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Astrocyte-derived interleukin-6 promotes specific neuronal differentiation of neural progenitor cells from adult hippocampus

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Abstract

The purpose of this study was to investigate the ability of astrocyte-derived factors to influence neural progenitor cell differentiation. We previously demonstrated that rat adult hippocampal progenitor cells (AHPCs) immunoreactive for the neuronal marker, class III β-tubulin (TUJ1) were significantly increased in the presence of astrocyte-derived soluble factors under non-contact co-culture conditions. Using whole cell patch clamp analysis, we observed that the co-cultured AHPCs displayed two prominent voltage-gated conductances - tetraethyl ammonium (TEA)-sensitive outward currents and fast transient inward currents. The outward and inward current densities of the co-cultured AHPCs were approximately 2.5-fold and 1.7-fold greater, respectively, than those of cells cultured alone. These results suggest that astrocyte-derived soluble factors induce neuronal commitment of AHPCs. To further investigate the activity of a candidate neurogenic factor on AHPC differentiation, we cultured AHPCs in the presence or absence of purified rat recombinant interleukin-6 (IL-6). We also confirmed that the astrocytes used in this study produced IL-6 by ELISA and RT-qPCR. When AHPCs were cultured with IL-6 for 6-7 days, the TUJ1-immunoreactive AHPCs and the average length of TUJ1-immunoreactive neurites were significantly increased, compared to the cells cultured without IL-6. Moreover, IL-6 increased the inward current density to a comparable extent as did co-culture with astrocytes, with no significant differences in the outward current density, apparent resting potential, or cell capacitance. These results suggest that astrocyte-derived IL-6 may facilitate AHPC neuronal differentiation. Our findings have important implications for understanding injury-induced neurogenesis and developing cell-based therapeutic strategies using neural progenitors.

Keywords

Neural stem cells; Interleukin-6; Neuronal differentiation; Electrophysiology

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Introduction

The cytokine interleukin-6 (IL-6) is best known as a regulator of hematopoiesis(Eglitis and Mezey 1997). In addition, IL-6 is found in the nervous system and in conjunction with its receptor complex can trigger cellular responses mediating inflammation, neurogenesis, gliogenesis, cell growth and survival, and myelination/demyelination(Gadient and Otten 1997; Gruol and Nelson 1997; Van Snick 1990; Van Wagoner and Benveniste 1999). In the brain, IL-6 is normally expressed at relatively low levels(Gadient and Otten 1997). However, during progression of inflammation or brain injury, IL-6 protein levels are elevated in the cerebral spinal fluid and brain homogenates(Gruol and Nelson 1997; Van Wagoner and Benveniste 1999). Chronic over-expression of IL-6 in transgenic mice caused neuroanatomical and neurophysiological alterations reminiscent of various neurological diseases(Campbell et al. 1993; Gruol and Nelson 1997; Vallieres et al. 2002). Interestingly, neurotrophins (NTs) such as nerve growth factor (NGF), are co-expressed with IL-6 at sites of nerve injury(Otten et al. 2000). IL-6 in combination with its specific receptor, IL-6R, has been reported to regulate a local supply of NTs that are critical for neuronal survival and protection(Gadient and Otten 1994; Gadient and Otten 1997; Otten et al. 2000). These reports suggest that IL-6 expression is up-regulated under diverse pathological and physiological conditions in the CNS, and also contributes to the production and function of NTs at the injured site of the CNS.

Astrocytes are an important source of IL-6 in the CNS(Gruol and Nelson 1997) and the expression levels vary for different brain regions. Barkho et al. (2006) demonstrated that astrocytes originating from neurogenic regions (newborn hippocampus, adult hippocampus, and newborn spinal cord) compared with cells from non-neurogenic regions (adult spinal cord astrocytes and adult skin fibroblasts) displayed different gene expression profiles(Barkho et al. 2006; Song et al. 2002). IL-6 mRNA was expressed at relatively higher levels in newborn hippocampal astrocytes than in non-neurogenic astrocytes. Furthermore, when adult hippocampus-derived neural progenitor cells were cultured in the presence of IL-6, neuronal promoter activity and neuronal marker-immunoreactivity were increased(Barkho et al. 2006). Our previous results confirmed that neuronal differentiation of adult hippocampal progenitor cells (AHPCs) was enhanced by soluble factors derived from neonatal astrocytes(Oh et al. 2009; Recknor et al. 2006). However, it was not known if astrocyte-derived IL-6 influenced NPC differentiation into functional neurons.

In the present study, we tested the hypothesis that IL-6 produced from neurogenic astrocytes is a key regulator in our culture system that stimulates functional differentiation of AHPCs to a neuronal fate. Using immunocytochemical analysis and whole-cell recording, in the presence of exogenous IL-6, we observed that (1) neuronal marker expression was increased, (2) the average length of neurites from neuronal-restricted AHPCs was increased and (3) voltage-gated inward current density was increased with no significant differences in voltage-gated outward current density, apparent resting membrane potential or cell capacitance. These results demonstrate that astrocyte-derived IL-6 enhances neuronal differentiation of AHPCs.

Materials and Methods

Cell culture

All animal procedures were conducted in accordance with and had the approval of the Iowa State University Committee on Animal Care. Astrocytes were isolated from cerebral cortices and hippocampi of postnatal day two Sprague-Dawley rat brains as described previously(Recknor et al. 2004). Adult hippocampal progenitor cells (AHPCs from F. Gage, Salk Institute, La Jolla, CA), isolated from the brains of adult Fischer 344 rats and infected

with retrovirus to express enhanced green fluorescent protein (GFP)(Gage et al. 1995; Ray et al. 1995) were maintained as described previously(Oh et al. 2009) (for details, see Supplementary Materials and Methods online).

Astrocyte-AHPC co-culture

Transwell® inserts (Corning, Inc., Corning, NY) were used to establish co-cultures of astrocytes and AHPCs growing together in the absence of physical contact (referred to as non-contact co-culture, NCCC) as described previously(Oh et al. 2009) (for details, see Supplementary Materials and Methods online).

IL-6 treatment

To examine the effect of IL-6 on AHPC differentiation, purified rat recombinant IL-6 (20 ng/ml; R&D Systems) and/or neutralizing antibody against IL-6 (anti-IL-6) produced in goat (10 ng/ml; R&D Systems) were added to the culture media. A concentration-response analysis was performed to determine optimal concentrations of purified IL-6 (Supplementary Figure 1). Based on this analysis and from the literature(Barkho et al. 2006; Hakkoum et al. 2007), an IL-6 concentration of 20 ng/ml was used in this study. The anti-IL-6 neutralizing antibody was tested at various concentrations (10, 100 and 1000 ng/ml in NCCCs of astrocyte-AHPC; 10 and 1000 ng/ml in AHPC alone cultures with 20 ng/ml of purified IL-6). There were no significant effects of these concentrations of blocking antibody on the percentages of TUJ1- or RIP-immunoreactive AHPCs (see 'Immunocytochemistry' section below) when the cells were cultured with the antibody in the absence of IL-6 (data not shown). Thus, the anti-IL-6 neutralizing antibody was used at 10 ng/ml in this study. As a control for the neutralizing antibody, a purified preimmune goat immunoglobulin G (goat IgG; Sigma-Aldrich) was used at the same concentration.

Immunocytochemistry

AHPCs cultured on laminin-coated substrates were processed for immunocytochemistry according to standard protocols described previously(Oh et al. 2009). Fixed AHPCs were labeled with antibodies against phenotypic markers, class III β-tubulin (TUJ1, mouse monoclonal IgG; R&D Systems), receptor interacting protein (RIP in concentrated form supplied by vendor, mouse monoclonal IgG; Developmental Studies Hybridoma Bank) and glial fibrillary acidic protein (GFAP, mouse monoclonal IgG; Lab Vision Corp., Fremont, CA) Biotinylated donkey anti-mouse secondary antibody and Cy3-conjuated streptavidin (Jackson ImmunoResearch, West Grove, PA) were used for visualizing the primary antibody labeling. Nuclei were counterstained with 1 μM 4′, 6-diamidino-2-phenylindole, dilactate (DAPI). Preparations were then mounted onto microscope slides using GelMount antifade mounting medium (Biomeda Corp., Foster City, CA) (for details, see Supplementary Materials and Methods online).

