed at the site of engraftment. Very little is known about the mechanisms instructing the terminal differentiation of stem cells in vivo; however, there is strong evidence that the local micro-environment might dictate the fate choice of transplanted uncommitted stem cells. In this respect, undifferentiated multipotent aNSCs or even totipotent ES cells, transplanted in different experimental neurological conditions, have shown considerable capacity to restrict their terminal fate to tissue-specific cues and replace nonfunctioning neural cells of different lineages, including myelin-forming cells (Brustle et al. 1999; Liu et al. 2000; McDonald et al. 1999; Pluchino et al. 2003). It has been shown that also BMSCs may give rise to myelin-forming cells once transplanted in vivo into demyelinated areas (Akiyama et al. 2002a; Inoue et al. 2003). However, developmental transdifferentiation of BMSCs into neural stem cells – although clearly described – has been recently disputed by studies showing that this is a rare event in vivo and that most of "transdifferentiated" BMSCs are transplanted cells whose nuclei are fused with those of endogenous resident neural cells (Alvarez-Dolado et al. 2003; Mezey et al. 2003; Priller et al. 2001; Weimann et al. 2003).

Finally, the functional integration of stem cells at the site of homing/transplantation is the most critical issue. Although indications that stem cells – whatever their tissue of origin – can reach the target organ and differentiate into the appropriate lineage exist, there is still scarce evidence that these cells can reconstruct the 3D brain architecture and give raise to properly functioning cells integrating into the brain circuitries. So far, most studies on aNSCs or BMSCs have relied strictly on morphological or immunohistochemical evidence (Doetsch 2003). Further studies fulfilling several strict criteria are therefore necessary to determine whether a stem cell has generated a functional neuronal or glial cell.

In conclusion, the intrinsic complex nature of MS – in particular its chronicity and multifocality and the presence of both inflammatory (acute myelin and axon destruction) and degenerative (chronic axonal loss) features – poses great limitations for cell-based remyelinating therapies. Although promising results have been obtained using stem cell-based therapies in preclinical settings, the great challenge for the future

is to understand how to use these cells in a reliable, safe, and reproducible fashion in order to hopefully achieve a complete functional and anatomical rescuing of myelin architecture.

References

Akiyama Y, Radtke C, Honmou O, Kocsis JD (2002a) Remyelination of the spinal cord following intravenous delivery of bone marrow cells. Glia 39:229–236

Akiyama Y, Radtke C, Kocsis JD (2002b) Remyelination of the rat spinal cord by transplantation of identified bone marrow stromal cells. J Neurosci 22:6623–6630

Alvarez-Dolado M, Pardal R, Garcia-Verdugo JM, Fike JR, Lee HO, Pfeffer K, et al. (2003) Fusion of bone-marrow-derived cells with Purkinje neurons, cardiomyocytes and hepatocytes. Nature 425:968–973

Ben-Hur T, Ben-Menachem O, Furer V, Einstein O, Mizrachi-Kol R, Grigoriadis N (2003a) Effects of proinflammatory cytokines on the growth, fate, and motility of multipotential neural precursor cells. Mol Cell Neurosci 24:623– 631

Ben-Hur T, Einstein O, Mizrachi-Kol R, Ben-Menachem O, Reinhartz E, Karussis D, et al. (2003b) Transplanted multipotential neural precursor cells migrate into the inflamed white matter in response to experimental autoimmune encephalomyelitis. Glia 41:73–80

Brustle O, Jones KN, Learish RD, Karram K, Choudhary K, Wiestler OD, et al. (1999) Embryonic stem cell-derived glial precursors: a source of myelinating transplants. Science 285:754–756

Brustle O, Spiro AC, Karram K, Choudhary K, Okabe S, McKay RD (1997) In vitro-generated neural precursors participate in mammalian brain development. Proc Natl Acad Sci 94:14809–14814

Bulte JW, Ben-Hur T, Miller BR, Mizrachi-Kol R, Einstein O, Reinhartz E, et al. (2003) MR microscopy of magnetically labeled neurospheres transplanted into the Lewis EAE rat brain. Magn Reson Med 50:201–205

Calabresi PA, Fields NS, Maloni HW, Hanham A, Carlino J, Moore J, et al. (1998) Phase 1 trial of transforming growth factor beta 2 in chronic progressive MS. Neurology 51:289–292

Chen LZ, Hochwald GM, Huang C, Dakin G, Tao H, Cheng C, et al. (1998) Gene therapy in allergic encephalomyelitis using myelin basic protein- specific T cells engineered to express latent transforming growth factor-beta1. Proc Natl Acad Sci 95:12516–12521

