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REVIEW

The more you have, the less you get: the functional role of inflammation on neuronal differentiation of endogenous and transplanted neural stem cells in the adult brain

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Abstract

The differentiation of neural stem cells toward a neuronal phenotype is determined by the extracellular and intracellular factors that form the neurogenic niche. In this review, we discuss the available data on the functional role of inflammation and in particular, pro- and anti-inflammatory cytokines, on neuronal differentiation from endogenous and transplanted neural stem/progenitor cells. In addition, we discuss the role of microglial cell activation on these processes and the fact that microglial cell activation is not univocally associated with a pro-inflammatory milieu. We conclude that brain cytokines could be regarded as part of the endogenous neurogenic niche. In addition, we propose that accumulating evidence suggests that pro-inflammatory cytokines have a negative effect on neuronal differentiation, while anti-inflammatory cytokines exert an opposite effect. The clarification of the functional role of cytokines on neuronal differentiation will be relevant not only to better understand adult neurogenesis, but also to envisage complementary treatments to modulate cytokine action that could increase the therapeutic benefit of future progenitor/stem cell-based therapies.

Keywords: cytokines, inflammation, neural stem cell, neuronal differentiation, Parkinson's disease.

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Adult neurogenesis

Adult neurogenesis is the process of generating new neurons from neural stem/progenitor cells (NSCs) in the adult. It encompasses different processes such as adult NSCs proliferation and differentiation, migration of neuronal precursors, and survival of newborn neurons. Shortly after the development of the [H^3]-thymidine incorporation technique to detect cell division in the 1960s, Altman showed that new cells with neuronal morphology could be found in the dentate gyrus (DG) of the hippocampus (Altman and Das 1965), neocortex (Altman and Das 1966), and olfactory bulb (Altman 1969). However, these results were not taken into consideration until the late 1970s, when Kaplan and Hinds (1977) showed that these new neurons could survive for long periods of time.

Adult neurogenesis is regulated by physiological and pathological conditions at different levels, including the activation and proliferation of adult NSCs, the migration,

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Abbreviations used: 6-OHDA, 6-hydroxydopamine; AdIL-1, IL-1 expressing recombinant adenoviral vector; Adβgal, β-galactosidase expressing recombinant adenoviral vector; BDNF, brain-derived neurotrophic factor; BrdU, 5-bromo-2′-deoxyuridine; DG, dentate gyrus; ED1, ectodermal dysplasia 1; FGF, fibroblast growth factor; GFAP, glial fibrillary acidic protein; IGF-1, insulin-like growth factor 1; IL, interleukin; IL-1R, IL-1 receptor; LPS, lipopolysaccharide; MCP-1, monocyte chemotactic protein-1; MHC, major histocompatibility complex; NeuN, neuronal neuron; NSCs, neural stem/progenitor cells; PD, Parkinson's disease; PD, Parkinson's disease; SCF, stem cell factor; SDF, stromal cell-derived factor; SGZ, subgranular zone of the dentate gyrus of the hippocampus; SN, substantia nigra pars compacta; SVZ, subventricular zone of the lateral ventricle; TGF-β1, transforming growth factor-β1; TH, tyrosine hydroxylase; TNF-R, TNF-α receptor; TNF-α, tumor necrosis factor-α; Tuj, type III β-tubulin.

differentiation and survival of the newly born neurons and, finally, the integration of these neurons to the existing neuronal circuits (Fuchs and Gould 2000; Duman *et al.* 2001; Kempermann 2002; Ming and Song 2005). In the adult mammalian CNS, adult NSCs reside in two specific regions, the subventricular zone of the lateral ventricle (SVZ) and the subgranular zone of the DG of the hippocampus (SGZ; Gage 2002). In the first case, neurogenesis occurs in the olfactory bulb after migration of the NSCs through the rostral migratory stream. In the second, newborn cells are produced in the DG (Gage 2002). In the normal brain, neurogenesis outside these regions appears to be extremely limited, or nonexistent. However, in pathological conditions affecting the brain, neurogenesis can be enhanced or suppressed. It is worth noticing that in the cases where neurogenesis is enhanced, formation of new neurons is also seen in non-neurogenic areas. In addition, NSCs could be cultivated from different brain areas where neurogenesis is not physiologically observed (Palmer *et al.* 1999). Finally, in general, transplanted NSCs fate will depend on whether the site of transplantation promotes neuronal differentiation or not (Lie *et al.* 2002). These observations suggest that NSCs proliferation and differentiation toward a specific phenotype are regulated by the characteristics of the microenvironment or niche in which they reside (Gage *et al.* 1995; Herrera *et al.* 1999; Gage 2002; Dziewczapolski *et al.* 2003; Alvarez-Buylla and Lim 2004; Chen *et al.* 2007).

Although there are structural differences in the architecture of each of the neurogenic niches, the main cellular and molecular components seem to be conserved. At a cellular level, compelling evidence suggests that astroglia plays a key role not only as a structural part of the neurogenic niche (Doetsch *et al.* 1997), but also in regulating almost every stage of the process of neurogenesis, including self-renewal, fate specification of adult NSCs, migration, differentiation, and integration of newly born neurons (Song *et al.* 2002; Barkho *et al.* 2006; Jiao and Chen 2008). Ependymal cells seem to regulate the quiescence and proliferation of adult NSCs (Ramirez-Castillejo *et al.* 2006). Also mature neurons near the SGZ are thought to function as niche cells, providing spatiotemporal regulation of adult neurogenesis in response to neuronal activity (Kempermann *et al.* 2000; Ma *et al.* 2009). Recent evidence supports a role for vascular/endothelial cells in regulating the proliferation of adult NSCs as they can enhance neurogenesis *in vitro* (Palmer 2002; Shen *et al.* 2004).

Molecular signals regulating adult neurogenic niche have also been identified. Physiological factors, including Wnt (Lie *et al.* 2005), Sonic Hedgehog (Lai *et al.* 2003; Ahn and Joyner 2005), bone morphogenetic proteins (Lim *et al.* 2000; Bonaguidi *et al.* 2008), membrane-associated Notch signaling (Nyfeler *et al.* 2005; Androutsellis-Theotokis *et al.* 2006), leukemia inhibitory factor (Bauer and Patterson 2006), transforming growth factor-α (TGF-α; Tropepe *et al.* 1997), and vascular endothelial growth factor (Jin *et al.* 2002; Schanzer *et al.* 2004) have been shown to promote NSCs proliferation and maintenance. Also, growth factors, including fibroblast growth factors (FGFs) and neurotrophins such as brain-derived neurotrophic factor (BDNF), also significantly contribute to proliferation, survival, and dendritic development of newborn neurons in the adult brain (Yoshimura *et al.* 2001; Schmidt and Duman 2007).

Recently, components of the immune response have been proposed as part of the neurogenic niche, affecting NSCs proliferation, differentiation and/or survival. Specially, the innate immune response and, in particular, inflammation have attracted attention as key players in modulating NSCs biology. In addition, transplantation of NSCs or stem-derived cells into the brain will elicit, at least, an inflammatory reaction. Therefore, the information on how signals coming from this response alters the endogenous niche and, in turn, affect the transplanted cells is crucial to enhance the potential benefits of transplanted cell-based therapies. Thus, this review will focus on the effects of brain inflammation on the biology of endogenous and transplanted NSCs. In particular, we will focus on the main source of cytokines in the brain, the activated microglia. Activated microglia can promote a pro- or anti-inflammatory milieu and therefore, would not always be equivalent to brain inflammation. This concept is important to prevent the idea that phenomena related with microglial cell activation are necessarily associated with production of pro-inflammatory cytokines. In addition, inflammatory reactivity varies among different brain regions according to their cellular composition. Thus, we will examine the features of these regions and suggest possible explanations to the differences observed in the modulation of NSCs biology by the local milieu.

Microglial cells

Microglia and astrocytes are the main mediators of inflammation in the brain. In addition, astrocytes are also considered part of the neurogenic niche (Song *et al.* 2002). This review will concentrate on describing the role of microglial cells which, from our perspective, should also be considered as one of the principal cellular components of the neurogenic niche. For a comprehensive review on the role of astrocytes on neurogenesis, see (Wang and Bordey 2008).

Microglia is typically described as the resident macrophages in the brain parenchyma (Gehrmann *et al.* 1995). They have no neural origin, but derive from invading monocytes during development (Perry and Gordon 1988). Microglial cells comprise up to 15% of total cells in different regions of the brain (Perry *et al.* 1993a). Under basal conditions, microglia exhibit a quiescent phenotype as indicated by a ramified morphology, down-regulation of activation antigens such as major histocompatibility complex

(MHC) II, and constitutive expression of macrophage antigens such as complement receptor 3 and ionized calcium binding adaptor protein-1 (Ladeby *et al.* 2005). Under CNS pathological conditions and systemic infectious processes, microglial cells undergo a phenotypic transformation characterized by up-regulation of antigen presentation molecules (MHC II, CD80, CD86, and CD40) and morphological changes. Activation proceeds through an activated ramified microglia, with long-shaped cell body and long but thick processes. Further activation leads to ameboid microglia, characterized by round-shaped cell body and few, short processes. In the final stage of activation, microglial cells are rounded cells with vacuolated cytoplasm and no visible processes which stain for ectodermal dysplasia 1 (ED1), a marker for phagocytic activity (see Fig. 1). At least four stages of activation characterized by morphology have been described (Kreutzberg 1996). These stages are dynamic and reversible (Perry *et al.* 2007).

Typically, microglial cell activation has been associated with production of pro-inflammatory cytokines, generation of reactive oxygen and nitrogen species, and the display of phagocytic activity (Streit *et al.* 1999). However, activated microglia may display a subset of these phenotypes or even anti-inflammatory effects depending on the type of activation (for a comprehensive review, see Perry *et al.* 2007). Microglia can enter an activation state, wherein they retain a ramified morphology, up-regulate activation antigens such as MHC II, and yet not secrete pro-inflammatory molecules

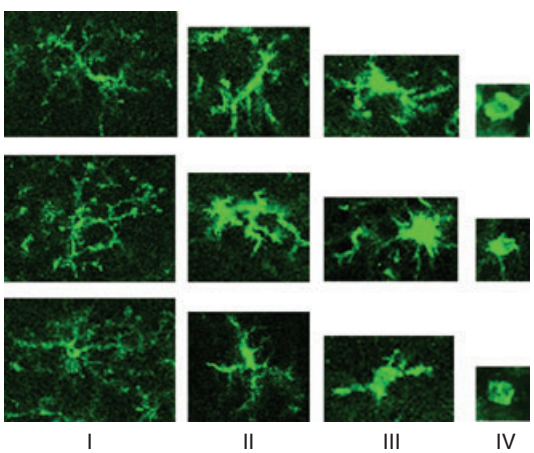

Fig. 1 Characteristic morphology of resting and activated microglia as seen by immunostaining with *Griffonia simplicifolia* isolectin-B4-positive cells (GSAI-B4) in adult rat brain tissue. The different morphology of the four defined activation stages of microglia is shown. Stage I: cells have rod-shaped cell bodies with fine, ramified processes and are defined as resting microglial cells. Stage II: activated microglia cells have elongated cell bodies with long thick processes. Stage III: activated microglia cells have small, thick processes, and a rounded morphology Stage IV: activated microglia cells have a rounded shape with barely any process, vacuolated cytoplasm.

(Betmouni *et al.* 1996; Walsh *et al.* 2001). For example, when microglia are activated by lipopolysaccharide (LPS; a well-known cytokine inducer), phagocytosis takes place with the release of pro-inflammatory factors (Hausler *et al.* 2002); in contrast when apoptosis is the trigger for microglial cell activation, anti-inflammatory cytokines are released (Magnus *et al.* 2001). Furthermore, LPS signaling can also promote different microglial cell activation phenotypes depending on whether it is an acute or a chronic stimulus (Cacci *et al.* 2008). In particular, in animal models of neurodegeneration, microglial cell activation has been defined as atypical (Depino *et al.* 2003; Perry *et al.* 2003; Godoy *et al.* 2008). These atypically activated microglia do not promote inflammation during neurodegeneration but are primed for a burst of secretion of pro-inflammatory molecules (Perry *et al.* 2003). Perry *et al.* (2003) developed a conceptual framework of microglia priming, in which a primary insult or disease process such as neurodegeneration induces this primed microglia phenotype. Upon subsequent induction of a peripheral or central pro-inflammatory process (secondary insult), previously primed microglia exhibit an exacerbated pro-inflammatory phenotype. This second, pro-inflammatory insult on primed microglia and the consequent increase in inflammation result in exacerbated neurodegeneration, increased behavioral or motor symptoms of the disease state as well as potentiate pro-inflammatory cytokine production in the CNS in models of prion, Alzheimer's and Parkinson's disease (PD; Depino *et al.* 2003; Perry *et al.* 2003; Godoy *et al.* 2008).