Neurite outgrowth assay

AHPCs were cultured in the absence or presence of purified IL-6 at different concentrations (from 0.00001 to 100 ng/ml; Figure 3 C) for 6 days. After fixation, cultured AHPCs were immunolabeled for TUJ1 and imaged using a 40X objective. From the images, 17 to 22 TUJ1-immunoreactive AHPCs per condition were analyzed for the length of neurites emanating from the cell body using ImageJ, image processing and analysis program (developed at the National Institutes of Health, http://rsbweb.nih.gov/ij/) (for details, see Supplementary Materials and Methods online).

Whole cell patch clamping

AHPCs were plated on a laminin-coated polystyrene substrate that was attached to the bottom of a recording chamber made of a 25-mm circular coverglass (Cat # 12-545-102, Fisher Scientific) with an attached silicone O-ring (Cat # 9396K22, McMaster Carr Supply, Atlanta, GA). After the cells were cultured in the recording chamber for a designated culture period, the chamber with cells was placed on the stage of a Nikon Diaphot inverted microscope (Tokyo, Japan). The cultures were bathed in an external solution containing (in mM): 145 NaCl, 3 KCl, 10 HEPES, 8 glucose, 2 $MgCl_2$, 3 $CaCl_2$ (pH 7.4, adjusted with NaOH). Patch pipettes were pulled from thick-walled borosilicate glass capillaries (Cat # PG52165-4, World Precision Instrument, Inc., Sarasota, FL) and fire-polished to resistances of 3 - 5 MΩ when filled with internal solution containing (in mM): 136.5 K-gluconate, 17.5 KCl, 9 NaCl, 10 HEPES, 8 glucose, 0.2 EGTA and 1 $MgCl_2$ (pH 7.2, adjusted with KOH). For the cultures maintained in the presence of basic fibroblast growth factor (bFGF), cells were randomly chosen for recording. For the cells cultured under differentiation condition, cells that possessed neuronal-like morphology typical of TUJ1-immunoreactive cells were selected(Ray et al. 1993; Sah et al. 1997).

Conventional whole cell recording was performed at room temperature using an Axopatch 200B patch clamp amplifier (Molecular Devices, Sunnyvale, CA) and IT-16 interface (Instrutech, Inc.) or an EPC9 (HEKA Instruments, Inc., Bellmore, NY). Current was filtered at 1 kHz and digitized at 5 kHz. Stimulation and acquisition were controlled with PULSE software (v8.78, HEKA Instruments, Inc.).

A liquid junction potential of 14.3 mV was estimated using JPCalc software (P. H. Barry, University of New South Wales). Membrane voltages were corrected after the experiment by subtraction of the liquid junction potential; the apparent resting potential was estimated 10 min after break-in to the whole cell mode as the zero current potential during current-clamp recording and corrected for the liquid junction potential. Ionic currents were measured by application of 100 ms depolarizing steps in 10 mV increments from the holding potential (V_H) of -100 mV. The interpulse interval was 5 s. Current densities were calculated by normalizing the peak currents to the membrane capacitance (Cm). Input resistance (Rin) was estimated from the reciprocal of the slope of voltage ramps at subthreshold potentials in the whole cell mode; because the values of Rin estimated this way were similar to seal resistances, we could only place a lower limit on the true Rin.

Pharmacological blockade of inward and outward currents was performed with 300 nM tetrodotoxin (TTX; Sigma-Aldrich) for voltage-gated sodium (Na^+) channels, or 5 mM tetraethylammonium (TEA; Sigma-Aldrich) for voltage-gated potassium (K^+) channels, respectively.

Statistical analysis

Statistical analysis was performed using GraphPad PRISM (ver. 3.0). All tests were two-tailed tests and p-values less than an alpha of 0.05 were considered significantly different.

Results

Astrocyte-derived soluble factors enhance AHPC neuronal differentiation

Our previous results demonstrated that astrocyte-derived soluble factors induced neuronal differentiation of AHPCs(Oh et al. 2009). When AHPCs were co-cultured with astrocytes (isolated from neonatal rat brain, cerebral hemispheres) in the absence of physical contact (referred to as non-contact co-culture, NCCC) using Transwell® inserts, neuronal differentiation of AHPCs was significantly augmented compared to AHPCs cultured alone

(without astrocytes) (Figure 1; 63.7% in NCCC *vs.* 13.1% in alone culture). To further delineate the source of neurogenic activity, we isolated separately hippocampal and cortical astrocytes. Immunocytochemical analysis revealed that under NCCC conditions, using either hippocampal or cortical astrocytes, the percentage of TUJ1-immunoreactive (IR) AHPCs was significantly higher compared to that when AHPCs were cultured alone (Figure 1). In addition, the percentage of TUJ1-IR cells was significantly greater for AHPCs when co-cultured with hippocampal astrocytes (NCCC with HC-Astro) than with cortical astrocytes (NCCC with CTX-Astro) (Figure 1; 54.4% in NCCC with HC-Astro *vs.* 34.2% in NCCC with CTX-Astro). These results suggest that the astrocyte-derived soluble factors induce neuronal differentiation of AHPCs, which is consistent with our previous results(Oh et al. 2009). Moreover, on a cell per cell basis, hippocampal astrocytes appear to possess significantly greater neurogenic activity, compared to cortical astrocytes.

The astrocyte-derived factors appeared specific for inducing AHPC neuronal differentiation, because no effect was observed on astroglial differentiation (Figure 1; RIP and GFAP immunoreactivities). Under NCCC using astrocytes from whole cerebral hemispheres (referred to as brain astrocytes, Brain-Astro), the percentage of oligodendrocytes (RIP-IR AHPCs) was greater than when AHPCs were cultured alone (Figure 1; 26.8% in NCCC with Brain-Astro *vs.* 13.0% in alone culture). However, under NCCC using either cortical astrocytes or hippocampal astrocytes, there was no significant difference in RIP immunoreactivity compared to the AHPCs cultured alone (Figure 1; 15.4% in NCCC with HC-Astro *vs.* 19.1% in NCCC with CTX-Astro *vs.* 13.0% in alone culture). This result demonstrates that the factors from cortical astrocytes or from hippocampal astrocytes have little effect on oligodendrocytic differentiation of AHPCs. However, whole brain astrocytes have a small incremental effect on oligodendrocytic differentiation of AHPCs.

To examine whether the AHPCs with neuronal morphology possessed membrane characteristics consistent with neuronal differentiation, patch clamp analysis in conventional whole cell mode was performed. AHPCs were cultured in the absence or presence of the astrocytes for 6-7 days or 9-10 days. AHPCs from both conditions had similar capacitance (Cm) values (Table 1) and input resistance (Rin, ≥ 2 GΩ) (data not shown). The apparent resting potential was more hyperpolarized under differentiation conditions compared to the proliferation conditions (Table 1). AHPCs at 6-7 DIV in co-culture with brain-derived astrocytes showed significantly greater current densities for both TEA-sensitive sustained outward currents (voltage-gated K^+ channel-mediated) and transient inward currents (voltage-gated Na^+ channel-mediated) in response to the voltage-step stimuli compared to the AHPCs cultured alone (Figure 2 A2 and B2; Table 1). These results demonstrate that astrocyte-derived neurogenic factors promoted neuronal differentiation with respect to the electrical membrane properties of AHPCs, as well as in cell morphology.

