Neural stem cells in inflammatory CNS diseases: mechanisms and therapy

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Received: February 18, 2005; Accepted: May 23, 2005

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Abstract

Autoimmune inflammatory diseases of the central nervous system (CNS) are highly complex in their interaction of different cell populations. The main therapy focus in the last years has been the inhibition of the immune system. Recent progress has shown that endogenous as well as transplanted neural stem cells might positively influence the outcome of such diseases. In this review, we discuss the current concept of the underlying pathogenesis with a specific focus on local CNS cells and potential treatment options.

Keywords: matrix metalloproteinases (MMPs) • angiogenesis • tumor angiogenesis • MMP inhibitors • extra-cellular matrix remodeling • tissue inhibitors of metalloproteinases (TIMPs) • thrombospondins

Introduction

For most of the twentieth century, it was thought that repair of central nervous system (CNS) lesions was impossible. At the beginning of the last century, Ramon y Cajal distinguished the mature CNS from the developing nervous system by the former's lack of growth and cellular regeneration; "Everything may die, nothing may regenerate" [1]. Although Cajal had observed that there might be a limited capacity for regeneration, it was not until the early 1960s that this could be substantiated by experimental evidence. By then, tagging tech-

niques such as thymidine-³H autoradiography had made it possible to trace newly formed cells. Altman and colleagues demonstrated limited post-natal neurogenesis in the adult rat brain [2]. In 1983 Goldman and Nottebohm were able to show that neurogenesis in the vocal control nucleus of the adult songbird was due to precursor cells migrating from the overlying ventricular zone [3]. Further evidence [4, 5], suggested that the subventricular zone (SVZ) was the likely source of adult neural stem cells. Today we know that a number of

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regions of the embryonic and adult mammalian CNS are sources of neural stem cells (NSC), such as the external germinal layer of the cerebellum, the subgranular zone of the dentate gyrus, and the ependymal layer of the spinal cord (reviewed in [6]). However, lineage restricted precursors (LRP) are also found as a source of new brain cells. These precursors can be found in greater numbers throughout the brain and are capable of differentiation and self-renewal as well (reviewed in [7]).

Knowledge gained from studying NSCs and LRPs in the adult and embryonic CNS has great implications with regard to all CNS diseases. Particularly for degenerative diseases like Alzheimer's disease, Parkinson's disease or amyotrophic lateral sclerosis, but also in autoimmune diseases such as multiple sclerosis, the potential activation of endogenous organ specific stem cells or transplantation of multipotent stem cells holds promise for future therapies. However, in almost every circumstance these cells will encounter parts of the immune system, which may either be potentially harmful or beneficial. In this review, we discuss the current understanding of therapy and potential treatment options using stem cells in chronic inflammatory CNS diseases.

Inflammatory diseases of the CNS

The most common autoimmune inflammatory, demyelinating CNS diseases are listed in Table 1. Among these, Multiple Sclerosis (MS) with an incidence of 100 in 100000 in the US and Europe, is by far the most frequent. Neurological deficits are progressively accumulated, so that 50% of untreated patients are wheelchair bound after 15 years [8].

The current pathophysiological concept of MS includes genetic and environmental factors as well as a dysfunction in immune regulation. In the initial phase, T cells (CD4 T helper cells and CD8 cytotoxic cells) play a key role in the directed immune attack against the CNS, causing inflammation, demyelination and axonal damage (Fig. 1). Over time this pattern is replaced by a chronic demyelination and axonal loss in which the immune mediated mechanism are of less importance [9–11]. It is believed that normally

