Neuroinflammatory signals enhance the immunomodulatory and neuroprotective properties of multipotent adult progenitor cells

Stylianos Ravanidis¹, Jeroen F. J. Bogie¹, Raf Donders¹, David Craeye², Robert W. Mays³, Robert Deans³, Kristel Gijbels², Annelies Bronckaers¹, Piet Stinissen¹, Jef Pinxteren² and Niels Hellings¹*

Abstract

Introduction: Stem cell-based therapies are currently widely explored as a tool to treat neuroimmune diseases. Multipotent adult progenitor cells (MAPC) have been suggested to have strong immunomodulatory and neuroprotective properties in several experimental models. In this study, we investigate whether MAPC are of therapeutic interest for neuroinflammatory disorders such as multiple sclerosis by evaluating their capacities to modulate crucial pathological features and gain insights into the molecular pathways involved.

Methods: Rat MAPC were treated with combinations of pro-inflammatory cytokines that are closely associated with neuroinflammatory conditions, a process called licensing. mRNA expression of immunomodulatory molecules, chemokines and chemokine receptors was investigated. The migratory potential of licensed rat MAPC towards a broad spectrum of chemokines was tested in a Transwell assay. Furthermore, the effect of licensing on the ability of rat MAPC to attract and suppress the proliferation of encephalitogenic T cells was assessed. Finally, neuroprotective properties of rat MAPC were determined in the context of protection from oxidative stress of oligodendrocytes. Therefore, rat MAPC were incubated with conditioned medium of OLN93 cells subjected to sublethal doses of hydrogen peroxide and the gene expression of neurotrophic factors was assessed.

Results: After licensing, a wide variety of immunomodulatory molecules and chemokines, including inducible nitric oxide synthase and fractalkine, were upregulated by rat MAPC. The migratory properties of rat MAPC towards various chemokines were also altered. In addition, rat MAPC were found to inhibit antigen-specific T-cell proliferation and this suppressive effect was further enhanced after pro-inflammatory treatment. This phenomenon was partially mediated through inducible nitric oxide synthase or cyclooxygenase-2. Activated rat MAPC secreted factors that led to attraction of myelin-specific T cells. Finally, exposure of rat MAPC to an in vitro simulated neurodegenerative environment induced the upregulation of mRNA levels of vascular endothelial growth factor and ciliary neurotrophic factor. Factors secreted by rat MAPC in response to this environment partially protected OLN93 cells from hydrogen peroxide-induced cell death.

Conclusions: Rat MAPC possess immune modulatory and neuroprotective properties which are enhanced in response to neuroinflammatory signals. These findings thereby warrant further research to evaluate MAPC transplantation as a therapeutic approach in diseases with an immunological and neurodegenerative component such as multiple sclerosis.

*Correspondence: niels.hellings@uhasselt.be
¹Hasselt University, Biomedical Research Institute/Transnational University Limburg, School of Life Sciences, Campus Diepenbeek, Agoralaan building C, 3590 Diepenbeek, Belgium
Full list of author information is available at the end of the article

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**Introduction**

Stem cell transplantation represents a promising therapeutic approach to treat neuroinflammatory and neurodegenerative disorders. By using experimental murine models of neuroinflammatory diseases, such as experimental autoimmune encephalomyelitis (EAE), traumatic brain injury (TBI) and spinal cord injury (SCI), several studies demonstrated that stem cells reduce neurological deterioration and protect the central nervous system (CNS) from further damage and even stimulate its repair [1–7]. In these studies both adult-derived bone marrow mesenchymal stem cells (MSCs) and newborn CNS-derived neural stem cells (NSCs) provided neurotrophic support and even replaced damaged oligodendrocytes and neurons [3–5]. Of note, the therapeutic action of transplanted cells did not relate to the route of administration—peripheral- or CNS-directed. In addition to the neuroprotective and regenerative potential, the immunomodulatory properties of NSCs and MSCs have been well established [1, 2, 8]. NSCs and MSCs were found to suppress the reactivity of encephalitogenic T cells in the EAE model, thereby likely ameliorating pathological features and clinical symptoms. Collectively, these findings indicate that stem cells can not only halt neuroinflammation but also stimulate CNS repair upon inflammatory neurodegeneration. These properties make them an interesting tool for the treatment of all pathophysiological facets of multiple sclerosis (MS). However, the use of CNS-derived NSCs for autologous transplantation is not a feasible option. Furthermore, although MSCs have been used in clinical trials for autoimmune diseases, the signs of replicative senescence that are demonstrated remain an obstacle for their use as a large-scale clinical product [9–13].

In 2002, another bone marrow-derived stem cell population of mesenchymal origin was initially described, namely multipotent adult progenitor cells (MAPC) [14]. Interestingly, in contrast to MSCs, MAPC do not show signs of replicative senescence and possess broader expansion capacities [9–11, 14]. MAPC, in contrast to MSCs, have an extensive differentiation potential towards cell types of all three germ layers depending on the expression levels of pluripotency genes such as oct-4 [14–16]. Importantly, recent studies indicate that MAPC have neuroprotective and immunosuppressive properties. Rat MAPC (rMAPC) were found to preserve hippocampal cell loss in an animal model of hypoxia-ischemia [17], while human MAPC (hMAPC) stimulated recovery in an animal model of TBI, likely through splenocyte-triggered modulation of microglia phenotype [18–20]. Yet other studies revealed that murine MAPC (mMAPC) attenuate alloreactive T-cell proliferation [21] while hMAPC suppress natural killer (NK) cell proliferation in an indoleamine 2,3-dioxygenase 1 (Ido-1)-dependent manner [22]. Therefore, while MAPC possess intrinsic immune modulating and neuroprotective properties, they show superior features over MSCs, such as the broader expansion rate without any obvious genetic abnormalities [9, 14]. These features make MAPC a more attractive candidate for potential stem cell transplantation therapies in CNS disorders, such as MS, TBI and SCI.

In this study, we defined the therapeutic potential of MAPC in neuroinflammatory diseases. For this purpose, we determined the basal immunomodulatory, migratory, chemoattractive and neuroprotective features of rMAPC. Next, the impact of pro-inflammatory and neurodegenerative stimuli on the physiology of rMAPC was assessed. It was previously reported that a pro-inflammatory milieu markedly alters the physiology of stem cells (a process called ‘licensing’) [23–26]. rMAPC were licensed with combinations of interferon gamma (IFNγ), tumor necrosis factor alpha (TNFα) and interleukin-1 beta (IL1β). These cytokines are highly expressed in the brain parenchyma and cerebrospinal fluid in CNS neuroinflammatory diseases and play a crucial role in their pathophysiology. Particularly, IFNγ secreted by Th1 cells leads to the activation of other immune cells whereas TNFα and IL1β secreted by activated macrophages and microglia lead to direct destruction of myelin sheath and oligodendrocytes [27–30]. We show that unstimulated rMAPC possess immunomodulatory and neuroprotective properties which are further enhanced when challenged with neuroinflammatory signals. Specifically, rMAPC suppressed autoreactive T-cell proliferation and protected oligodendrocytes from hydrogen peroxide (H2O2)-induced damage. We further present that licensing increases the capacity of rMAPC to attract T cells, while they themselves adopt an enhanced migratory profile. Collectively these findings show that rMAPC, when challenged in vitro with signals that are overexpressed in a neuroinflammatory environment, acquire a phenotype which may limit disease activity in vivo.