From these studies, it is also clear that whereas microglial cell activation can elicit pro- and anti-inflammatory effects, *brain inflammation does not equal microglial cell activation.* Our definition of inflammation includes microglial cell activation, but the reverse does not always apply. Thus, the brain inflammatory reaction that includes peripheral immune cell recruitment into the brain should be clearly differentiated from the endogenous response of the brain where only microglial cell activation takes place.

It is also worth noticing that the brain can also be classified in different compartments according to its reactivity to inflammation. There are a number of stimuli that fail to elicit an inflammatory response in the parenchyma, which can elicit a rapid response when they are placed in the ventricles or subarachnoid space (Perry *et al.* 1993b). This can be because of differences in the cellular composition of the different compartments. Cells of the monocyte/macrophage system can be divided into three populations: parenchymal microglia, perivascular macrophages, and macrophages located in the meninges and the choroids plexus (Lassmann *et al.* 1991). Macrophages in the choroid plexus and meninges show an up-regulated phenotype when compared with parenchymal microglia, expressing OX62 and MHC II (Matyszak *et al.* 1992). Moreover, there is a large number of mast cells in the meninges, and these cells are not observed

in other regions of the brain (Perry *et al.* 1985). Furthermore, ventricular ependymal cells are abundant in the SVZ (20–50% of the overall cell number; Doetsch *et al.* 1997) and represent an important barrier to protect neuronal tissue by an innate immune response. Different receptors allow ependymal cells to sample the cerebrospinal fluid and respond to various infectious challenges. Ependymal cells also express a number of factors that sustain NSCs proliferation and differentiation (e.g. FGF-2, Notch-I, and bone morphogenetic proteins; reviewed in Hauwel *et al.* 2005).

With the previous information in mind, several hypotheses can be proposed. NSCs in the SVZ have contact with ventricular macrophages with an up-regulated phenotype while NSC in the SGZ of the hippocampal formation are preferentially surrounded by parenchymal microglial cells. These differences allow us to hypothesize that in the presence of the same pro-inflammatory stimulus, ventricular macrophages will elicit and inflammatory response while parenchymal microglial cells will not. Thus, NSC from the SVZ will be affected by an inflammatory milieu, while NSC from the SGZ will not. Following a similar line of thinking, a stem cell transplant will elicit a particular inflammatory response according to the local microenvironment of the host tissue, which will influence cell proliferation, differentiation and survival. For example, an exacerbated inflammatory response to transplantation is expected to happen in the ventricles in comparison to the brain parenchyma.

Thus, information on the brain compartment of study should be kept in mind to avoid drawing general conclusions from experiments performed only in one brain compartment.

Microglial cell activation and neurogenesis

In 2003, two independent studies showed that an LPS challenge in the brain led to microglial cell activation and a decline in hippocampal neurogenesis (Ekdahl *et al.* 2003; Monje *et al.* 2003). Furthermore, the administration of anti-inflammatory drugs restored hippocampal neurogenesis. In these reports, a negative association between the number of activated microglia and the number of new neurons was found. Also, proliferation of NSCs was significantly reduced only when cultured in the presence of conditioned media from activated microglia, but not from resting ones. The effect was thought to be mediated by the production of pro-inflammatory cytokines and chemokines such as interleukin (IL)-1β, tumor necrosis factor-α (TNF-α), IL-6, IL-18, and monocyte chemotactic protein-1 (MCP-1), and various reactive nitrogen and oxygen species (Rock *et al.* 2004). Recent evidence suggests that activated microglia are not always detrimental for neurogenesis. In fact, under certain conditions they can even be beneficial (Hanisch and Kettenmann 2007; Ekdahl *et al.* 2009). For example, microglial cell activation to stage 3, but not stage 4 (phagocytic) has been associated with enhanced neurogenesis and not pro- but anti-inflammatory cytokine production (Battista *et al.* 2006).

It is well known that the final effect of microglial cell activation upon NSCs biology will depend on the resulting profile of molecules they secrete. This review will concentrate on one of the main families of molecules derived from microglia and that has also been extensively studied in relation to NSCs proliferation and neurogenesis, i.e. pro- and anti-inflammatory cytokines.

Cytokines involved in adult neurogenesis

Cytokines are considered main components of the inflammatory/immune response. Pro- and anti-inflammatory cytokines are constitutively expressed and induced under a variety of stimuli in the brain (Schöbitz *et al.* 1994). These cytokines can be endogenously expressed by brain cells without the infiltration of immune cells from the periphery (Pitossi *et al.* 1997). Cytokines and their receptors are expressed in brain cells, both in glia and in neurons (Hopkins and Rothwell 1995; Rothwell *et al.* 1996). These endogenous molecules not only serve as communicators of immune signals into the brain and modulators of the glial response, but they also appear to contribute to exclusive brain function (e.g. long-term potentiation and synaptic plasticity; Besedovsky and Rey 2007). This particular fact gains relevance if cytokines derived from brain cells are to be considered as a part of the endogenous neurogenic niche without the intervention of the immune system.

It is worth to mention the differences found in the dynamics of cytokine expression in the brain when it is compared with the periphery. For example, contrary to the periphery, central IL-1 fails to induce TNF-α expression, and TNF-α does not induce either itself or IL-1 in the brain (Blond *et al.* 2002; Ferrari *et al.* 2004; Depino *et al.* 2005). This altered cytokine network reflects a specific level of control on the final effects of cytokines in the brain.

We here summarize the current literature showing a role of specific cytokines in modulating NSCs proliferation, differentiation, and neuronal survival. We have concentrated our analysis on prototypic pro- (IL-1, IL-6, and TNF-α) and anti-inflammatory cytokines (IL-4 and TGF-β) and not in other immune-related molecules such as interferons. We have also not intended to review the beneficial and toxic effects of cytokines in the brain, which could be found elsewhere (Liberto *et al.* 2004). In addition, we will not describe effects of the adaptive immune system on NSCs biology (for a review on the subject see (Rolls *et al.* 2009). For the considerations given before and as most cytokines have context-dependent functions, we differentiate between NSCs of SVZ and SGZ origin, and whether experiments were performed *in vivo* or *in vitro* (Table 1).

Table 1 Cytokines involved in modulation of NSCs

Cytokine	Type of NSC[a]	Experimental model used	Role in proliferation[b]	Role on neuronal differentiation[b]	Role on survival[b]	References
IL-1β	SGZ	*In vivo*	↓	=	=	Koo and Duman (2008)
			ND	↓	ND	Fig. 2
		In vitro	↓	ND	=	Koo and Duman (2008)
			ND	=	ND	Fig. 2
			ND	=	ND	Monje *et al.* (2003)
	SVZ	*In vivo*	=	ND	ND	Koo and Duman (2008)
TNF-α	SGZ	*In vivo*	↓ (TNF-R1)[c]	↓ (TNF-R1)[c]	(TNF-R2)[d]	Iosif *et al.* (2006)
		In vitro	ND	ND	↓	Cacci *et al.* (2005)
			ND	↓	ND	Monje *et al.* (2003)
	SVZ	*In vivo*	↓	ND	ND	Iosif *et al.* (2008)
			←	ND	ND	Wu *et al.* (2000)
		In vitro	↓	=	←	Ben-Hur *et al.* (2003)
			←	=	↓	Widera *et al.* (2006)
			=	=	↓	Wong *et al.* (2004)
IL-6	SGZ	*In vivo*	↓	↓	↓	Vallières *et al.* (2002)
		In vitro	=	↓	↓	Monje *et al.* (2003)
IL-4	SVZ	*In vitro*	ND	=	ND	Butovsky *et al.* (2006)
		In vitro (microglia co-culture)[e]	=	←	=	Butovsky *et al.* (2006)
TGF-β1	SGZ	*In vivo*	=	←	ND	Battista *et al.* (2006)
			↓	=	ND	Wachs *et al.* (2006)
		In vivo (Tg)[f]	↓	ND[g]	↓	Buckwalter *et al.* (2006)
		In vitro	↓	←	↓	Battista *et al.* (2006)
			↓	ND	=	Buckwalter *et al.* (2006)
	SVZ	*In vivo*	←	←	ND	Ma *et al.* (2008)
			←	←	ND	Mathieu *et al.* unpublished data
			↓	=	ND	Wachs *et al.* (2006)
		In vitro	↓	=	ND	Wachs *et al.* (2006)

IL, interleukin; NSCs, neural stem/progenitor cells; SDF, stromal cell-derived factor; SGZ, subgranular zone of the dentate gyrus of the hippocampus; TNF-R, TNF-α receptor; TNF-α, tumor necrosis factor-α. [a]Type of NSCs refers to their origin: SGZ or SVZ. [b]↓, reduced; ← , increased; =, non-affected; ND, no data; for proliferation, differentiation, or survival. [c]TNF-R1, in this study, a role of TNF-α through the TNF-R1 was shown. [d]TNF-R2, in this study a role of TNF-α through the TNF-R2 was shown. [e]NSCs were co-cultured with microglial cells. [f]Tg, transgenic mice overexpressing TGF-β1. [g]In this report, proliferation is reduced, and as differentiation data are not normalized, the effect on differentiation is not clear.

Interleukin-1

Interleukin-1 and IL-1 receptor (IL-1R) are constitutively expressed in the hippocampus (Ban *et al.* 1991; Pitossi *et al.* 1997). In fact, the hippocampus is the brain region with the highest density of IL-1R (Kabiersch *et al.* 1988; Rothwell and Hopkins 1995). IL-1β alone is capable of overriding the intrinsic resistance of the CNS to leukocyte infiltration, resulting in acute cellular recruitment to the brain parenchyma (Anthony *et al.* 1997; Proescholdt *et al.* 2002; Ferrari *et al.* 2004; Ching *et al.* 2005; Depino *et al.* 2005). Additionally, blocking the actions of IL-1β using the IL-1R antagonist results in significant reductions in parenchymal leukocyte infiltration after injury (Garcia *et al.* 1995; Yang *et al.* 1998). IL-1β can also have autocrine effects in the CNS and has been proposed as a mechanistic link between a potential beneficial inflammatory response and detrimental glutamate excitotoxicity (Fogal and Hewett 2008).

It has been reported that IL-1 also show non-immune effects in the brain. For example, endogenous IL-1 modulates long-term potentiation (Schneider *et al.* 1998) and learning (Depino *et al.* 2004). Moreover, exogenous, high levels of IL-1β block memory formation (Pugh *et al.* 1999, 2001).

Interleukin-1 and its receptors are expressed in the DG (Koo and Duman 2008; Arguello *et al.* 2009), but IL-1R1 was not detected in progenitor cells derived from SVZ (Ben-Hur *et al.* 2003). Administration of exogenous IL-1β protein (20 or 100 ng, i.c.v.) significantly decreased the number of 5-bromo-2′-deoxyuridine (BrdU)-positive cells in the DG, but did not affect the number of proliferating cells in the SVZ (Koo and Duman 2008). Moreover, the administration of IL-1R antagonist protein (250 ng, i.c.v.) blocked the decrease in proliferating cells in the DG caused by acute stress. Similar

results were obtained *in vitro*, as a 2 h treatment with 10 ng/mL of IL-1β protein reduced the proportion of proliferating NSC, and 100 ng/mL of IL-1ra protein blocked this effect (Koo and Duman 2008).

Concerning the effects of IL-1β on adult neurogenesis, we have studied the effects of the chronic expression of IL-1β in the DG of adult rats. To achieve chronic expression of human IL-1β, we administered a low dose (10^7 plaque formation units) of a replication-deficient, recombinant adenoviral vector expressing human IL-1β (AdIL-1β) to the right DG as described before (Ferrari *et al.* 2004, 2006). As control, a similar dose of β-galactosidase expressing adenovirus was used (Adβgal). At these doses, control vectors do not elicit a long-lasting inflammatory response in the brain (Ferrari *et al.* 2004).

At the peak of transgenic expression (14 days after the injection of the vectors; Ferrari *et al.* 2004), the tissue response was analyzed. Long-lasting expression of IL-1β elicited microglial cell activation accompanied by ED1 expression in the SGZ and the hilus and some tissue damage (17% of the DG; data not shown). No ED1-positive cells were observed in the contralateral hippocampus or in control animals (data not shown).

To analyze the effect of long-lasting IL-1β expression on neurogenesis, animals were injected intraperitoneally with BrdU during four consecutive days ending 3 days before adenoviral injection. In this way, we analyzed the effect of IL-1β on the fate of cells proliferating before adenovirus administration and not the population of cells such as microglia and astrocytes that are caused to proliferate by IL-1β expression. We performed double immunofluorescence analysis of BrdU and the neuronal neuron marker (NeuN), indicative of neurogenesis (Fig. 2a), or glial fibrillary acidic protein, indicative of astrogliogenesis (Fig. 2b). Cell counts were performed as described before (Battista *et al.* 2006).