controlled autoimmune T cells are activated by the peripheral immune system (*e.g.* in a lymph node). The exact reason for this activation is largely unknown. Two distinct causes have been suggested: 1) Molecular mimicry, which is defined by T cell cross recognition of microbial structures and potential auto-antigens [12, 13] and 2) the T cell activation through presentation of self-antigen. Once activated, T cells can cross the blood brain barrier (BBB) to reach the CNS. The mechanism of transendothelial migration is regulated by a complex interaction of chemokines and their receptors, adhesion molecules and matrix metalloproteinases. In the CNS, CD4 and probably also cytotoxic CD8 positive T cells are locally reactivated [14]. The release of pro-inflammatory Th1 cytokines results in the destruction of myelin sheaths by different types of inflammatory cells. Furthermore, CD4 positive T helper cells can activate antibody-producing B cells. The binding of antibodies to auto-antigens triggers activation of the complement cascade and increases the myelin damage [15]. In general, two different mechanisms can be distinguished [16]. One is a T cell and macrophage based myelin and neuron destruction, while antibodies and complement mediate destruction *via* a second complementary and possibly synergistic pathway.

In this auto-aggressive, inflammatory state early axonal damage and loss can be detected [17]. So far, it is not clear if the axonal damage is a result of direct destruction by cytotoxic T cells and/or macrophages or a secondary phenomenon of demyelination due to the lack of trophic support [18]. More recently, the importance of the local environment, especially the glial cells, has been realized. Key functions even in the initiation process of the disease have been attributed to microglial cells [19], but also astrocytes serve major functions regulating homeostasis and neurotrophic support [20]. Furthermore, changes in the local environment might support the development of chronic disease course, which is not only true for inflammatory CNS diseases.

Based on this scenario several interventions seem possible:

1. Targeting the immune response
2. Targeting the local environment especially reactive glia

Table 1 Inflammatory diseases of the CNS

Focal inflammatory demyelination:
Optic neuritis
Transverse myelitis
Devic's disease
Disseminated diseases:
Acute disseminated encephalomyelitis
Acute haemorrhagic encephalomyelitis
Multiple sclerosis
Relapsing-remitting and secondary progressive multiple sclerosis
Primary progressive multiple sclerosis
Marburg disease
Schilder's disease
Balò's disease

- Using cell therapy as a means of repair and replacement

Targeting the immune response

Changing the direct immune response has been the main strategy of multiple studies over the last decades. Several different target areas can be distinguished:

- Immune suppression, immune modulation or bone marrow transplantation
- Interference during antigen presentation and T cell activation
- Inhibition of chemotaxis, adherence or migration into the CNS
- Inhibition of B cell activation and antibody production

The current treatment of MS is based on immune modulators or immune suppressors (reviewed in [21]). The immune modulation especially by glatiramer acetate is believed to be a

switch from a dominantly Th1 response to an anti-inflammatory Th2 response [22].

The only immune suppressant currently approved for treatment in MS is mitoxantrone [23]. However, immune suppressive treatment is accompanied by typical side effects such as bone marrow suppression, a higher risk of infection, a greater risk of secondary risk of leukemia and cardiac toxicity, which is specific for the case of mitoxantrone.

An even more radical approach to immune therapy is the eradication of the bone marrow followed by transplantation of autologous hematopoietic stem cells, with the hope of eliminating all auto-aggressive cells and thus potentially cure patients. There has been good evidence of potential benefits in the animal model and from a small retrospective study on MS patients [24] but there is a high trade off with a mortality rate of 5–8% in some patient groups.

Therefore, it seems likely that more focused approach would have less side effects. For example, one could interfere in the process of antigen presentation. Based on the two-signal model of T cell

Fig. 1 Schematic figure of the immune pathogenesis of MS. In the systemic immune compartment auto-reactive T cells are activated through the recognition of their specific antigen in the context of the appropriate MHC molecule on the antigen presenting cell (APC) and the additional presentation of co-stimulatory molecules (CD28-B7, CD40-CD40L). Once activated, the T cells cross the blood brain barrier (BBB) and re-encounter their antigen. This encounter leads to a reactivation and expansion of the auto-reactive T cells which in turn stimulates macrophages/microglia to increase their phagocytic activity, to release more pro-inflammatory cytokines such as TNF- α and lymphotoxin (LT) or matrix metalloproteinases (MMP) and toxic mediators like nitric oxide (NO). The release of these mediators induces demyelination and axonal degeneration that is further enhanced by the secretion of auto-antigen binding antibodies and activation of the complement cascade. Also, cytotoxic CD8 positive cells directly destroy oligodendrocytes and neurons. Anti-inflammatory cytokines such as interleukin (IL)-10 and transforming growth factor (TGF)- β or prostaglandin E (PGE) as well as the mechanism of apoptosis help balance the inflammatory response. Differential effects are seen in the modulation of the immune response by the major classes present in the CNS such as microglial cells and astrocytes.