**Materials and methods**

**rMAPC culture and inflammatory licensing**

Lewis rMAPC and culture medium were provided by ReGenesys (Leuven, Belgium). rMAPC were isolated and maintained according to a previously described protocol [15]. Briefly, bone marrow from tibiae and femur from Lewis rats was excised and flushed using phosphate-buffered saline (PBS; Lonza, Verviers, Belgium). The single cell suspension was centrifuged and washed using rMAPC culture and inflammatory licensing medium. rMAPC medium consisted of 60 % Dulbecco's modified Eagle's medium (DMEM; Gibco, Life Technologies Europe B.V., Gent, Belgium) low glucose (1 g/l), 40 % MCDB-201 medium (pH 7.2), 1x linoleic acid-bovine serum albumin (BSA), 10−4 M L-Ascorbic acid, 0.05 μM dexamethasone, 55 μM 2-mercapto-ethanol (all from Sigma Aldrich, Diegem, Belgium), 100 IU/ml penicillin and 100 μg/ml streptomycin (Invitrogen, Life Technologies).
α/β+ TNF-α, 5 units/ml mouse leukemia inhibitory factor (Millipore). Cells were seeded in fibronectin (10 ng/ml; Sigma Aldrich) coated plates for 1 month in increasing densities. After 1 month of culturing, negative control selection was performed to eliminate CD45+ hematopoietic cells. The resulted cell fraction was subcloned until a homogeneous population of small, spindle-shaped cells remained. The cells with MAPC morphology were maintained in culture in order for colonies to be formed. Frozen stock was created with cells in medium containing rMAPC medium, fetal bovine serum and dimethyl sulfoxide (DMSO). Cells were maintained in T175 flasks (Cellstar, Greiner Bio-One, Vilvoorde, Belgium) or petri dishes (Nunc, VWR, Leuven, Belgium) according to the purposes needed and maintained at 37 °C/5 % O₂.

All experiments were performed with rMAPC that reached 10 population doublings maximum.

To assess the impact of pro-inflammatory treatment (licensing) on rMAPC properties, cells were treated with 100 ng/ml of combinations of rat recombinant cytokines (IFNγ + TNFα, IFNγ + IL1β, TNFα + IL1β; all from Peprotech, London, UK) or PBS as vehicle control for 12 or 24 hours. Specific licensing incubation times are described in each section.

Colony forming unit fibroblast assay
Cells were seeded as 10 cells/cm² in six-well plates. Medium was changed every 2 days. After 12 days, medium was removed and cells were fixed with 4 % paraformaldehyde (PFA) for 20 minutes and then were washed twice with PBS. A solution of 0.5 % crystal violet (Sigma Aldrich) in methanol was added for 30 minutes. Following the incubation time, cells were washed three times with PBS, rinsed with tap water and allowed to air dry before measuring the colonies. To explore the impact of licensing on the ability of rMAPC to form colonies, cells were pre-treated for 24 hours with the three combinations of cytokines and vehicle.

Flow cytometry
Cells were detached with 0.25 % trypsin (Gibco), harvested and washed with fluorescence-activated cell sorting (FACS) buffer (PBS supplemented with 2 % fetal calf serum (FCS; Gibco)) and incubated in the dark for 30 minutes at 4 °C with surface antibodies. Phenotypic analysis of the cells was performed using CD11b/c, CD31 (BD Biosciences, Erembodegem, Belgium), CD44 (ImmunoTools, Friesoythe, Germany), CD80, CD86 (eBioscience, Vienna, Austria), RT-1a and RT-1b (Biolegend, San Diego, CA, USA). Cells were positive for CD44 and RT-1a, expressed low to negligible levels of CD80, while they were completely negative for CD11b/c, CD31, CD86 and RT-1b.

Generation of myelin basic protein-specific T cells
Myelin basic protein (MBP)-specific T cells were isolated as described previously [31]. Briefly, 8-week-old female Lewis rats (Janvier, France) were injected subcutaneously with a 0.1 ml solution of 250 µg/ml guinea pig MBP, 2.5 mg/ml H37RA heat-killed mycobacterium tuberculosis (Difco, Detroit, USA) and 60 µl Complete Freund’s adjuvant (Sigma Aldrich) in both hind paws. Ten days post-immunization, popliteal and inguinal lymph nodes were removed and single cell suspensions were obtained by grinding the tissues through a 70 µm cell strainer with a syringe plunger. The isolated cells were seeded initially in T cell medium consisting of RPMI-1640 medium, 1 % penicillin-streptomycin mixture, 1 % non-essential amino acids, 1 % sodium pyruvate (all from Invitrogen, Life Technologies Europe B.V.), 20 µM 2-mercapto-ethanol (Sigma Aldrich) supplemented with 2 % heat-inactivated autologous serum and 33 µg/ml MBP. After 48 hours, T cells were collected, washed and seeded in T cell medium supplemented with 10 % FCS (Gibco) and 6.5 % CAS medium (supernatant of Concanavalin A (Sigma Aldrich) activated spleen cells) for another 48 hours. Next, cells were collected, washed and seeded in T cell medium supplemented with 10 % FCS for 3 days. All animal experiments were approved by the Ethical Committee for Animal Experiments of Hasselt University.

Co-cultures
Prior to co-culture with rMAPC, T cells were labeled with 4 µM carboxyfluorescein diacetate succinimidyl ester (CFSE; Invitrogen) at a concentration of 2 × 10⁶ cells/ml in PBS/0.1 % BSA (Millipore, Merck Chemicals N.V./S.A., Overijse, Belgium) solution. CFSE-labeled T cells (7.5 × 10⁵ cells/well) were seeded alongside rMAPC in ratios ranging from 1:0.5 to 1:2 (T cells/rMAPC). Irradiated thymocytes (7.5 × 10⁴ cells/well, 3000 rad) were added to each well as antigen-presenting cells. The medium of the co-cultures consisted of a 1:1 mixture of rMAPC medium and T cell medium with 2 % autologous serum supplemented with 10 µg/ml MBP. To explore the effect of licensing on their suppressive capacity, rMAPC were pre-treated with the three combinations of cytokines and vehicle for 12 hours prior to the co-culture. To define the involvement of nitric oxide (NO), cyclooxygenase (COX)-2 and Id0-1 in suppressive activity, respectively 1.5 mM L-N(ω)-monomethyl arginine citrate (L-NMMA;VWR), 10 µM indomethacin (Sigma Aldrich) and 200 µM 1-methyl-L-tryptophan/1-methyl-D-tryptophan (1-Mt-L and 1-Mt-D; Sigma
Aldrich) were added at day 0 and day 2 of the co-culture. After 4 days, flow cytometry was used to assess proliferation and cell death of lymphocytes. Therefore, cells were stained with phycoerythrin-conjugated mouse anti-rat CD3 (eBioscience) and 7 aminoactinomycin D (7AAD; BD Biosciences). T-cell proliferation was determined based on CFSE dye dilution of CD37AAD− cells using flow cytometry (FACSCalibur).

Migration assays
To explore the chemoattractive properties of rMAPC, we seeded MBP-specific T cells (2.5 × 10^5 cells/insert) in the upper chamber of a transwell plate with a 5-μm pore size (Sigma Aldrich). In the bottom chamber supernatant of (licensed) rMAPC was placed. To obtain the supernatants, rMAPC were seeded in a mixture of media (30 % MAPC medium, 70 % RPMI-1640) and licensed for 24 hours with respective cytokine combinations. The supernatant was aspirated and filtered through a 0.45-μm filter. Following 4 hours of culture at 37 °C, migrated cells were collected from the bottom chamber and counted using a hemocytometer. Non-conditioned medium served as negative control.