IL-6 enhances neuronal differentiation of AHPCs

IL-6 has been suggested as a candidate astrocyte-derived factor that can induce AHPC differentiation(Barkho et al. 2006). To investigate the molecular mechanism(s) by which astrocytes promote neurogenesis, a neutralizing antibody against rat IL-6 (referred to as anti-IL-6) was added to the AHPC cultures. As a control, equal concentrations of affinity-purified goat IgG were added to parallel cultures. Addition of anti-IL-6 to the hippocampal NCCC resulted in a significant decrease in neuronal differentiation of the AHPCs (percentage of TUJ1-IR AHPCs: 54.4% without anti-IL-6 *vs.* 27.7% with anti-IL-6) (Figure 3 A). In contrast, the blocking antibody had no effect on TUJ1 immunoreactivity in cortical NCCC (34.2% without anti-IL-6 *vs.* 35.2% with anti-IL-6) (Figure 3 A). These results suggest that IL-6 derived from neurogenic hippocampal astrocytes may play a critical role in neuronal differentiation of AHPCs.

To examine further whether IL-6 itself can directly influence AHPC differentiation, we cultured AHPCs (alone, in the absence of astrocytes) with or without rat recombinant IL-6 for 6 days. Incubation with purified IL-6 specifically increased the percentage of TUJ1-IR AHPCs (38.5% with IL-6 *vs.* 13.1% without IL-6) with no effect on the percentages of RIP- or GFAP-IR AHPCs (RIP immunoreactivity: 13.6% with IL-6 *vs.* 13.0% without IL-6; GFAP immunoreactivity: 1.5% with IL-6 *vs.* 1.6% without IL-6) (Figure 3 B). Moreover, the IL-6-mediated augmentation of TUJ1 immunoreactivity was eliminated by addition of anti-IL-6 neutralizing antibody (Figure 3 B). These results suggest that IL-6 is a key molecule that can induce neuronal differentiation of AHPCs.

In addition, IL-6 influences neurite outgrowth of AHPCs. When AHPCs were cultured in the presence of various concentrations of purified IL-6, the average length of TUJ1-IR processes extending from the soma of TUJ1-IR AHPCs was positively correlated with the concentration of IL-6 (Figure 3 C). At concentrations greater than 1 ng/ml of IL-6, the average process length per cell was significantly longer than that of the control cultures (AHPCs alone with no IL-6). The average length of neurites analyzed from the cells cultured in the presence of IL-6 was approximately 40 μm, which is comparable to the process length of AHPCs co-cultured with enriched postnatal astrocytes as demonstrated previously(Oh et al. 2009). However there was no significant difference observed in the total length of processes per cell with increasing IL-6 concentrations (Supplementary Figure 2). These data indicate that IL-6 increases the length of TUJ1-expressing neurites from AHPCs undergoing neuronal differentiation.

To investigate the influence of IL-6 on electrical membrane properties, AHPCs were cultured under differentiation conditions in the absence or presence of IL-6. The AHPCs cultured in the presence of IL-6 showed significantly greater transient inward currents compared to cells cultured in the absence of IL-6 (Figure 4; Table 2). In three out of three IL-6-treated AHPCs for which it was tested, 300 nM TTX inhibited the transient inward currents, which suggests mediation by TTX-sensitive Na^+ channels. Augmentation of the transient inward current in the AHPCs cultured in the presence of IL-6 was comparable to that seen with the AHPCs co-cultured with astrocytes. The effect appears to be selective for Na^+ current, as no enhancement of a presumed K^+ current (TEA-sensitive current) was apparent (Table 2). The observed changes in Cm and apparent resting potential due to exogenous IL-6 were statistically insignificant (Table 2). These results suggest that IL-6 can facilitate expression of voltage-gated Na^+ channels during AHPC differentiation into neurons.

IL-6 is produced from the co-cultured astrocytes

To quantify the amount of IL-6 protein secreted into the culture media, we performed enzyme-linked immunosorbent assays (ELISA) (Supplementary Figure 3 A). These results demonstrate that (a) the major source of IL-6 production in the non-contact co-culture system was the astrocytes, rather than AHPCs, and (b) the IL-6 production capability of astrocytes appeared to be independent of the AHPCs co-cultured with them. The expression levels of IL-6, its specific receptor, IL-6Rα (transmembrane and soluble) and gp130 in AHPCs and astrocytes were analyzed by RT-qPCR (Supplementary Figure 3 B, C) (for details, see Supplementary Results and Discussion online).

Discussion

The local microenvironment is crucial in regulating the survival, proliferation and differentiation of NPCs(Conover and Notti 2008; Garcion et al. 2004; Soen et al. 2006; Watt and Hogan 2000; Wurmser et al. 2004). In addition to the extracellular matrix (ECM), astrocytes surrounding NPCs can provide various biological cues for regulating

neurogenesis *in vivo*(Lim and Alvarez-Buylla 1999; Recknor et al. 2006; Song et al. 2002). Our previous results demonstrated that astrocyte-derived soluble factors promote neuronal differentiation of AHPCs, in combination with three-dimensional spatial control(Oh et al. 2009). In the present study, we identified a candidate molecule, IL-6, which specifically induces neuronal differentiation of adult NPCs by demonstrating that (1) newborn hippocampal astrocytes produced more IL-6 protein than non-neurogenic skin-derived fibroblasts and (2) IL-6 treatment increased the fraction of TUJ1-immunoreactive cells. These results support the earlier findings by Barkho and colleagues(Barkho et al. 2006). Furthermore, in our study we reported for the first time electrophysiological properties of AHPCs undergoing differentiation in the presence of IL-6. Our findings demonstrate that IL-6 derived from neurogenic astrocytes promotes functional differentiation of AHPCs and accelerates neurite outgrowth.

Astrocyte-derived IL-6 promoting neuronal differentiation of AHPCs

Previously we demonstrated that the percentage of TUJ1-IR AHPCs was dramatically increased when AHPCs were co-cultured with astrocytes compared to the cells cultured alone(Oh et al. 2009). This result suggested that astrocyte-derived soluble factors enhance neuronal differentiation of AHPCs. To identify the source of neurogenic activity of the astrocytes, we separated the hippocampi, a neurogenic region of the brain, from the surrounding cerebral cortex, thus generating two distinct populations of astrocytes. A significantly higher percentage of AHPCs differentiated into neurons (TUJ1 immunoreactivity) when co-cultured with hippocampal astrocytes compared to those co-cultured with cortical astrocytes. This suggests that the soluble factors derived from hippocampal astrocytes are in part responsible for the increase in neuronal differentiation of AHPCs, which agrees with the previous finding that astrocytes from adult hippocampus can instruct the NPCs to adopt a neuronal fate(Song et al. 2002).

The increased neuronal differentiation of AHPCs co-cultured with hippocampal astrocytes was diminished in the presence of neutralizing antibody against IL-6. However, the neutralizing antibody had no effect on the TUJ1 immunoreactivity of AHPCs co-cultured with cortical astrocytes. These results might be explained by the combined action of multiple factors produced by astrocytes and/or AHPCs. For example, hippocampal astrocytes may produce additional neurogenic factors that promote neuronal differentiation of AHPCs. Recently, metalloprotease-9, all-*trans* retinoic acid, Wnt3 and neurogenesin-1 were reported as factors derived from neurogenic astrocytes that can induce neuronal differentiation of NPCs or oligodendrocyte fate-restricted progenitor cells(Barkho et al. 2008; Barkho et al. 2006; Gaughwin et al. 2006; Kornyei et al. 2007; Lie et al. 2005; Ueki et al. 2003). Furthermore, cortical astrocytes appear to express developmental signals, such as Notch, Noggin, sonic hedgehog and thrombospondin-1 which can participate in generating a neurogenic microenvironment surrounding NPCs(Lu and Kipnis; Taupin 2006). Factors derived from cortical astrocytes may also suppress neuronal differentiation of AHPCs. For instance, insulin-like growth factor (IGF)-1 is known to be important for early neural development as well as maintenance of the adult CNS (Russo et al. 2005). In the adult CNS, IGF-1, which is expressed both in neurons and astrocytes, binds to IGF binding proteins (IGFBPs) that are mainly produced by astrocytes(Chesik et al. 2007; Feldman et al. 1997; Russo et al. 2005; Ye et al. 2004). IGFBP-6, in particular, was reported to be expressed at much higher levels in non-neurogenic astrocytes than in neurogenic-astrocytes, so it is thought to inhibit neuronal differentiation of NPCs(Barkho et al. 2006). Bone morphogenic proteins (BMPs), having multiple roles in embryonic brain development, are also known to influence glial and neuronal differentiation of NPCs(Gross et al. 1996; Taga and Fukuda 2005; Zhang and Li 2005). Therefore, the neurogenic effect of IL-6 released from cortical astrocytes may be prevented by other cortical astrocyte-derived factors. Taken together,

reduced neuronal differentiation by neutralizing antibody against IL-6 demonstrates that an astrocyte-derived soluble factor, IL-6, is a critical soluble factor that can enhance neuronal differentiation of AHPCs. Furthermore, astrocytes isolated from the hippocampal region, a neurogenic niche, may be more potent in inducing neuronal differentiation of AHPCs than the general astrocyte population.