activation, a necessary prerequisite for T cell activation is T cell receptor engagement by MHC peptides (signal 1) together with a second antigen-independent signal mediated by co-stimulatory receptors (signal 2) [25]. In theory, the co-stimulatory molecule CD28, which interacts with CD80 and CD86 on the presenting cell, provides a good target. Blockade of this interaction with a fusion protein-cytotoxic T-lymphocyte-associated antigen

4-IgG1 (CTLA4Ig), which also binds CD80 and CD86 with a high affinity, can prevent T cell activation and has been shown to improve the symptoms of rheumatoid arthritis in early clinical trials [26]. However, treatment of MS patients during a double blind placebo controlled trial (Bristol Myers Squibb, BMS 188667) had to be stopped due to increased disease activity. In a preliminary analysis the increased disease activity in the treatment group

was present prior to study enrollment, therefore it may not have been treatment induced.

Another co-stimulatory pair is the CD40/CD40L, which is similarly important in the initiation process of MS [27]. A study in humans with an anti-CD40L antibody (Anti-CD154, IDEC131, IDEC Pharmaceuticals) had to be stopped due to an increased risk of thrombotic emboli.

In analogue to immune suppression, T cell can be targeted by monoclonal antibodies against T cell differentiation markers. Campath-1 (Alemtuzumab) is an example of a monoclonal antibody that recognizes CD52 (a leukocyte differentiation marker) on T cells. Its use in patients with secondary progressive multiple sclerosis led to reduction of inflammatory lesions without influencing the progression of disability and brain atrophy [28, 29]. This supports the theory that inflammation in the later stages of MS does not play such an important role. Additionally, one third of the patients developed antibodies against the thyrotropin receptor and an autoimmune thyroid disease.

Another way to block T cell activation is to inhibit the function of IL-2, a cytokine essential for T cell proliferation. Current investigations of a monoclonal anti-IL-2 receptor antibody (Daclizumab) are ongoing.

A different approach has been to interfere with the migration of T cells into the CNS. Three basic concepts are feasible: interfering with the chemo attractant signal, blocking the adherence, and inhibiting the matrix metalloproteinases (MMPs).

It is widely accepted that the cells in an inflammatory lesions are recruited by chemotatic cytokines the so-called chemokines [30]. Blocking specific chemokine receptors in an inflammatory process in the CNS is likely to result in a favorable outcome [31–33]. One pilot study is testing the effects of a CCR-1 receptor antagonist [34].

Adhesion molecules and their ligands mediate the entrance of activated lymphocytes into the brain. The most promising trial has been with a monoclonal antibody (Natalizumab, Antgren®) against the leukocyte integrin α 4-integrin, one of main ligands that enable leukocytes to adhere to adhesion molecules expressed on the surface of vascular endothelia cells. In this study, 213

patients with relapsing remitting or secondary progressive MS were enrolled and treated with 3 mg/kg, 6 mg/kg Natalizumab or placebo. MRI scans showed a significant reduction in new lesions over 6 months. Two big multi center trials are currently ongoing (AFFIRM, SENTINEL), investigating in part the additional synergistic effect of Natalizumab and interferon (IFN)- β 1a (SENTINEL). However, the use of monoclonal antibodies always carries the risk of developing neutralizing or anti-idiotypic antibodies.

To migrate through the extra-cellular matrix, lymphocytes use MMPs. MMPs have been shown to be important in cell migration, destruction of the BBB, demyelination, and cytokine activation [35]. Through blocking of MMPs in the experimental autoimmune encephalomyelitis (EAE) model, demyelination and inflammation could be decreased and, therefore, could be potential treatment options [36]. No clinical trials have been conducted yet.