To explore the functionality of chemokine receptors expressed by rMAPC, rat recombinant CCL2, CCL5, CX3CL1, CXCL10 and CXCL12a (100, 250 and 500 ng/ml, all from Peprotech) were administered in the bottom chamber of a transwell plate with an 8-μM pore size (Sigma Aldrich). Cells were seeded in the upper chamber at a concentration of 5 × 10^5 cells/insert and allowed to migrate for 16 hours. Recombinant chemokines were diluted in DMEM-low glucose 1 g/l (Gibco). DMEM (glucose 1 g/l) was used as negative control. As a positive control, rMAPC medium (ReGenesys) was used, as it was optimized to be more effective than the more traditionally used FCS containing solutions (20 % FCS in DMEM 1 g/l solution (see Additional file 1)). To explore the impact of inflammatory conditions on the migration profile of rMAPC, cells were licensed for 12 hours before allowing them to migrate. For the quantification of the migrated fraction we used a protocol by Bronckaers et al. [32] with minor modifications. An illustration of the protocol is depicted in Additional file 2. Briefly, rMAPC were seeded in a 96-well flat bottom plate (Greiner Bio-One) (5 × 10^4 cells/well) and then treated with 1 mM H₂O₂ to induce sublethal cell damage. After 2 hours, H₂O₂ was removed and rMAPC were allowed to condition fresh medium for 24 hours (OLN-CM \( \text{H}_2\text{O}_2 \)). Non-damaged rMAPC cells provided the OLN-CM. The OLN-CM \( \text{H}_2\text{O}_2 \) and OLN-CM were applied to rMAPC in a 96-well flat bottom plate (5 × 10^4 cells/well) for 18 hours resulting in double-conditioned media (DCM). These media are designated as DCM \( \text{H}_2\text{O}_2 \) and DCMnull respectively. rMAPC treated with OLN-CM \( \text{H}_2\text{O}_2 \) and OLN-CM were also processed for RNA extraction (5 × 10^6 cells/well per 24-well plate) to evaluate alterations in gene expression levels of neurotrophic factors. All the aforementioned conditioned media were collected and filtered through a 0.45-μm filter. OLN93 cells were allowed to adhere in a 96-well flat bottom plate (5 × 10^3 cells/well) and exposed to DCM \( \text{H}_2\text{O}_2 \) and DCMnull for 6 hours (see Additional file 2). Subsequently, cells were subjected to H₂O₂-induced oxidative stress (1 mM, 1.5 mM and 2 mM H₂O₂ for 24 hours). OLN93 cell viability was determined using the MTT assay.

Gene expression analysis
rMAPC licensed for 12 hours and rMAPC treated with OLN93-derived media (OLN-CM \( \text{H}_2\text{O}_2 \) and OLN-CM) for 18 hours were processed for gene expression analysis. Cells were detached, centrifuged and stored in lysis buffer of RNeasy mini kit (QIAGEN, Venlo, The Netherlands) at −80 °C until later use.

RNA was isolated with the RNeasy mini kit (QIAGEN) and was reversely transcribed into complementary DNA (cDNA) using the Quanta kit (VWR, Leuven, Belgium) following the manufacturer’s instructions. cDNA was subsequently used for semi quantitative real-time polymerase chain reaction (RT-PCR). Quantitative PCR reactions were performed with a StepOnePlus™ Real-Time PCR System (Applied Biosystems) in micro-AMP Fast Optical 96-well reaction plates in a total volume of 10 μl per reaction. The reaction mix contained 1× Fast SYBR green master mix (Applied Biosystems), 10 mM of each primer (designed with Primer 3 [35]; Eurogentec, Liege, Belgium), nuclease-free water and 12.5 ng of cDNA template. The amplification protocol used was the following:
20 seconds at 95 °C, followed by 40 cycles of 3 seconds at 95 °C and 30 seconds at 60 °C, and subsequent melting-curve analysis. All the primer sequences used are listed in Additional file 3. Relative quantification of gene expression was calculated using the $2^{-ΔΔCt}$ method [36] and data were normalized to the most stable reference genes for each experiment according to geNorm [37]. For visualization of mRNA transcript presence, PCR amplified products (Hoffmann-La Roche, Basel, Switzerland) were separated in a 1.5 % agarose gel and were visualized with etidium bromide.

**Nitrite formation and cytokine release**

Supernatants of licensed rMAPC (24 hours) and co-cultures were collected and release of NO was determined using the Griess reagent system (Promega, Leuven, Belgium) following the manufacturer's instructions. Absorbance was measured at 550 nm using a microplate reader (Bio-Rad Benchmark, Bio-Rad Laboratories, Hercules, CA, USA).

IFNγ release was measured in the co-culture supernatant using Rat IFNy enzyme-linked immunosorbent assay (Peprotech) following the manufacturer’s instructions. Absorbance was measured at 415 nm.

**Cell viability assay (MTT)**

Cell survival and proliferation was assessed with MTT assay (Sigma Aldrich). Briefly, 12.5 μl MTT (3-(4,5)-dihydroxy-2-(5-[(2-carboxyethyl)-2-carboxyethyl]amino)-2-nitro-5-naphthylamine) dissolved in 100 μl medium per well was added for 4 hours at 37 °C. After incubation, MTT was removed and a mixture of 25 μl glycine and 150 μl DMSO/well was added and the absorbance was determined at 540 nm.

**Statistical analysis**

Data were analyzed with the GraphPad Prism version 5.00 for Windows, (GraphPad Software, San Diego California USA [38]) and are presented as mean ± SEM. D’Agostino and Pearson omnibus normality test was used to test normal distribution. Parametrical data were analyzed using unpaired student t test or one way analysis of variance followed by Dunnett multiple comparison test. Data that did not follow normal distribution were analyzed using Mann Whitney and Kruskal-Wallis followed by Dunns multiple comparison test. Differences with $P$ value ≤0.05 were considered significant.

**Results**

**In vitro licensing does not affect the viability and colony forming ability of rMAPC**

When stem cells are to be used in treating inflammatory diseases, they will be challenged by a high pro-inflammatory environment. To evaluate whether inflammatory conditions lead to alterations of basic biological functions, we assessed cell viability and colony formation ability following in vitro treatment with combinations of IFNγ, TNFα and IL1β. We show that the viability of rMAPC was not affected after 24 hours treatment (Fig. 1a). Similar, the ability of rMAPC to form colonies was not hampered following pre-treatment with pro-inflammatory cytokines (Fig. 1b). These findings indicate that an inflammatory environment does not affect basic biological functions of rMAPC.

**rMAPC suppress proliferation of encephalitogenic T cells**

To elucidate whether rMAPC are suppressive towards T cells, we performed co-culture experiments with MBP-specific T cells as a model of encephalitogenic T cells. MBP-reactive T cells are evident in diverse neurological
disorders such as MS, TBI, SCI and stroke [45–47]. We demonstrate that rMAPC potently suppressed T-cell proliferation in response to MBP even at the lowest ratio (1:0.5). Increasing the number of rMAPC did not further enhance the inhibition of T-cell proliferation (Fig. 3a). The observed effect was not due to cell death, as the percentage of CD3⁺7AAD⁺ cells was not altered (data not shown). In addition, a decrease in IFNγ was detected when increasing amounts of rMAPC were added (Fig. 3b). Of note, NO also decreased in a similar way, in line with the observation that IFNγ induces release of NO by MAPC (Fig. 2a, b). Activated T cells or thymocytes alone did not produce NO (Fig. 3c).

Inflammatory treatment enhances the suppression of myelin-specific T-cell proliferation

Next, we investigated whether the pro-inflammatory cytokine treatment enhances the suppressive properties of rMAPC towards MBP-specific T cells in line with previous findings for MSCs [40, 43]. Inhibition of antigen-specific T-cell proliferation was enhanced when cells were pre-licensed with either IFNγ + TNFα or TNFα + IL1β. Of note, pre-licensed rMAPC completely inhibited the antigen-specific T-cell proliferation reaching the negative control levels. For IFNγ + IL1β, no increased suppression was found (Fig. 4a).