Direct application of IL-6 enhanced AHPC neuronal but not glial differentiation. Moreover, IL-6-mediated neuronal differentiation was neutralized by anti-IL-6 function-blocking antibody. This result suggests that AHPCs are capable of responding with neuronal differentiation to exogenous IL-6. RIP immunoreactivity of AHPCs under co-culture conditions tended to be slightly higher compared to that of AHPCs cultured alone. This result is not surprising because numerous soluble factors derived from the astrocytes may influence NPC fate specification during CNS development or inflammation response(Martino and Pluchino 2006; Taupin 2008). For example, sonic hedgehog secreted from reactive astrocytes can induce NPCs to differentiate into oligodendrocytes as well as neurons(Martino and Pluchino 2006). Platelet-derived growth factor produced by astrocytes has been known to stimulate the proliferation of oligodendrocyte precursors in the CNS(Abe 2000; Martino and Pluchino 2006). Interestingly, oligodendrocyte differentiation (assessed by RIP immunoreactivity) from AHPCs co-cultured with astrocytes from the whole cerebral hemispheres (including the hippocampus) was significantly higher than that under control conditions (AHPCs cultured alone). However, under co-culture conditions with either cortical or hippocampal astrocytes, the RIP immunoreactivity was not significantly different compared to that under control conditions. Region-specific factors may be produced only from the cortical or hippocampal astrocytes that potentially promote oligodendrocyte differentiation of AHPCs. The oligodendrocyte differentiation-promoting activity of those factors may be reinforced by juxtacrine or paracrine stimulation when the cortical and hippocampal astrocytes are in close proximity.

Neurite extension in the presence of IL-6

IL-6 application specifically influenced TUJ1 immunoreactivity of AHPCs in a concentration-dependent manner, with no effect on RIP immunoreactivity. Above 1 ng/ml of IL-6, the percentage of TUJ1-IR AHPCs appeared to have reached saturation. In addition, the average length of processes extended from the TUJ1-IR AHPCs was significantly longer than that for the cells in the control condition (0 ng/ml of IL-6), although neither the average number of processes per cell (Supplementary Table 1) nor the total length of processes (Supplementary Figure 2) was significantly different.

Marz et al. (1999) reported that the application of the IL-6/IL-6R fusion protein (covalently linked by an intermediate peptide(Fischer et al. 1997)) increased expression levels of neurotrophins (NTs) in brain-derived astrocytes(Marz et al. 1999). Both hippocampal and cortical astrocytes express NGF at high levels following IL-6 treatment(Marz et al. 1999). NGF can suppress the activation of STAT3 which itself inhibits neurite outgrowth of PC12 cells(Ihara et al. 1997). Moreover, hippocampal and cortical astrocytes exclusively express NT-3 and NT-4/5, respectively(Marz et al. 1999). NT-3 facilitates neuronal differentiation and neurite outgrowth of mouse NPCs through the MAPK-ERK pathway(Lim et al. 2007). Thus, the astrocyte-derived IL-6 may stimulate neurogenic astrocytes that express IL-6R to produce NTs, such as NGF and NT-3. Such NTs might then inhibit STAT3 activation and induce ERK activation following MAPKK activation. Therefore, the IL-6 released from the astrocytes may be a key molecule that influences neuronal differentiation and neurite outgrowth of AHPCs.

Electrophysiological properties of AHPCs

For whole cell patch clamp analysis, only cells possessing a neuronal morphology were selected for recording. In this analysis, we examined resting potential, capacitance and current density. AHPCs cultured in the absence or presence of IL-6 showed similar apparent resting potentials, −79 ~ −82 mV on average. Schmidt-Hieber et al. (2004) reported that the resting membrane potentials for PSA-NCAM-expressing newborn hippocampal granule cells were about −75 mV which was similar to that for PSA-NCAM immunonegative mature cells (about −81 mV)(Schmidt-Hieber et al. 2004). Resting potentials of AHPCs cultured under differentiation condition for 1 week were comparable to the values for the granule cells in the hippocampus. AHPCs cultured without or with IL-6 also showed similar membrane capacitance values in the range of 10 ~ 15 pF. These values are comparable to those reported for rat hippocampus-derived NPCs cultured for less than 2 weeks *in vitro* with various factors(Sah et al. 1997). Considering that cell capacitance is proportional to cell surface area, more mature dentate granule cells extending longer dendrites, as expected, have greater cell capacitance than do their immature counterparts(Schmidt-Hieber et al. 2004; van Praag et al. 2002). In brain slices, 4 week-old hippocampal neurons displaying complex morphologies with highly branched dendritic arborizations exhibit 3.5-times higher capacitance values(van Praag et al. 2002) than do AHPCs cultured for 6 days. From our result TUJ1-IR AHPCs cultured for 6 days in the presence of exogenous IL-6 displayed a total length of presumed axonal processes per cell of approximately 150 μm regardless of IL-6 concentrations. This insignificant difference in the total length of processes between cells cultured without and with IL-6 is consistent with the result of no significant difference in cell capacitance values between the two conditions.

Interestingly, the average length of TUJ1-IR processes for AHPCs cultured with IL-6 was about 1.5-fold greater than that for cells in control condition without IL-6. However total length of TUJ1-IR processes and the membrane capacitance were similar between the two conditions. In the presence of IL-6, it is possible that some TUJ1-IR neurites that may be presumptive axons were extended while others that were destined to become dendrites were retracted compared to the control condition(Ferreira and Caceres 1992; Laferriere et al. 1997; Winckler 2007). This result suggests that IL-6 may be involved in neurite specification during neuronal development. The remodeling of neurites during neuronal polarization (formation of axons and dendrites) is an important aspect of neuronal differentiation(Craig and Banker 1994; Da Silva et al. 2005). Axons can be distinguished from the dendrites by the expression of distinct molecules, such as plasma membrane ganglioside sialidase (PMGS) found in the axon of mature neurons(Da Silva et al. 2005) and higher glycogen synthase kinase (GSK)-3β activity in dendrites than in axons of polarized hippocampal neurons(Jiang et al. 2005). Immunocytochemically TUJ1 (class III β-tubulin) is commonly used as an axonal marker(Joshi and Cleveland 1989; Lee et al. 1990). Our result demonstrating longer average length of TUJ1-IR neurites of AHPCs cultured with IL-6 than without IL-6, with no effect on total TUJ-IR neurite length, suggests that IL-6 presumably facilitated axonal specification. However it remains to be elucidated how IL-6 influences the selection of neurites to be remodeled in conjunction with microtubule reorganization(Cao et al. 2006; Hakkoum et al. 2007; Zhang et al. 2007).

AHPCs differentiating along a neuronal path exhibited two kinds of macroscopic conductances - sustained outward currents (sensitive to TEA, which blocks delayed rectifying potassium channels) and transient inward currents. Our electrophysiological data demonstrate that the presumed Na^+ current density of AHPCs cultured in the presence of IL-6 was 2-fold greater than that in the absence of IL-6, whereas there was no significant difference in K^+ current density between the two conditions; co-culture with astrocytes at 6-7 DIV, in contrast, significantly amplified K^+ current density 2.5-fold and appeared to increase Na^+ current density 1.7-fold although this difference was not statistically

significant. This result suggests that IL-6 application may selectively enhance expression of voltage-gated Na^+ currents and that other astrocyte products may enhance the K^+ currents.

