Stem Cell Research (2014) 13, 227–239

Available online at www.sciencedirect.com

ScienceDirect

www.elsevier.com/locate/scr

Signaling through three chemokine receptors triggers the migration of transplanted neural precursor cells in a model of multiple sclerosis

Mikhal E. Cohen[a,1], Nina Fainstein[a,1], Iris Lavon[a,b], Tamir Ben-Hur[a,*]

[a] Department of Neurology, The Agnes Ginges Center for Human Neurogenetics, Hadassah University Medical Center, Jerusalem, Israel
[b] Leslie and Michael Gaffin Centre for Neuro-Oncology, Hadassah University Medical Center, Jerusalem, Israel

Received 7 May 2013; received in revised form 5 May 2014; accepted 13 June 2014
Available online 21 June 2014

Abstract Multiple sclerosis (MS) is a multifocal disease, and precursor cells need to migrate into the multiple lesions in order to exert their therapeutic effects. Therefore, cell migration is a crucial element in regenerative processes in MS, dictating the route of delivery, when cell transplantation is considered. We have previously shown that inflammation triggers migration of multi-potential neural precursor cells (NPCs) into the white matter of experimental autoimmune encephalomyelitis (EAE) rodents, a widely used model of MS. Here we investigated the molecular basis of this attraction.

NPCs were grown from E13 embryonic mouse brains and transplanted into the lateral cerebral ventricles of EAE mice. Transplanted NPC migration was directed by three tissue-derived chemokines. Stromal cell-derived factor-1α, monocyte chemo-attractant protein-1 and hepatocyte growth factor were expressed in the EAE brain and specifically in microglia and astrocytes. Their cognate receptors, CXCR4, CCR2 or c-Met were constitutively expressed on NPCs. Selective blockage of CXCR4, CCR2 or c-Met partially inhibited NPC migration in EAE brains. Blocking all three receptors had an additive effect and resulted in profound inhibition of NPC migration, as compared to extensive migration of control NPCs. The inflammation-triggered NPC migration into white matter tracts was dependent on a motile NPC phenotype. Specifically, depriving NPCs from epidermal growth factor (EGF) prevented the induction of glial commitment and a motile phenotype (as indicated by an in vitro motility assay), hampering their response to neuroinflammation.

In conclusion, signaling via three chemokine systems accounts for most of the inflammation-induced, tissue-derived attraction of transplanted NPCs into white matter tracts during EAE.

Introduction

Multiple sclerosis (MS) is a chronic immune mediated disease of the central nervous system (CNS) characterized by perivascular immune cell infiltrates, myelin destruction and axonal loss, resulting in permanent neurological disability.

* Corresponding author at: Department of Neurology, Hadassah University Medical Center, Ein-Kerem, PO Box 12000, Jerusalem 91120, Israel.
E-mail address: tamir@hadassah.org.il (T. Ben-Hur).
[1] These two authors contributed equally to this work.

http://dx.doi.org/10.1016/j.scr.2014.06.001

Current therapies help prevent recurrent immune attacks on the CNS by an immunomodulatory or immunosuppressive effect, but are inefficient in reversing impaired neurological functions. Therefore stem cell therapy has been suggested as a replacement therapy aimed to promote axon remyelination. Since in MS the immune attack is widespread in the CNS white matter, transplanted cells need to reach the multiple lesion sites in order to achieve beneficial local effects.

The experimental autoimmune encephalomyelitis (EAE) model in mice and rats is widely used to study potential therapies for MS as it reconstitutes an inflammatory environment in the white matter. Previous experiments in our laboratory have shown that migration of transplanted multi-potential neural precursor cells (NPCs) from the lateral cerebral ventricles into white matter tracts is triggered by neuroinflammation: Following intraventricular transplantation in healthy rodents, the NPCs remained confined to the ventricles, whereas NPCs that were transplanted into the ventricles of EAE rodents migrated extensively into the inflamed white matter (Ben-Hur et al., 2003, 2007; Bulte et al., 2003; Einstein et al., 2006; Muja et al., 2011) In addition, transplanted cells are attracted to migrate towards blood vessels surrounded by immune cell infiltrates (Muja et al., 2011). While NPC migration into the white matter is driven by inflammation, the molecular mechanism and specific tissue-derived inflammatory molecules that play a role in this attraction during EAE and MS have not been characterized.

Several cytokines and chemokines possess important chemo-attractant functions during fetal CNS development (Stumm and Hollt, 2007; Li and Ransohoff, 2008). This suggests that neural stem and precursor cells may respond to cues by these mediators of inflammation in immune-mediated pathological conditions. In vitro migration assays showed that stromal cell-derived factor 1α (SDF-1α, CXCL12) enhanced NPC migration in a dose-dependent manner (Robin et al., 2006). Monocyte chemo-attractant protein-1 (MCP-1, CCL2) induces migration of adult neural stem cells in-vitro (Widera et al., 2004) and in an inflammation induced model in hippocampal slices (Belmadani et al., 2006). In vitro studies showed that hepatocyte growth factor (HGF) is secreted by microglia upon TGF-β treatment, attracts oligodendrocyte precursor cells (Lalive et al., 2005) and is also a powerful chemo-attractant molecule for neural stem cells (Heese et al., 2005).

Several pathological conditions lead to the release of chemokines which trigger the migration of neural stem cells in vivo. In a stroke model, SDF-1α plays a critical role in recruiting endogenous stem cells to the lesion site (Imitola et al., 2004). Brain hypoxia leads to the up-regulation of vascular-endothelial growth factor (VEGF), stem cell factor (SCF), SDF-1α, MCP-1, HGF and urokinase-type plasminogen activator and triggers migration of NPCs (Xu et al., 2007; Zhao et al., 2008). Brain tumors attract NPCs through SDF-1α/CXCR4 signaling (van der Meulen et al., 2009), MCP-1/CCR2 signaling (Magge et al., 2009), or HGF/c-Met signaling pathways (Heese et al., 2005). In view of these studies, we examined the role of these factors in mediating EAE-induced NPC migration.

Herein, we show that inflammation-triggered NPC migration in white matter tracts during EAE depends on specific tissue-derived chemokine signaling. Most of NPC migration into white matter tracts during EAE was accounted for by signaling through CXCR4, CCR2 and c-Met receptors on NPCs, by tissue astrocyte- and microglial-derived chemokines. A pre-requisite to the ability of NPCs to migrate in response to neuroinflammatory signals is being motile. We show that exposure to epidermal growth factor induces a motile phenotype in NPCs.

Methods

Induction and clinical scoring of EAE

All animal experiments were approved by our institutional committee. Female 6–7 weeks old SJL mice were immunized by subcutaneous injection of 150 μg of proteolipid protein (PLP) peptide 139–151, diluted in normal saline (0.9% NaCl) and emulsified with complete Freund's adjuvant. Female 6–7 weeks old C57Bl/6 mice were immunized by subcutaneous injection of 300 μg of myelin oligodendrocyte glycoprotein (MOG) peptide corresponding to amino acids 35–55, diluted in normal saline (0.9% NaCl) and emulsified with complete Freund's adjuvant. Bordetella pertussis toxin (300 ng; List Biological Laboratories, Inc.) was diluted in 0.2 ml normal saline and injected intraperitoneally immediately after PLP or MOG immunization and two days after MOG immunization in C57Bl/6 mice. Clinical signs of EAE typically appeared 10–12 days post-immunization, reaching the peak neurological disability within additional 2–3 days for SJL mice and 6–10 days for C57Bl/6 mice. Disease progression and severity was scored daily as follows: 0 = normal; 1 = partial tail weakness; 2 = flaccid tail 3 = difficulty attaining upright posture; 4 = hind limb paralysis; 5 = weakness or paralysis in all limbs/moribund, and 6 = dead. The cumulative score was calculated as the sum of disease scores of the individual animals in each experimental group.

Isolation, growth and characterization of mouse NPCs

NPCs were isolated from the cerebral hemispheres of C57BL/6 E13 embryos of wild type, GFP transgenic or CCR2 knock-out mice and grown as free-floating neurospheres. Briefly, cerebral hemispheres were dissociated using EBSS containing 0.25 mg/ml trypsin (5 min at 37 °C) and 10 μg/ml DNase. Tissue was further dispersed by trituration with a 5 ml pipette. Dissociated cells were transferred to T-75 flasks and expanded for 4 days as neurospheres in serum-free DMEM/F-12 medium supplemented with B27 without vitamin A (Gibco). Cells were supplemented daily with basic fibroblast growth factor (FGF2, 10 ng/ml, R&D Biosystem) and epidermal growth factor (EGF, 20 ng/ml, Peprotech). In some experiments NPC spheres were grown in the presence of only FGF2. For characterization of NPCs, 200–300 neurospheres were plated on poly-D-lysine (10 μg/ml, Sigma) and fibronectin (0.1% from bovine plasma, Sigma) coated sterile plates. Immunostaining was performed after 1 h of adhesion or 5 days of differentiation. Blocking was performed with 3% normal goat serum (NGS) in phosphate buffered saline (PBS). Primary antibodies included goat IgG anti-PDGFR-α (1:150, Santa Cruz Biotech.), rabbit IgG anti-GFAP (1:200, Dako), mouse IgG anti-βIII-tubulin (1:2000, Sigma), goat anti-c-Met (1:100, R&D Biosystem), goat anti-CCR2 (1:1000, Abcam) and rabbit

anti-CXCR4 (1:500, Abcam). Secondary antibodies used included goat anti-mouse IgG Alexa Fluor 488 (1:200, Molecular Probes), goat anti-rabbit IgG Alexa Fluor 488 (1:200, Molecular Probes), and donkey anti-goat IgG Alexa Fluor 488 (1:200, Molecular Probes), as appropriate. Cells were fixed with a solution of 5% acetic acid and 95% ethanol after immunostaining for membranal markers and prior to staining for cytoplasmic markers and covered with mounting medium containing DAPI (Santa Cruz Biotech.)