To identify the mechanisms involved, we applied selective inhibitors for immune modulatory molecules, taking into consideration the upregulation of immunomodulatory genes reported in this study (Fig. 2a). Specifically, we used inhibitors for iNOS (L-NMMA), COX-2 (indomethacin) and Ido-1 (two isoforms; 1-Mt-L, 1-Mt-D). Blockade of NO reversed the observed enhancement of T-cell suppression to the levels observed when MAPC were not pre-treated with pro-inflammatory cytokines (Fig. 4b, c). Furthermore, COX-2 inhibition abrogated the enhanced suppression observed when MAPC were pre-treated with TNFα + IL1β (Fig. 4c). Ido-1 inhibitors did not have any reversible effect (Fig. 4b). The inhibitors alone did not have any effect on T-cell proliferation following exposure to MBP (see Additional file 5). Together, these results demonstrate that rMAPC are able to suppress the proliferation of encephalitogenic T cells when restimulated with cognate antigen.

rMAPC acquire a chemoattractive profile following licensing

T cell chemotaxis is crucial for the suppression of T-cell proliferation by MSCs [43]. rMAPC constitutively express mRNA copies of certain chemokines that are massively increased after inflammatory treatment. IFNγ + TNFα led to a significant induction of four out of five chemokines tested. TNFα + IL1β upregulated three out of five of the chemokines, while IFNγ + IL1β only significantly induced CXCL10 (Fig. 5a, Additional file 4).

To confirm the secretion of chemokines in the culture medium following inflammatory treatment, we allowed MBP-specific T cells to migrate towards supernatants of conditioned medium from licensed rMAPC. Enhanced T-cell migration was observed towards supernatant of rMAPC after treatment with TNFα + IL1β and to a lesser extent after IFNγ + TNFα pre-treatment. No
Fig. 2 (See legend on next page.)
significant T-cell migration towards supernatant of IFNγ + IL1β treated rMAPC was observed (Fig. 5b). These results match with the massive upregulation of chemokine mRNA expression (Fig. 5a). Chemokine receptors were all detected on myelin-specific T cells used in the migration assay (Fig. 5c).

We conclude that rMAPC are able to exert chemotraction towards T cells when challenged with pro-inflammatory stimuli.

**rMAPC express a set of functional chemokine receptors**

Chemokine receptors are potentially involved in MSC homing when injected in pathological conditions [48]. Therefore, we sought to explore the chemokine receptor repertoire of rMAPC. We demonstrate that rMAPC express mRNA levels of CCR1, CCR2, CCR9, CXCR3, CXCR4, CXCR5, CXCR6, CCR7 and CX3CR1. rMAPC did not express detectable levels of CCR3, CCR5, CCR6, CCR7 and CXCR2 (Fig. 6a).

To confirm the functionality of the receptors expressed, we explored the migration of rMAPC towards chemokines known to be expressed during neuroinflammation [49]. rMAPC were able to migrate to all chemokines tested, namely CCL2, CCL5, CXCL1, CXCL12α and CXCL10 (Fig. 6b). Generally we observed a dose-dependent increase in chemotaxis in all chemokines used. These results indicate that rMAPC are attracted to neuroinflammatory chemokines.

**Licensing affects the expression of chemokine receptors**

Systemic inflammation in the periphery could potentially affect rMAPC migratory behavior and thus dictate migration towards ongoing inflammation in the CNS and lymphoid organs. Following licensing we saw a differential upregulation of chemokine receptors (Fig. 6c, Additional file 4). Specifically, while IFNγ + TNFα treatment increased only the expression of CXCR3, IFNγ + IL1β treatment upregulated CCR9, CXCR3, CXCR5 and CX3CR1. Finally TNFα + IL1β treatment induced the upregulation of CCR7 and CX3CR1 while it was the only condition where CCR3 expression was induced (data not shown). Of interest, IFNγ + TNFα significantly downregulated the expression of CCR1.

We further assessed the migration of rMAPC following inflammatory treatment. We observed selective alterations in the migration pattern of rMAPC upon licensing. In particular, TNFα + IL1β treatment induced an enhanced migration of rMAPC towards CCL5, CXCL12α and CX3CL1, while IFNγ + IL1β enhanced migration towards CXCL10 (Fig. 6d). The observed results correlate partially with the alterations in the expression of CCR1, CXCR7, CX3CR1 and CXCR3 following inflammatory treatment. Of note, IFNγ + TNFα treated rMAPC migrated in a lesser extent towards CCL5, correlating with the significant downregulation in the expression of CCR1. The three combinations did not confer significant alterations on rMAPC migration towards the various concentrations of chemokines, besides the ones illustrated in Fig. 6d. These results indicate that the inflammatory milieu may modify the migratory ability of rMAPC thereby affecting the expression of certain chemokine receptors and subsequent migratory activity.

**rMAPC enhance their neuroprotective activity features when challenged with a neurodegenerative environment**

Neurodegeneration-induced secretion of growth factors by bone marrow-derived stem cells leads to protection of oligodendrocytes and neurons [34, 44]. To assess potential neuroprotective features of rMAPC in response to neurodegeneration, we challenged rMAPC with secreted factors from the sublethally damaged (H2O2) oligodendroglia cell line, OLN93 [50]. H2O2 generates free radicals leading to apoptotic death after oxidative stress, a situation which is prominent within the CNS during neurodegenerative events [51]. We demonstrated that secreted factors released by rMAPC in response to H2O2-treated OLN93 cells (DCM12O2) are able to partially protect OLN93 cells in all three concentrations of H2O2 used. rMAPC-derived soluble factors in response to non-damaged OLN93 cells (DCMnull) showed no evidence of protecting OLN93 cells from H2O2 damage (Fig. 7a). rMAPC viability under the influence of OLN-CM12O2 was not affected due to possible toxic factors secreted by early apoptotic cells or H2O2 remnants (data not shown). OLN93 cells did not show any differences in cell proliferation when allowed to proliferate for 24 hours in normal culture medium after the pre-treatment
period (Fig. 7b). This finding indicates that the effect of soluble factors secreted by rMAPC is actually protective and does not induce an excessive proliferation resulting in higher cell numbers. Next, we further characterized the behavior of rMAPC in the in vitro generated neurodegenerative environment. We analyzed the mRNA expression of neurotrophic factors known to enhance the survival and proliferation of oligodendrocytes and oligodendrocytes progenitors, as well as those that enhance myelin formation [44, 52–55]. We demonstrate that rMAPC when cultured in supernatant derived from sublethally damaged OLN93 cells (OLN-CM_H2O2) upregulate the expression of the neurotrophic factors VEGFα and CNTF but not GDNF (Fig. 7c). HGF was not detected under any condition. Collectively, these results indicate that the interaction with neurodegenerative signals derived by damaged oligodendrocytes enhances the neuroprotective features of rMAPC.

Discussion

Stem cell transplantation represents a promising therapeutic approach to treat neuroinflammatory and neurodegenerative disorders such as MS, TBI, SCI and stroke. In this study, we show that rMAPC, a stem cell population similar but distinct to MSCs, possess immunomodulatory and neuroprotective properties which are further enhanced when challenged with selected neuroinflammatory stimuli. These findings point out that MAPC can be considered as a promising therapeutic option for neuroinflammatory and neurodegenerative disorders.

In concordance with other studies, rMAPC exhibit a low immunogenic profile, being negative for co stimulatory molecules and RT-1b. Expression of RT-1a, though, makes rMAPC prone to lysis by NK cells in case...
of transplantation as hMAPC [22]. The low immunogenic profile of rMAPC was further confirmed by the low, if any, increase of pro-inflammatory genes following licensing. Upregulation of TNFα by rMAPC should be investigated further, although TNFα can also act as an inhibitory mechanism for CD4 T-cell proliferation [56].
Human and rodent MAPC have been reported to inhibit T-cell proliferation after polyclonal stimulation or allogeneic responses [21, 57, 58]. We demonstrate here that rMAPC effectively suppress proliferation of myelin-reactive T cells in vitro and that licensing enhances the suppressive effect of rMAPC. This finding suggests that transplanted MAPC can suppress the autoantigen-specific adaptive inflammatory response in MS. In line with other studies with hMAPC, rMAPC suppressed IFNγ release from autoreactive T cells in co-culture, indicating an attenuation of Th1 polarization [57].