During rapid current clamp recording, depolarizing current steps failed to trigger action potentials, although slow regenerative events (maximal rate of depolarizing phase, < 12 V/s) with modest amplitude were elicited. The maximal slope of the upswing is set by the Na^+ current density, which was about 10-fold lower in AHPCs than in mature granule cells of the dentate gyrus(van Praag et al. 2002), which are thought to arise from AHPCs *in vivo*. Steady-state inactivation of the Na^+ current is also sufficiently depolarized to limit availability of Na^+ current from the resting potential in some cells including glia(Barres et al. 1989). Detailed biophysical and molecular characterization of Na^+ channels induced by IL-6 awaits further study.

AHPCs are multipotent NPCs in the hippocampus thought to give rise to mainly granule cells and possibly glial cells in the dentate gyrus(Gage et al. 1998; Namba et al. 2005). In rodent hippocampi, NPCs expressing PSA-NCAM and GFP driven by a Nestin promoter undergo biphasic changes in input resistance upon commitment and maturation along the neuronal lineage; neural progenitors in the subgranular zone have a low Rin (< 100 MΩ), restricted progenitors and immature granule cells a high Rin (~ 4 GΩ) and mature granule cells a 20-fold lower Rin of ~ 200 MΩ(Overstreet-Wadiche and Westbrook 2006; Schmidt-Hieber et al. 2004). AHPCs cultured under proliferation or differentiation conditions, without or with IL-6, had Rin values ≥ 2 GΩ. By this criterion AHPCs are consistent either with cells that have committed to a neuronal lineage or with lineage-restricted progenitors.

AHPCs that have differentiated in response to IL-6 may also be at an ‘intermediate’ stage of neuronal and glial differentiation (as bipotent ‘neuro-glial’ precursors). Past studies have reported that O2A glial precursors(Barres et al. 1990), type-1 and type-2 astrocytes(Barres et al. 1989) and immature hippocampal astrocytes(Sontheimer et al. 1991) express sodium currents that are comparable to those in neurons. However, based on our immunocytochemical results and the striking morphological differences between neuronal and glial cells, it is unlikely that recordings were made from AHPCs differentiating into astrocytes because only a few cells express GFAP in our culture system (< 2%) and their morphology is clearly distinguishable from the other cell types. In addition, it is not likely that the AHPCs examined in this study were oligodendrocytes because the capacitance that is normally measured in oligodendrocytes is much greater than 10 pF(Barres et al. 1990).

Conclusion

Soluble factors secreted from rat hippocampal astrocytes promoted neuronal differentiation of multipotent AHPCs. Under non-contact co-culture conditions, the percentage of AHPCs expressing a neuronal marker, TUJ1, was dramatically increased compared to that when cultured alone without astrocytes. In addition to the neuronal morphology, AHPCs under non-contact co-culture conditions developed membrane properties that are consistent with those of differentiated neurons. When the AHPCs were cultured in the presence of exogenous IL-6, a candidate for a neurogenic astrocyte-derived soluble factor, the percentage of TUJ1-IR AHPCs was significantly greater than that in the absence of IL-6. Moreover, AHPCs cultured with IL-6 developed a subset of neuronal-like membrane properties at an early time point. These results suggest that IL-6 is an astrocyte-derived cytokine that can induce neuronal differentiation of the AHPCs.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Abbreviation

AHPC	adult hippocampal progenitor cell
bFGF	basic fibroblast growth factor
Brain-Astro	astrocytes isolated from whole cerebral hemispheres of the brain
CCM	co-culture medium
CNS	central nervous system
CTX-Astro	astrocytes isolated from cortical region of the brain
DIV	days in vitro
GFAP	glial fibrillary acidic protein
HC-Astro	astrocytes isolated from hippocampal region of the brain
IL-6	interleukin-6
MEM	minimal essential medium
MMEM	modified MEM
NCCC	non-contact co-culture
NPC	neural progenitor cell
NGF	nerve growth factor
NT	neurotrophin
PSA-NCAM	polysialic acid neural cell adhesion molecule
RIP	receptor interacting protein
TEA	tetraethyl ammonium
TUJ1	class III β-tubulin
TTX	tetrodotoxin

References

Abe K. Therapeutic potential of neurotrophic factors and neural stem cells against ischemic brain injury. J Cereb Blood Flow Metab. 2000; 20(10):1393–1408. [PubMed: 11043902]

Barkho BZ, Munoz AE, Li X, Li L, Cunningham LA, Zhao X. Endogenous Matrix Metalloproteinase (MMP)-3 and MMP-9 Promote the Differentiation and Migration of Adult Neural Progenitor Cells in Response to Chemokines. Stem Cells. 2008

Barkho BZ, Song H, Aimone JB, Smrt RD, Kuwabara T, Nakashima K, Gage FH, Zhao X. Identification of astrocyte-expressed factors that modulate neural stem/progenitor cell differentiation. Stem Cells Dev. 2006; 15(3):407–421. [PubMed: 16846377]

Barres BA, Chun LL, Corey DP. Glial and neuronal forms of the voltage-dependent sodium channel: characteristics and cell-type distribution. Neuron. 1989; 2(4):1375–1388. [PubMed: 2560639]

Barres BA, Koroshetz WJ, Swartz KJ, Chun LL, Corey DP. Ion channel expression by white matter glia: the O-2A glial progenitor cell. Neuron. 1990; 4(4):507–524. [PubMed: 1691005]

Campbell IL, Abraham CR, Masliah E, Kemper P, Inglis JD, Oldstone MB, Mucke L. Neurologic disease induced in transgenic mice by cerebral overexpression of interleukin 6. Proc Natl Acad Sci U S A. 1993; 90(21):10061–10065. [PubMed: 7694279]

Cao Z, Gao Y, Bryson JB, Hou J, Chaudhry N, Siddiq M, Martinez J, Spencer T, Carmel J, Hart RB, Filbin MT. The cytokine interleukin-6 is sufficient but not necessary to mimic the peripheral conditioning lesion effect on axonal growth. J Neurosci. 2006; 26(20):5565–5573. [PubMed: 16707807]

Chesik D, De Keyser J, Wilczak N. Insulin-like growth factor binding protein-2 as a regulator of IGF actions in CNS: implications in multiple sclerosis. Cytokine Growth Factor Rev. 2007; 18(3-4): 267–278. [PubMed: 17485236]

Conover JC, Notti RQ. The neural stem cell niche. Cell Tissue Res. 2008; 331(1):211–224. [PubMed: 17922142]

Craig AM, Banker G. Neuronal polarity. Annu Rev Neurosci. 1994; 17:267–310. [PubMed: 8210176]

Da Silva JS, Hasegawa T, Miyagi T, Dotti CG, Abad-Rodriguez J. Asymmetric membrane ganglioside sialidase activity specifies axonal fate. Nat Neurosci. 2005; 8(5):606–615. [PubMed: 15834419]

Eglitis MA, Mezey E. Hematopoietic cells differentiate into both microglia and macroglia in the brains of adult mice. Proc Natl Acad Sci U S A. 1997; 94(8):4080–4085. [PubMed: 9108108]

Feldman EL, Sullivan KA, Kim B, Russell JW. Insulin-like growth factors regulate neuronal differentiation and survival. Neurobiol Dis. 1997; 4(3-4):201–214. [PubMed: 9361296]

Ferreira A, Caceres A. Expression of the class III beta-tubulin isotype in developing neurons in culture. J Neurosci Res. 1992; 32(4):516–529. [PubMed: 1527798]

Fischer M, Goldschmitt J, Peschel C, Brakenhoff JP, Kallen KJ, Wollmer A, Grotzinger J, Rose-John S. I. A bioactive designer cytokine for human hematopoietic progenitor cell expansion. Nat Biotechnol. 1997; 15(2):142–145. [PubMed: 9035138]