In vitro migration assay

NPCs isolated from wild type C57Bl/6 E13 hemispheres (as described above) were expanded as spheres in B27 medium supplemented daily with either EGF (20 μg/ml) and FGF2 (10 μg/ml) or with FGF2 (10 μg/ml) alone. Four days old NPC spheres were centrifuged and re-suspended in fresh B27 medium. 150 NPC spheres of each group were plated on separate poly-D-lysine (10 μg/ml, Sigma) and fibronectin (0.1% from bovine plasma, Sigma) coated sterile plates in B27 medium without growth factors. After one hour incubation at 37 °C, the outward migration of neurosphere cells was evaluated using an inverted microscope. Computer microscopic images (×100) of individual spheres were analyzed and classified as spheres with no outward migration, spheres with migration of up to 2 cell diameters (approximately 20 μm) from the sphere's border, and spheres with extensive outward cell migration (over 2 cell diameters). Statistical analysis was performed by chi-square on a total of 100 spheres per experimental condition, in two independent experiments. Error bars represent standard deviations.

BrdU labeling and blocking chemokine receptors

Neural precursor cell spheres that were grown from wild type or CCR2 knock-out fetal brain were labeled with BrdU (2 mM, Sigma) 48 h before transplantation. On the day of transplantation, neurospheres were incubated for 30 min at 37°C with anti-mouse c-Met (HGF-receptor) antibody (2.5 μg/ml, R&D Systems) or at room temperature with AMD3100, a specific CXCR4 (SDF-1α receptor) antagonist (Hatse et al., 2002) (5 μg/ml, Sigma), or both agents at 37 °C.

TUNEL

200–300 neurospheres were plated on poly-D-lysine and fibronectin coated sterile plates for adhesion for 1 h. The In Situ Cell Detection Kit TMR red (Roche) was used to compare cell death rate with the different treatments.

ICV transplantation

2500 neurospheres in 5 μl DMEM/F12 medium were injected using a Hamilton syringe (catalog #702) on day 8 or 14 post-EAE induction into each lateral ventricle, using a stereotactic device (coordinates Bregma A = 0, L = 1 mm, H = 2.2 mm). The procedure was performed under ketamine (80 mg/kg) and xylazine (20 mg/kg) anesthesia.

Histopathology

Three days after ICV transplantation (day 17 after PLP-EAE induction, or 21 days after MOG-EAE induction), mice were deeply anesthetized (sodium pentobarbital, 200 mg/kg i.p.) and transcardially perfused with ice-cold PBS followed by 4% formalin. The brains were removed and further fixated by immersion in 4% formalin for 24 h. Coronal 10 μm thick frozen sections were cut, and every 6th section was stained with DAPI to enable anatomic orientation using a mouse brain atlas. Brain sections were blocked for 1 h using PBS with 5% normal goat serum and 5% BSA and then incubated overnight with the primary antibody at 4 °C, followed by 1 h incubation with the secondary antibody at room temperature. Primary antibodies included rabbit anti-CD11b (1:200, IBA1, Wako), rabbit IgG anti-GFAP (1:200, Dako), rabbit anti-SDF-1α (1:200, Abcam), rabbit anti-MCP-1 (1:200, Abcam), rabbit anti-HGF (1:200, Abcam), and rat anti-BrdU (1:200, AbD Serotec). Secondary antibodies used included goat anti-mouse IgG Alexa Fluor 488 (1:200, Molecular Probes), goat anti-rabbit IgG Alexa Fluor 488 (1:200, Molecular Probes), goat anti-rat IgG and IgM Alexa Fluor 555 (1:200, Molecular Probes). Brain slices were covered with mounting medium containing DAPI (Santa Cruz Biotech.). Images of specific immunofluorescent reactivity were obtained using a Nikon DXm1200F camera attached to an Olympus BX51 microscope or using a confocal microscope (Zeiss, Feldbach, Switzerland).

Migration measurements

The migration of transplanted cells was evaluated microscopically by GFP fluorescence and BrdU immunostaining. Serial 10 μm coronal brain sections (105 sections per brain) were obtained spanning the ventricular system between Bregma −2.7 and +0.1 mm. All sections were observed under the fluorescent microscope to identify those with transplants and parenchymal migration. Measurements were performed in the corpus callosum of 3 representative sections for each brain. An ocular grid of determined scale was used to count the number of squares spanning the entire distance of migration. The maximal distance of migration was determined in each section as the distance from the edge of the ventricle to the most distant migrating cell. Then, the segment representing the distal third of the entire migrating cell tract was defined in each section using an ocular grid. The numbers of GFP^+ and BrdU labeled cells were counted in this defined segment. The number of chemokine receptor blocked ($BrdU^+$) cells was divided by the number of control (GFP^+) cells, such that an equal number of $BrdU^+$ and GFP^+ cells (representing equal migration) would result in a proportion of 1.

RNA extraction, cDNA preparation RT-PCR and PCR

Total RNA was extracted from neurospheres using the SV total RNA kit (Promega). cDNA was prepared from 1 μg of total RNA using MuLV reverse transcriptase (Applied Biosystems) and random hexamers according to the manufacturer's instructions for first-strand cDNA synthesis. The PCR reaction mixture included 1.5 μl of cDNA, 300 nmol/l concentrations of the appropriate forward and reverse primers (Syntezza, Israel)

and 9.5 μl of the master mix buffer containing nucleotides and Red Load Taq polymerase (Larova) in a total volume of 15 μl. Gene amplification was carried out using the GeneAmp 9700 Sequence Detection System (Applied Biosystems). Amplification included one stage of 10 min at 95 °C, followed by 40 cycles of a two-step loop: 20 s at 95 °C and 1 min at 60 °C.

For real time PCR the reaction mixture included 1 μl of cDNA, 300 nmol/l concentrations of the appropriate forward and reverse primers (Syntezza, Israel), and 7.5 μl of SYBR green mix (SYBR Green Master Mix, Applied Biosystems) in a total volume of 15 μl. Gene amplification was carried out using the GeneAmp 7000 PCR system (Applied Biosystems). The gene expression results were normalized to the HPRT gene. Two independent experiments were repeated in triplicate and are presented as the standard error (SE) of mean. For the mRNA quantification, the fold change was normalized to the HPRT transcript (ΔCT). Following normalization, the fold change of each mRNA was calculated between the EAE, CNS tissue and naïve brain reference (ΔΔCT). Statistical significance of induction of gene expression as compared to its respective naïve control was calculated using 2-tailed *t* test.

The following primers were used:

Gene	Forward	Reverse
CXCR4	5′-CGGCTGTAGA GCGAGTGTTG-3′	5′-GACCCCACTTCTT CAGAGTAGTTATCA-3′
CCR2	5′-CCATGCAAGT TCAGCTGCCT-3′	5′-TGCCGTGGATGA ACTGAGG-3′
c-Met	5′-TGCAGCGCGT CGACTTATT-3′	5′-TGAGGTGTGCT GTTCGAGAGAG-3′
18S	5′-GGCCCTGTAA TTGGAA-3′	5′-CCCTCCAATGG ATCCTCGTT-3′

Western blot analysis

The cortex, corpus callosum and spinal cord from 4 naïve and 5 EAE (13 days post-induction) SJL mice were homogenized to 10% (w/v) in cold homogenization buffer (320 mM sucrose; 10 mM EDTA in PBS), followed by centrifugation at 3000 rpm for 15 min at 4 °C. Homogenates from each group were pooled and normalized for total protein level using BCA assay (Pierce); 500 μg from each sample was extracted in 2% Sarkosyl and boiled for 5 min at 100 °C in sample buffer containing β-mercaptoethanol. Samples were applied to 8% (for HGF and PRP detection) or 18% (for SDF-1α and MCP-1 detection) SDS-PAGE and electrotransferred to nitrocellulose membrane in a Tris/glycine buffer. Membranes were blocked with 5% milk in TBST (10 mM Tris–HCl, pH 7.4, 150 mM NaCl, 0.05% Tween 20), incubated overnight at 4 °C with rabbit anti-SDF-1α (Abcam, 1:1000), with goat anti-MCP-1 (R&D systems, 1:1000), with goat anti-HGF (R&D systems, 1:1000) or with a rabbit polyclonal anti-prion protein RTC (Canello et al., 2010, 1:250) primary antibodies, washed and incubated for 1 h with anti-rabbit or anti-goat AP secondary antibodies (Promega 1:15,000).