CNS neurodegeneration and peripheral inflammation represent microenvironments that potentially affect the behavior of transplanted cells. We show that pro-inflammatory cytokines markedly increased the expression of immunomodulatory markers previously reported for MSCs and hMAPC, such as iNOS, Ido-1, PD-L1, TSG-6 and prostaglandin E2 (PGE2) [23, 39, 40, 42, 43, 59, 60]. IFNγ-driven upregulation of these molecules in MAPC and MSCs collectively has been demonstrated [23, 24, 40, 41, 43, 57]. We now provide evidence that a combination of inflammatory cytokines and, in particular,
Fig. 6 (See legend on next page.)
Fig. 6 rMAPC migration is altered following licensing. a Detection of chemokine receptor mRNA in rMAPC. Amplified cDNAs are visualized in a 1.5 % agarose gel. YWHAZ and HMBS were used as loading controls. One representative experiment out of five is shown. b rMAPC migration towards important MS pathogenesis chemokines. Three different concentrations of chemokines were used in the lower compartment (100, 250 and 500 ng/ml). The percentage of area covered by the migrated cells is shown. Values represent mean ± SEM from four independent experiments, with duplicates per experiment. Asterisks show statistical significance between the different concentrations of each chemokine and the negative control. No statistically significant differences were found between the different concentrations for each chemokine. Positive control values showed statistically significant differences with all the other conditions. Data were analyzed with one way analysis of variance comparison test, followed by Dunnett’s test for significant differences between the groups. c Gene expression of chemokine receptors by rMAPC following inflammatory treatment. Fold differences compared to control (PBS treated, dotted line) are shown. Significant differences with the control condition are indicated with asterisks. Values represent mean ± SEM from four independent experiments, with duplicates per experiment. Asterisks show the statistical significant difference between the pre-treated and not treated (naïve) migrated fraction towards specific concentrations of each chemokine. All values differ significantly with n.c. Mean of positive control is statistically significant to all the other conditions. Data were analyzed with one way analysis of variance comparison test, followed by Dunnett’s test for differences between the groups. *P ≤ 0.05, **P ≤ 0.01, ***P ≤ 0.001. IFN interferon, IL interleukin, n.c. negative control, p.c. positive control, rMAPC rat multipotent adult progenitor cells, TNF tumor necrosis factor.

Fig. 7 Neurodegeneration enhances the neuroprotective features of rMAPC. a Neuroprotection assay where OLN93 cells were pre-treated (with the indicated (double) conditioned media and then three concentrations of H2O2 were added (pos. control). Optical densities are shown (±SEM) from five experiments. Negative control (dotted line) represents OLN93 cells without damage insult. One way analysis of variance followed by Dunnet’s test for multiple comparisons between groups was used. b OLN93 cells were pre-treated for 5 hours with rMAPC double conditioned media (DCM) and then allowed to proliferate for 24 hours. Optical densities are shown (±SEM), n = 5 experiments. c rMAPC gene expression of neurotrophic factors (VEGFα, CNTF, GDNF) when treated with OLN-CM or OLN-CM. Expressions were relatively quantified against the expression of YWHAZ and CycA. The fold differences are shown in comparison to OLN-CM (±SEM) from five experiments. Mann Whitney t-test was used for comparisons between two groups. Significant differences are shown with asterisks: *P ≤ 0.05, **P ≤ 0.01, ***P ≤ 0.001. CM conditioned medium, CNTF ciliary neurotrophic factor, GDNF glial cell line-derived neurotrophic factor, OLN med OLN93 medium, VEGF vascular endothelial growth factor.
the combinations of IFNγ/TNFα and TNFa/IL1β induce the increase of iNOS, TSG-6 and COX-2 mRNA transcripts. We demonstrate that the increase of NO and PGE2 is functionally involved in the suppression of T-cell proliferation by licensed rMAPC as suppression was abrogated when iNOS and COX-2 were inhibited in the cocultures.

While PGE2 has already been reported to partially underlie the suppressive effect of mMAPC on T-cell proliferation [21, 41], we now provide evidence that NO is crucial in this process as well. Notably, other studies using mMAPC have excluded PGE2 and NO as possible mechanisms for the modulation of alloreactive T-cell responses [21, 61]. Moreover, human and rat MAPC have been reported to modulate alloreactive T-cell responses by an Ido-dependent mechanism [57, 58]. Jacobs et al. showed that IFNγ pre-treatment of hMAPC did not confer an additional suppressive effect as it was already of a great extent without pre-licensing [57]. Overall, there is a pluripotency and species-related variation regarding the mechanisms involved in T-cell suppression, as is apparent for MSCs as well [62, 63].

We found that licensing of rMAPC markedly increased the expression of a large number of chemokines, such as CXCL2, CXCL10, CCL2, CCL5 and fractalkine. These chemokines are well known for their ability to attract encephalitogenic CD4+ T cells in the context of neuroinflammation [64–67], as well as CD8+ T cells [68]. The ability of stem cells to migrate towards inflammatory chemokines and to attract leukocytes is a crucial aspect of their potential therapeutic use. For instance, an IFNγ-mediated increase in chemokine release by murine MSCs, such as CXCL10, is key to their immunomodulatory properties [43]. In line with this chemoattractive transcriptional profile, we demonstrated that rMAPC effectively attracted myelin-specific T cells, especially when challenged with IFNγ + TNFα and TNFα + IL1β. In this project we only assessed the capacity of rMAPC to attract T cells. Others have shown that licensing enhances the chemoattractive ability of human MSCs towards other immune cells and, mainly, neutrophils [69]. Future studies should determine whether MAPC also attract other immune cell subsets that play an important role in inflammatory pathophysiology, such as monocytes and dendritic cells. Increased attraction of other immune cell types such as macrophages, for instance, would be crucial for the immunomodulatory mechanisms of MAPC, as it has already been demonstrated that hMAPC suppress their classically activated phenotype in vivo [18].

In addition to their ability to attract T cells, we characterized rMAPC regarding their migratory profile. We observed that rMAPC were able to migrate towards chemokines typical for a neuroinflammatory environment. Of interest, the motility of rMAPC towards these chemokines was further enhanced following licensing, except of the CCL2/CCR1 interaction following IFNγ + TNFα treatment. Overall, these results point out the potential of rMAPC in transplantation experiments where inflammation is already established. Directed injection of NSCs within the CNS in the EAE model showed that inflammation triggered the migration of the transplanted cells towards the white matter tracts, with CXCL12α and CCL2 being important inflamed tissue-derived chemoattractive stimuli [70]. Furthermore, in other neuroinflammatory models, both peripheral- and CNS-targeted injected stem cells are being attracted by local sites of inflammation [71]. While the lack of expression of CCR7 could limit the migration of rMAPC to lymphoid organs, previously reported experimental set up with intravenous administration of hMAPC showed no real obstacle [19]. This points out that other molecules in addition to chemokine receptors are implicated [72]. Overall, these features highlight the migratory potential of rMAPC when injected either in the periphery or within the CNS.

MSCs and NSCs possess neuroprotective features which are induced from inflamed CNS microenvironment [4, 34]. Our findings show that rMAPC that are exposed to sublethally damaged OLN93 cells gain neuroprotective properties. This suggests that damaged oligodendrocytes release mediators that promote the neuroprotective capacity of rMAPC. Similar findings have been reported for rat MSCs [34]. The fact that rMAPC increase their expression of trophic factors confirms this notion. It has already been suggested that human and rodent MAPC provide neuroprotection and vascular regeneration in vivo through the secretion of trophic factors [73–75]. Oligodendrocytes in normal appearing white matter (NAWM) seem to actively participate in immune regulation within the CNS during MS pathology as they are elevating the expression of transcription factors such as STAT-6 and STAT-4, which are important for the activation of anti- and pro-inflammatory pathways, respectively [76]. IL-4 and IL1β, which have been detected in oligodendrocytes in MS NAWM, could prime rMAPC in the same way as do the combinations of pro-inflammatory cytokines. In this way, rMAPC could be effectively triggered by damaged oligodendrocytes or even neurons to secrete trophic factors and thus provide neuroprotection.