After AdIL-1β injection, we observed a 50% reduction in the percentage of BrdU–NeuN-positive neurons among the total BrdU-positive cell population (*$p < 0.05$, Fig. 2a). On the contrary, we did not observe any changes in astrocytic differentiation after AdIL-1β injection ($p = 0.8$, Fig. 2b). When contralateral hemispheres were compared, there was no difference in the percentage of double-labeled cells for

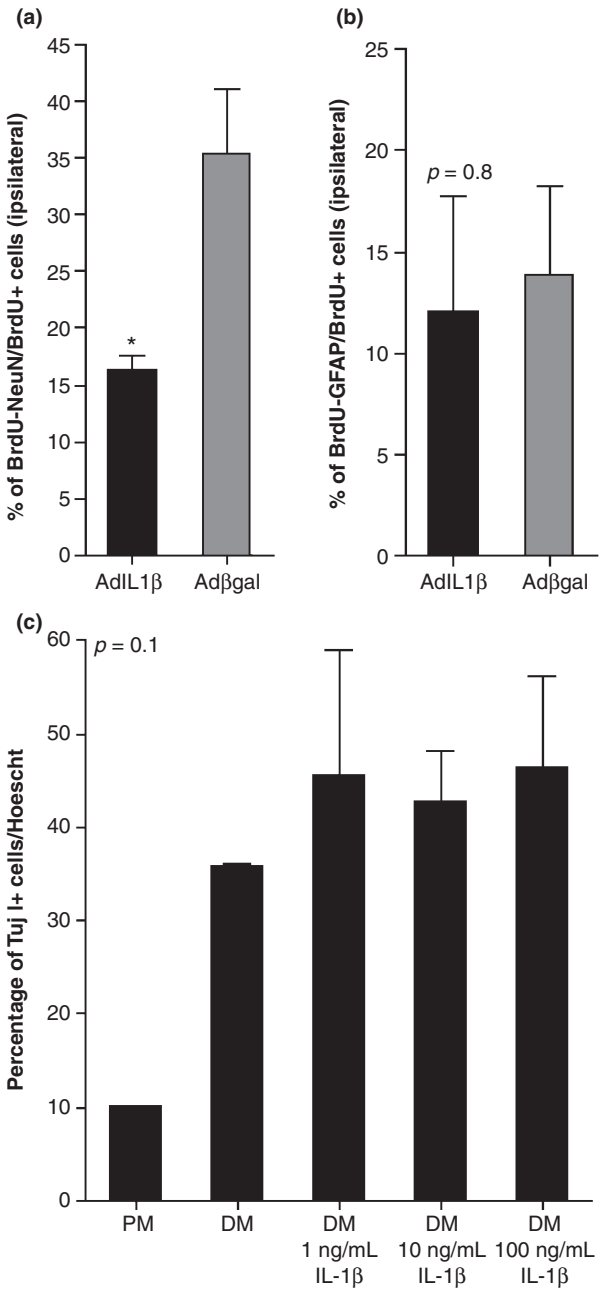

Fig. 2 Effects of the chronic expression of human IL-1β on neurogenesis *in vivo* and *in vitro*. Quantification of the percentage of double BrdU/NeuN (a) or BrdU/glial fibrillary acidic protein (GFAP) (b) positive cells among the BrdU-positive population in the SGZ of the DG. Data are presented as the percentage of double-labeled cells in the ipsilateral hemispheres of AdIL-1 and Adβgal-injected animals. Animals received intraperitoneal BrdU injections during four consecutive days; the last injection was 3 days before the adenoviral injection. A decrease in neurogenesis is observed (a; *$p < 0.05$) after chronic expression of IL-1β. No effect on astrogliogenesis (b; $p = 0.8$) is observed. Values are the mean ± SEM (Student's *t*-test; AdIL-1 $n = 4$ and Adβgal $n = 4$). (c) NSCs were kept in proliferation medium (PM) or treated with differentiation medium (DM) with or without different rat IL-1β concentrations (1, 10, and 100 ng/mL) during 5 days. NSCs cultures were stained for nuclear presence with Hoescht and for type III β-tubulin (Tuj) expression by immunofluorescence. The percentage of Tuj-positively stained cells over the total number of cells visualized with Hoescht was quantified. IL-1β had no effect on neurogenesis *in vitro*. All treatments were performed in triplicate in three independent experiments. Values are the mean ± SEM (ANOVA, $p = 0.1$). DM, DMEM/F12 supplemented with N_2 (GIBCO; Invitrogen, Carlsbad, CA, USA) and 20 ng/mL of FGF-2. PM, DMEM/F12 supplemented with N_2 (GIBCO; Invitrogen), 0.5 ng/mL FGF-2, 0.5 nM forskolin, and 1% fetal bovine serum.

BrdU–NeuN over total BrdU-positive cells (33.9 ± 8.3% vs. 44.3 ± 11.2%, AdIL-1β vs. Adβgal, respectively; $p = 0.33$) or BrdU–glial fibrillary acidic protein over total BrdU-positive cells (14.9 ± 5.5% vs. 17.8 ± 6.6%, AdIL-1β vs. Adβgal; $p = 0.75$). This indicates that fewer cells acquired a neuronal phenotype because of long-lasting IL-1β expression in the DG.

To analyze a possible direct effect of IL-1β on NSC differentiation, primary hippocampal NSCs were cultured with 1, 10, or 100 ng/mL of rat IL-1β during 5 days. The number of type III β-tubulin (Tuj)-positive cells was quantified as indicative of neuronal lineage. There was no difference in the number of Tuj-positive cells in any of the IL-1β concentrations tested, compared with control cultures (Fig. 2c). A similar observation was made by Monje *et al.* (2003) using doublecortin as a marker of neuronal precursor.

Thus, IL-1 had no direct effect on neurogenesis *in vitro* under the experimental conditions tested and this suggests that additional factors should be present to observe the anti-neurogenic effect detected *in vivo*. It is interesting to stress the observation that the anti-neurogenic effects of IL-1 were observed in a DG that has also been affected by the adenoviral injection.

Tumor necrosis factor-α

Tumor necrosis factor-α is expressed at very low levels in basal conditions in the CNS (Pitossi *et al.* 1997). Astrocytes, microglia, and neurons have been described to produce increased TNF-α upon exposure to multiple physiological and pathological stimuli (e.g. CNS infection and brain injury; Munoz-Fernandez and Fresno 1998). TNF-α is present in two isoforms in the CNS, as a soluble molecule and as a transmembrane one. Upon released, TNF-α exerts its action through binding to TNF receptors (TNF-R1 and TNF-R2). Different actions of TNF-α can partly be explained by the combination of TNF-α isoforms and TNFR expression (McCoy and Tansey 2008).

Transgenic mice specifically over-expressing murine TNF-α in the CNS, either in neurons or in astrocytes, and at different stages of development, spontaneously develop chronic CNS inflammation. This is characterized by widespread reactive astrocytosis and microgliosis, meningeal and parenchymal infiltration by activated T cells and macrophages, loss of white matter, and neurodegeneration (Probert *et al.* 1995). On the contrary, mice lacking TNF-α or both receptors show exacerbated neuronal damage elicited by different neurotoxic stimuli (Bruce *et al.* 1996; Liu *et al.* 1998). Therefore, TNF-α can be neurotoxic or have neuroprotective effects depending on the level of expression, the type of neurons affected and other unknown factors.

Tumor necrosis factor-α in the brain has also been observed to modulate CNS functions such as peripheral activation of neuroendocrine axes, release of neurotransmitters or neuronal firing in a paracrine or autocrine fashion (Turnbull *et al.* 1997; Besedovsky and Rey 2007).

Tumor necrosis factor-α and its receptors are expressed in the DG (Harry *et al.* 2008) and the SVZ (Iosif *et al.* 2008). Hippocampal NSCs express both TNF-R1 and TNF-R2 (Cacci *et al.* 2005). As a neurotoxic factor, TNF-α increases the death of a hippocampal NSCs line *in vitro* (Cacci *et al.* 2005). In line with this, Monje *et al.* (2003) showed that exposure of hippocampal NSCs *in vitro* to recombinant TNF-α (20 ng/mL for 60 h) significantly decreased neurogenesis. TNF-α reduces proliferation of NSCs derived from the SVZ, without affecting their differentiation (1–100 ng/mL for 48 h; Ben-Hur *et al.* 2003). Moreover, these *in vitro* studies showed that 100 ng/mL TNF-α increases NSCs migration.

However, recent work has highlighted the role of TNF-α as a positive regulator of neurogenesis. *In vitro* treatment of neurospheres grown from adult rat SVZ with TNF-α increased the proliferation of these spheres, without affecting differentiation (10 ng/mL for 24–72 h; Widera *et al.* 2006). Moreover, an *in vivo* study showed an increase in SVZ progenitor cells 6 and 24 h after injection of TNF-α protein above the lateral ventricle (355 ng per rat; Wu *et al.* 2000).

Using mice in which TNF-R1 and/or TNF-R2 have been deleted, Iosif *et al.* (2006) showed a differential effect of these receptors on hippocampal neurogenesis. Signaling through TNF-R1 suppresses neural progenitor proliferation and neurogenesis in the adult brain *in vivo*, whereas binding of TNF-α to TNF-R2 increases the survival of newly formed neurons. This suggests that the effect of TNF-α would depend not only on the type of NSC studied, but also on the levels of the cytokine and the relative expression of TNF-R1 and TNF-R2 on the NSCs (see Table 1).

Interleukin-6

Interleukin-6 regulates immune responses, acute phase reactions and hematopoiesis, and plays a central role in host defense mechanisms. IL-6 is a pleiotropic cytokine that is produced in a variety of cells and acts on a wide range of tissues, exerting growth-inducing, growth-inhibitory and differentiation-inducing effects, depending on the nature of the target cells (Hirano *et al.* 1990). In the CNS, IL-6 and its specific receptor are both expressed on neurons and glial cells including astrocytes (Gadient and Otten 1997).

Interleukin-6 exerts pronounced effects on the CNS: stimulates neuronal growth (Satoh *et al.* 1988; Hama *et al.* 1991), reduction of food intake (Plata-Salaman 1988), induction of fever (LeMay *et al.* 1990) and activation of the hypothalamic-pituitary-adrenal system (Naitoh *et al.* 1988). Transgenic mice with high levels of IL-6 expression develop severe neurologic disease characterized by tremor, ataxia, and seizure (Campbell *et al.* 1993). These mice also exhibit neurodegeneration, astrocytosis, and angiogenesis. In

contrast, IL-6 also supports neuroregeneration of hippocampal neurons in the CNS (Hakkoum *et al.* 2007).

Interleukin-6 receptor is expressed in the DG (Gadient and Otten 1994; Vallieres and Rivest 1997). Monje *et al.* (2003) revealed that the negative effect of activated microglial cells on neurogenesis was blocked upon addition of IL-6R antibody. Interestingly, when IL-6 is chronically expressed in astroglia of young transgenic mice, a remarkable decrease in the proliferation, survival and differentiation of NSCs was observed (Vallieres and Rivest 1997). These data suggest a clear anti-neurogenic effect of IL-6 *in vitro* and *in vivo* (see Table 1).

Interleukin-4

Functional IL-4Rs are expressed in rat hippocampal neurons and IL-4 is secreted by glial cells (Nolan *et al.* 2005). IL-4 can act as a neurotrophic factor increasing the survival of hippocampal neuronal cultures (Araujo and Cotman 1993). It has been proposed that IL-4 exert an anti-inflammatory effect in the brain, by down-regulating IL-1R1 and IL-1β expression (Nolan *et al.* 2005). Moreover, IL-4 can modulate microglial cell activation (Lyons *et al.* 2007), and induces a neuroprotective phenotype of microglia (Butovsky *et al.* 2005). This protective state appears to correlate with the down-regulation of TNF-α and with the up-regulation of insulin-like growth factor 1 (IGF-1).

Interleukin-4 had no direct effect on cultures of SVZ-derived NSCs. However, IL-4-treated microglia induces neurogenesis and oligodendrogenesis in the NSCs by different mechanisms (Butovsky *et al.* 2006). IL-4-treated microglia induce differentiation to oligodendrocytes via the IGF-1 signaling pathway, but the effect on neurogenesis is independent of IGF-1. In the same study it was shown that IL-4 have only limited effects on NSCs proliferation (< 10%) and co-culture of NSCs with IL-4-treated microglia had no effect on proliferation. Moreover, IL-4 treatment neither directly nor in microglia co-cultures affected NSCs survival. Thus, IL-4 seem to have a pro-neurogenic effect (see Table 1).

Transforming growth factor-β1

Transforming growth factor-β1 is normally present at low levels in the healthy adult CNS cells, and is rapidly up-regulated after injury, inducing the expression of many injury responsive genes (Finch *et al.* 1993). The main sources of TGF-β1 in the injured brain are astrocytes and microglia (Finch *et al.* 1993), but neurons can produce it as well (Flanders *et al.* 1998).

Transforming growth factor-β1 inhibits the synthesis of pro-inflammatory cytokines such as TNF-α, prostaglandins, nitric oxide, and eicosanoids from macrophages, adding to the protective effect of phagocytosing apoptotic cells and reducing the inflammatory response (Freire-de-Lima *et al.* 2006). The phagocytosis of apoptotic cells is closely linked to the down-regulation of inflammatory cytokines and also promotes the resolution of inflammation, contributing to CNS immune privilege. Microglial phagocytosis of apoptotic inflammatory T cells leads to down-regulation of microglial immune activation (Magnus *et al.* 2001). In line with this, it was previously proposed that TGF-β1 could contribute to the region-specific refractory response to inflammatory stimuli in specific brain regions (Depino *et al.* 2005). In summary, TGF-β1 generates an anti-inflammatory milieu in the brain, which contributes to the resistance of brain to inflammation.