The importance of B cells and myelin specific antibodies in the pathogenesis has been increasingly recognized [37, 38]. New enthusiasm is being generated for depleting B-lymphocytes with antibodies to CD20, an approach that has been successful for treating lymphoma. Initial trials in rheumatoid arthritis (RA) are promising, and trials are being initiated in MS and systemic lupus erythematosus (SLE) [39].

But not in all instances inflammation is viewed as detrimental. For example, in injury models it has been suggested that the lack of communication with the immune system results in poor recovery. Improved outcomes in the spinal injury model were observed with transplanted macrophages that had been exposed to peripheral myelin or an activation of an autoimmune T cell response [40, 41]. This is in part contributed to secretion of neurotrophic factors secreted by inflammatory cells [42].

While still speculative, some investigators have suggested bone marrow stem cells (BMSCs) may modulate or suppress the immune response. In culture when mixed leukocyte reactions (MLR) are examined in the presence or absence of BMSCs a significant difference is observed and additionally a BMSC triggered abrogation of EAE has been reported [43]. This function of BMSCs is distinct from its potential to integrate into the brain or transdifferentiate that is discussed elsewhere [44].

Table 2 Dichotomy of microglial immune responses

	Pro-inflammatory	Anti-inflammatory
Cytokines	IL-1, TNF- α , IL-12, IL-23	TGF- β , IL-10, IL-1 receptor antagonist
Chemokines	MCP-1, MIP-1 α , MIP-1 β , RANTES, IL-8, MIG, IP-10, SDF-1, fractaline	
Matrix metalloproteinases (MMPs)	MMP-2, MMP-3, MMP-9	TIMP-1
Neurotrophic factors		BDNF, NGF, FGF
Free radicals	Superoxide, nitric oxide	
Eicosanoids	Leukotriene C ₄	PGD ₂
Proteases	Elastase, plasminogen	
Complement factors	C1, C3, C4	
T cell activation	T _H 1	T _H 2
Co-stimulatory molecules	B7-1, B7-2, CD40	B7-H1, PD-L2

Reactive glial cells

In recent years, the disease modifying capacities of the CNS have been increasingly in the scope of investigations. Antigen presentation in the CNS is thought to play a critical role in the initiation and perpetuation of neuroinflammation. It is becoming apparent that microglial cells, the resident immune cells of the brain, differ considerably from peripheral APCs in terms of their responses to inflammatory stimuli and their abilities to modulate immune responses. Microglial cells can express MHC class II molecules and have the potential to act as effective APC to T-cells [45]. Under virtually all inflammatory conditions microglia markedly upregulate MHC class II expression. Even under physiological conditions or on resting human microglial cells, MHC class II expression can be detected [46]. In MS a close interactions of MHC class II-positive microglial cells with T cells are visible within the inflammatory lesions [47].

Evidence suggests that during CNS inflammation activated microglia express co-stimulatory molecules such as CD80 (B7.1), CD86 (B7.2) and CD40 in addition to certain adhesion molecules (reviewed in [48]). Their regulated expression of

costimulatory molecules, combined with their capacity for efficient antigen processing, enables microglia to act as professional antigen-presenting cells to T-cells. Existing studies have elucidated the relevance of stimulatory second signals on microglial cells (such as CD80, CD86, CD40) including their contribution to modulate or amplify acute or chronic neuroinflammation [46, 49–51]. Recently in a very elegant study using bone marrow chimerics in transgenic mice with the thymidine kinase of herpes simplex driven by the CD11b promoter (expressed by cells of the myeloid origin including macrophages and microglia), the fundamental role of microglial cells in the initiation process of CNS immune reactions was described [19].

The modulation of inflammation by microglial cells entails an important dichotomy (Table 2): on the one hand microglial cells are capable of secreting high amounts of pro-inflammatory cytokines (e.g. IL-1, TNF- α , IL-12), chemokines, MMPs, and toxic mediators (e.g. NO), factors critically involved in chronic inflammatory demyelination and axonal injury in multiple sclerosis. However, they can also participate in the down modulation of inflammation by producing anti-inflammatory cytokines such as TGF- β , IL-10 or IL-1 receptor antagonist [52] or

co-inhibitory molecules such as B7-H1 [53]. Microglia secrete potent growth and differentiation factors, such as brain derived neurotrophic factor (BDNF) and IL-6, with effects that reach far beyond the immune system and may be central players in repairing brain tissue and maintaining its integrity [54]. Moreover, human microglia are now recognized to have the capacity to drive both Th1 and Th2 response [55].