**Conclusions**

MAPC possess the required properties needed to consider the development of a therapeutic scheme for neuroinflammatory disorders such as MS, TBI and SCI, as was established previously for MSCs [6, 7, 77]. We show that rMAPC are lowly immunogenic and possess numerous potential mechanisms which could facilitate MAPC
action, even if they cannot transdifferentiate towards damaged CNS cells. While the microenvironment in neuroinflammatory disorders is likely more complex, we show that typical pro-inflammatory cytokines and mediators released by damaged oligodendrocytes strongly enhance the immunomodulatory properties of rMAPC. Apart from affecting T cells and oligodendrocytes, MAPC may also affect other immune and CNS-resident cell types that are important drivers of neuroinflammation, such as macrophages, microglia and astrocytes. Future studies should define the impact of (licensed) rMAPC on these cell types. Collectively, our findings suggest that MAPC represent an interesting therapeutic tool for the treatment of neuroinflammatory disorders. Yet, future experiments should reinforce the notion that MAPC can reduce neuroinflammation and neurodegeneration in animal models of MS, TBI and SCI [18–20].

Additional files

Additional file 1: Selection of suitable positive control for migration assays. (TIFF 274 kb)
Additional file 2: Schematic illustration of the generation of double conditioned media and the neuroprotection assay. (TIFF 55 kb)
Additional file 3: Sequences of primers used for quantitative PCR. (DOCX 18 kb)
Additional file 4: rMAPC gene expression analysis following inflammatory treatment with real-time PCR. (DOCX 15 kb)
Additional file 5: Antigen-specific proliferation of T cells is not affected by the inhibitors alone. (TIFF 3085 kb)

Abbreviations
1-Mt-D: 1-methyl-droxy-tryptophan; 1-Mt-L: 1-methyl-levo-tryptophan; 7AAD: 7 aminoactinomycin D; BSA: Bovine serum albumin; CCL: Chemokine (C-C motif) ligand; CCR: Chemokine (C-C motif) receptor; CD: Cluster of differentiation; cDNA: Complementary deoxyribonucleic acid; CSF: Cerebrospinal fluid; COX: Cyclooxygenase; CXCL: Chemokine (C-X-C motif) ligand; CXCR: Chemokine (C-X-C motif) receptor; CyA: Cyclophilin A; DCM: Double conditioned medium; DMEM: Dulbecco’s modified Eagle’s medium; DMSO: Dimethyl sulfoxide; EAE: Experimental autoimmune encephalomyelitis; FACS: Fluorescence-activated cell sorting; FCS: Fetal calf serum; GDNF: Glial cell line-derived neurotrophic factor; H2O2: Hydrogen peroxide; HGF: Hepatocyte growth factor; hMAPC: Human multipotent adult progenitor cells; HMBS: Hydroxymethylbilane synthase; HO-1: Heme oxygenase 1; Ido-1: Indoleamine 2,3-dioxygenase 1; IFN: Interferon gamma; IL1β: Interleukin-1 beta; iNOS: Inducible nitric oxide synthase; L-NMMA: L-N α-arginine; L-NMMA: L-N α-arginine; Macrophage: Mature multipotent adult progenitor cells; mRNA: Messenger ribonucleic acid; MS: Multiple sclerosis; MSC: Mesenchymal stem cell; MTT: 3-(4,5-dimethylthiazol-2-yl)-5,5-diphenyltetrazolium bromide; NAWM: Normal appearing white matter; NAWM: Normal appearing white matter; NK: Natural killer cell; NO: Nitric oxide; NSC: Neural stem cell; PBS: Phosphate-buffered saline; PD-L1: Programmed death-1 ligand; PFA: Parafomoldehyde; PGE2: Prostaglandin E2; rMAPC: Rat multipotent adult progenitor cells; RPML: Roswell Park Memorial Institute; RT-PCR: Real time polymerase chain reaction; SCI: Spinal cord injury; SEM: Standard error of the mean; TBI: Traumatic brain injury; TNFα: Tumor necrosis factor alpha; TSG-6: TNF-stimulated gene 6 protein; VEGF: Vascular endothelial growth factor alpha; VWAH2: 14-3-3 protein zeta/delta.

Competing interests
DC, KG and JP are employees of ReGenesys BVBA, the European subsidiary of Athersys Inc. RWM and RD are employees of Athersys Inc. The other authors declare that they have no competing interests.

Authors’ contributions
SR performed the experiments and wrote the manuscript. SR, JFJB and NH designed the experiments. SR, JFJB and RD performed the analysis of data. DC, KG and JP contributed to the design of the experiments. DC, RWM, RD, KG, PS and JP revised the manuscript for important intellectual content. RWM, RD, AB and PS contributed to data interpretation and editing of the manuscript. AB contributed to the design and interpretation of migration assays. JFJB and NH revised the manuscript. All authors read and approved the final manuscript.

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Author details
1Hasselt University, Biomedical Research Institute/Transnational University Limburg, School of Life Sciences, Campus Diepenbeek, Agoralaan building C, 3590 Diepenbeek, Belgium. 2ReGenesys BV, Leuven, Belgium. 3Department of Regenerative Medicine, Athersys Inc., Cleveland, OH, USA.

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References
1. Einstein O, Fainstein N, Vaknin I, Mizrahi-Kol R, Reihartz E, Grigoriadis N, et al. Neural precursors attenuate autoimmune encephalomyelitis by peripheral immunosuppression. Ann Neurol. 2007;61:209–18.
2. Gerdoni E, Gallo B, Casazza S, Musio S, Bonanni I, Pedemonte E, et al. Mesenchymal stem cells effectively modulate pathogenic immune response in experimental autoimmune encephalomyelitis. Ann Neurol. 2007;61:219–27.
3. Kassis I, Grigoriadis N, Gowda-Kurkalli B, Mizrahi-Kol R, Ben-Hur T, Slavin S, et al. Neuroprotection and immunomodulation with mesenchymal stem cells in chronic experimental autoimmune encephalomyelitis. Arch Neurol. 2008;65:753–61.
4. Puchino S, Quattrini A, Brambilla E, Gritti A, Salani D, Gina G, et al. Injection of adult neurospheres induces recovery in a chronic model of multiple sclerosis. Nature. 2003;422:688–94.
5. Puchino S, Zancotti L, Rossi B, Brambilla E, Ottoboni L, Salani G, et al. Neurosphere-derived multipotent precursors promote neuroprotection by an immunomodulatory mechanism. Nature. 2005;436:266–71.
6. Anbari F, Khalili MA, Bahrami AR, Khorademhr A, Sadeghian F, Fesahat F, et al. Intravenous transplantation of bone marrow mesenchymal stem cells promotes neural regeneration after traumatic brain injury. Neural Regen Res. 2014;9:519–23.
7. Wilcox JT, Satkunendraranjek K, Zuccato JA, Nasriri F, Fehlings MG. Neural precursor cell transplantation enhances functional recovery and reduces astrogliosis in bilateral compressive/contusive cervical spinal cord injury. Stem Cells Transl Med. 2014;3:1148–59.
8. Zhang R, Liu Y, Yan K, Chen L, Chen XR, Li P, et al. Anti-inflammatory and immunomodulatory mechanisms of mesenchymal stem cell transplantation in experimental traumatic brain injury. J Neuroinflammation. 2013;10:106.
9. Jacobs SA, Roobrouck VD, Verfaillie CM, Van Gool SW. Immunological characteristics of human mesenchymal stem cells and multipotent adult progenitor cells. Immunol Cell Biol. 2013;91:32–9.
10. Sindberg GM, Lindborg BA, Wang Q, Clarkson C, Graham M, Donahue R, et al. Comparisons of phenotypic and immunomodulatory capacity among rhesus bone-marrow-derived mesenchymal stem/stromal cells, multipotent adult progenitor cells, and dermal fibroblasts. J Med Primatol. 2014;43:231–41.
11. Prokop DJ, Olson SD. Clinical trials with adult stem/progenitor cells for tissue repair: let’s not overlook some essential precautions. Blood. 2007;109:3147–51.
12. Karussi D, Karageorgiou C, Vaknin-Dembinsky A, Gowda-Kurkalii B, Gomori JM, Kassis I, et al. Safety and immunological effects of mesenchymal stem cell transplantation in patients with multiple sclerotic and allergic lateral sclerosis. Arch Neurool. 2010;67:1187–94.