Transforming growth factor-β1 is expressed in the DG (Bravo *et al.* 2006), and its expression is augmented during aging (Bye *et al.* 2001). Battista *et al.* 2006 showed in a model of enhanced neurogenesis by adrenalectomy that there was a positive correlation between increased neurogenesis, the number of activated microglia, and the levels of TGF-β1. Moreover, neutralizing antibody to TGF-β1 reduced the increase in adrenalectomy-induced neurogenesis, providing evidence for a positive role of this cytokine in this process. This same treatment did not significantly alter NSCs proliferation (Battista *et al.* 2006).

However, adult transgenic mice over-expressing TGF-β1 as embryonic days, exhibit decreased cell proliferation (BrdU-positive cells), lower number of NSCs (nestin-positive) and neural progenitor cells (doublecortin-positive) in the hippocampus (Buckwalter *et al.* 2006). The number of proliferating progenitor and newborn neurons was also decreased, most likely because of the dramatic effect of TGF-β1 expression on cell proliferation (60% reduction). In these animals, a reduction on the pool of NSCs because of effects of TGF-β1 during development that could account for these results could not be discarded. In a third study, the chronic intracerebroventricular infusion of TGF-β1 (6 ng/day for 7 days) reduced NSCs proliferation in the DG, but it did not change the differentiation fate to neurons in this region (Wachs *et al.* 2006).

In the SVZ, TGF-β1 transgenic expression or chronic intracerebroventricular infusion (6 ng/day for 7 days) resulted in decreased cell proliferation and had no effect on NSCs differentiation to a neuronal phenotype (Buckwalter *et al.* 2006; Wachs *et al.* 2006). However, TGF-β1 was able to increase proliferation and neuronal differentiation if the SVZ area was affected previously by a stroke lesion (intranasal delivery of 1 μg of recombinant human TGF-β1; Ma *et al.* 2008). In addition, adenoviral mediated delivery of TGF-β1 to the SVZ (a treatment that also results in a minor injury to the region injected) also resulted in an increased cell proliferation and differentiation toward a neuronal phenotype (Mathieu *et al.,* unpublished observations). Thus, as for other cytokines, TGF-β1 effects on adult NSCs proliferation and differentiation *in vivo* are context-dependent. However, a

tendency toward inhibiting cell proliferation (either during development or in the adult) and increasing neurogenesis in the intact DG and SVZ emerges (see Table 1). In addition, the contribution of other stimuli to the experimental set-up such as infarction or adenoviral vector delivery could have an opposite effect on NSCs proliferation.

In vitro, all studies revealed an inhibitory effect of TGF-β1 on NSCs proliferation irrespective of their origin (SVZ or DG) and culture conditions. The proportion of cells expressing a neuronal marker increased after incubating adult NSCs from the DG or the SVZ in the presence of human TGF-β1 and low (0.5 ng/mL) FGF-2 with or without 1% Fetal calf serum and 0.5 mM forskolin (Battista *et al.* 2006 and unpublished observations). In another study, NSCs isolated from the SVZ did not show any change in cell differentiation to any phenotype in the presence of 10 ng/mL of TGF-β1 and 20 ng/mL of FGF-2 or 5% fetal calf serum. Thus, culture conditions are important to visualize the pro-neurogenic potential of TGF-β1 *in vitro* (see Table 1).

Effects of inflammation on transplanted stem cells

The knowledge on the functional role of brain cytokines on the proliferation, differentiation and/or survival of NSCs should also help to understand the effects of cytokines on transplanted NSCs.

Following cell transplantation in a neurodegenerating brain, the host recognition of the transplanted cells will activate an innate immune response and liberate cytokines, among other mediators. This response will not only be affected by the cell type, cell number and architecture of the transplant but also by the previous inflammatory characteristics of the diseased host tissue. Increasing complexity, transplanted NSCs could also have immunomodulatory effects *per se*. Thus, there are three basic components of the inflammatory response to transplantation: the transplanted cell, the diseased host tissue and the interactions between them. To identify and study the functional role of key inflammatory mediators of these three components is crucial to understand and try to predict this response and, in turn, imagine possible complementary treatments to transplantation that could enhance the possibility of therapeutic success. The previously reviewed effects of cytokines on NSCs biology highlight the relevance of this knowledge to optimize the functional outcome of a cell-based therapy.

Here, we will first review the basic characteristics of the inflammatory component in a well-known neurodegenerative disease, PD, then the effects of transplantation in this disease and the consequences of the inflammatory environment in transplanted cell survival, differentiation and migration. Finally we will discuss the inherent immunomodulatory components of NSCs.

Inflammation in Parkinson's disease

Parkinson's disease is characterized by a progressive degeneration of dopaminergic neurons in the substantia nigra pars compacta (SN). The degenerated neurons present intracytoplasmatic inclusions or protein aggregates called Lewy's bodies, a feature characteristic of PD. The loss of dopaminergic neurons causes most of the motor symptoms of the disease, which can be alleviated by restoring neurotransmission with the dopamine precursor levodopa or with dopaminergic agonists (Lees *et al.* 2009).

It has been demonstrated that microglial cell activation is a primary component in the pathogenesis of the disease. In PD, robust microglial cell activation is consistently found in animal models and PD patients (McGeer *et al.* 1988; Hunot *et al.* 1999; Mogi and Nagatsu 1999; Mirza *et al.* 2000; Vila *et al.* 2001; Cicchetti *et al.* 2002; Depino *et al.* 2003; Hirsch *et al.* 2003). In addition, the substantia nigra is the brain region with the highest density of microglial cells in the brain (Lawson *et al.* 1990), neurons from the substantia nigra are particularly susceptible to microglial-mediated toxicity *in vitro* and *in vivo* (Castano *et al.* 1998, 2002; Kim *et al.* 2000; Liu *et al.* 2000) and this effect could be reversed by the administration of anti-inflammatory drugs (Liu *et al.* 2000; Castano *et al.* 2002; Sanchez-Pernaute *et al.* 2004; Hald and Lotharius 2005; Marchetti and Abbracchio 2005). In patients, microglia seem to be activated for long periods of time (Langston *et al.* 1999).

In animal models, we have previously shown that during neurodegeneration in the substantia nigra, microglial cells change to a 'primed' state in which pro-inflammatory cytokine transcription is increased but translation is blocked (Depino *et al.* 2003). This microglia overreacts when a central or peripheral pro-inflammatory stimulus is elicited, leading to an exacerbation of neurodegeneration, motor symptoms and increased production of IL-1β (Godoy *et al.* 2008). If cells are transplanted in an area with ongoing neurodegeneration and primed microglia, it can be speculated that anti-inflammatory measures had to be enforced to prevent a deleterious effect from the reactivation of primed microglia.

Transplantation in Parkinson's disease

The first transplants performed in human brains included solid pieces of human fetal or embryonic ventral mesencephalon. One of these studies showed clinical improvement at 5 months up to at least 3 years after surgery (Li *et al.* 2008). Its postmortem analysis demonstrated grafted cells survival and dopaminergic differentiation. Even though symptomatic relief was observed, the grafts have shown characteristics of the disease such as α-synuclein- and ubiquitin-positive Lewy's bodies (Lindvall *et al.* 1989; Li *et al.* 2008). In 2001, Fred *et al.* reported a clinical trial focus

on transplanted human embryonic dopamine neurons into the brains of PD patients. The evaluation 1 year post-grafting revealed some clinical improvements in younger but not in older patients (Freed *et al.* 2001). It is important to comment that besides the partial benefit, five over 40 patients of the trial develop dyskinesias more than a year after the transplant.

Likewise, in a double-blind, placebo-controlled trial of fetal nigral transplantation using one or four donors, the motor features of PD patients did not significantly improve in comparison to the placebo group but there was an increase in striatal fluorodopa uptake on positron emission tomography scan and evidence of surviving transplanted cells (Olanow *et al.* 2003). Other studies had similar results with an initially marked improvement that gradually deteriorates. This deterioration may have been because of graft failure caused by the PD process or an immune reaction, as suggested by the extensive microglial cell infiltration of the graft (Kordower *et al.* 2008). However, a different study where three subjects with advanced idiopathic PD received intracerebral transplantation of fetal ventral midbrain cell suspension grafts showed reduced inflammation response, grafted dopamine and serotonin neurons survival and not significant degeneration for a long term (Mendez *et al.* 2008). These results from trials of human transplants in PD patients suggest that grafted cells might be affected by the disease environment and *that the variables that affect the inflammatory response are still unclear.*

Transplant survival, differentiation, and migration

To assess the immunogenicity of allogeneic fetal neural dopamine tissue Widner *et al.* (1989) developed different donor–host transplants combinations in animal models; 15 weeks after grafting, there were no signs of rejection regardless whether grafted cells were syngeneic or allogeneic, or when different histocompatible donor–host combinations were performed. None of the animals showed considerable infiltration of immune cells and there were no difference in the amount of immune cells between the different groups. Another study showed that transplants of allogeneic or syngeneic embryonic mesencephalic rat cells in an intact or toxin-induced inflammated animal tissue displayed similar graft survival. More abundant infiltrates of macrophages, activated microglia, and astrocytes where present around both syngeneic and allogeneic grafts in toxin-lesioned striatum, than around cells transplanted in intact tissue (Duan *et al.* 1998). These studies proved that the immune privilege of the brain permits a different response to syngeneic and allogeneic grafts than the one expected in the periphery.

In the case of multiple transplantations*, timing between interventions seems* to affect the immune response and the final outcome of the treatments. A first, intracerebral allograft into unilateral 6-hydroxydopamine (6-OHDA) lesioned animals increased the host immune response against a second allograft 8 weeks later, resulting in a lower survival of the second graft (Duan *et al.* 1993). On the contrary, when repeated allografts were made with 14 weeks in between them, there was no significant difference between the survival of the first and the second allogeneic graft. Moreover, the immune infiltration around the second grafts was low and displayed a microglia/macrophage-like morphology (Duan *et al.* 1993; Widner and Brundin 1993).

The environment where cells are transplanted could have an effect on the differentiation of cells. For example, in a previous work it was demonstrated that cells obtained from adult rat hippocampus transplanted into an adult neurogenic area such as the hippocampus differentiated in much greater number into mature neurons or glia compared with *in vitro* assays. This finding indicates that there are signals within the adult brain that are crucial for the differentiation of the progenitors (Gage *et al.* 1995). In 1999, Fricker *et al.* demonstrated that human neuronal progenitors from embryonic brain tissue transplanted in adult rat SVZ migrated to the olfactory bulb, and expressed neural markers. When these cells were grafted in the striatum, part of them differentiated into glia, but they also underwent neurogenesis in this non-neurogenic environment and assumed neuronal phenotype similar to those normally present there. The transplanted cells remain close to the injection site in absence of suitable substrate for migration. The authors concluded that the long-term propagated embryonic neuronal progenitors are capable of migration, integration and site-specific differentiation in the adult brain (Fricker *et al.* 1999). Other work, where adult brain subventricular zone progenitor cells were transplanted into the striatum of adult rats showed that adult progenitor cells can also differentiate in a region-specific manner to striatal neurons (Zhang *et al.* 2003). In contrast to these studies, a plethora of evidence proved that adult NSCs from hippocampus or subventricular zone can only produce neurons when they are transplanted into neurogenic regions, and mainly produce glial cells when transplanted into non-neurogenic regions, such as the striatum (Gage *et al.* 1995; Herrera *et al.* 1999; Dziewczapolski *et al.* 2003; Chen *et al.* 2007). The differences could be explained in *part by the neuronal compromise of the cells* before transplant, as was characterized by Zhang *et al.* (2003) were the majority of the cells were already committed neuronal precursors (Tuj-positive-cells). It is still unclear whether NSCs and progenitor cells at different stages of differentiation elicit different inflammatory responses when transplanted in the brain. There is also evidence lacking on the differences on the inflammatory response elicited by a certain type of transplanted cell depending on the brain region transplanted.

In the same way, the environment generated by brain lesions could also dictate the fate of transplanted cells. Embryonic rat striatal cells transplanted into the adult rat

striatum lesioned by excitotoxicity showed extensive migration into the surrounding host tissue and increased cellular proliferation as a response to signals from the lesion. The majority of grafted cells assumed glial-like morphology and only a very small fraction developed neuron-like characteristics (Lundberg *et al.* 1997). On the other hand, low numbers of transplanted mouse embryonic stem cells developed into mature ventral midbrain-like dopaminergic neurons and successfully reduced motor asymmetry when cells were transplanted into the lesioned rat striatum. The applications of low numbers of embryonic stem cells might result in dilution of epidermal growth factors and other germ layer-inducing signals, and cell–cell contacts that would facilitate neuronal differentiation (Bjorklund *et al.* 2002).