Statistical analysis

Chi square test was used to compare the in vivo and in vitro motility of NPCs cultured with daily supplementation of FGF2 versus FGF2 and EGF. Comparisons between treated and untreated NPC's maximal distance of migration and number of treated cells in the last portion of migration (+/− S.E.M.) were performed using the paired 2-tailed Student's *t*-test. The effects of NPC spheres on the cumulative daily score of EAE severity was compared to sham-operated controls using 2-tailed Student's *t*-test. Error bars in the real-time PCR graphs represent the standard error of mean.

Results

Intracerebroventricular transplanted NPCs migrate in response to neuroinflammation

Our previous studies examined the migratory response of NPCs to neuroinflammation in MOG-induced EAE in C57Bl/6 mice. Therefore we first examined whether this is a universal response and whether this occurs in a different model. Neural precursor cells were isolated from E13 embryonic brains of GFP transgenic C57Bl/6 mice and expanded in spheres for 4 days in B27 medium supplemented daily with FGF2 and EGF. The cells grew into floating neurospheres and were transplanted into the lateral cerebral ventricles of either naïve or EAE induced SJL mice (5 animals per group) on day 8 post induction. EAE was induced by immunization with PLP peptide. Mice brains were examined histologically at day 22. In naïve mice, transplanted NPCs remained in the ventricles (Fig. 1A), while in EAE mice NPC spheres migrated extensively into the corpus callosum (Fig. 1B).

NPCs express chemokine receptors

In view of the role of CXCR4 (receptor of SDF-1α), CCR2 (receptor of MCP-1, MCP-5 and other chemokines) and c-Met (receptor of HGF) in cell migration during development and other pathological conditions, we examined their expression on NPCs. PCR showed the expression of all three receptors on NPCs (Fig. 2A). In addition, strong immunofluorescent staining for CXCR4, CCR2 and c-Met was detected on the vast majority of sphere cells (Figs. 2B–D).

Migration of NPCs in the EAE brain is inhibited by blocking chemokine receptors on NPCs

Our previous studies indicated that migration of ICV transplanted NPCs in EAE is a highly variable phenomenon. Specifically, there was significant variability across individual animals in the side, tract, direction and extent of NPC migration, making it difficult to perform a comparison between groups (Ben-Hur et al., 2007; Muja et al., 2011). Therefore, in order to evaluate the role of chemokines in determining NPC migration during EAE we designed an experiment in which the migration of NPCs whose chemokine receptors were blocked was directly compared to that of control untreated NPCs that were co-transplanted into the same animal. First, untreated BrdU-labeled NPC spheres were mixed with an equal number of GFP^+ NPC spheres and co-transplanted into the lateral ventricle of PLP-EAE SJL mice. The two cell populations exhibited similar migratory properties. This enabled us to compare the migration of BrdU-labeled NPCs in which chemokine receptors were

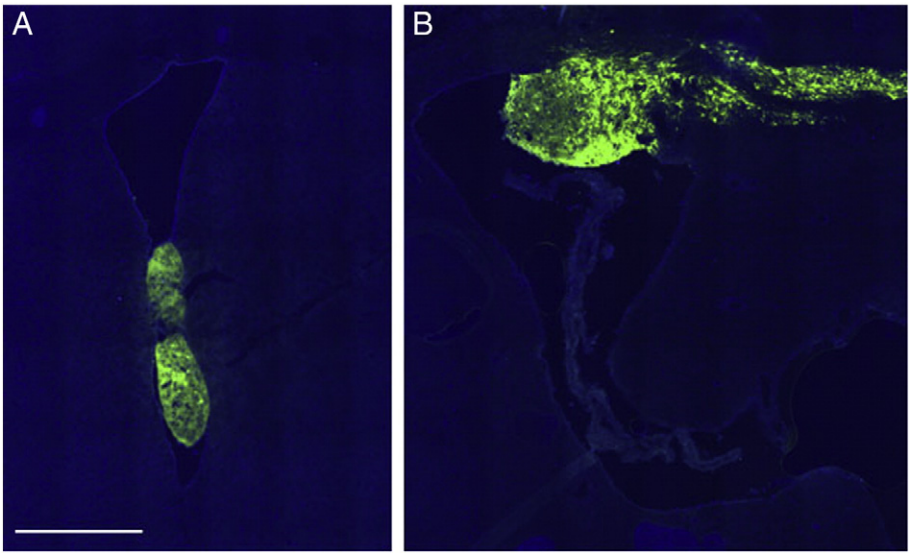

Figure 1 NPCs migrate into white matter tracts in response to neuro-inflammation. NPC spheres ICV transplanted to naïve mice were found at 22 days after the injection in the ventricles without migrating into the tissue (A). NPCs that were transplanted on day 8 after EAE induction were observed at day 22 to have migrated extensively into the corpus callosum (B), Scale bar 1 mm.

blocked to that of control GFP^+ NPCs. BrdU labeled NPC spheres were incubated prior to transplantation with either an anti-c-Met blocking antibody in order to prevent response to HGF, or with the CXCR4 antagonist AMD3100, to prevent response to SDF-1α. The blocking agents did not affect NPC viability as examined with a TUNEL assay (Supplementary data 1). A third group of NPC spheres was grown from E13 fetal brains of CCR2 knock-out mice and labeled with BrdU. In addition, a fourth group of NPC spheres from CCR2 knock-out fetal brains was incubated with both anti-c-Met antibody and AMD3100 to inhibit response to all three chemokines. Given the time-limited effect of blocking agents on the receptors (Hatse et al., 2002), we performed a short term experiment and evaluated the extent of migration at 72 h post-transplantation. It should

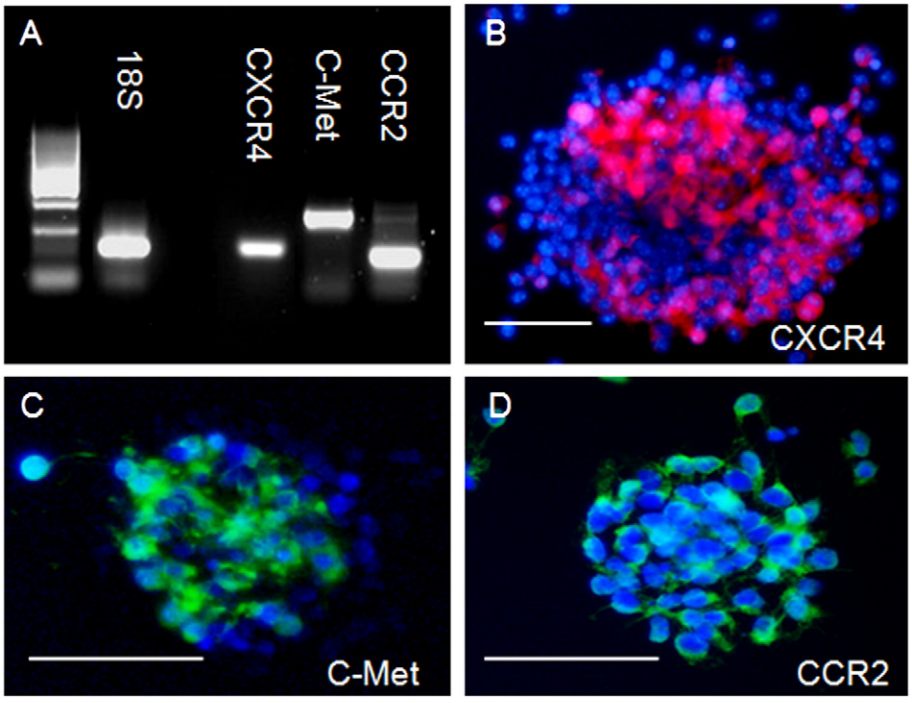

Figure 2 Neural precursor cells express several chemokine receptors. Neurosphere cells express the receptors CXCR4 (SDF-1α receptor), c-Met (HGF receptor) and CCR2 (MCP-1 receptor), as shown by PCR (A). Strong expression of chemokine receptors in NPCs was also detected by immunofluorescence staining (B–D), Scale bar: 0.1 mm.