Gadient RA, Otten U. Expression of interleukin-6 (IL-6) and interleukin-6 receptor (IL-6R) mRNAs in rat brain during postnatal development. Brain Res. 1994; 637(1-2):10–14. [PubMed: 8180786]

Gadient RA, Otten UH. Interleukin-6 (IL-6)--a molecule with both beneficial and destructive potentials. Prog Neurobiol. 1997; 52(5):379–390. [PubMed: 9304698]

Gage FH, Coates PW, Palmer TD, Kuhn HG, Fisher LJ, Suhonen JO, Peterson DA, Suhr ST, Ray J. Survival and differentiation of adult neuronal progenitor cells transplanted to the adult brain. Proc Natl Acad Sci U S A. 1995; 92(25):11879–11883. [PubMed: 8524867]

Gage FH, Kempermann G, Palmer TD, Peterson DA, Ray J. Multipotent progenitor cells in the adult dentate gyrus. J Neurobiol. 1998; 36(2):249–266. [PubMed: 9712308]

Garcion E, Halilagic A, Faissner A, ffrench-Constant C. Generation of an environmental niche for neural stem cell development by the extracellular matrix molecule tenascin C. Development. 2004; 131(14):3423–3432. [PubMed: 15226258]

Gaughwin PM, Caldwell MA, Anderson JM, Schwiening CJ, Fawcett JW, Compston DA, Chandran S. Astrocytes promote neurogenesis from oligodendrocyte precursor cells. Eur J Neurosci. 2006; 23(4):945–956. [PubMed: 16519659]

Gross RE, Mehler MF, Mabie PC, Zang Z, Santschi L, Kessler JA. Bone morphogenetic proteins promote astroglial lineage commitment by mammalian subventricular zone progenitor cells. Neuron. 1996; 17(4):595–606. [PubMed: 8893018]

Gruol DL, Nelson TE. Physiological and pathological roles of interleukin-6 in the central nervous system. Mol Neurobiol. 1997; 15(3):307–339. [PubMed: 9457704]

Hakkoum D, Stoppini L, Muller D. Interleukin-6 promotes sprouting and functional recovery in lesioned organotypic hippocampal slice cultures. J Neurochem. 2007; 100(3):747–757. [PubMed: 17144903]

Ihara S, Nakajima K, Fukada T, Hibi M, Nagata S, Hirano T, Fukui Y. Dual control of neurite outgrowth by STAT3 and MAP kinase in PC12 cells stimulated with interleukin-6. Embo J. 1997; 16(17):5345–5352. [PubMed: 9311994]

Jiang H, Guo W, Liang X, Rao Y. Both the establishment and the maintenance of neuronal polarity require active mechanisms: critical roles of GSK-3beta and its upstream regulators. Cell. 2005; 120(1):123–135. [PubMed: 15652487]

Joshi HC, Cleveland DW. Differential utilization of beta-tubulin isotypes in differentiating neurites. J Cell Biol. 1989; 109(2):663–673. [PubMed: 2503525]

Kornyei Z, Gocza E, Ruhl R, Orsolits B, Voros E, Szabo B, Vagovits B, Madarasz E. Astroglia-derived retinoic acid is a key factor in glia-induced neurogenesis. Faseb J. 2007; 21(10):2496–2509. [PubMed: 17438145]

Laferriere NB, MacRae TH, Brown DL. Tubulin synthesis and assembly in differentiating neurons. Biochem Cell Biol. 1997; 75(2):103–117. [PubMed: 9250358]

Lee MK, Rebhun LI, Frankfurter A. Posttranslational modification of class III beta-tubulin. Proc Natl Acad Sci U S A. 1990; 87(18):7195–7199. [PubMed: 2402501]

Lie DC, Colamarino SA, Song HJ, Desire L, Mira H, Consiglio A, Lein ES, Jessberger S, Lansford H, Dearie AR, Gage FH. Wnt signalling regulates adult hippocampal neurogenesis. Nature. 2005; 437(7063):1370–1375. [PubMed: 16251967]

Lim DA, Alvarez-Buylla A. Interaction between astrocytes and adult subventricular zone precursors stimulates neurogenesis. Proc Natl Acad Sci U S A. 1999; 96(13):7526–7531. [PubMed: 10377448]

Lim MS, Nam SH, Kim SJ, Kang SY, Lee YS, Kang KS. Signaling pathways of the early differentiation of neural stem cells by neurotrophin-3. Biochem Biophys Res Commun. 2007; 357(4):903–909. [PubMed: 17466268]

Lu Z, Kipnis J. Thrombospondin 1--a key astrocyte-derived neurogenic factor. Faseb J.

Martino G, Pluchino S. The therapeutic potential of neural stem cells. Nat Rev Neurosci. 2006; 7(5): 395–406. [PubMed: 16760919]

Marz P, Heese K, Dimitriades-Schmutz B, Rose-John S, Otten U. Role of interleukin-6 and soluble IL-6 receptor in region-specific induction of astrocytic differentiation and neurotrophin expression. Glia. 1999; 26(3):191–200. [PubMed: 10340760]

Namba T, Mochizuki H, Onodera M, Mizuno Y, Namiki H, Seki T. The fate of neural progenitor cells expressing astrocytic and radial glial markers in the postnatal rat dentate gyrus. Eur J Neurosci. 2005; 22(8):1928–1941. [PubMed: 16262632]

Oh J, Recknor JB, Recknor JC, Mallapragada SK, Sakaguchi DS. Soluble factors from neocortical astrocytes enhance neuronal differentiation of neural progenitor cells from adult rat hippocampus on micropatterned polymer substrates. J Biomed Mater Res A. 2009; 91(2):575–585. [PubMed: 18985780]

Otten U, Marz P, Heese K, Hock C, Kunz D, Rose-John S. Cytokines and neurotrophins interact in normal and diseased states. Ann N Y Acad Sci. 2000; 917:322–330. [PubMed: 11268359]

Overstreet-Wadiche LS, Westbrook GL. Functional maturation of adult-generated granule cells. Hippocampus. 2006; 16(3):208–215. [PubMed: 16411232]

Ray J, Peterson DA, Schinstine M, Gage FH. Proliferation, differentiation, and long-term culture of primary hippocampal neurons. Proc Natl Acad Sci U S A. 1993; 90(8):3602–3606. [PubMed: 8475109]

Ray J, Raymon HK, Gage FH. Generation and culturing of precursor cells and neuroblasts from embryonic and adult central nervous system. Methods Enzymol. 1995; 254:20–37. [PubMed: 8531687]

Recknor JB, Recknor JC, Sakaguchi DS, Mallapragada SK. Oriented astroglial cell growth on micropatterned polystyrene substrates. Biomaterials. 2004; 25(14):2753–2767. [PubMed: 14962554]

Recknor JB, Sakaguchi DS, Mallapragada SK. Directed growth and selective differentiation of neural progenitor cells on micropatterned polymer substrates. Biomaterials. 2006; 27(22):4098–4108. [PubMed: 16616776]

Russo VC, Gluckman PD, Feldman EL, Werther GA. The insulin-like growth factor system and its pleiotropic functions in brain. Endocr Rev. 2005; 26(7):916–943. [PubMed: 16131630]

Sah DW, Ray J, Gage FH. Regulation of voltage- and ligand-gated currents in rat hippocampal progenitor cells in vitro. J Neurobiol. 1997; 32(1):95–110. [PubMed: 8989666]

Schmidt-Hieber C, Jonas P, Bischofberger J. Enhanced synaptic plasticity in newly generated granule cells of the adult hippocampus. Nature. 2004; 429(6988):184–187. [PubMed: 15107864]

Soen Y, Mori A, Palmer TD, Brown PO. Exploring the regulation of human neural precursor cell differentiation using arrays of signaling microenvironments. Mol Syst Biol. 2006; 2:37. [PubMed: 16820778]