These evidences support the knowledge that the survival and differentiation of transplanted cells are affected by the milieu where they are transplanted. There is still systematic studies lacking to show what cytokines during an inflammatory state have a main impact on the fate or survival of transplanted cells. On the other hand, the role of chemokines on migration of transplanted cells has been better documented.

Experiments with human neural stem cells from fetal brain tissue showed that cells transplanted into the cortical region of adult uninjured rats showed no spontaneous migration, but grafts performed in injured brains by a distal middle cerebral artery occlusion showed migration toward the lesion. Although human neural stem cells can moved appreciable distances to regions of pathology and migrated within the impaired region, their migration depended on the distance between the graft and the lesion. If this distance is too large there will not be targeted migration (Guzman *et al.* 2007; Bjugstad *et al.* 2008). Migration toward the area of lesion was described in different disease models; adult NSCs migrated to sites of demyelination when they were intravenous injected into an animal model of Multiple Sclerosis (Pluchino *et al.* 2003), or NSCs lines transplanted in a mouse model of cerebral ischemia showed migration toward the infarcted area (Imitola *et al.* 2004). In contrast to these results, it was demonstrated that SVZ cells from adult rats grafted into the intact striatum migrated a distance about 1.5 mm from their site of transplant in the adult brain. The authors suggest that in addition to extrinsic cues, during culture conditioning, these cells may lose the intrinsic factors that inhibit their migration in a heterotypical region (Zhang *et al.* 2003). The molecules that have been implied in migration of neural stem cells are chemoattractants such as stromal cell-derived factor-1α (SDF-1) and MCP-1. Human neural stem cells express the chemokine receptor 4, the receptor for SDF-1α, and transplanted in hypoxic-ischemic cerebral injured mice showed migration that correlated with the area of SDF-1α expression. Inflammation may also activate astrocytes and endothelial cells. These glial cells produce SDF-1α that may direct the migration of NSCs toward the injure site (Imitola *et al.* 2004). MCP-1 is important for leukocyte traffic into the brain and might play a similar role in directing migration of progenitor cells. The up-regulation of MCP-1 synthesis is produced by neuroinflammation and together with the activation of its receptor, the chemokine receptor 2 expressed by neural progenitors, may be important in guiding the NSCs migration (Belmadani *et al.* 2006). Another factor that was proposed to be involved in transplant migration is stem cell factor (SCF). SCF expression is induced by brain injury in neurons surrounding the lesion. Besides, it was demonstrated that recombinant SCF triggers the migration of NSCs *in vivo* and *in vitro*, and c-kit (the SCF receptor) was detected on NSCs and was activated after SCF exposure (Sun *et al.* 2004).

One main feature to obtain a true regenerative therapy is to reestablish damaged circuits in the CNS. One main, although not exclusive, area of neurodegeneration is the SN. Loss of neurons in this area affects their target area, the caudate–putamen, resulting in a deficit in dopaminergic transmission in that area. Because of the lack of information to force transplanted cells in the SN to connect to the target area, the main site of transplanted strategies in PD is the site where dopamine is required (i.e. the caudate–putamen). Thus, most therapeutic strategies against PD are focused on replacing dopamine locally in the caudate–putamen, lacking the physiological control of dopamine release of a functional nigrostriatal system. Seminal work by Alberto Aguayo presented the proof of principle that axonal elongation is inducible under defined conditions (Benfey and Aguayo 1982). Yet, there is still not enough knowledge available to design efficient and reliable strategies to guide axons to target areas *in vivo*.

Immunomodulation by neural stem/progenitor cell

Stem cells from different origins were proved to reduce the inflammation response in distinct neurodegenerative models. Human NSCs lines from fetal brain and postnatal SVZ cells injected intravenously in an intracerebral hemorrhage model displayed anti-inflammatory functions that protected the tissue by interrupting the splenic response after intracerebral hemorrhage (Lee *et al.* 2008). The neuroprotective effect was associated with modulation of immune recognition, as these cells produced TGF-β under basal conditions. Similarly, an anti-inflammatory action was observed when mouse neural stem cells injected intravenously in a mouse model of Sandhoff's disease migrated extensively trough the animal body and distributed mainly in systemic organs as liver, spleen and lung. However, only a small number of injected cells were found in the brain. The authors proved that NSCs reduced mRNA levels of TNF-α and IL-6 in the brain and spleen, and it a lower number of microglia in NSCs injected animals was found. So, in this model cerebral inflammation was modified by the interaction of intravenously injected

NSCs with the peripheral inflammatory system (Lee *et al.* 2008). Moreover, the injection of adult NSCs either i.v. or i.c.v. in an animal model of multiple sclerosis, experimental autoimmune encephalomyelitis, showed that transplanted cells can reach the brain and produce functional recovery (Pluchino *et al.* 2003). The amelioration of the symptoms was demonstrated to be because of a double effect: a direct remyelination by the differentiation of transplanted NSCs into oligodendrocytes and by a bystander regulation of endogenous oligodendroglia and the reactive astrogliosis. The authors detected levels of mRNA for FGF-2, TGF-β, ciliary neurotrophic factor, glial-derived neurotrophic factor, nerve growth factor, BDNF, leukemia inhibitory factor, and neurotrophin-3 in cultured neural precursors, which might be responsible for the bystander effects (Pluchino *et al.* 2003). In another example, human NSCs transplanted in MPTP-treated, dopamine depleted monkeys produce behavioral improvements up to 60 days after transplant. The results showed prolonged cell survival (more than 7 months) but only a 5.47–8.03% of tyrosine hydroxylase (TH)-positive, dopaminergic grafted cells. This percentage of TH-positive neurons should not be enough to explain the behavioral benefit observed. Thus, human neural stem cells appeared to exert protective effects that may be displayed by the transplanted cells that acquired astrocyte phenotype and expressed glial-derived neurotrophic factor (Redmond *et al.* 2007). Also in an middle cerebral artery occlusion model, adult or embryonic neural stem cells transplanted differentiated into astrocytes and the therapeutic effects detected might also be because of a trophic factor secretion rather than replacement of damaged neurons (Takahashi *et al.* 2008). Human fetal-derived neural stem cells line transplanted after 6-OHDA injection in the middle forebrain produced behavior improvement in apomorphine-induced rotations 4 weeks after the graft. TH staining of the striatum and the SN was also significantly preserved. Even though the graft survival was 1.1% and there were no TH-positive transplanted cells, in an *in vitro* assay the authors observed neuroprotection by conditioned NSCs medium against 6-OHDA neurotoxicity. This effect could be explained by a reduction in apoptosis. In this conditioned medium it was demonstrated the presence of SCF and BDNF, two growth factors that could be responsible of the observed beneficial effects (Yasuhara *et al.* 2006).

Final remarks

As reviewed here, cytokines can modulate NSCs proliferation, differentiation, and survival. As brain cytokines can be constitutively expressed in neurogenic regions, and NSCs express cytokine receptors, endogenous NSCs can be subjected to the functional influence of endogenous brain cytokines. We believe that enough evidence is available to consider brain cytokines as part of the endogenous neurogenic niche. This field of research is just emerging and as most cytokine actions are context-dependent, data are not consistent for all models studied. However, when all available data are gathered together, a picture emerges where pro-inflammatory cytokines have mainly an anti-neurogenic effect and anti-inflammatory cytokines have pro-neurogenic functions (see Fig. 3). This concept will be validated or proved wrong by accumulating data obtained in future studies.

In addition, cytokines will be produced after NSCs grafting in the brain. As in the case with endogenous NSCs, cytokine production triggered by cell transplantation will undoubtedly affect the proliferation, differentiation, and survival of the grafted cells, and the final therapeutic effect of cell transplantation. The study of the inflammatory reaction expected after grafting remains largely unexplored. We believe that considerable efforts should be taken to try to elucidate main basic rules governing the inflammatory reaction after grafting. Here, we show evidences that these rules will depend upon the type of NSCs studied and their degree of differentiation, the microglial state of activation of the diseased brain area to be grafted, the timing between

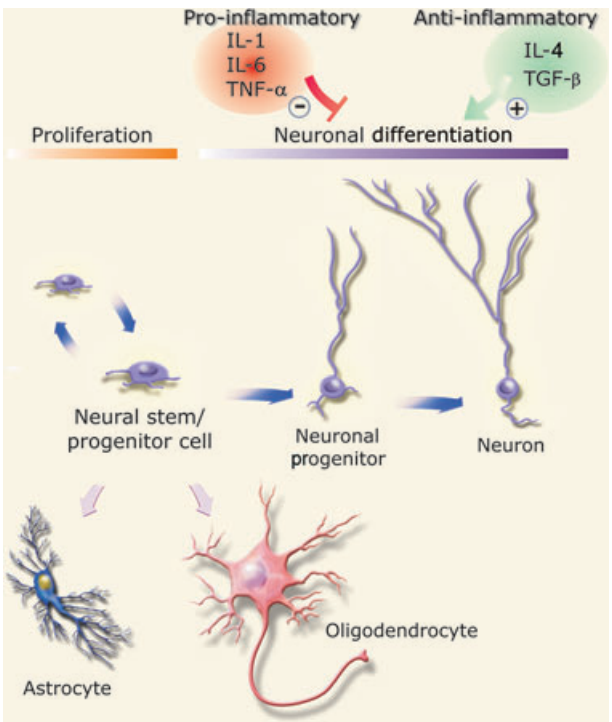

Fig. 3 Proposed role of pro- and anti-inflammatory cytokines on neuronal differentiation. Although cytokine action is context-dependent and their effects are sometimes seen only in coordination with other molecules, increasing data suggest a different functional role for pro- and anti-inflammatory cytokines on neuronal differentiation in the adult brain. Anti-inflammatory cytokines (IL-4, IFN-γ, and TGF-β1) seem to promote neuronal differentiation. Pro-inflammatory cytokines (IL-1, IL-6, and TNF-α) tend to diminish this process.

graftings and the dilution of the number of cells transplanted, among other factors. The discovery of the factors determining the type of inflammatory reaction after transplant is challenging as basic rules governing CNS inflammation are just starting to be elucidated. As a first step, we propose to clearly differentiate microglial cell activation, which could be anti- or pro-inflammatory to brain inflammation which also includes pro-inflammatory immune cell recruitment from the periphery. This view will help to prevent the automatic extrapolation of results showing microglial cell activation to a pro-inflammatory mileu. In addition, microglial cell activation should be studied in concert with the identification of its secreted molecules, which will define its net effect on a given phenomenon. Furthermore, the effect of the grafted NSC *per se* on the fate of the graft by secreting neuroprotective and growth factors should not be disregarded.

Brain transplantation of NSC at different levels of neuronal differentiation is regarded as a promising new avenue of therapeutic opportunities against many neurodegenerative diseases. Certainly, many challenges should be faced to translate this type of approach to the clinic. No doubt, that the better we understand the inflammatory response after grafting and its modulatory role on the transplant, the closer we will be to design therapeutically effective interventions. Studies to elucidate not only the role of the transplanted cell, the state of the brain tissue at the moment of grafting and the interactions between transplanted cells and host tissue, but also basic studies on the functional role of endogenous cytokines on adult neurogenesis should pave the way for a better planning of immunomodulatory treatments aiming at increasing therapeutic efficacy.

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References

Ahn S. and Joyner A. L. (2005) *In vivo* analysis of quiescent adult neural stem cells responding to Sonic hedgehog. *Nature* **437**, 894–897.

Altman J. (1969) Autoradiographic and histological studies of postnatal neurogenesis. IV. Cell proliferation and migration in the anterior forebrain, with special reference to persisting neurogenesis in the olfactory bulb. *J. Comp. Neurol.* **137**, 433–457.

Altman J. and Das G. D. (1965) Autoradiographic and histological evidence of postnatal hippocampal neurogenesis in rats. *J. Comp. Neurol.* **124**, 319–335.

Altman J. and Das G. D. (1966) Autoradiographic and histological studies of postnatal neurogenesis. I. A longitudinal investigation of the kinetics, migration and transformation of cells incorporating tritiated thymidine in neonate rats, with special reference to postnatal neurogenesis in some brain regions. *J. Comp. Neurol.* **126**, 337–389.

Alvarez-Buylla A. and Lim D. A. (2004) For the long run: maintaining germinal niches in the adult brain. *Neuron* **41**, 683–686.

Androutsellis-Theotokis A., Leker R. R., Soldner F. *et al.* (2006) Notch signalling regulates stem cell numbers *in vitro* and *in vivo*. *Nature* **442**, 823–826.

Anthony D., Bolton S., Fearn S. and Perry V. (1997) Age-related effects of interleukin-1β on polymorphonuclear neutrophil-dependent increases in blood-brain barrier permeability in rats. *Brain* **120**, 435–444.

Araujo D. M. and Cotman C. W. (1993) Trophic effects of interleukin-4, -7 and -8 on hippocampal neuronal cultures: potential involvement of glial-derived factors. *Brain Res.* **600**, 49–55.