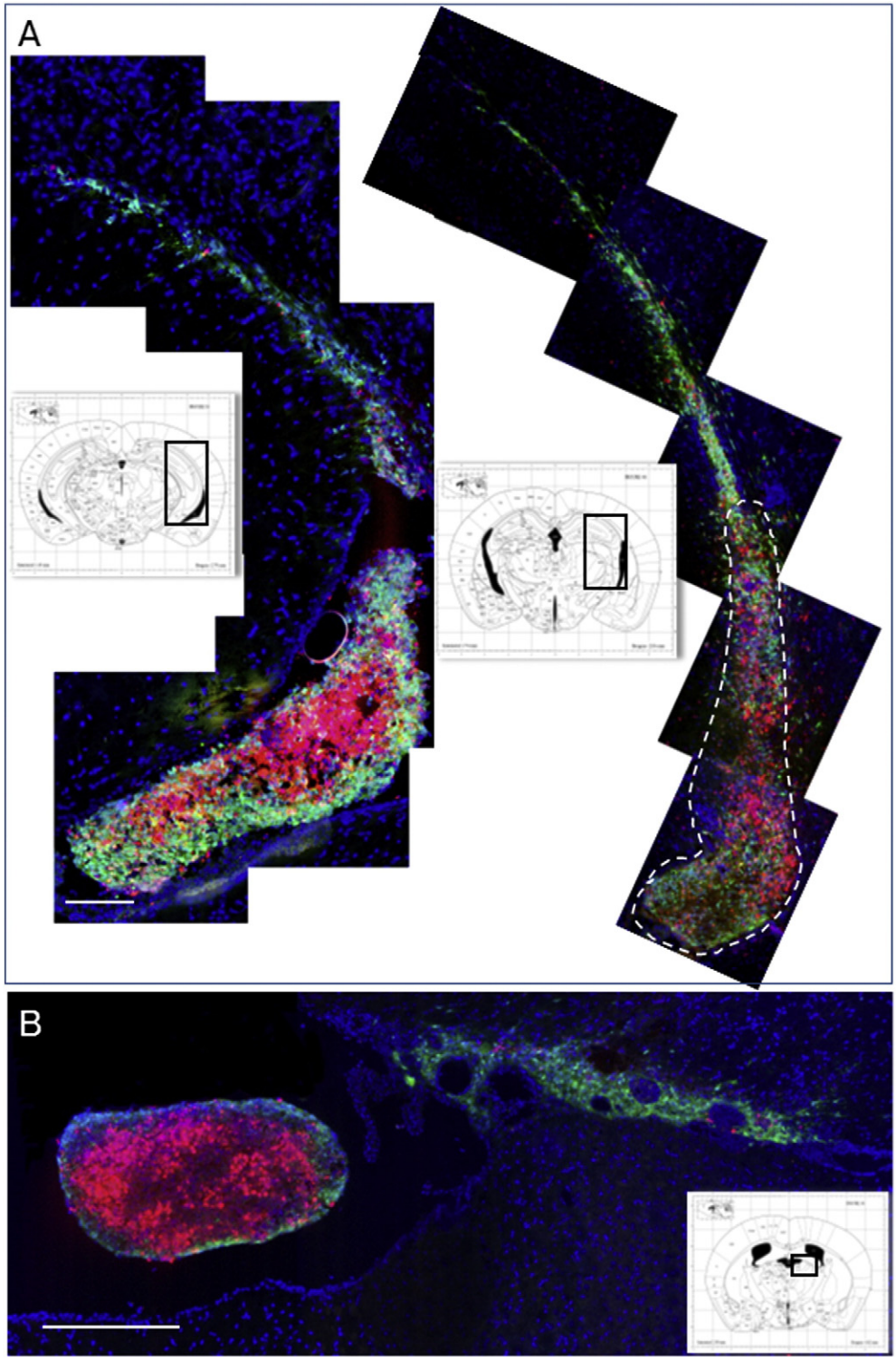

Figure 3 Blocking of CXCR4, CCR2 and c-Met on NPCs inhibits their migration in the EAE brain. BrdU labeled NPCs from CCR2 ko mice which were incubated prior to transplantation with the CXCR4 inhibitor AMD3100 and with an anti-c-Met blocking antibody were co-transplanted with GFP^+ NPCs (as controls) into the lateral ventricles of PLP-induced EAE SJL mice. While many GFP^+ control cells (green) exited the lateral ventricles and migrated extensively in the corpus callosum, the blocked $BrdU^+$ cells (red) remained mostly in the ventricle (A). Similarly, in MOG-induced EAE in C57Bl/6 mice, GFP^+ control NPCs (green) exited the ventricle and migrated extensively into the fimbria, whereas BrdU labeled NPCs (red) from CCR2 ko mice which were incubated with the AMD3100 and with anti-c-Met antibody remained mostly in the ventricle (B). Scale bars: 0.25 mm.

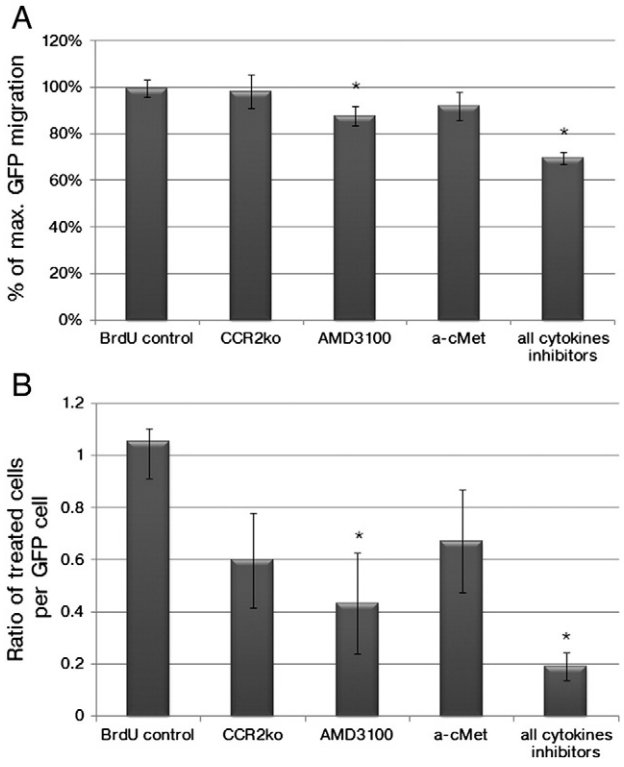

Figure 4 Quantification of the inhibition of NPC migration by blocking chemokine receptors. The maximal distance of migration in the corpus callosum was compared in individual animals between control GFP NPCs and BrdU-labeled NPCs, non-treated or in which one or all three chemokine receptors were blocked. Blocking CCR2 or c-Met alone did not induce a significant effect on migration. Blocking CXCR4 caused a mild inhibition of migration. Blocking all three chemokine receptors induced a significant 31% inhibition of NPC migration (A). The proportion of BrdU labeled cells as per control GFP^+ cells was assessed in the distal third segment of migration. An equal number of $BrdU^+$ and GFP^+ cells will result in a proportion of 1. Blocking each chemokine receptor alone caused a 33–57% inhibition of NPC migration. When all three receptors were blocked there was massive (81%) inhibition of NPC migration (B). Number of animals per group: BrdU control: 2 mice, CXCR2: 5 mice, AMD3100: 4 mice, α-cMet: 4 mice and all chemokines: 7 mice.

be noted that given the short term transplantation experiment (72 h), the low immunogenicity of stem cells (Hori et al., 2003; Mammolenti et al., 2004) and the universal nature of NPC migration in response to neuroinflammation (Ben-Hur et al., 2007), the allogeneic experimental design (transplantation of C57Bl/6 derived NPCs into PLP-EAE SJL mice) did not pose any issues of graft rejection. For each of these groups NPC migration was compared individually to co-transplanted control GFP^+ NPCs. When all three blocking agents were used, profound inhibition on NPC migration was observed (Fig. 3A). The extent of migration was compared between treated and control NPCs by two measures. First, the maximal distance of migration represents the distance from the edge of the lateral ventricle into which NPC spheres were transplanted to the most distant migrating cell. The maximal distance of migration of CCR2 k-o NPCs that were also incubated with both AMD3100 and α-c-Met antibody was significantly reduced by 31% ($p < 0.001$) in comparison to control GFP cells (Fig. 4A). It was evident that these measurements did not reflect the profound effect that was observed microscopically, probably since some NPCs evaded the effect of chemokine-receptor blockers. We therefore quantified also the number of migrating cells, and specifically in the segment representing the last third of the migration chain of control NPCs. When all three chemokine receptors were blocked, the number of cells reaching the last segment was reduced by 81% ($p < 0.001$) as compared to control GFP^+ NPCs (Fig. 4B). To examine the relative role of each chemokine signaling system, we measured the migration of NPCs in which only one chemokine receptor was blocked. The maximal distance of migration of CCR2 k-o NPCs was not significantly reduced as compared to that of control GFP^+ cells. Incubation of wild type NPCs with AMD3100 reduced the maximal distance of migration by 12% ($p = 0.02$). Incubation with anti-c-Met reduced NPC migration by 8% (not significant). Blocking the effect of each chemokine resulted in partial reduction in number of migrating cells. The proportion of CCR2 k-o NPCs was reduced by 40% (not significant), AMD3100-treated NPCs by 57% ($p = 0.002$), and anti-c-Met-treated NPCs by 33% ($p = 0.087$). In order to verify the consistency of these results in another EAE model, we transplanted CCR2 k-o BrdU-labeled NPCs incubated with AMD3100 and α-c-Met together with GFP^+ NPCs in 3 MOG-EAE C57Bl/6 mice at the peak of the disease (day 18). The mice were euthanized after 72 h. Migration of the BrdU labeled NPCs was massively inhibited compared to that of the control GFP^+ NPCs (Fig. 3B).