But microglial cells may also be substantially involved in the development of a chronic disease course. For example, there seems to be an inverse relationship between microglial activation and neurogenesis [56–58]. Microglial cells express receptors for stem cell factors and can be skewed toward a more dendritic cell-like profile. CD11c-positive brain DCs can be isolated from an experimental model of nervous system inflammation [59]. Furthermore, adult mouse microglia exhibited a functional maturation comparable to that of DCs after stimulation with granulocyte macrophage-colony stimulating factor (GM-CSF) and CD40 ligation. GM-CSF stimulation alone resulted in an immature phenotype, while the addition of CD40 ligand led to cell maturation [59] and antigen presenting capacity to stimulate antigen specific T cells [50]. These cells behave differently when compared to unstimulated microglial cells [60], so it might be that these matured cells can drive an inflammation independently of other influences.

The question of how to influence microglia cells is a very difficult one. Monje and colleagues showed that blocking inflammation elicited by either irradiation or injection of bacterial lipopolysaccharide (LPS) with indomethacin restores hippocampal neurogenesis [58], which might be one explanation why clinical treatment with indomethacin and other NSAIDs ameliorates the risk and/or progression of memory loss [61]. Also minocycline, which inhibits microglia activation, increases hippocampal neurogenesis that was impaired by inflammation [56]. In addition, the inflammatory mediators released by microglia during an innate immune response strongly influence neurons and their ability to process information, which can be influenced through interference with a CXCR4-dependent astrocyte microglia signaling [62].

The other major cell type involved in every CNS inflammation is the astrocyte. The main function of astrocytes in the undamaged CNS is thought to be

the regulation of homeostasis. Astrocytes can respond to and regulate virtually all known neuroactive compounds, including neurotransmitters, neuropeptides, growth factors, cytokines, small molecules and toxins. They serve key functions in establishing and maintaining CNS boundaries, including the BBB and the glial limitans, through interactions with endothelial and leptomeningeal cells. More than any other cell in the CNS, astrocytes sense an inflammatory disruption of homeostasis. They modulate their metabolic activity, as well as their production of growth and trophic factors in trying to protect other brain cells from energy depletion [20], toxic-free radicals, ammonia, metals and calcium overload. Additionally, after injury, cytokine-activated astrocytes promote the reformation of essential barriers and reestablishment of CNS ionic homeostasis, which in turn can also lead to astrocytic scarring inhibiting neurite outgrowth [63–65]. Reactive astrocytes may also exacerbate tissue damage as they can release pro-inflammatory cytokines such as TNF- α which can inhibit neurite outgrowth and kill oligodendrocytes [66, 67], and they can produce and release arachidonic acid metabolites, nitric oxide (NO) and reactive oxygen species that can adversely affect cell survival after injury (Table 3) [68].

But on the other hand, astrocytes in response to injury, disease, and inflammatory activation of cytokine receptors produce an enormous array of neurotrophic factors, including nerve growth factor (NGF), BDNF, ciliary neurotrophic factor (CNTF), glial derived neurotrophic factor (GDNF) and fibroblast growth factor (FGF)-2 [69–75], which promote neuronal and oligodendrocyte survival and differentiation. Furthermore, T cell-driven autoimmune inflammatory assaults are suppressed [76] or modulated by astrocytes into Th2 type of reaction [77]. When transplanted hippocampal astrocytes even induce neurogenesis [78]. Evidence has suggested that fetal astrocytes behave differently than reactive astrocytes and may ameliorate scar formation and reduce inflammatory activity thus preserving some of the positive aspects while reducing the potential side effects. More importantly recent studies have suggested that repair of myelin may require the activity of both oligodendrocyte precursors as well as astrocytes [79] highlighting the importance of considering this cell type in modulating inflammatory diseases of the CNS.