Arguello A. A., Fischer S. J., Schonborn J. R., Markus R. W., Brekken R. A. and Eisch A. J. (2009) Effect of chronic morphine on the dentate gyrus neurogenic microenvironment. *Neuroscience* **159**, 1003–1010.

Ban E., Milon G., Prudhomme N., Fillion G. and Haour F. (1991) Receptors for interleukin-1 (α and β) in mouse brain: mapping and neuronal localization in hippocampus. *Neuroscience* **43**, 21–30.

Barkho B. Z., Song H., Aimone J. B., Smrt R. D., Kuwabara T., Nakashima K., Gage F. H. and Zhao X. (2006) Identification of astrocyte-expressed factors that modulate neural stem/progenitor cell differentiation. *Stem Cells Dev.* **15**, 407–421.

Battista D., Ferrari C. C., Gage F. H. and Pitossi F. J. (2006) Neurogenic niche modulation by activated microglia: transforming growth factor beta increases neurogenesis in the adult dentate gyrus. *Eur. J. Neurosci.* **23**, 83–93.

Bauer S. and Patterson P. H. (2006) Leukemia inhibitory factor promotes neural stem cell self-renewal in the adult brain. *J. Neurosci.* **26**, 12089–12099.

Belmadani A., Tran P. B., Ren D. and Miller R. J. (2006) Chemokines regulate the migration of neural progenitors to sites of neuroinflammation. *J. Neurosci.* **26**, 3182–3191.

Benfey M. and Aguayo A. J. (1982) Extensive elongation of axons from rat brain into peripheral nerve grafts. *Nature* **296**, 150–152.

Ben-Hur T., Cialic R. and Weidenfeld J. (2003) Virus and host factors that mediate the clinical and behavioral signs of experimental herpetic encephalitis. A short auto-review. *Acta Microbiol. Immunol. Hung.* **50**, 443–451.

Besedovsky H. O. and Rey A. D. (2007) Physiology of psychoneuroimmunology: a personal view. *Brain Behav. Immun.* **21**, 34–44.

Betmouni S., Perry V. and Gordon J. (1996) Evidence for an early inflammatory response in the central nervous system of mice with scrapie. *Neuroscience* **74**, 1–5.

Bjorklund L. M., Sanchez-Pernaute R., Chung S. *et al.* (2002) Embryonic stem cells develop into functional dopaminergic neurons after transplantation in a Parkinson rat model. *Proc. Natl Acad. Sci. USA* **99**, 2344–2349.

Bjugstad K. B., Teng Y. D., Redmond D. E. Jr, Elsworth J. D., Roth R. H., Cornelius S. K., Snyder E. Y. and Sladek J. R. Jr (2008) Human neural stem cells migrate along the nigrostriatal pathway in a primate model of Parkinson's disease. *Exp. Neurol.* **211**, 362–369.

Blond D., Campbell S. J., Butchart A. G., Perry V. H. and Anthony D. C. (2002) Differential induction of interleukin-1beta and tumour necrosis factor-alpha may account for specific patterns of leukocyte recruitment in the brain. *Brain Res.* **958**, 89–99.

Bonaguidi M. A., Peng C. Y., McGuire T., Falciglia G., Gobeske K. T., Czeisler C. and Kessler J. A. (2008) Noggin expands neural stem cells in the adult hippocampus. *J. Neurosci.* **28**, 9194–9204.

Bravo J. A., Parra C. S., Arancibia S., Andres S., Morales P., Herrera-Marschitz M., Herrera L., Lara H. E. and Fiedler J. L. (2006) Adrenalectomy promotes a permanent decrease of plasma corticoid

levels and a transient increase of apoptosis and the expression of transforming growth factor beta1 (TGF-beta1) in hippocampus: effect of a TGF-beta1 oligo-antisense. *BMC Neurosci.* **7**, 40.

Bruce A. J., Boling W., Kindy M. S., Peschon J., Kraemer P. J., Carpenter M. K., Holtsberg F. W. and Mattson M. P. (1996) Altered neuronal and microglial responses to excitotoxic and ischemic brain injury in mice lacking TNF receptors. *Nat. Med.* **2**, 788–794.

Buckwalter M. S., Yamane M., Coleman B. S., Ormerod B. K., Chin J. T., Palmer T. and Wyss-Coray T. (2006) Chronically increased transforming growth factor-beta1 strongly inhibits hippocampal neurogenesis in aged mice. *Am. J. Pathol.* **169**, 154–164.

Butovsky O., Talpalar A. E., Ben-Yaakov K. and Schwartz M. (2005) Activation of microglia by aggregated beta-amyloid or lipopolysaccharide impairs MHC-II expression and renders them cytotoxic whereas IFN-gamma and IL-4 render them protective. *Mol. Cell. Neurosci.* **29**, 381–393.

Butovsky O., Ziv Y., Schwartz A., Landa G., Talpalar A. E., Pluchino S., Martino G. and Schwartz M. (2006) Microglia activated by IL-4 or IFN-gamma differentially induce neurogenesis and oligodendrogenesis from adult stem/progenitor cells. *Mol. Cell. Neurosci.* **31**, 149–160.

Bye N., Zieba M., Wreford N. G. and Nichols N. R. (2001) Resistance of the dentate gyrus to induced apoptosis during ageing is associated with increases in transforming growth factor-beta1 messenger RNA. *Neuroscience* **105**, 853–862.

Cacci E., Claasen J. H. and Kokaia Z. (2005) Microglia-derived tumor necrosis factor-alpha exaggerates death of newborn hippocampal progenitor cells *in vitro*. *J. Neurosci. Res.* **80**, 789–797.

Cacci E., Ajmone-Cat M. A., Anelli T., Biagioni S. and Minghetti L. (2008) *In vitro* neuronal and glial differentiation from embryonic or adult neural precursor cells are differently affected by chronic or acute activation of microglia. *Glia* **56**, 412–425.

Campbell I. L., Abraham C. R., Masliah E., Kemper P., Inglis J. D., Oldstone M. B. A. and Mucke L. (1993) Neurologic disease induced in transgenic mice by cerebral overexpression of interleukin 6. *Proc. Natl Acad. Sci. USA* **90**, 10061–10065.

Castano A., Herrera A. J., Cano J. and Machado A. (1998) Lipopolysaccharide intranigral injection induces inflammatory reaction and damage in nigrostriatal dopaminergic system. *J. Neurochem.* **70**, 1584–1592.

Castano A., Herrera A. J., Cano J. and Machado A. (2002) The degenerative effect of a single intranigral injection of LPS on the dopaminergic system is prevented by dexamethasone, and not mimicked by rh-TNF-alpha, IL-1beta and IFN-gamma. *J. Neurochem.* **81**, 150–157.

Chen K., Hughes S. M. and Connor B. (2007) Neural progenitor cells derived from the adult rat subventricular zone: characterization and transplantation. *Cell Transplant.* **16**, 799–810.

Ching S., He L., Lai W. and Quan N. (2005) IL-1 type I receptor plays a key role in mediating the recruitment of leukocytes into the central nervous system. *Brain Behav. Immunol.* **19**, 127–137.

Cicchetti F., Brownell A. L., Williams K., Chen Y. I., Livni E. and Isacson O. (2002) Neuroinflammation of the nigrostriatal pathway during progressive 6-OHDA dopamine degeneration in rats monitored by immunohistochemistry and PET imaging. *Eur. J. Neurosci.* **15**, 991–998.

Depino A. M., Earl C., Kaczmarczyk E., Ferrari C., Besedovsky H., del Rey A., Pitossi F. J. and Oertel W. H. (2003) Microglial activation with atypical proinflammatory cytokine expression in a rat model of Parkinson's disease. *Eur. J. Neurosci.* **18**, 2731–2742.

Depino A. M., Alonso M., Ferrari C., del Rey A., Anthony D., Besedovsky H., Medina J. H. and Pitossi F. (2004) Learning modulation by endogenous hippocampal IL-1: blockade of endogenous IL-1 facilitates memory formation. *Hippocampus* **14**, 526–535.

Depino A., Ferrari C., Pott Godoy M. C., Tarelli R. and Pitossi F. J. (2005) Differential effects of interleukin-1beta on neurotoxicity, cytokine induction and glial reaction in specific brain regions. *J. Neuroimmunol.* **168**, 96–110.

Doetsch F., Garcia-Verdugo J. M. and Alvarez-Buylla A. (1997) Cellular composition and three-dimensional organization of the subventricular germinal zone in the adult mammalian brain. *J. Neurosci.* **17**, 5046–5061.

Duan W. M., Widner H., Bjorklund A. and Brundin P. (1993) Sequential intrastriatal grafting of allogeneic embryonic dopamine-rich neuronal tissue in adult rats: will the second graft be rejected? *Neuroscience* **57**, 261–274.

Duan W. M., Widner H., Cameron R. M. and Brundin P. (1998) Quinolinic acid-induced inflammation in the striatum does not impair the survival of neural allografts in the rat. *Eur. J. Neurosci.* **10**, 2595–2606.

Duman R. S., Malberg J. and Nakagawa S. (2001) Regulation of adult neurogenesis by psychotropic drugs and stress. *J. Pharmacol. Exp. Ther.* **299**, 401–407.

Dziewczapolski G., Lie D. C., Ray J., Gage F. H. and Shults C. W. (2003) Survival and differentiation of adult rat-derived neural progenitor cells transplanted to the striatum of hemiparkinsonian rats. *Exp. Neurol.* **183**, 653–664.

Ekdahl C. T., Claasen J. H., Bonde S., Kokaia Z. and Lindvall O. (2003) Inflammation is detrimental for neurogenesis in adult brain. *Proc. Natl Acad. Sci. USA* **100**, 13632–13637.

Ekdahl C. T., Kokaia Z. and Lindvall O. (2009) Brain inflammation and adult neurogenesis: the dual role of microglia. *Neuroscience* **158**, 1021–1029.

Ferrari C. C., Depino A. M., Prada F., Muraro N., Campbell S., Podhajcer O., Perry V. H., Anthony D. C. and Pitossi F. J. (2004) Reversible demyelination, blood-brain barrier breakdown, and pronounced neutrophil recruitment induced by chronic IL-1 expression in the brain. *Am. J. Pathol.* **165**, 1827–1837.

Ferrari C. C., Pott Godoy M. C., Tarelli R., Chertoff M., Depino A. M. and Pitossi F. J. (2006) Progressive neurodegeneration and motor disabilities induced by chronic expression of IL-1beta in the substantia nigra. *Neurobiol. Dis.* **24**, 183–193.

Finch C. E., Laping N. J., Morgan T. E., Nichols N. R. and Pasinetti G. M. (1993) TGF-beta 1 is an organizer of responses to neurodegeneration. *J. Cell. Biochem.* **53**, 314–322.

Flanders K., Ren R. and Lippa C. (1998) Transforming growth factor-βs in neurodegenerative disease. *Prog. Neurobiol.* **54**, 71–85.

Fogal B. and Hewett S. J. (2008) Interleukin-1beta: a bridge between inflammation and excitotoxicity? *J. Neurochem.* **106**, 1–23.

Freed C. R., Greene P. E., Breeze R. E. *et al.* (2001) Transplantation of embryonic dopamine neurons for severe Parkinson's disease. *N. Engl. J. Med.* **344**, 710–719.

Freire-de-Lima C. G., Xiao Y. Q., Gardai S. J., Bratton D. L., Schiemann W. P. and Henson P. M. (2006) Apoptotic cells, through transforming growth factor-beta, coordinately induce anti-inflammatory and suppress pro-inflammatory eicosanoid and NO synthesis in murine macrophages. *J. Biol. Chem.* **281**, 38376–38384.

Fricker R. A., Carpenter M. K., Winkler C., Greco C., Gates M. A. and Bjorklund A. (1999) Site-specific migration and neuronal differentiation of human neural progenitor cells after transplantation in the adult rat brain. *J. Neurosci.* **19**, 5990–6005.

Fuchs E. and Gould E. (2000) Mini-review: *in vivo* neurogenesis in the adult brain: regulation and functional implications. *Eur. J. Neurosci.* **12**, 2211–2214.

Gadient R. A. and Otten U. (1994) Identification of interleukin-6 (IL-6)-expressing neurons in the cerebellum and hippocampus of normal adult rats. *Neurosci. Lett.* **182**, 243–246.

Gadient R. A. and Otten U. H. (1997) Interleukin-6 (IL-6) – a molecule with both beneficial and destructive potentials. *Prog. Neurobiol.* **52**, 379–390.

Gage F. H. (2002) Neurogenesis in the adult brain. *J. Neurosci.* **22**, 612–613.

Gage F. H., Coates P. W., Palmer T. D., Kuhn H. G., Fisher L. J., Suhonen J. O., Peterson D. A., Suhr S. T. and Ray J. (1995) Survival and differentiation of adult neuronal progenitor cells transplanted to the adult brain. *Proc. Natl Acad. Sci. USA* **92**, 11879–11883.