Chemokine expression in the CNS during EAE

Our findings indicated a central role of CCR2, CXCR4 and c-Met in mediating NPC migration in EAE. We therefore examined the expression of their main related chemokines. Stromal cell derived factor 1α (SDF-1α) and hepatocyte growth factor (HGF) are the only known ligand of CXCR4 and c-MET, respectively (Birchmeier et al., 2003). Monocyte chemo-attractant protein 1 (MCP-1) is the major CCR2 ligand in EAE mice (Huang et al., 2001). Immunofluorescent staining was performed on brain slices to compare the actual presence of these chemokines in naïve versus PLP-EAE conditions. Very low levels of SDF-1α, MCP-1 and HGF were detected in naïve brains. At the peak of EAE an increase in tissue staining of these chemokines was observed, and mainly in white matter tracts, such as the corpus callosum and fimbria (Figs. 5A–B). We quantified also the chemokines by western blot analysis (Fig. 5C). MCP-1 expression was significantly increased in EAE brain tissue. SDF-1α was undetectable in naïve tissues and barely detectable in the EAE corpus callosum and spinal cord tissues, indicating a slight increase. Western blot analysis did not show clear differences in HGF quantity between naïve and EAE brains (Fig. 5C). Chemokine staining was not limited to foci of perivascular inflammation but was induced in resident glial cells throughout the white matter tracts (Figs. 5A–B). Specifically, double immunofluorescent staining showed that the expression of all three chemokines was induced in tissue $Iba\text{-}1^+$ and $Mac\text{-}3^+$ macrophages/microglia (Fig. 6A) and in $GFAP^+$ astrocytes (Fig. 6B).

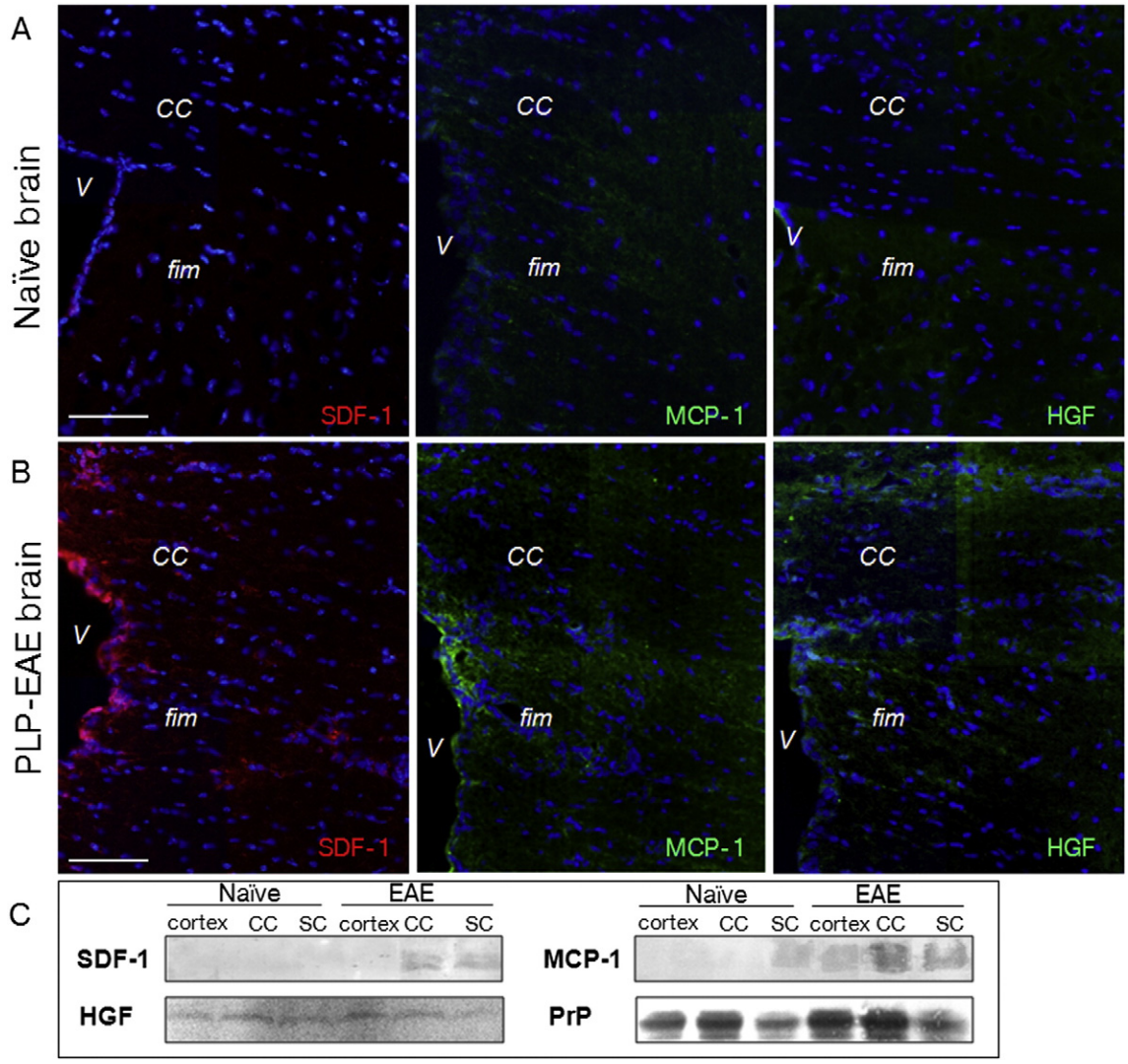

Figure 5 Immunofluorescent staining of SDF-1α, MCP-1, and HGF in naïve and EAE brains. There was negligible staining of the three cytokines in naïve brains (A). There was diffuse tissue expression in the corpus callosum and fimbria of EAE mice (B). Western blot analysis showed an increase in MCP-1 and SDF-1α protein levels in the spinal cord and corpus callosum of EAE mice (C). Prion protein content was analyzed as a housekeeping protein. V = ventricle, CC = corpus callosum, fim = fimbria, SC = spinal cord. Scale bars: 0.1 mm.

NPC migratory response depends on a motile phenotype, induced by EGF

After establishing the role of tissue factors that trigger NPC migration we examined whether intrinsic characteristics of the NPCs affect their ability to respond to such environmental cues. Specifically, we examined whether NPC migratory response depends on a motile phenotype which is acquired by EGF supplementation.

Real time PCR was performed to examine the effect of EGF deprivation on the expression of these receptors. There was a non-significant change in the expression of CCR2 and c-Met. In spheres grown only in FGF2 there was a 4-fold increase in CXCR4 expression (Fig. 7A). However, immunofluorescent staining showed a similar expression of these receptors on NPCs grown only in FGF2 (Supplementary data 2).

In order to characterize the effect of EGF deprivation on NPC motility they were plated on a poly-D-lysine and fibronectin coated substrate in vitro. Within 1 h after plating there was strong outward migration of cells from spheres that had been grown in EGF and FGF2 supplemented media, but only negligible cell migration from spheres that had been grown in FGF2 alone (Fig. 7B, chi square = 45.2, df = 2, $p < 0.001$). This indicates that EGF improves motility of NPCs regardless of any external cue. After an additional 4 days of culture without growth factor supplementation, outward-migrating NPC sphere cells that were previously grown with FGF2 alone differentiated into neurons (38% ± 21%), astrocytes (28% ± 5%) and oligodendrocytes (34% ± 8%), whereas outward-migrating cells from NPC spheres that had been grown with FGF2 and EGF generated only astrocytes (32% ± 3%) and oligodendrocytes (58% ± 13%), and did not give rise to neurons (less than 1%) (Figs. 7C–H). Therefore, EGF also directs fetal NPC fate into glial lineages.

In view of EGF effect on NPC motility in-vitro, we examined whether EGF deprivation affects NPC migratory

response to neuroinflammation. GFP$^+$ NPC spheres that were grown in FGF2 + EGF or FGF2 alone were ICV transplanted at the peak of the disease (days 13–15 post immunization in SJL EAE mice). The mice were euthanized at 3 days post-transplantation and their brains processed for histopathology. Brain sections were examined for cell migration in the corpus callosum and other white matter tract regions. NPCs grown in the presence of EGF and FGF2 migrated extensively in white matter tracts (corpus callosum and fimbria) of 47.5% of the brains (19 of 40 mice), whereas migration of NPCs grown in the presence of FGF2 alone was detected in only 12.5% of the brains (5 of 40 mice, Fig. 7I and J, chi square = 25.13, df = 1, $p < 0.001$). In brains where NPC migration took place, the average maximal distance reached by the cells was not significantly different between the two groups (882 ± 484 μm for the FGF2 group and 955 ± 421 μm for the NPCs grown in EGF and FGF2, $p > 0.1$).