Chu K, Kim M, Jeong SW, Kim SU, Yoon BW (2003) Human neural stem cells can migrate, differentiate, and integrate after intravenous transplantation in adult rats with transient forebrain ischemia. Neurosci Lett 343:129–133

Coulombel L, Auffray I, Gaugler MH, Rosemblatt M (1997) Expression and function of integrins on hematopoietic progenitor cells. Acta Haematol 97:13–21

Croxford JL, O'Neill JK, Baker D (1997) Polygenic control of experimental allergic encephalomyelitis in Biozzi ABH and BALB/c mice. J Neuroimmunol 74:205–211

Deacon T, Dinsmore J, Costantini LC, Ratliff J, Isacson O (1998) Blastula-stage stem cells can differentiate into dopaminergic and serotonergic neurons after transplantation. Exp Neurol 149:28–41

Doetsch F (2003) The glial identity of neural stem cells. Nat Neurosci 6:1127–1134

Einstein O, Karussis D, Grigoriadis N, Mizrachi-Kol R, Reinhartz E, Abramsky O, et al. (2003) Intraventricular transplantation of neural precursor cell spheres attenuates acute experimental allergic encephalomyelitis. Mol Cell Neurosci 24:1074–1082

Franklin RJ (2002) Remyelination of the demyelinated CNS: the case for and against transplantation of central, peripheral and olfactory glia. Brain Res Bull 57:827–832

Franklin RJ, Hinks GL, Woodruff RH, O'Leary MT (2001) What roles do growth factors play in CNS remyelination? Prog Brain Res 132:185–193

Furlan R, Brambilla E, Ruffini F, Poliani PL, Bergami A, Marconi PC, et al. (2001) Intrathecal delivery of IFN-gamma protects C57BL/6 mice from chronic-progressive experimental autoimmune encephalomyelitis by increasing apoptosis of central nervous system-infiltrating lymphocytes. J Immunol 167:1821–1829

Furlan R, Pluchino S, Marconi PC, Martino G (2003) Cytokine gene delivery into the central nervous system using intrathecally injected nonreplicative viral vectors. Methods Mol Biol 215:279–289

Furlan R, Poliani PL, Galbiati F, Bergami A, Grimaldi LM, Comi G, et al. (1998) Central nervous system delivery of interleukin 4 by a nonreplicative herpes simplex type 1 viral vector ameliorates autoimmune demyelination. Hum Gene Ther 9:2605–2617

The IFNB Multiple Sclerosis Study Group (1993) Interferon beta-1b is effective in relapsing-remitting multiple sclerosis. I. Clinical results of a multicenter, randomized, double-blind, placebo-controlled trial. Neurology 43:655–661

The IFNB Multiple Sclerosis Study Group and the University of British Columbia MS/MRI Analysis Group (1996) Neutralizing antibodies during treatment of multiple sclerosis with interferon beta-1b: experience during the first three years. Neurology 47:889–894

Inoue M, Honmou O, Oka S, Houkin K, Hashi K, Kocsis JD (2003) Comparative analysis of remyelinating potential of focal and intravenous administration of autologous bone marrow cells into the rat demyelinated spinal cord. Glia 44:111–118

Khan OA, Xia Q, Bever CT, Jr., Johnson KP, Panitch HS, Dhib- Jalbut SS (1996) Interferon beta-1b serum levels in multiple sclerosis patients following subcutaneous administration. Neurology 46:1639–1643

Kieseier BC, Storch MK, Archelos JJ, Martino G, Hartung HP (1999) Effector pathways in immune mediated central nervous system demyelination. Curr Opin Neurol 12:323–336

Klassen HJ, Imfeld KL, Kirov, II, Tai L, Gage FH, Young MJ, et al. (2003) Expression of cytokines by multipotent neural progenitor cells. Cytokine 22:101–106

The Lenercept Multiple Sclerosis Study Group and The University of British Columbia MS/MRI Analysis Group 1999) TNF neutralization in MS: results of a randomized, placebo-controlled multicenter study. Neurology 53:457–465

Liu S, Qu Y, Stewart TJ, Howard MJ, Chakrabortty S, Holekamp TF, et al. (2000) Embryonic stem cells differentiate into oligodendrocytes and myelinate in culture and after spinal cord transplantation. Proc Natl Acad Sci 97:6126–6131

Lucchinetti CF, Bruck W, Rodriguez M, Lassmann H (1996) Distinct patterns of multiple sclerosis pathology indicates heterogeneity on pathogenesis. Brain Pathol 6:259–274