Garcia J. H., Liu K. F. and Relton J. K. (1995) Interleukin-1 receptor antagonist decreases the number of necrotic neurons in rats with middle cerebral artery occlusion. *Am. J. Pathol.* **147**, 1477–1486.

Gehrmann J., Matsumoto Y. and Kreutzberg G. (1995) Microglia: intrinsic immuneffector cell of the brain. *Brain Res. Rev.* **20**, 269–287.

Godoy M. C., Tarelli R., Ferrari C. C., Sarchi M. I. and Pitossi F. J. (2008) Central and systemic IL-1 exacerbates neurodegeneration and motor symptoms in a model of Parkinson's disease. *Brain* **131**, 1880–1894.

Guzman R., Uchida N., Bliss T. M. *et al.* (2007) Long-term monitoring of transplanted human neural stem cells in developmental and pathological contexts with MRI. *Proc. Natl Acad. Sci. USA* **104**, 10211–10216.

Hakkoum D., Stoppini L. and Muller D. (2007) Interleukin-6 promotes sprouting and functional recovery in lesioned organotypic hippocampal slice cultures. *J. Neurochem.* **100**, 747–757.

Hald A. and Lotharius J. (2005) Oxidative stress and inflammation in Parkinson's disease: is there a causal link? *Exp. Neurol.* **193**, 279–290.

Hama T., Kushima Y., Miyamoto M., Kubota M., Takei N. and Hatanaka H. (1991) Interleukin-6 improves the survival of mesencephalic catecholaminergic and septal cholinergic neurons from postnatal, two-week-old rats in cultures. *Neuroscience* **40**, 445–452.

Hanisch U. K. and Kettenmann H. (2007) Microglia: active sensor and versatile effector cells in the normal and pathologic brain. *Nat. Neurosci.* **10**, 1387–1394.

Harry G. J., Lefebvre d'Hellencourt C., McPherson C. A., Funk J. A., Aoyama M. and Wine R. N. (2008) Tumor necrosis factor p55 and p75 receptors are involved in chemical-induced apoptosis of dentate granule neurons. *J. Neurochem.* **106**, 281–298.

Hausler K. G., Prinz M., Nolte C., Weber J. R., Schumann R. R., Kettenmann H. and Hanisch U. K. (2002) Interferon-gamma differentially modulates the release of cytokines and chemokines in lipopolysaccharide- and pneumococcal cell wall-stimulated mouse microglia and macrophages. *Eur. J. Neurosci.* **16**, 2113–2122.

Hauwel M., Furon E., Canova C., Griffiths M., Neal J. and Gasque P. (2005) Innate (inherent) control of brain infection, brain inflammation and brain repair: the role of microglia, astrocytes, 'protective' glial stem cells and stromal ependymal cells. *Brain Res. Brain Res. Rev.* **48**, 220–233.

Herrera D. G., Garcia-Verdugo J. M. and Alvarez-Buylla A. (1999) Adult-derived neural precursors transplanted into multiple regions in the adult brain. *Ann. Neurol.* **46**, 867–877.

Hirano T., Akira S., Taga T. and Kishimoto T. (1990) Biological and clinical aspects of interleukin 6. *Immunol. Today* **11**, 443–449.

Hirsch E. C., Breidert T., Rousselet E., Hunot S., Hartmann A. and Michel P. P. (2003) The role of glial reaction and inflammation in Parkinson's disease. *Ann. NY Acad. Sci.* **991**, 214–228.

Hopkins S. J. and Rothwell N. J. (1995) Cytokines and the nervous system. I: expression and recognition. *Trends Neurosci.* **18**, 83–88.

Hunot S., Dugas N., Faucheux B., Hartmann A., Tardieu M., Debre P., Agid Y., Dugas B. and Hirsch E. C. (1999) FcepsilonRII/CD23 is expressed in Parkinson's disease and induces, *in vitro*, production of nitric oxide and tumor necrosis factor-alpha in glial cells. *J. Neurosci.* **19**, 3440–3447.

Imitola J., Raddassi K., Park K. I. *et al.* (2004) Directed migration of neural stem cells to sites of CNS injury by the stromal cell-derived factor 1alpha/CXC chemokine receptor 4 pathway. *Proc. Natl Acad. Sci. USA* **101**, 18117–18122.

Iosif R. E., Ekdahl C. T., Ahlenius H., Pronk C. J., Bonde S., Kokaia Z., Jacobsen S. E. and Lindvall O. (2006) Tumor necrosis factor receptor 1 is a negative regulator of progenitor proliferation in adult hippocampal neurogenesis. *J. Neurosci.* **26**, 9703–9712.

Iosif R. E., Ahlenius H., Ekdahl C. T., Darsalia V., Thored P., Jovinge S., Kokaia Z. and Lindvall O. (2008) Suppression of stroke-induced progenitor proliferation in adult subventricular zone by tumor necrosis factor receptor 1. *J. Cereb. Blood Flow Metab.* **28**, 1574–1587.

Jiao J. and Chen D. F. (2008) Induction of neurogenesis in non-conventional neurogenic regions of the adult central nervous system by niche astrocyte-produced signals. *Stem Cells* **26**, 1221–1230.

Jin K., Zhu Y., Sun Y., Mao X. O., Xie L. and Greenberg D. A. (2002) Vascular endothelial growth factor (VEGF) stimulates neurogenesis *in vitro* and *in vivo*. *Proc. Natl Acad. Sci. USA* **99**, 11946–11950.

Kabiersch A., del Rey A., Honegger C. G. and Besedovsky H. O. (1988) Interleukin-1 induces changes in norepinephrine metabolism in the rat brain. *Brain Behav. Immunol.* **2**, 267–274.

Kaplan M. and Hinds J. (1977) Neurogenesis in the adult rat: electron microscopic analysis of light radioautographs. *Science* **197**, 1092–1094.

Kempermann G. (2002) Why new neurons? Possible functions for adult hippocampal neurogenesis *J. Neurosci.* **22**, 635–638.

Kempermann G., van Praag H. and Gage F. H. (2000) Activity-dependent regulation of neuronal plasticity and self repair. *Prog. Brain Res.* **127**, 35–48.

Kim W. G., Mohney R. P., Wilson B., Jeohn G. H., Liu B. and Hong J. S. (2000) Regional difference in susceptibility to lipopolysaccharide-induced neurotoxicity in the rat brain: role of microglia. *J. Neurosci.* **20**, 6309–6316.

Koo J. W. and Duman R. S. (2008) IL-1beta is an essential mediator of the antineurogenic and anhedonic effects of stress. *Proc. Natl Acad. Sci. USA* **105**, 751–756.

Kordower J. H., Chu Y., Hauser R. A., Freeman T. B. and Olanow C. W. (2008) Lewy body-like pathology in long-term embryonic nigral transplants in Parkinson's disease. *Nat. Med.* **14**, 504–506.

Kreutzberg G. (1996) Microglia: a sensor for pathological events in the CNS. *Trends Neurosci.* **19**, 312–318.

Ladeby R., Wirenfeldt M., Garcia-Ovejero D., Fenger C., Dissing-Olesen L., Dalmau I. and Finsen B. (2005) Microglial cell population dynamics in the injured adult central nervous system. *Brain Res. Brain Res. Rev.* **48**, 196–206.

Lai K., Kaspar B. K., Gage F. H. and Schaffer D. V. (2003) Sonic hedgehog regulates adult neural progenitor proliferation *in vitro* and *in vivo*. *Nat. Neurosci.* **6**, 21–27.

Langston J. W., Forno L. S., Tetrud J., Reeves A. G., Kaplan J. A. and Karluk D. (1999) Evidence of active nerve cell degeneration in the substantia nigra of humans years after 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine exposure. *Ann. Neurol.* **46**, 598–605.

Lassmann H., Zimprich F., Vass K. and Hickey W. F. (1991) Microglial cells are a component of the perivascular glia limitans. *J. Neurosci. Res.* **28**, 236–243.

Lawson L. J., Perry V. H., Dri P. and Gordon S. (1990) Heterogeneity in the distribution and morphology of microglia in the normal adult mouse brain. *Neuroscience* **39**, 151–170.

Lee S. T., Chu K., Jung K. H. *et al.* (2008) Anti-inflammatory mechanism of intravascular neural stem cell transplantation in haemorrhagic stroke. *Brain* **131**, 616–629.

Lees A. J., Hardy J. and Revesz T. (2009) Parkinson's disease. *Lancet* **373**, 2055–2066.

LeMay L. G., Vander A. J. and Kluger M. J. (1990) Role of interleukin 6 in fever in rats. *Am. J. Physiol.* **258**, R798–R803.

Li J. Y., Englund E., Holton J. L. *et al.* (2008) Lewy bodies in grafted neurons in subjects with Parkinson's disease suggest host-to-graft disease propagation. *Nat. Med.* **14**, 501–503.

Liberto C. M., Albrecht P. J., Herx L. M., Yong V. W. and Levison S. W. (2004) Pro-regenerative properties of cytokine-activated astrocytes. *J. Neurochem.* **89**, 1092–1100.

Lie D. C., Dziewczapolski G., Willhoite A. R., Kaspar B. K., Shults C. W. and Gage F. H. (2002) The adult substantia nigra contains progenitor cells with neurogenic potential. *J. Neurosci.* **22**, 6639–6649.

Lie D. C., Colamarino S. A., Song H. J. *et al.* (2005) Wnt signalling regulates adult hippocampal neurogenesis. *Nature* **437**, 1370–1375.

Lim D. A., Tramontin A. D., Trevejo J. M., Herrera D. G., Garcia-Verdugo J. M. and Alvarez-Buylla A. (2000) Noggin antagonizes BMP signaling to create a niche for adult neurogenesis. *Neuron* **28**, 713–726.

Lindvall O., Rehncrona S., Brundin P. *et al.* (1989) Human fetal dopamine neurons grafted into the striatum in two patients with severe Parkinson's disease. A detailed account of methodology and a 6-month follow-up. *Arch. Neurol.* **46**, 615–631.

Liu J., Marino M. W., Wong G., Grail D., Dunn A., Bettadapura J., Slavin A. J., Old L. and Bernard C. C. (1998) TNF is a potent anti-inflammatory cytokine in autoimmune-mediated demyelination. *Nat. Med.* **4**, 78–83.

Liu B., Du L. and Hong J. S. (2000) Naloxone protects rat dopaminergic neurons against inflammatory damage through inhibition of microglia activation and superoxide generation. *J. Pharmacol. Exp. Ther.* **293**, 607–617.

Lundberg C., Martinez-Serrano A., Cattaneo E., McKay R. D. and Bjorklund A. (1997) Survival, integration, and differentiation of neural stem cell lines after transplantation to the adult rat striatum. *Exp. Neurol.* **145**, 342–360.

Lyons A., Downer E. J., Crotty S., Nolan Y. M., Mills K. H. and Lynch M. A. (2007) CD200 ligand receptor interaction modulates microglial activation *in vivo* and *in vitro*: a role for IL-4. *J. Neurosci.* **27**, 8309–8313.

Ma M., Ma Y., Yi X., Guo R., Zhu W., Fan X., Xu G., Frey W. H. II and Liu X. (2008) Intranasal delivery of transforming growth factor-beta1 in mice after stroke reduces infarct volume and increases neurogenesis in the subventricular zone. *BMC Neurosci.* **9**, 117.

Ma D. K., Jang M. H., Guo J. U. *et al.* (2009) Neuronal activity-induced Gadd45b promotes epigenetic DNA demethylation and adult neurogenesis. *Science* **323**, 1074–1077.

Magnus T., Chan A., Grauer O., Toyka K. V. and Gold R. (2001) Microglial phagocytosis of apoptotic inflammatory T cells leads to down-regulation of microglial immune activation. *J. Immunol.* **167**, 5004–5010.

Marchetti B. and Abbracchio M. P. (2005) To be or not to be (inflamed) – is that the question in anti-inflammatory drug therapy of neurodegenerative disorders? *Trends Pharmacol. Sci.* **26**, 517–525.

Matyszak M. K., Lawson L. J., Perry V. H. and Gordon S. (1992) Stromal macrophages of the choroid plexus situated at an interface between the brain and peripheral immune system constitutively express major histocompatibility class II antigens. *J. Neuroimmunol.* **40**, 173–181.

McCoy M. K. and Tansey M. G. (2008) TNF signaling inhibition in the CNS: implications for normal brain function and neurodegenerative disease. *J. Neuroinflammation* **5**, 45.

McGeer P. L., Itagaki S., Boyes B. E. and McGeer E. G. (1988) Reactive microglia are positive for HLA-DR in the substantia nigra of Parkinson's and Alzheimer's disease brains. *Neurology* **38**, 1285–1291.

Mendez I., Vinuela A., Astradsson A. *et al.* (2008) Dopamine neurons implanted into people with Parkinson's disease survive without pathology for 14 years. *Nat. Med.* **14**, 507–509.

Ming G. L. and Song H. (2005) Adult neurogenesis in the mammalian central nervous system. *Annu. Rev. Neurosci.* **28**, 223–250.