13. Yamout B, Hourani R, Salii H, Barada W, El-Halal T, Al-Kutoubi A, et al. Bone marrow mesenchymal stem cell transplantation in patients with multiple sclerosis: a pilot study. J Neuroimmunol. 2010;227:185–9.

14. Jiang Y, Jahagirdar BD, Reinhardt RL, Schwartz RE, Keene CD, Ortiz-Gonzalez XR, et al. Pluripotency of mesenchymal stem cells derived from adult marrow. Nature. 2002;418:41–9.

15. Subramanian K, Geraets M, Pauwelyn KA, Park Y, Owens DJ, Muijtjens M, et al. Isolation procedure and characterization of multipotent adult progenitor cells from rat bone marrow. Mol Biol. 2010;63:55–78.

16. Uboh-Montoya F, Kiddier BL, Pauwelyn KA, Chase LG, Lutton A, Crabbe A, et al. Comparative transcriptome analysis of embryonic and adult stem cells with expanded and limited differentiation capacity. Genome Biol. 2007;8:R163.

17. Yasuhara T, Hara K, Maki M, Mays RW, Deans RJ, Hess DC, et al. Intravenous grafts recapitulate the neurorestoration afforded by intracerebrally delivered multipotent adult progenitor cells in neonatal hypoxic-ischemic rats. J Cereb Blood Flow Metab. 2008;28:1804–10.

18. Walker PA, Bedi SS, Shah SK, Jimenez F, Xue H, Hamilton JA, et al. Intravenous multipotent adult progenitor cell therapy after traumatic brain injury: modulation of the resident microglial population. J Neuroinflammation. 2012;9.

19. Walker PA, Shah SK, Jimenez F, Gerber MH, Xue H, Cutrone R, et al. Intravenous multipotent adult progenitor cell therapy for traumatic brain injury: preserving the blood brain barrier via an interaction with splenocytes. Exp Neurol. 2010;225:341–52.

20. Bedi SS, Hetz R, Thomas C, Smith P, Olsen AB, Williams S, et al. Intravenous multipotent adult progenitor cell therapy attenuates microglial/macrophage response and improves spatial learning after traumatic brain injury. Stem Cells Transl Med. 2013;2:953–60.

21. Luycke A, De Somer L, Jacobs S, Rutgeuts Q, Lenaerts C, Roodbrock VD, et al. Oct-4-negative multipotent adult progenitor cells and mesenchymal stem cells as regulators of T cell alloreactivity in mice. Immunol Lett. 2011;137:78–81.

22. Jacobs SA, Plessers J, Pinxtener J, Roodbrock VD, Verfaillie CM, Van Godd SW. Mutual interaction between human multipotent adult progenitor cells and NK cells. Cell Transplant. 2014;23:1099–110.

23. Krampera M. Mesenchymal stromal cell ‘licensing’: a multistep process. Leukemia. 2011;25:1408–14.

24. English K, Barry FP, Field-Corbett CP, Mahon BP. IFN-gamma and TNF-alpha in priming MSC-mediated suppression of T cell proliferation. Immunol Lett. 2007;110:91–6.

25. Sheng H, Wang Y, Jin Y, Zhang Q, Zhang Y, Wang L, et al. A critical role of IFN-gamma in priming MSC-mediated suppression of T cell proliferation following up-regulation of B7-1. Cell Res. 2008;18:466–57.

26. Croitoru-Lamoury J, Lamoury FM, Zaunders JJ, Veas LA, Brew BJ. Human mesenchymal stem cells constitutively express chemokines and chemokine receptors that can be upregulated by cytokines, IFN-beta, and C-Coxane. J Interferon Cytokine Res. 2007;27:53–64.

27. di Penta A, Moreno B, Reix S, Fernandez-Diez B, Villanueva M, Erea O, et al. Oxidative stress and proinflammatory cytokines contribute to demyelination and axonal damage in a cerebellar culture model of neuroinflammation. Plos One. 2013;8:e54722.

28. Filon LG, Gazzani-Boverie G, Matocevics D, Freedman MS. Monocyte-derived cytokines in multiple sclerosis. Clin Exp Immunol. 2003;131:324–34.

29. Lovett-Racke AE, Yang Y, Racke MK. Th1 versus Th17: are T cell cytokines relevant in multiple sclerosis? Biochim Biophys Acta. 2011;1812:246–51.

30. Zhao R, Zhou H, Su SB. A critical role for interleukin-1beta in the progression of autoimmune diseases. Int Immunopharmacol. 2013;17:658–69.

31. Bogie JF, Stinissen P, Helling S, Hendriks JJ. Myelin-phagocytosing macrophages modulate autoreactive T cell proliferation. J Neuroinflammation. 2011;8:83.

32. Bronkeraas A, Hikkila K, Fanto Y, Struyf T, Gervois P, Politis C, et al. Angiogenic properties of human dental pulp stem cells. Plos One. 2013;8:e71104.

33. Schneider CA, Rasband WS, Eliceiri KW. NIH image to ImageJ: 25 years of geometric averaging of multiple internal control genes. Genome Biol. 2002;3:RESEARCH0034.

34. GraphPad Prism Software. www.graphpad.com.

35. Untergasser A, Cutcutache I, Koressaar T, Ye Y, Faircloth BC, Remm M, et al. Primer3--new capabilities and interfaces. Nucleic Acids Res. 2012;40,e115.

36. Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2−ΔΔCT method. Methods. 2001;25:402–8.

37. Vandesompele J, De Preter K, Pattyn F, Poppe B, Van Roy N, De Paepe A, et al. Accurate normalization of real-time quantitative RT-PCR data by geometric averaging of multiple internal control genes. Genome Biol. 2002;3:RESEARCH0034.

38. Highfill SL, Kelly RM, O'Shaughnessy MJ, Zhou Q, Xia L. Panoskaltsis-Mortari A, et al. Multipotent adult progenitor cells can suppress graft-versus-host disease via prostaglandin E2 synthesis and only if localized to sites of allografting. Blood. 2009;114:693–701.

39. Kota DJ, Wiggins LL, Yoon N, Lee RH. TGF-6 produced by mMSCs delays the onset of autoimmune diabetes by suppressing Th1 development and enhancing tolerogeneity. Diabetes. 2013;62:240–8.

40. Ren GX, Zhang LY, Zhao X, Xu GW, Zhang YY, Roberts AI, et al. Mesenchymal stem cell-mediated immunosuppression occurs via concerted action of chemokines and nitric oxide. Cell Stem Cell. 2008;2:141–50.

41. Bai L, Lennon DP, Caplan AI, DeChant A, Hecker J, Kranos J, et al. Hepatocyte growth factor mediates mesenchymal stem cell-induced recovery in multiple sclerosis models. Nat Neurosci. 2012;15:862–70.

42. Jones TB, Basso DM, Sodhi A, Pan JZ, Hart RP, MacGillum RC, et al. Pathological CNS autoimmune disease triggered by traumatic spinal cord injury: implications for autoimmune vaccine therapy. J Neurosci. 2002;22:690–700.

43. Kil K, Zang YC, Yang D, Markowski M, Juco G, Vendetti GC, et al. T cell responses to myelin basic protein in patients with spinal cord injury and multiple sclerosis. J Neuroimmunol. 1999;98:201–7.