Luo Y, Cai J, Liu Y, Xue H, Chrest FJ, Wersto RP, et al. (2002) Microarray analysis of selected genes in neural stem and progenitor cells. J Neurochem 83:1481–1497

Martino G, Furlan R, Comi G, Adorini L (2001) The ependymal route to the CNS: an emerging gene-therapy approach for MS. Trends Immunol 22:483--490

Martino G, Hartung HP (1999) Immunopathogenesis of multiple sclerosis: the role of T cells. Curr Opin Neurol 12:309–321

McDonald JW, Liu XZ, Qu Y, Liu S, Mickey SK, Turetsky D, et al. (1999) Transplanted embryonic stem cells survive, differentiate and promote recovery in injured rat spinal cord. Nat Med 5:1410–1412

Mezey E, Key S, Vogelsang G, Szalayova I, Lange GD, Crain B (2003) Transplanted bone marrow generates new neurons in human brains. Proc Natl Acad Sci 100:1364–1369

Papayannopoulou T (2003) Bone marrow homing: the players, the playfield, and their evolving roles. Curr Opin Hematol 10:214–219

Pluchino S, Furlan R, Martino G (2004) Cell-based remyelinating therapies in multiple sclerosis: evidence from experimental studies. Curr Opin Neurol 17:247–255

Pluchino S, Quattrini A, Brambilla E, Gritti A, Salani G, Dina G, et al. (2003) Injection of adult neurospheres induces recovery in a chronic model of multiple sclerosis. Nature 422:688–694

Poliani PL, Brok H, Furlan R, Ruffini F, Bergami A, Desina G, et al. (2001) Delivery to the central nervous system of a nonreplicative herpes simplex type 1 vector engineered with the interleukin 4 gene protects rhesus monkeys from hyperacute autoimmune encephalomyelitis. Hum Gene Ther 12:905–920

Priller J, Persons DA, Klett FF, Kempermann G, Kreutzberg GW, Dirnagl U (2001) Neogenesis of cerebellar Purkinje neurons from gene-marked bone marrow cells in vivo. J Cell Biol 155:733–738

Prineas JW, Barnard RO, Kwon EE, Sharer LR, Cho ES (1993) Multiple sclerosis: remyelination of nascent lesions. Ann Neurol 33:137–151

Reubinoff BE, Itsykson P, Turetsky T, Pera MF, Reinhartz E, Itzik A, et al. (2001) Neural progenitors from human embryonic stem cells. Nat Biotechnol 19:1134–1140

Ruffini F, Furlan R, Poliani PL, Brambilla E, Marconi PC, Bergami A, et al. (2001) Fibroblast growth factor-II gene therapy reverts the clinical course and the pathological signs of chronic experimental autoimmune encephalomyelitis in C57BL/6 mice. Gene Ther 8:1207–1213

Schmid RS, Anton ES (2003) Role of integrins in the development of the cerebral cortex. Cereb Cortex 13:219–224

Selmaj K, Walczak A, Mycko M, Berkowicz T, Kohno T, Raine CS (1998) Suppression of experimental autoimmune encephalomyelitis with a TNF binding protein TNFbp) correlates with down-regulation of VCAM-1/VLA-4. Eur J Immunol 28:2035–2044

Shimamura M, Sato N, Oshima K, Aoki M, Kurinami H, Waguri S, et al. (2004) Novel therapeutic strategy to treat brain ischemia: overexpression of hepatocyte growth factor gene reduced ischemic injury without cerebral edema in rat model. Circulation 109:424–431

Villoslada P, Hauser SL, Bartke I, Unger J, Heald N, Rosenberg D, et al. (2000) Human nerve growth factor protects common marmosets against autoimmune encephalomyelitis by switching the balance of T helper cell type 1 and 2 cytokines within the central nervous system. J Exp Med 191:1799–1806

Weimann JM, Charlton CA, Brazelton TR, Hackman RC, Blau HM (2003) Contribution of transplanted bone marrow cells to Purkinje neurons in human adult brains. Proc Natl Acad Sci 100:2088–2093

Yanai J, Doetchman T, Laufer N, Maslaton J, Mor-Yosef S, Safran A, et al. (1995) Embryonic cultures but not embryos transplanted to the mouse's brain grow rapidly without immunosuppression. Int J Neurosci 81:21–26

Zhang SC, Wernig M, Duncan ID, Brustle O, Thomson JA (2001) In vitro differentiation of transplantable neural precursors from human embryonic stem cells. Nat Biotechnol 19:1129–1133