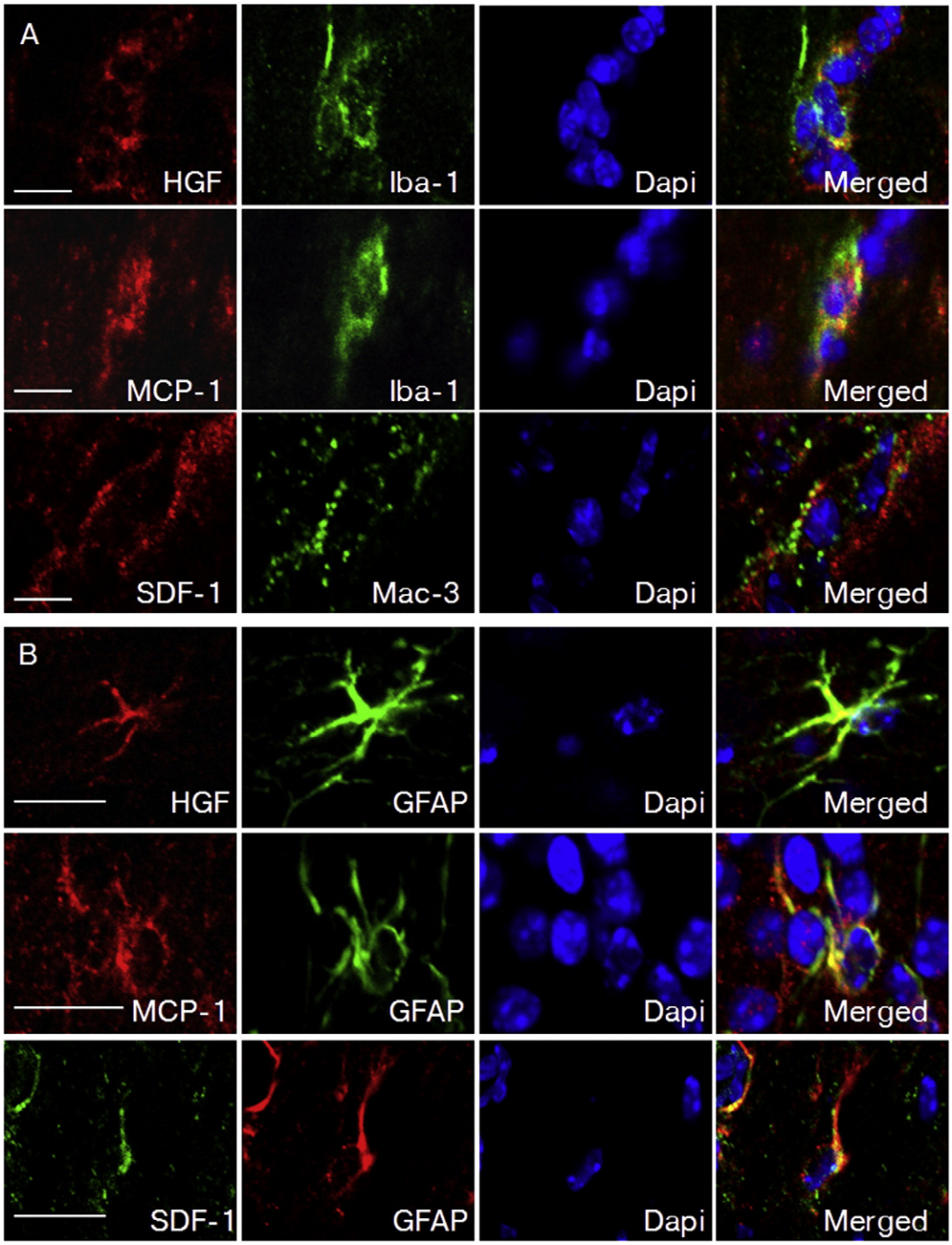

Figure 6 Expression of chemokines by tissue glial cells. Confocal microscopy of double immunofluorescent staining showed the expression of SDF-1α, MCP-1 and HGF in tissue Iba-1$^+$/Mac-3$^+$ microglia (A) and in GFAP$^+$ astrocytes (B). Scale bars: 25 μm.

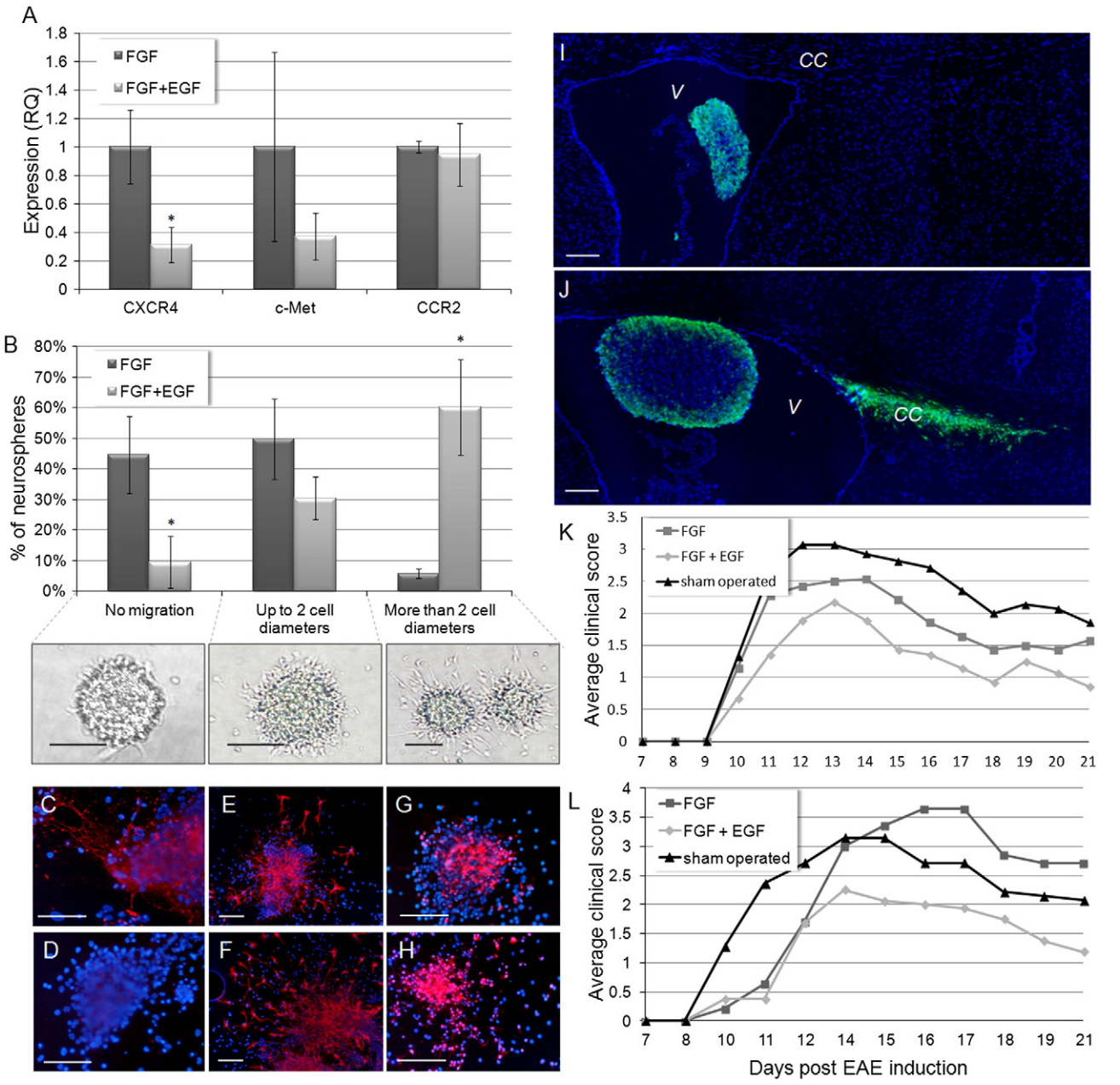

Figure 7 EGF confers a motile and glial-only phenotype to NPCs, associated with improved therapeutic properties. There was no significant change in the mRNA level of CCR2 and c-Met expression in neurospheres deprived of EGF, while expression of CXCR4 was mildly increased (A). An in vitro motility assay was used to examine the effect of EGF by measurement of outward NPC migration from the neurosphere after 1 h of adherence to coated plates. NPCs grown in FGF2 supplemented medium alone did not exhibit any migration whereas in most of the NPC spheres grown in FGF2 and EGF supplemented medium there was an extensive outward migration of more than two cell diameters (B). Neurosphere cells cultured with FGF2 alone express the neuronal marker βIII-tubulin (C) whereas neurospheres cultured with FGF2 and EGF do not (D). Neurospheres grown with FGF2 and neurospheres grown with FGF2 and EGF express the oligodendrocyte progenitor marker PDGFR-α (respectively E and F) and the astrocyte marker GFAP (respectively G and H). Scale bar: 0.1 mm. The migration of intracerebroventricular-transplanted GFP^+ NPC spheres into white matter tracts in EAE mice was examined by fluorescence microscopy. In the vast majority of EAE mice transplanted with NPC spheres grown in FGF2 only, GFP^+ cells remained in the ventricular space and did not migrate into the parenchyma (I). When NPC spheres were grown in FGF2 and EGF there was an extensive migration into the white matter (J). V = ventricle, CC = corpus callosum, Scale bars: 0.1 mm. Transplanted NPCs grown in FGF2 and EGF are superior to NPCs grown in FGF2 alone in ameliorating EAE clinical score. Mice were transplanted with neurospheres cultured either with FGF2 or with FGF2 and EGF, 8 days after EAE induction. Neurospheres cultured with FGF2 and EGF attenuated the severity of EAE, while neurospheres that were cultured in FGF2 alone exhibited a non-significant effect. This was observed both in SJL mice PLP-EAE model (K) and in C57Bl/6 mice MOG-EAE model (L).