44. Popovich PG, Stokes BT, Whitacre CC. Concept of autoimmunity following spinal cord injury: possible roles for T lymphocytes in the traumatized central nervous system. J Neurosci Res. 1996;45:349–63.

45. Chamberlain G, Fox J, Ashton B, Middleton J. Concise review: mesenchymal stem cells: their phenotype, differentiation capacity, immunological features, and potential for homing. Stem Cells. 2007;25:2379–49.

46. Rice CM, Scolding NJ. Adult human mesenchymal cells proliferate and migrate in response to chemokines expressed in demyelination. Cell Adh Migr. 2010;4:235–40.

47. Richter-Landsberg C, Heinrich M. OLN-93: a new permanent oligodendroglia cell line derived from primary rat brain glial cultures. J Neurosci Res. 1996;45:161–73.

48. Jana A, Pahan K. Oxidative stress kills human primary oligodendrocytes via neutral sphingomyelinase: implications for multiple sclerosis. J Neuroimmunol Pharmacol. 2007;2:184–93.

49. Vondran MW, Clinton-Luke P, Honeywell JZ, Dreyfus CF. BDNF+/− mice exhibit deficits in oligodendroglia lineage cells of the basal forebrain. Glia. 2010;58:848–56.

50. Akerud P, Canals JM, Snyder EY, Atanas M. Neuroprotection through delivery of gial cell line-derived neurotrophic factor by neural stem cells in a mouse model of Parkinson’s disease. J Neurosci. 2001;21:8108–20.

51. Kim HM, Hwang DH, Lee JE, Kim SJ, Kim BG. Ex vivo VEGF delivery by neural stem cells enhances proliferation of gial progenitors, angiogenesis, and tissue sparing after spinal cord injury. Plos One. 2009;4:e4987.

52. Lu Z, Hu X, Zhu C, Wang D, Zheng X, Liu Q. Overexpression of CNITF in mesenchymal stem cells reduces demyelination and induces clinical recovery in experimental autoimmune encephalomyelitis mice. J Neuroimmunol. 2009;206:658–69.

53. Said EA, Dupuy FP, Trautmann L, Zhang Y, Shi Y, El-Far M, et al. Programmed death-1–induced interleukin-10 production by monocytes impairs CD4+ T cell activation during HIV infection. Nat Med. 2010;16:452–9.

54. Jacobs SA, Pinxtenner J, Roodbrock VD, Luyckx A, van’t Hof W, Deans R, et al. Human multipotent adult progenitor cells are immunonegative and exert...
potent immunomodulatory effects on alloreactive T-cell responses. Cell Transplant. 2013;22:1915–28.

58. Kovacsics-Bankowski M, Streeter PR, Mauch KA, Frey MR, Raber A, van't Hof W, et al. Clinical scale expanded adult pluripotent stem cells prevent graft-versus-host disease. Cell Immunol. 2009;255:55–60.

59. Carrade Holt DD, Wood JA, Granick JL, Walker NJ, Clark KC, Borjeson DL. Equine mesenchymal stem cells inhibit T cell proliferation through different mechanisms depending on tissue source. Stem Cells Dev. 2014;23:1258–65.

60. Meisel R, Zibert A, Lanyea M, Gobel U, Daubener W, Dilloo D. Human bone marrow stromal cells inhibit allogenic T-cell responses by indoleamine 2,3-dioxygenase-mediated tryptophan degradation. Blood. 2004;103(4619–21.

61. Luyckx A, De Somer L, Rutgeerts O, Waer M, Verfaillie CM, Van Gool S, et al. Mouse MAPC-mediated immunomodulation: cell-line dependent variation. Exp Hematol. 2010;38:1–2.

62. Ren G, Su J, Zhang L, Zhao X, Ling W, L’Huillie A, et al. Species variation in the mechanisms of mesenchymal stem cell-mediated immunosuppression. Stem Cells. 2006;24:1554–62.

63. Schnabel LV, Abratte CM, Schimenti JC, Felippe MJ, Cassano JM, Southard TL, et al. Induced pluripotent stem cells have similar immunogenic and more potent immunomodulatory properties compared with bone marrow-derived stromal cells in vitro. Regen Med. 2014;9:621–35.

64. Balashov KE, Rottman JB, Weiner HL, Hancock WW. CCR5(+) and CXCR3(+) T cells are increased in multiple sclerosis and their ligands MIP-1alpha and IP-10 are expressed in demyelinating brain lesions. Proc Natl Acad Sci U S A. 1999;96:6875–8.

65. Broux B, Pannemans K, Zhang X, Markovic-Plese S, Broekmans T, Eijnde BO, et al. CX(3)CR1 drives cytotoxic CD4(+CD28(−) T cells into the brain of multiple sclerosis patients. J Autoimmun. 2012;38:10–9.

66. Simpson J, Rezaie P, Newcombe J, Cuzner ML, Male D, Woodrooffe MN. Expression of the beta-chemokine receptors CCR2, CCR3 and CCR5 in multiple sclerosis central nervous system tissue. J Neuroimmunol. 2000;108:192–200.

67. Sorensen TL, Trebst C, Kivisakk P, Klaege KL, Majmudar A, Ravid R, et al. Multiple sclerosis: a study of CXCL10 and CXCR3 co-localization in the inflamed central nervous system. J Neuroimmunol. 2002;127:59–68.

68. Chuntharapai A, Lee J, Hebert CA, Kim KJ. Monoclonal antibodies detect different distribution patterns of IL-8 receptor A and IL-8 receptor B on human peripheral blood leukocytes. J Immunol. 1994;153:5682–8.

69. Carrero R, Cerrada I, Lledo E, Dopazo J, Garcia-Garcia F, Rubio MP, et al. IL1beta induces mesenchymal stem cells migration and leucocyte chemotaxis through NF-kappaB. Stem Cell Rev. 2012;8:905–16.

70. Cohen ME, Faiststein N, Lavon I, Ben-Hur T. Signaling through three chemokine receptors triggers the migration of transplanted neural precursor cells in a model of multiple sclerosis. Stem Cell Res. 2014;10:227–39.

71. Jackson JS, Golding JP, Chapon C, Jones WA, Bhakoo KK. Homing of stem cells to sites of inflammatory brain injury after intracerebral and intravenous administration: a longitudinal imaging study. Stem Cell Res Ther. 2010;1:17.

72. Ries C, Egea V, Karow M, Kolb H, Jochum M, Neth P. MMP-2, MT1-MMP, and TIMP-2 are essential for the invasive capacity of human mesenchymal stem cells: differential regulation by inflammatory cytokines. Blood. 2007;109:4055–63.

73. Aranguren XL, Pelacho B, Penuelas I, Abizanda G, Utit M, Ecay M, et al. MAPC transplantation confers a more durable benefit than AC133+ cell transplantation in severe hind limb ischemia. Cell Transplant. 2011;20:259–69.

74. Busch SA, Hamilton JA, Horn KP, Cusack FX, Cunstone R, Lehman N, et al. Multipotent adult progenitor cells prevent macrophage-mediated axonal dieback and promote regrowth after spinal cord injury. J Neurosci. 2011;31:944–53.

75. Pelacho B, Nakamura Y, Zhang J, Ross J, Heremans Y, Nelson-Holte M, et al. Multipotent adult progenitor cell transplantation increases vasculature and improves left ventricular function after myocardial infarction. J Tissue Eng Regen Med. 2007;1:51–9.

76. Zeis T, Graumann U, Reynolds R, Schaeren-Wiemers N. Normal-appearing white matter in multiple sclerosis is in a subtle balance between inflammation and neuroprotection. Brain. 2008;131:288–303.

77. Freedman MS, Bar-Or A, Atkins HJ, Kurass D, Frassoni F, Lazarus H, et al. The therapeutic potential of mesenchymal stem cell transplantation as a treatment for multiple sclerosis: consensus report of the International MSCT Study Group. Mult Scler. 2010;16:503–10.