Mirza B., Hadberg H., Thomsen P. and Moos T. (2000) The absence of reactive astrocytosis is indicative of a unique inflammatory process in Parkinson's disease. *Neuroscience* **95**, 425–432.

Mogi M. and Nagatsu T. (1999) Neurotrophins and cytokines in Parkinson's disease. *Adv. Neurol.* **80**, 135–139.

Monje M. L., Toda H. and Palmer T. D. (2003) Inflammatory blockade restores adult hippocampal neurogenesis. *Science* **302**, 1760–1765.

Munoz-Fernandez M. A. and Fresno M. (1998) The role of tumour necrosis factor, interleukin 6, interferon-gamma and inducible nitric oxide synthase in the development and pathology of the nervous system. *Prog. Neurobiol.* **56**, 307–340.

Naitoh Y., Fukata J., Tominaga T., Nakai Y., Tamai S., Mori K. and Imura H. (1988) Interleukin-6 stimulates the secretion of adrenocorticotropic hormone in conscious, freely-moving rats. *Biochem. Biophys. Res. Commun.* **155**, 1459–1463.

Nolan Y., Maher F. O., Martin D. S., Clarke R. M., Brady M. T., Bolton A. E., Mills K. H. and Lynch M. A. (2005) Role of interleukin-4 in regulation of age-related inflammatory changes in the hippocampus. *J. Biol. Chem.* **280**, 9354–9362.

Nyfeler Y., Kirch R. D., Mantei N., Leone D. P., Radtke F., Suter U. and Taylor V. (2005) Jagged1 signals in the postnatal subventricular zone are required for neural stem cell self-renewal. *EMBO J.* **24**, 3504–3515.

Olanow C. W., Goetz C. G., Kordower J. H. *et al.* (2003) A double-blind controlled trial of bilateral fetal nigral transplantation in Parkinson's disease. *Ann. Neurol.* **54**, 403–414.

Palmer T. D. (2002) Adult neurogenesis and the vascular Nietzsche. *Neuron* **34**, 856–858.

Palmer T. D., Markakis E. A., Willhoite A. R., Safar F. and Gage F. H. (1999) Fibroblast growth factor-2 activates a latent neurogenic program in neural stem cells from diverse regions of the adult CNS. *J. Neurosci.* **19**, 8487–8497.

Perry V. H. and Gordon S. (1988) Macrophages and microglia in the nervous system. *Trends Neurosci.* **11**, 273–277.

Perry V. H., Hume D. A. and Gordon S. (1985) Immunohistochemical localization of macrophages and microglia in the adult and developing mouse brain. *Neuroscience* **15**, 313–326.

Perry V., Andersson P.-B. and Gordon S. (1993a) Macrophages and inflammation in the central nervous system. *Trends Neurosci.* **16**, 268–273.

Perry V., Newman T. and Cunningham C. (2003) The impact of systemic infection on the progression of neurodegenerative disease. *Nat. Rev. Neurosci.* **4**, 103–112.

Perry V. H., Cunningham C. and Holmes C. (2007) Systemic infections and inflammation affect chronic neurodegeneration. *Nat. Rev. Immunol.* **7**, 161–167.

Pitossi F., del Rey A., Kabiersch A. and Besedovsky H. (1997) Induction of cytokine transcripts in the central nervous system and pituitary following peripheral administration of endotoxin to mice. *J. Neurosci. Res.* **48**, 287–298.

Plata-Salaman C. R. (1988) Food intake suppression by growth factors and platelet peptides by direct action in the central nervous system. *Neurosci. Lett.* **94**, 161–166.

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

Probert L., Akassoglou K., Pasparakis M., Kontogeorgos G. and Kollias G. (1995) Spontaneous inflammatory demyelinating disease in transgenic mice showing central nervous system-specific expression of tumor necrosis factor alpha. *Proc. Natl Acad. Sci. USA* **92**, 11294–11298.

Proescholdt M. G., Chakravarty S., Foster J. A., Foti S. B., Briley E. M. and Herkenham M. (2002) Intracerebroventricular but not intravenous interleukin-1beta induces widespread vascular-mediated leukocyte infiltration and immune signal mRNA expression followed by brain-wide glial activation. *Neuroscience* **112**, 731–749.

Pugh C. R., Nguyen K. T., Gonyea J. L., Fleshner M., Wakins L. R., Maier S. F. and Rudy J. W. (1999) Role of interleukin-1 beta in impairment of contextual fear conditioning caused by social isolation. *Behav. Brain Res.* **106**, 109–118.

Pugh R. C., Fleshner M., Watkins L. R., Maier S. F. and Rudy J. W. (2001) The immune system and memory consolidation: a role for the cytokine IL-1beta. *Neurosci. Biobehav. Rev.* **25**, 29–41.

Ramirez-Castillejo C., Sanchez-Sanchez F., Andreu-Agullo C., Ferron S. R., Aroca-Aguilar J. D., Sanchez P., Mira H., Escribano J. and Farinas I. (2006) Pigment epithelium-derived factor is a niche signal for neural stem cell renewal. *Nat. Neurosci.* **9**, 331–339.

Redmond D. E. Jr, Bjugstad K. B., Teng Y. D. *et al.* (2007) Behavioral improvement in a primate Parkinson's model is associated with multiple homeostatic effects of human neural stem cells. *Proc. Natl Acad. Sci. USA* **104**, 12175–12180.

Rock R. B., Gekker G., Hu S., Sheng W. S., Cheeran M., Lokensgard J. R. and Peterson P. K. (2004) Role of microglia in central nervous system infections. *Clin. Microbiol. Rev.* **17**, 942–964 (table of contents).

Rolls A., Shechter R. and Schwartz M. (2009) The bright side of the glial scar in CNS repair. *Nat. Rev. Neurosci.* **10**, 235–241.

Rothwell N. J. and Hopkins S. J. (1995) Cytokines and the nervous system II: actions and mechanisms of action. *Trends Neurosci.* **18**, 130–136.

Rothwell N. J., Luheshi G. and Toulmond S. (1996) Cytokines and their receptors in the central nervous system: physiology, pharmacology, and pathology. *Pharmacol. Ther.* **69**, 85–95.

Sanchez-Pernaute R., Ferree A., Cooper O., Yu M., Brownell A. L. and Isacson O. (2004) Selective COX-2 inhibition prevents progressive dopamine neuron degeneration in a rat model of Parkinson's disease. *J. Neuroinflammation* **1**, 6.

Satoh T., Nakamura S., Taga T., Matsuda T., Hirano T., Kishimoto T. and Kaziro Y. (1988) Induction of neuronal differentiation in PC12 cells by B-cell stimulatory factor 2/interleukin 6. *Mol. Cell. Biol.* **8**, 3546–3549.

Schanzer A., Wachs F. P., Wilhelm D. *et al.* (2004) Direct stimulation of adult neural stem cells *in vitro* and neurogenesis *in vivo* by vascular endothelial growth factor. *Brain Pathol. (Zurich, Switzerland)* **14**, 237–248.

Schmidt H. D. and Duman R. S. (2007) The role of neurotrophic factors in adult hippocampal neurogenesis, antidepressant treatments and animal models of depressive-like behavior. *Behav. Pharmacol.* **18**, 391–418.

Schneider H., Pitossi F., Balschun D., Wagner A., del Rey A. and Besedovsky H. O. (1998) A neuromodulatory role of interleukin-1beta in the hippocampus. *Proc. Natl Acad. Sci. USA* **95**, 7778–7783.

Schöbitz B., Holsboer F. and de Kloet E. R. (1994) Cytokines in the healthy and diseased brain. *NIPS* **9**, 138–142.

Shen Q., Goderie S. K., Jin L., Karanth N., Sun Y., Abramova N., Vincent P., Pumiglia K. and Temple S. (2004) Endothelial cells stimulate self-renewal and expand neurogenesis of neural stem cells. *Science* **304**, 1338–1340.

Song H., Stevens C. and Gage F. (2002) Astroglia induce neurogenesis from adult neural stem cells. *Nature* **417**, 39–44.

Streit W. J., Walter S. A. and Pennell N. A. (1999) Reactive microgliosis. *Prog. Neurobiol.* **57**, 563–581.

Sun L., Lee J. and Fine H. A. (2004) Neuronally expressed stem cell factor induces neural stem cell migration to areas of brain injury. *J. Clin. Invest.* **113**, 1364–1374.

Takahashi K., Yasuhara T., Shingo T. *et al.* (2008) Embryonic neural stem cells transplanted in middle cerebral artery occlusion model of rats demonstrated potent therapeutic effects, compared to adult neural stem cells. *Brain Res.* **1234**, 172–182.

Tropepe V., Craig C. G., Morshead C. M. and van der Kooy D. (1997) Transforming growth factor-alpha null and senescent mice show decreased neural progenitor cell proliferation in the forebrain subependyma. *J. Neurosci.* **17**, 7850–7859.

Turnbull A. V., Pitossi F. J., Lebrun J. J., Lee S., Meltzer J. C., Nance D. M., del Rey A., Besedovsky H. O. and Rivier C. (1997) Inhibition of tumor necrosis factor-alpha action within the CNS markedly reduces the plasma adrenocorticotropin response to peripheral local inflammation in rats. *J. Neurosci.* **17**, 3262–3273.

Vallieres L. and Rivest S. (1997) Regulation of the genes encoding interleukin-6, its receptor, and gp130 in the rat brain in response to the immune activator lipopolysaccharide and the proinflammatory cytokine interleukin-1beta. *J. Neurochem.* **69**, 1668–1683.

Vallières L., Campbell I., Gage F. and Sawchenko P. (2002) Reduced hippocampal neurogenesis in adult transgenic mice with chronic astrocytic production of interleukin-6. *J. Neurosci.* **22**, 486–492.

Vila M., Jackson-Lewis V., Guegan C., Wu D. C., Teismann P., Choi D. K., Tieu K. and Przedborski S. (2001) The role of glial cells in Parkinson's disease. *Curr. Opin. Neurol.* **14**, 483–489.

Wachs F. P., Winner B., Couillard-Despres S., Schiller T., Aigner R., Winkler J., Bogdahn U. and Aigner L. (2006) Transforming growth factor-beta1 is a negative modulator of adult neurogenesis. *J. Neuropathol. Exp. Neurol.* **65**, 358–370.

Walsh D., Betmouni S. and Perry V. (2001) Absence of detectable IL-1β production in murine prion disease: a model of chronic neurodegeneration. *J. Neuropathol. Exp. Neurol.* **60**, 173–182.

Wang D. D. and Bordey A. (2008) The astrocyte odyssey. *Prog. Neurobiol.* **86**, 342–367.

Widera D., Mikenberg I., Elvers M., Kaltschmidt C. and Kaltschmidt B. (2006) Tumor necrosis factor alpha triggers proliferation of adult neural stem cells via IKK/NF-kappaB signaling. *BMC Neurosci.* **7**, 64.

Widner H. and Brundin P. (1993) Sequential intracerebral transplantation of allogeneic and syngeneic fetal dopamine-rich neuronal tissue in adult rats: will the first graft be rejected? *Cell Transplant.* **2**, 307–317.

Widner H., Brundin P., Bjorklund A. and Moller E. (1989) Survival and immunogenicity of dissociated allogeneic fetal neural dopamine-rich grafts when implanted into the brains of adult mice. *Exp. Brain Res.* **76**, 187–197.

Wong G., Goldshmit Y. and Turnley A. M. (2004) Interferon-gamma but not TNF alpha promotes neuronal differentiation and neurite outgrowth of murine adult neural stem cells. *Exp. Neurol.* **187**, 171–177.

Wu J. P., Kuo J. S., Liu Y. L. and Tzeng S. F. (2000) Tumor necrosis factor-alpha modulates the proliferation of neural progenitors in the subventricular/ventricular zone of adult rat brain. *Neurosci. Lett.* **292**, 203–206.

Yang G. Y., Liu X. H., Kadoya C., Zhao Y. J., Mao Y., Davidson B. L. and Betz A. L. (1998) Attenuation of ischemic inflammatory response in mouse brain using an adenoviral vector to induce overexpression of interleukin-1 receptor antagonist. *J. Cereb. Blood Flow Metab.* **18**, 840–847.

Yasuhara T., Matsukawa N., Hara K., Yu G., Xu L., Maki M., Kim S. U. and Borlongan C. V. (2006) Transplantation of human neural stem cells exerts neuroprotection in a rat model of Parkinson's disease. *J. Neurosci.* **26**, 12497–12511.

Yoshimura S., Takagi Y., Harada J., Teramoto T., Thomas S. S., Waeber C., Bakowska J. C., Breakefield X. O. and Moskowitz M. A. (2001) FGF-2 regulation of neurogenesis in adult hippocampus after brain injury. *Proc. Natl Acad. Sci. USA* **98**, 5874–5879.

Zhang R. L., Zhang L., Zhang Z. G., Morris D., Jiang Q., Wang L., Zhang L. J. and Chopp M. (2003) Migration and differentiation of adult rat subventricular zone progenitor cells transplanted into the adult rat striatum. *Neuroscience* **116**, 373–382.