Table 3 The role of astrocyte in modulating repair in the CNS

	Improving	Inhibiting
Cytokines	TNF- α , TGF- β , IL-10	TNF- α , TGF- β
Repair	Induce neurogenesis, support oligodendrocyte precursors in myelination	Form scar tissue
Neurotrophic factors	BDNF, NGF, GDNF, CNTF, FGF-2	
Clearance of neurotoxic transmitters	Glutamate, superoxide, nitric oxide	Fail to clear glutamate, superoxide, nitric oxide
Substrate support	Lactate, glucose	
T cell activation	Suppress activation, bias T _H 2 response	

Cell repair and replacement therapy

Stem cells are defined through their capability to divide, self-renew, and differentiate into cells characteristic of a particular tissue. Stem cells come in different varieties, depending on when and where they occur during development [80–82]. One major question is if one should try to activate the endogenous capacities or transplant new stem cell into the brain?

Endogenous repair

The endogenous stem cells of the CNS are the neural stem cells (NSCs). NSCs arise from specific germinative zones in the embryonic and adult mammalian CNS [83]. During the early stages of the mammalian CNS development, neurons and glia are generated from these cells in the ventricular zone and migrate out using radial glia as support to reach to the cortex. NSCs seemed to have a proliferative response mainly to epidermal growth factor (EGF) and basic fibroblast growth factor (bFGF), although other factors might have an additional influence [84]. These multipotent cells give rise to more restricted precursor cells, which in turn generate the main cell classes in the brain (Fig. 2, [85]). When considering neural stem cells as a source of endogenous repair in a multi localized inflammatory brain, several questions arise. Keeping in mind that this is a small cell

population, which can only be found in certain areas, the question of migration and generation of sufficient numbers of differentiated cells becomes a key issue. Furthermore, how do these cells behave in an inflammatory environment?

In the EAE model, NSCs proliferate in the SVZ and in some instances migrate into adjacent lesions, where they differentiate into all three cell types: neurons, oligodendrocytes, and astrocytes [86]. The observed proliferation in the SVZ might be in part triggered by tumor necrosis factor- α (TNF- α [87], a mechanism probably mediated by FGF and EGF. A migratory response in neurospheres can be achieved through addition of microglia-conditioned medium [88]. However, the actual signal that guides the migration of precursor cells is largely unknown. In the rostral migratory stream, the secreted Slit protein has been suggested as a chemorepellant signal for the outgrowth of immature neurons [89, 90]. Other molecules shown to influence migration of neuronal precursors include meningeally secreted SDF-1 [91, 92] or BDNF [93]. In a stroke model, it was also SDF-1 expressed by astrocytes that attracted NSCs *via* CXC chemokine receptor 4 to the site of the lesion [94]. In contrast to astrocytes, injured neurons seemed to rely on stem cell factor to direct NSCs to the lesion site [95].

Once the NSCs reach the lesion site, they come into direct contact with inflammatory cells. The communication with the immune system will be twofold: cellular and humoral. The cell contact is dependent on the expression of MHC com-

Fig. 2 Depicts the progressive restriction during the development of a stem cell. Markers for different stages of neural stem cells are given.

plexes. Some evidence suggests that NSCs especially after stimulation with pro-inflammatory cytokines or after exposure to a foreign environment express notable levels of MHC I and MHC II [96–100]. So far, only one study has looked for expression of co-stimulatory molecules on NSC [101]. In this study, expression of B7-1 on nestin positive cells in the SVZ could be detected that increased during EAE. After IFN- γ stimulation, B7-1 as well as B7-2 expression increased *in vitro*. In contrast to the other groups, the analyzed nestin positive cells even after exposure to IFN- γ were only capable of expressing MHC I and therefore only able to interact with CD8 positive T cells, a hint that these cells are particular susceptible to cytotoxic T cells. Surprisingly, the cross-linking of B7-1 resulted in apoptosis of the NSCs, a so far not described mechanism for the induction of apoptosis.