Song H, Stevens CF, Gage FH. Astroglia induce neurogenesis from adult neural stem cells. Nature. 2002; 417(6884):39–44. [PubMed: 11986659]

Sontheimer H, Ransom BR, Cornell-Bell AH, Black JA, Waxman SG. Na(+)-current expression in rat hippocampal astrocytes in vitro: alterations during development. J Neurophysiol. 1991; 65(1):3–19. [PubMed: 1999729]

Taga T, Fukuda S. Role of IL-6 in the neural stem cell differentiation. Clin Rev Allergy Immunol. 2005; 28(3):249–256. [PubMed: 16129909]

Taupin P. Adult neural stem cells, neurogenic niches, and cellular therapy. Stem Cell Rev. 2006; 2(3): 213–219. [PubMed: 17625257]

Taupin P. Adult neurogenesis, neuroinflammation and therapeutic potential of adult neural stem cells. Int J Med Sci. 2008; 5(3):127–132. [PubMed: 18566676]

Ueki T, Tanaka M, Yamashita K, Mikawa S, Qiu Z, Maragakis NJ, Hevner RF, Miura N, Sugimura H, Sato K. A novel secretory factor, Neurogenesin-1, provides neurogenic environmental cues for neural stem cells in the adult hippocampus. J Neurosci. 2003; 23(37):11732–11740. [PubMed: 14684875]

Vallieres L, Campbell IL, Gage FH, Sawchenko PE. Reduced hippocampal neurogenesis in adult transgenic mice with chronic astrocytic production of interleukin-6. J Neurosci. 2002; 22(2):486–492. [PubMed: 11784794]

van Praag H, Schinder AF, Christie BR, Toni N, Palmer TD, Gage FH. Functional neurogenesis in the adult hippocampus. Nature. 2002; 415(6875):1030–1034. [PubMed: 11875571]

Van Snick J. Interleukin-6: an overview. Annu Rev Immunol. 1990; 8:253–278. [PubMed: 2188664]

Van Wagoner NJ, Benveniste EN. Interleukin-6 expression and regulation in astrocytes. J Neuroimmunol. 1999; 100(1-2):124–139. [PubMed: 10695723]

Watt FM, Hogan BL. Out of Eden: stem cells and their niches. Science. 2000; 287(5457):1427–1430. [PubMed: 10688781]

Winckler B. BDNF instructs the kinase LKB1 to grow an axon. Cell. 2007; 129(3):459–460. [PubMed: 17482540]

Wurmser AE, Palmer TD, Gage FH. Neuroscience. Cellular interactions in the stem cell niche. Science. 2004; 304(5675):1253–1255. [PubMed: 15166350]

Ye P, Popken GJ, Kemper A, McCarthy K, Popko B, D'Ercole AJ. Astrocyte-specific overexpression of insulin-like growth factor-I promotes brain overgrowth and glial fibrillary acidic protein expression. J Neurosci Res. 2004; 78(4):472–484. [PubMed: 15468174]

Zhang J, Li L. BMP signaling and stem cell regulation. Dev Biol. 2005; 284(1):1–11. [PubMed: 15963490]

Zhang PL, Levy AM, Ben-Simchon L, Haggiag S, Chebath J, Revel M. Induction of neuronal and myelin-related gene expression by IL-6-receptor/IL-6: a study on embryonic dorsal root ganglia cells and isolated Schwann cells. Exp Neurol. 2007; 208(2):285–296. [PubMed: 17963753]

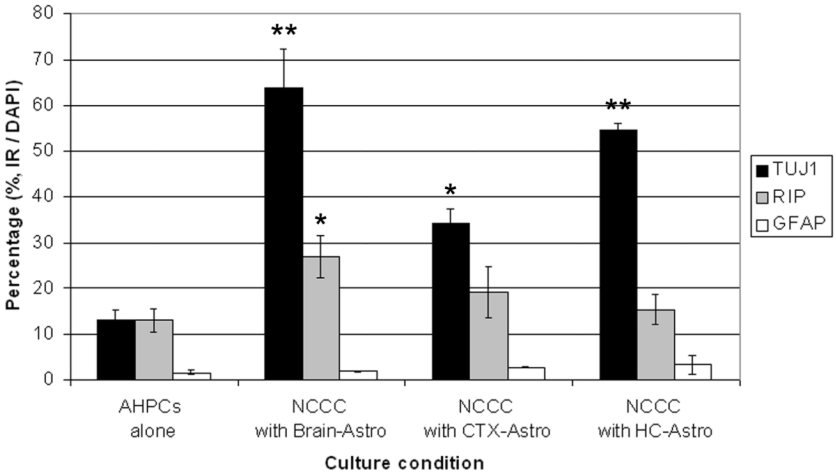

Figure 1.

Differentiation of AHPCs under non-contact co-culture conditions (NCCC). AHPCs were cultured under four different culture conditions: (1) AHPCs cultured alone without astrocytes (AHPCs alone), (2) non-contact co-culture with astrocytes isolated from cerebral hemispheres of neonatal rat brain (NCCC with Brain-Astro), (3) non-contact co-culture with cortical astrocytes (NCCC with CTX-Astro) and (4) non-contact co-culture with hippocampal astrocytes (NCCC with HC-Astro). Differentiation of AHPCs was characterized using cell-type specific antibodies: TUJ1 for young neurons, RIP for oligodendrocytes and GFAP for astrocytes. Values represent the average percentage of AHPCs immunolabeled by each antibody. N (number of independent experiments) = 6 for AHPCs alone and N = 3 for each NCCC. Error bars represent standard error of the mean (SEM). Asterisks represent significant difference from the control culture of AHPCs alone (*t* test; *, $p < 0.05$; **, $p < 0.0001$). TUJ1 immunoreactivity of AHPCs under all NCCC conditions was significantly greater compared to AHPCs cultured alone. The average percentage of TUJ1-IR AHPCs co-cultured with hippocampal astrocytes was significantly greater than that of the AHPCs co-cultured with cortical astrocytes. The RIP-IR AHPCs were significantly increased only when the AHPCs were co-cultured with Brain-Astro, compared to the AHPCs cultured alone or co-cultured with either CTX-Astro or HC-Astro. The percentage of GFAP-IR AHPCs did not vary significantly under these conditions.

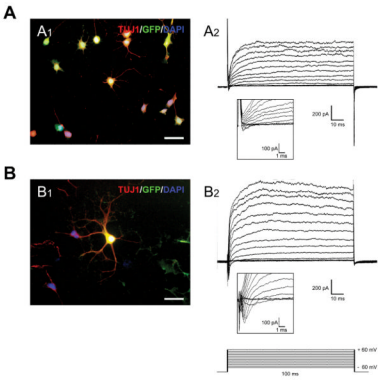

Figure 2.

Comparison of electrophysiological properties of AHPCs cultured alone or co-cultured with neonatal astrocytes. AHPCs with neuronal-like morphologies were selected for whole cell recording in voltage-clamp mode. (A) AHPCs cultured in the absence of astrocytes under differentiation condition. (A1) A superimposed image of TUJ1-IR shown in red, GFP in green and DAPI-stained nuclei in blue. (A2) A representative whole cell recording trace showing sustained outward currents and small transient inward currents elicited by a family of depolarizing voltage steps (inset, magnified inward currents). (B) AHPCs co-cultured with astrocytes. (B1) A superimposed image of TUJ1-IR AHPCs. (B2) A representative trace displaying sustained outward currents and substantial transient inward currents. Scale bars in (A1) and (B1) represent 20 μm.