In order to examine whether induction of motility and glial lineage preference was associated with a functional phenotype, we examined the ability of NPCs grown in FGF2 alone or in FGF2 and EGF to attenuate PLP-EAE following ICV transplantation in comparison to sham operated EAE SJL mice. NPC spheres were transplanted (5000 spheres per mouse, 7 SJL mice per experimental group) at day 8 post EAE induction and animals were followed for 21 days. Disease severity was compared between experimental groups (Fig. 7K). In mice transplanted with NPCs grown in FGF2 alone there was a mild, non-significant attenuation of disease severity as compared to sham-operated EAE mice. In mice that were transplanted with NPCs grown in FGF2 and EGF there was a significant amelioration of disease severity, as indicated by cumulative disease scores: 28.46 ± 16.07 for the control EAE, 21.4 ± 10.94 for the FGF2 alone group ($p = 0.18$) and 16.1 ± 9.85 for the FGF2 + EGF group ($p = 0.05$). A similar trend was observed when NPC spheres were ICV transplanted into EAE C57BL/6 mice induced by MOG33–55 peptide. Cumulative disease score was 24.5 ± 14.2 for the EAE control group, 24.5 ± 7.8 for EAE mice transplanted with FGF2-spheres, and 15.0 ± 12.7 for EAE mice transplanted with FGF2 + EGF-spheres ($p = 0.09$, Fig. 7L).

Discussion and conclusion

In this report we show for the first time an additive role of three chemokine receptor signaling systems, accounting for most of the robust migratory response of neural precursor cells to brain inflammation in the EAE model.

In vivo cell migration depends on three factors: cell motility, environmental signals and the ability of the cell to respond to these signals. In this study we examined the cell- and tissue-derived factors that affect NPC migration in response to brain inflammation in an experimental model of MS.

First, cytokines and chemokines are mediators of inflammation and strong chemo-attractant proteins. Specifically, SDF-1α, MCP-1 and HGF play important roles in the pathogenesis of EAE. MCP-1 is involved in mediating the infiltration of macrophages and T-cells into the CNS during EAE. MCP-1 deficient mice are resistant to EAE induction (Huang et al., 2001) and the MCP-1/CCR2 signaling pathway has become a potential target for MS therapy (Giraud et al., 2010; Karpus et al., 2008). SDF-1α regulates the severity of EAE (Meiron et al., 2008). It is thought to maintain the integrity of the blood–brain barrier in normal condition and its effect is disrupted in EAE (McCandless et al., 2006). Furthermore, a recent study suggested that SDF-1α is involved in the migration of oligodendrocyte progenitors during EAE (Banisadr et al., 2011). The role of HGF in EAE is controversial with reports on the promotion of macrophage proliferation and a pro-inflammatory role (Moransard et al., 2010) versus an immunomodulatory and neuroprotective effect (Benkhoucha et al., 2010).

We observed that NPCs express the cognate receptors to these chemokines. The absence of MCP-1 receptor CCR2 on NPCs, or blockage of HGF receptor c-Met or of SDF-1α receptor CXCR4 resulted in the partial inhibition of NPC migration. Only the blockage of all three receptors resulted in a dramatic inhibition of NPC migration, indicating an additive effect of the three chemokine receptor signaling. Thus, CXCR4, CCR2 and c-MET signaling accounts for most of the NPC migratory performance in the EAE mouse brain. It is technically difficult to achieve total inhibition since the blocking antibodies and specific receptor inhibitors are limited in the length of action in time and some cells at the core of the neurospheres can evade exposure to the agents. However we do not exclude that additional inflammatory mediators may be involved in the induction of NPC migration.

The blockage of the three chemokine receptors resulted in the inhibition of migration in two different mouse strains, SJL and C57Bl/6 with two different EAE models induced by the proteins PLP and MOG respectively. This suggests that the role of these chemokine systems may be universal in the EAE model.

Since inflammation-induced NPC migration was dependent on the three chemokine receptors' signaling, we examined the expression of their major ligands. There was an increase in tissue immuno-reactivity of SDF-1α, MCP-1 and HGF in the corpus callosum during EAE. Specifically, induction of EAE was associated with chemokine staining in activated tissue microglia and astrocytes. Our findings are in agreement with previous reports on increased tissue immuno-reactivity of SDF-1α in EAE and MS brain tissue derived astrocytes and microglia (Banisadr et al., 2011; Moll et al., 2009), and with the correlation between the Iba-1$^+$ microglia density and NPC migration in EAE brain (Ben-Hur et al., 2007).

A motile phenotype is required for enabling cellular migratory response to these chemokine signaling. Epidermal growth factor (EGF) is a powerful mitogen for NPCs and was shown to increase motility of several cell populations (Ayuso-Sacido et al., 2010; Boockvar et al., 2003; Fricker-Gates et al., 2000). We show here that without EGF supplementation, transplanted NPCs fail to respond to inflammatory cues in almost 90% of EAE mice. The motile phenotype induced by EGF was not due to the induction of chemokine receptors (Fig. 7A and Supplementary Fig. 2) and not dependent on any additional external signal as observed in vitro (Fig. 7B). However, a motile phenotype is essential for cell migration in response to inflammatory cues. In addition, EGF supplementation influences NPC fate commitment towards glial differentiation (Gonzalez-Perez et al., 2009; Sanalkumar et al., 2010). Thus, glial committed NPCs acquire superior migratory properties over multi-potential NPCs in response to inflammation. This may be an important property since regenerative therapy in MS is mostly aimed at promoting remyelination. Therefore the final differentiation of NPCs into mature myelinating oligodendrocytes is highly sought (Ben-Hur, 2011; Gonzalez-Perez and Alvarez-Buylla, 2011). Indeed, NPCs grown in the presence of EGF and FGF2 achieved a stronger clinical effect when transplanted to EAE mice than NPCs grown with FGF2 alone. Multiple studies have shown that the main therapeutic effect of transplanted NPCs in EAE is via their immunomodulatory and neurotrophic properties, rather than participating directly in regeneration (Ben-Hur et al., 2007; Einstein et al., 2006; Pluchino et al., 2005). The improved therapeutic properties of FGF2-EGF NPCs may be partly related to their increased motility and glial preference, bringing them in close proximity to the site of inflammation and injury.

In MS, tissue injury is mediated by the inflammatory process. CNS invasion by T cells and macrophages leads to myelin sheaths' damage and loss of neural function. However, inflammatory mediators may have a dual role, in which the acute detrimental effects are followed by the initiation of processes of repair (Lucas et al., 2006). If SDF-1α and HGF indeed regulate EAE severity (Benkhoucha et al., 2010) as well as mobilize tissue precursors, they may be considered as therapeutic targets for regenerative therapy in MS. For MCP-1, which seems to play a central role both in trafficking of detrimental immune cells into the CNS and in inducing NPC migration, then this apparent contradiction may be solved by proper timing of its inhibition or induction. A better understanding of such dual functions of inflammatory mediators could lead to the development of more effective therapies in MS.

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.scr.2014.06.001.

Acknowledgments

We thank Avital Granit and Daniel Zrihan for their help with the Real-Time PCR assay and Prof. Ruth Gabizon and Kati Frid for their help with the western blot assay. We thank Prof. Eitan Galun for generously providing the CCR2 ko mice. This study was supported in part by The Taubman Foundation, The Judy and Sidney Swartz Fund for research in multiple sclerosis and The Jacob and Hilda Blaustein Fund.

References

Ayuso-Sacido, A., Moliterno, J.A., Kratovac, S., Kapoor, G.S., O'Rourke, D.M., Holland, E.C., Garcia-Verdugo, J.M., Roy, N.S., Boockvar, J.A., 2010. Activated EGFR signaling increases proliferation, survival, and migration and blocks neuronal differentiation in post-natal neural stem cells. J. Neurooncol. 97, 323–337.

Banisadr, G., Frederick, T.J., Freitag, C., Ren, D., Jung, H., Miller, S.D., Miller, R.J., 2011. The role of CXCR4 signaling in the migration of transplanted oligodendrocyte progenitors into the cerebral white matter. Neurobiol. Dis. 44, 19–27.

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

Ben-Hur, T., 2011. Cell therapy for multiple sclerosis. Neurotherapeutics 8, 625–642.

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

Ben-Hur, T., van Heeswijk, R.B., Einstein, O., Aharonowiz, M., Xue, R., Frost, E.E., Mori, S., Reubinoff, B.E., Bulte, J.W., 2007. Serial in vivo MR tracking of magnetically labeled neural spheres transplanted in chronic EAE mice. Magn. Reson. Med. 57, 164–171.

Benkhoucha, M., Santiago-Raber, M.L., Schneiter, G., Chofflon, M., Funakoshi, H., Nakamura, T., Lalive, P.H., 2010. Hepatocyte growth factor inhibits CNS autoimmunity by inducing tolerogenic dendritic cells and CD25+ Foxp3+ regulatory T cells. Proc. Natl. Acad. Sci. U. S. A. 107, 6424–6429.

Birchmeier, C., Birchmeier, W., Gherardi, E., Vande Woude, G.F., 2003. Met, metastasis, motility and more. Nat. Rev. Mol. Cell Biol. 4, 915–925.

Boockvar, J.A., Kapitonov, D., Kapoor, G., Schouten, J., Counelis, G.J., Bogler, O., Snyder, E.Y., McIntosh, T.K., O'Rourke, D.M., 2003. Constitutive EGFR signaling confers a motile phenotype to neural stem cells. Mol. Cell. Neurosci. 24, 1116–1130.