Secondly, humoral factors have shown to influence NSCs in an inflammatory environment. Two of the main cytokines secreted during inflammations are the Th1 cytokines IFN- γ and TNF- α . Some groups have investigated the effects of these cytokines on neurospheres derived from postnatal brains. The *in vitro* effect seems to be mainly pro-apoptotic and anti-mitotic [102–104], in contrast to the proliferation inducing effect of TNF- α in the SVZ [87]. Under some circumstances IFN- γ induces neuronal differentiation and neurite outgrowth [104], in agreement with previous observations that IFN- γ differentiates embryonic cortical and hippocampal neurons [105]. However, one has to keep in mind that neurospheres are not a homogenous cell population and therefore it is likely these effects are mediated through other cells (e.g. astrocytes and LRP) and factors rather than a direct effect.

Even though promising, the overall number of newly generated cells by NSCs is low and new cells are usually found in close proximity to the SVZ. These limitations are not found in another CNS precursor cell population, the glial restricted precursor cells (GRP).

GRPs are defined by their ability to generate astrocytes and oligodendrocytes. This ability is not limited to the *in vitro* situation but can also be found after transplantation [106, 107]. These cells most likely resemble a continuum with even more committed cells such as oligodendrocyte and astrocyte precursor cells (reviewed in [108]). To date, the exact timing when a GRP becomes an oligodendrocyte precursor cell or astrocyte precursor cell is not known. It is likely that once an oligodendrocyte precursor cell acquires the GalC antigen (Fig. 2) (reviewed in [109]) that it will generate an oligodendrocyte. It seems possible that adult brain cells expressing the chondroitin sulfate NG2 [110] can generate more than just oligodendrocytes or type 2 astrocytes in culture [111, 112].

NG2 expressing cells constitute a major distinct glial population that depending on the CNS region comprises 3–10% of total cells [113]. In case of injury, it is this cell population that together with microglial cells responds immediately. They react to all types of injuries such as stab injuries [114], kainite-induced lesions [115], stroke [116, 117], pseudorabies [118] and especially demyelination [119–121]. In the initial phase, NG2 positive cells exhibit reactive changes but an increase in numbers is mainly observed with either demyelination or substantial tissue loss [122, 123]. NG2 positive cells can be found in MS lesions [124, 125, 119] and recent work suggests that there is no lack of these cells even in chronic lesions [126]. The reasons why they fail to sufficiently myelinate are not completely understood. Different mechanisms have been suggested. For example, PSA-NCAM on axons that is re-expressed in MS negatively regulates myelination [127, 128]. Also in a demyelinated lesion, the absence of Jagged or Delta ligand signaling from oligodendrocytes through the Notch receptor on oligodendrocyte precursor cells [129] could lead to further activation of the NG2 positive cell population. A mechanism that might be in part reversed in multiple sclerosis through expression of Jagged1 on reactive astrocytes possibly mediated by TGF- β [130, 131]. Furthermore, other factors

such as growth factors or thyroid hormone regulate remyelination and are in part regulated during inflammation [132–145].

However, the exact role of inflammation remains unclear. New data indicates that an acute inflammation is needed to trigger remyelination in areas of chronic demyelination [146]. One way by which inflammation drives this remyelination might be TNF- α a cytokine closely tied to oligodendrocyte apoptosis, that *in vivo* promotes remyelination [147], in contrast to observations *in vitro*, where TNF- α usually induces apoptosis in oligodendrocyte precursors [148–150]. These differential effects can to some extent be explained by involvement of different TNF- α receptors of which p75 (TNFR2) would support remyelination rather than destruction [147].

There are many factors influencing remyelination, but they act at different stages and through different cell types. It will become increasingly important to study the combination of these factors to understand the failure of remyelination in chronic human diseases like multiple sclerosis.