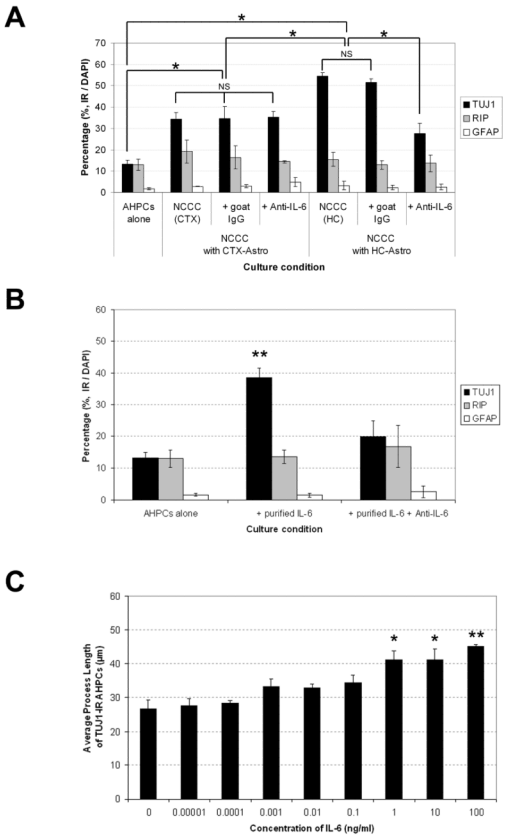

Figure 3.

Interleukin (IL)-6 enhances expression of neuronal marker protein in AHPCs. (A) Average percentages (± SEM) of cell-type specific antibody labeling of AHPCs under three different NCCCs in the presence of goat anti-IL-6 neutralizing antibody (Anti-IL-6) or non-specific IgG produced from goat (goat IgG) as a control. Goat IgG was added at an equal concentration of Anti-IL-6 added (10 ng/ml). With addition of Anti-IL-6 to the hippocampal NCCC, TUJ1-IR AHPCs were significantly decreased (54.4% in control and 51.5% with goat IgG *vs.* 27.7% with Anti-IL-6). The blocking antibody had no effect on TUJ1 immunoreactivity in cortical NCCC (34.2% in control and 34.4% with goat IgG *vs.* 35.2% with Anti-IL-6). N (number of independent experimental sessions) = 3 for each co-culture session. Asterisks represent statistical difference at $p < 0.05$. NS, no significant difference. (B) Recombinant rat IL-6 (20 ng/ml) specifically induced neuronal differentiation of AHPCs (increased percentage of TUJ1 immunoreactivity). N = 6. The double asterisks represent a significant difference from the control culture (AHPCs alone) at $p < 0.0001$. The percentage of TUJ-IR AHPCs was significantly greater when cultured in the presence of purified IL-6, compared to that in the absence of IL-6 (38.5% with IL-6 *vs.* 13.1% without IL-6). Addition of Anti-IL-6 blocking antibody dramatically reduced the percentage of TUJ1-IR AHPCs when cultured with IL-6. (C) IL-6 enhanced primary neurite growth. AHPCs were cultured under differentiation condition for 6 days in the presence of various concentrations of purified IL-6 from 0 to 100 ng/ml (see 'IL-6 treatment' in 'Methods and Materials' section). The average length of primary neurites emanating from a TUJ1-IR AHPC was measured. From each condition of an experimental set, 17 to 23 TUJ1-IR AHPCs were analyzed. N = 3 independent experiments. Values are mean ± SEM. Asterisks indicate statistical difference (*t* test; *, $p < 0.05$; **, $p < 0.0001$).

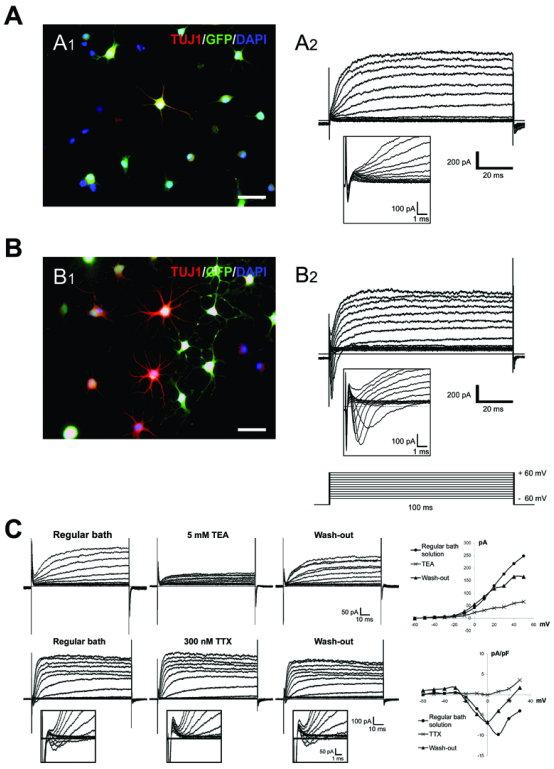

Figure 4.

IL-6 promotes the development of sodium currents in AHPCs undergoing differentiation. AHPCs were cultured alone without astrocytes under differentiation condition in the absence (A) or presence (B) of IL-6. AHPCs cultured in the presence of IL-6 developed membrane properties consistent with those of functional neurons. The AHPCs possessing neuronal morphologies were analyzed using whole cell patch clamping. (A) AHPCs cultured without IL-6. (A1) A superimposed image of TUJ1-IR AHPCs shown in red, GFP in green and DAPI-stained nuclei in blue. (A2) A representative family of traces showing TEA-sensitive sustained outward currents and very small transient inward currents elicited by depolarizing voltage steps (inset, magnified inward currents). (B) AHPCs cultured with IL-6. (B1) A superimposed image of TUJ1-IR AHPCs. (B2) A representative traces showing TEA-sensitive outward currents and larger TTX-sensitive, transient inward currents. (C) IV-plot showing the relationship between stimulating voltage and current density. (Upper) Recording showing TEA-sensitive outward currents (N = 5). (Lower) Recording showing TTX-sensitive inward currents (N = 3). Scale bars in (A1) and (B1) represent 20 μm.

Table 1

Passive membrane properties of AHPCs cultured alone or under non-contact co-culture conditions

Culture condition		N (cells)	Apparent RMP (mV)	Cm (pF)	Outward current density # (pA*/pF)	Inward current density (pA/pF)
AHPCs alone	Proliferation (3-4 DIV)	14	−71.1 ± 4.5	12.6 ± 1.3	42.5 ± 7.9 *a,b*	−3.7 ± 1.0 *d,e*
	Differentiation (6-7 DIV)	22	−79.6 ± 5.0	10.9 ± 0.7	47.6 ± 6.0 *c*	−6.6 ± 1.5
AHPCs in NCCC	Differentiation (6-7 DIV)	19		9.9 ± 0.8	120.9 ± 18.1 *a,c*	−10.9 ± 2.7 *d*
	Differentiation (9-10 DIV)	11	−85.8 ± 5.1	9.9 ± 1.0	92.2 ± 26.0 *b*	−17.0 ± 9.0 *e*

DIV, days in vitro; **RMP**, resting membrane potential; **Cm**, cell capacitance; **mV**, millivolt; **pF**, picofarad; **pA**, picoampere

Current densities were calculated by normalizing the peak currents to the membrane capacitance.

* Maximum outward currents were measured at +60 mV-step depolarization for 100 milliseconds in voltage-clamp mode (holding potential, $V_H = -100$mV).

a Statistically significant difference ($p < 0.05$, *t* test)

b Statistically significant difference ($p < 0.05$, *t* test)

c Statistically significant difference ($p < 0.05$, *t* test)

d Statistically significant difference ($p < 0.05$, *t* test)

e Statistically significant difference ($p < 0.05$, *t* test)

f Statistically significant difference ($p < 0.05$, *t* test)

Table 2

Passive membrane properties of AHPCs cultured in the absence or presence of IL-6 (1 ng/ml)

Culture condition		N (cells)	Apparent RMP (mV)	Cm (pF)	Outward current density (pA/pF)	Inward current density (pA/pF)
Differentiation (6-7 DIV)	− **IL-6**	22	−79.6 ± 5.0	10.9 ± 0.7	47.6 ± 6.0	−6.6 ± 1.5 [a]
	+ **IL-6**	21	−82.1 ± 4.3	12.9 ± 1.8	47.8 ± 6.0	−13.1 ± 3.3 [a]

[a] Statistically significant difference ($p < 0.05$, *t* test)

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