Bulte, J.W., Ben-Hur, T., Miller, B.R., Mizrachi-Kol, R., Einstein, O., Reinhartz, E., Zywicke, H.A., Douglas, T., Frank, J.A., 2003. MR microscopy of magnetically labeled neurospheres transplanted into the Lewis EAE rat brain. Magn. Reson. Med. 50, 201–205.

Canello, T., Frid, K., Gabizon, R., Lisa, S., Friedler, A., Moskovitz, J., Gasset, M., Gabizon, R., 2010. Oxidation of helix-3 methionines precedes the formation of PK resistant PrP. PLoS Pathog. 6, e1000977.

Einstein, O., Ben-Menachem-Tzidon, O., Mizrachi-Kol, R., Reinhartz, E., Grigoriadis, N., Ben-Hur, T., 2006. Survival of neural precursor cells in growth factor-poor environment: implications for transplantation in chronic disease. Glia 53, 449–455.

Fricker-Gates, R.A., Winkler, C., Kirik, D., Rosenblad, C., Carpenter, M.K., Bjorklund, A., 2000. EGF infusion stimulates the proliferation and migration of embryonic progenitor cells transplanted in the adult rat striatum. Exp. Neurol. 165, 237–247.

Giraud, S.N., Caron, C.M., Pham-Dinh, D., Kitabgi, P., Nicot, A.B., 2010. Estradiol inhibits ongoing autoimmune neuroinflammation and NFkappaB-dependent CCL2 expression in reactive astrocytes. Proc. Natl. Acad. Sci. U. S. A. 107, 8416–8421.

Gonzalez-Perez, O., Alvarez-Buylla, A., 2011. Oligodendrogenesis in the subventricular zone and the role of epidermal growth factor. Brain Res. Rev. 67, 147–156.

Gonzalez-Perez, O., Romero-Rodriguez, R., Soriano-Navarro, M., Garcia-Verdugo, J.M., Alvarez-Buylla, A., 2009. Epidermal growth factor induces the progeny of subventricular zone type B cells to migrate and differentiate into oligodendrocytes. Stem Cells 27, 2032–2043.

Hatse, S., Princen, K., Bridger, G., De Clercq, E., Schols, D., 2002. Chemokine receptor inhibition by AMD3100 is strictly confined to CXCR4. FEBS Lett. 527, 255–262.

Heese, O., Disko, A., Zirkel, D., Westphal, M., Lamszus, K., 2005. Neural stem cell migration toward gliomas in vitro. Neuro Oncol. 7, 476–484.

Hori, J., Ng, T.F., Shatos, M., Klassen, H., Streilein, J.W., Young, M. J., 2003. Neural progenitor cells lack immunogenicity and resist destruction as allografts. Stem Cells 21, 405–416.

Huang, D.R., Wang, J., Kivisakk, P., Rollins, B.J., Ransohoff, R.M., 2001. Absence of monocyte chemoattractant protein 1 in mice leads to decreased local macrophage recruitment and antigen-specific T helper cell type 1 immune response in experimental autoimmune encephalomyelitis. J. Exp. Med. 193, 713–726.

Imitola, J., Raddassi, K., Park, K.I., Mueller, F.J., Nieto, M., Teng, Y.D., Frenkel, D., Li, J., Sidman, R.L., Walsh, C.A., 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. U. S. A. 101, 18117–18122.

Karpus, W.J., Reynolds, N., Behanna, H.A., Van Eldik, L.J., Watterson, D.M., 2008. Inhibition of experimental autoimmune encephalomyelitis by a novel small molecular weight proinflammatory cytokine suppressing drug. J. Neuroimmunol. 203, 73–78.

Lalive, P.H., Paglinawan, R., Biollaz, G., Kappos, E.A., Leone, D.P., Malipiero, U., Relvas, J.B., Moransard, M., Suter, T., Fontana, A., 2005. TGF-beta-treated microglia induce oligodendrocyte precursor cell chemotaxis through the HGF–c-Met pathway. Eur. J. Immunol. 35, 727–737.

Li, M., Ransohoff, R.M., 2008. Multiple roles of chemokine CXCL12 in the central nervous system: a migration from immunology to neurobiology. Prog. Neurobiol. 84, 116–131.

Lucas, S.M., Rothwell, N.J., Gibson, R.M., 2006. The role of inflammation in CNS injury and disease. Br. J. Pharmacol. 147 (Suppl. 1), S232–S240.

Magge, S.N., Malik, S.Z., Royo, N.C., Chen, H.I., Yu, L., Snyder, E.Y., O'Rourke, D.M., Watson, D.J., 2009. Role of monocyte chemoattractant protein-1 (MCP-1/CCL2) in migration of neural progenitor cells toward glial tumors. J. Neurosci. Res. 87, 1547–1555.

Mammolenti, M., Gajavelli, S., Tsoulfas, P., Levy, R., 2004. Absence of major histocompatibility complex class I on neural stem cells does not permit natural killer cell killing and prevents recognition by alloreactive cytotoxic T lymphocytes in vitro. Stem Cells 22, 1101–1110.

McCandless, E.E., Wang, Q., Woerner, B.M., Harper, J.M., Klein, R. S., 2006. CXCL12 limits inflammation by localizing mononuclear infiltrates to the perivascular space during experimental autoimmune encephalomyelitis. J. Immunol. 177, 8053–8064.

Meiron, M., Zohar, Y., Anunu, R., Wildbaum, G., Karin, N., 2008. CXCL12 (SDF-1alpha) suppresses ongoing experimental autoimmune encephalomyelitis by selecting antigen-specific regulatory T cells. J. Exp. Med. 205, 2643–2655.

Moll, N.M., Cossoy, M.B., Fisher, E., Staugaitis, S.M., Tucky, B.H., Rietsch, A.M., Chang, A., Fox, R.J., Trapp, B.D., Ransohoff, R.M., 2009. Imaging correlates of leukocyte accumulation and CXCR4/CXCL12 in multiple sclerosis. Arch. Neurol. 66, 44–53.

Moransard, M., Sawitzky, M., Fontana, A., Suter, T., 2010. Expression of the HGF receptor c-Met by macrophages in experimental autoimmune encephalomyelitis. Glia 58, 559–571.

Muja, N., Cohen, M.E., Zhang, J., Kim, H., Gilad, A.A., Walczak, P., Ben-Hur, T., Bulte, J.W., 2011. Neural precursors exhibit distinctly different patterns of cell migration upon transplantation during either the acute or chronic phase of EAE: a serial MR imaging study. Magn. Reson. Med. 65, 1738–1749.

Pluchino, S., Zanotti, L., Rossi, B., Brambilla, E., Ottoboni, L., Salani, G., Martinello, M., Cattalini, A., Bergami, A., Furlan, R., et al., 2005. Neurosphere-derived multipotent precursors promote neuroprotection by an immunomodulatory mechanism. Nature 436, 266–271.

Robin, A.M., Zhang, Z.G., Wang, L., Zhang, R.L., Katakowski, M., Zhang, L., Wang, Y., Zhang, C., Chopp, M., 2006. Stromal cell-derived factor 1alpha mediates neural progenitor cell motility after focal cerebral ischemia. J. Cereb. Blood Flow Metab. 26, 125–134.

Sanalkumar, R., Vidyanand, S., Lalitha Indulekha, C., James, J., 2010. Neuronal vs. glial fate of embryonic stem cell-derived neural progenitors (ES-NPs) is determined by FGF2/EGF during proliferation. J. Mol. Neurosci. 42, 17–27.

Stumm, R., Hollt, V., 2007. CXC chemokine receptor 4 regulates neuronal migration and axonal pathfinding in the developing nervous system: implications for neuronal regeneration in the adult brain. J. Mol. Endocrinol. 38, 377–382.

van der Meulen, A.A., Biber, K., Lukovac, S., Balasubramaniyan, V., den Dunnen, W.F., Boddeke, H.W., Mooij, J.J., 2009. The role of CXC chemokine ligand (CXCL)12-CXC chemokine receptor (CXCR) 4 signalling in the migration of neural stem cells towards a brain tumour. Neuropathol. Appl. Neurobiol. 35, 579–591.

Widera, D., Holtkamp, W., Entschladen, F., Niggemann, B., Zanker, K., Kaltschmidt, B., Kaltschmidt, C., 2004. MCP-1 induces migration of adult neural stem cells. Eur. J. Cell Biol. 83, 381–387.

Xu, Q., Wang, S., Jiang, X., Zhao, Y., Gao, M., Zhang, Y., Wang, X., Tano, K., Kanehara, M., Zhang, W., et al., 2007. Hypoxia-induced astrocytes promote the migration of neural progenitor cells via vascular endothelial factor, stem cell factor, stromal-derived factor-1alpha and monocyte chemoattractant protein-1 upregulation in vitro. Clin. Exp. Pharmacol. Physiol. 34, 624–631.

Zhao, D., Najbauer, J., Garcia, E., Metz, M.Z., Gutova, M., Glackin, C.A., Kim, S.U., Aboody, K.S., 2008. Neural stem cell tropism to glioma: critical role of tumor hypoxia. Mol. Cancer Res. 6, 1819–1829.