Stem cell transplantation

The potential of several promising cell types to serve as an effective transplant has been evaluated for their ability to survive, differentiate, and migrate in an inflammatory milieu of the adult CNS. Many different cell types, including neural stem cells and precursors, have been suggested as candidate cells for therapy [151]. It has been demonstrated that transplantation of myelin forming cells can result in extensive myelin sheath formation when undertaken during development or in association with acute demyelination (reviewed in [152]). Transplanted GRP cells into a lateral funiculus injury are able to migrate extensively and to differentiate into oligodendrocytes and astrocytes while transplanted neuronal restricted precursors differentiate into neurons [153, 154].

Many inflammatory diseases are widespread and diffuse and as such injection into the parenchyma is an unlikely strategy, intraventricular injection in the adult do not result in significant engraftment but paradoxically the inflammatory response may enhance the process of cell invasion [155]. More success has been reported *via* intravenous or

Fig. 3 During the course of an autoimmune inflammatory CNS disease several interventions are conceivable. Some of those are shown in this picture

intraarterial injection. Intravenous injections have the advantage of diffuse delivery with some possibility of targeting. However, the problem for distribution to other tissues or organs must be evaluated. Intraarterial delivery has been attempted as it has in other systems but the potential of localized emboli and infarcts have raised caution. It is also important to note that while it is possible that this strategy may work for cells that can traverse or do traverse the BBB it is unclear if neural stem or progenitor cells will behave the same way. Recently, some of these issues have been addressed in a chronic model of EAE in the mouse where intravenously transplanted neurospheres induced recovery [156]. This model exhibits pathologically with significant immune mediated demyelination and axonal loss that lead to chronic neurologic disability in the affected mice. In the chronic EAE, neurosphere transplantation was associated with remyelination,

both by transplanted as well as by endogenous cells. Also new neurons and astrocytes could be detected within lesions improving the overall disease scores of the animals. The acute EAE model is a transient inflammatory disease without significant demyelination, and the observed positive clinical effect of the NPC spheres seems to be mainly mediated via anti-inflammatory mechanisms [157].

It is important to consider the immunological aspects of transplantations. Stems cells are in general considered low immunogenic, however NSCs for example, express increased levels of MHC I and MHC II after stimulation with pro-inflammatory cytokines or after exposure to a foreign environment [96–100]. Intracerebral neural xenografts have shown to trigger response involving host cytotoxic T-lymphocytes, microglia/macrophages, immunoglobulin M and complement [158] and therefore it is not surprising that rejections of neu-

ral allografts and xenografts were observed in several studies [159]. Furthermore, it is likely that studies of transplanted inbred animals do not provide us with a correct picture of the expected immune reaction in humans, where rejections are much more likely to occur. There will be the need for strict immune suppressive treatment even if MHC compatibility between donor and recipient has been optimized. Apart from the problems faced in neurological disease of appropriate stem cell niches, appropriate regional specific differentiation, functional integration and establishment of connectivity with distant targets, appropriate and organized remyelination the inflammatory environment offers several additional challenges. Cells themselves may either provoke or inhibit the ongoing immune process; cells may respond inappropriately to the toxic milieu, the normal pattern of differentiation may be altered. Especially in autoimmune diseases such as MS, the underlying immunological process may attack the transplanted cells and vice versa, the transplanted cells could reactivate the auto-aggressive inflammatory process thus worsening the disease.

Conclusions

How does all this information affect our outlook on the future? It has become clear in recent years that inflammatory responses include both aspects: one destructive and one reparative. All in all, it will be hard to manipulate one without affecting the other, but it seems that a more focussed approach might be more able to dissect the harmful from beneficial aspects and to target those solely. Even though several strategies, global and focal exist (Fig. 3), it will take more studies to understand these interactions in chronic autoimmune diseases such as MS. Cell therapy is one component in the therapeutic armamentarium. Cell therapies of different kinds may be useful either singly or as a complement to existing therapies. The cell type we choose, the time we deliver will depend on which aspect of the disease process we would target. For example, it may be better to transplant lineage restricted cells, since their fate commitment make them less dependent on environmental differentiating signals. Clearly many approaches are experimental and in their

infancy and it will be important to follow their evolution. As technology evolves, other possibilities for cell therapy such as the local delivery of beneficial molecules might be further options.

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