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IL-9 signaling affects central nervous system resident cells during inflammatory stimuli

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Abstract

Interleukin (IL) 9, a dominant cytokine in Th9 cells, has been proven to play a pathogenic role in experimental autoimmune encephalomyelitis (EAE), an animal model of multiple sclerosis (MS), by augmenting T cell activation and differentiation; however, whether IL-9 signaling affects central nervous system (CNS)-resident cells during CNS autoimmunity remains unknown. In the present study, we found that the IL-9 receptor (IL-9R) was highly expressed in astrocytes, oligodendrocyte progenitor cells (OPCs), oligodendrocytes and microglia cells, and that its expression was significantly upregulated in brain and spinal cord during EAE. In addition, IL-9 increased chemokine expression, including CXCL9, CCL20 and MMP3, in primary astrocytes. Although IL-9 had no effect on the proliferation of microglia cells, it decreased OPC proliferation and differentiation when in combination with other pro-inflammatory cytokines, but not with IFN- γ . IL-9 plus IFN- γ promoted OPC proliferation and differentiation. These findings indicate that CNS-restricted IL-9 signaling may be involved in the pathogenesis of MS/EAE, thus providing a potential therapeutic target for future MS/EAE treatment through disruption of CNS cell-specific IL-9 signaling.

Keywords

IL-9; IL-9 receptor; CNS resident cells; Experimental autoimmune encephalomyelitis

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1. Introduction

Interleukin (IL) 9 (IL-9) was originally described as a growth factor for T lymphocytes (Uyttenhove et al., 1988). CD4⁺ T cells have been shown to be a major source of IL-9 (Monteyne et al., 1997). Although it has been reported that Th2, Th17, regulatory T cells (Tregs), mast cells, or even natural killer T cells can produce IL-9 (reviewed in Noelle and Nowak, 2010), the major source of IL-9 are Th9 cells. The T9 cell owes its name to its secretion of dominant cytokine IL-9, and it is recognized as a distinct helper T cell subset as it neither co-expresses cytokines IL-4, IL-5, IL-13 (Th2), IL-17a (Th17) or IFN- γ (Th1) with IL-9 upon activation (Chang et al., 2010; Dardalhon et al., 2008; Staudt et al., 2010; Veldhoen et al., 2008) nor subset-determining transcription factors including T-bet (Th1), GATA3 (Th2), ROR γ t (Th17), and FoxP3 Treg cells (Dardalhon et al., 2008; Veldhoen et al., 2008).

Despite a high amount of IL-10 secretion, Th9 cells are not known to have regulatory properties (Dardalhon et al., 2008). Th9 cells exert their biological role through the dominant cytokine IL-9, which has been reported to be involved in many diseases. Accumulating data indicate that IL-9 plays a role in the pathogenic process of allergy, in particular asthma (Knoops et al., 2005; Nicolaides et al., 1997; Wilhelm et al., 2011). IL-9 could drive T-cell mediated colitis by working with its receptor in the intestinal epithelial cells (Gerlach et al., 2014), and IL-9/IL-9 receptor signaling is involved in the pathogenesis of ulcerative colitis (Nalleweg et al., 2014). Functional interactions between IL-9 and mast cells that lead to VEGF release contribute to the initiation/propagation of the pathogenesis of atopic dermatitis (Sismanopoulos et al., 2012).

In addition, Th9/IL-9 involvement in several autoimmune diseases has been reported (Li and Rostami, 2010), e.g., increased IL-9 and Th9 cells have been found in systemic lupus erythematosus (Ouyang et al., 2013). Our previous work showed that IL-9 is important for T cell activation and differentiation in the central nervous system (CNS) autoimmune disease (Li et al., 2011); neutralization of IL-9 would thus ameliorate experimental autoimmune encephalomyelitis (EAE) (Li et al., 2010). It has been reported that CNS-restricted inflammatory signaling plays an important role in EAE (Ding et al., 2015; Yan et al., 2012); however, whether the IL-9 signal pathway in CNS cells is involved in disease development remains unknown. As the actions of IL-9 are mediated through its interaction with IL-9 receptor (IL-9R) (Renauld et al., 1992), in this study, we compared IL-9R expression in CNS tissues during EAE and characterized its expression on different CNS cells. In order to further investigate the pathophysiological mechanisms of MS/EAE, we then tested the effect of IL-9 stimulation of various CNS-resident cells in combination with other inflammatory cytokines.

2. Materials and methods

2.1. Mice

Female C57BL/6 mice, 8–10 weeks of age, were purchased from the Jackson Laboratory (Bar Harbor, ME). All animal procedures were performed in accordance with the guidelines of the Institutional Animal Care and Committee of Thomas Jefferson University.

2.2. Antibodies and reagents

Antibodies for CNS-resident cell staining were from the following companies: anti-GFAP (2A5) from StemCell Technologies (BC, Canada); anti-NeuN from Millipore (Billerica, MA); anti-CD11b, antiA2B5 and anti-OSP (oligodendrocyte-specific protein) from Abcam (Cambridge, MA) and antibody for IL-9R from Biolegend (San Diego, CA). Fluorescent-conjugated secondary antibodies were from Jackson ImmunoResearch Laboratories (West Grove, PA). Recombinant IL-9, IL-17, IL-1 β , IFN- γ , TNF- α , PDGF, bFGF, NT3 and M-CSF were from PeproTech (Rocky Hill, NJ). T3 and T4 were from Sigma-Aldrich (St. Louis, MO).

2.3. EAE induction

For EAE induction, mice were immunized subcutaneously (s.c.) on the back with 200 μ g of MOG_{35–55} (MEVGWYRSPFSRVVHLYRNGK) emulsified in CFA (Difco Lab, Detroit, MI) containing 4 mg/ml Mycobacterium tuberculosis H37Ra (Difco). Two hundred nanograms of pertussis toxin (List Biological Lab, Epsom, England) was given i.p. on days 0 and 2 post-immunization (p.i.). Mice were scored daily for appearance of clinical signs of EAE on a scale from 0 to 5: 0, no clinical signs; 1, fully limp tail; 2, paralysis of one hind limb; 3, paralysis of both hind limbs; 4, paralysis of trunk; and 5, moribund or death.

2.4. Primary CNS cell purification, culture and cytokine treatment

For oligodendrocyte progenitor cell (OPC) culture, the whole brain of mouse embryos (E16) was harvested and dissociated with a Neural Tissue Dissociation Kit purchased from Miltenyi Biotec Inc. (Auburn, CA) following the manufacturer's instructions. OPCs were purified with anti-A2B5 microbeads (Miltenyi Biotec Inc.) following the manufacturer's MACS instructions. The purified OPCs were centrifuged at 300 g for 10 min, then resuspended with D-MEM/F12 (Mediatech, Inc., Manassas, VA), plus B27 (Invitrogen, Grand Island, NY), 20 ng/ml PDGF, 20 ng/ml bFGF, 2 mmol/L L-Glutamine, 100 I.U./ml penicillin and 100 μ g/ml Streptomycin (Mediatech, Inc., Manassas, VA), and seeded on poly-lysine pre-coated 60-mm dishes at a density of 1×10^6 cells/dish. For differentiation, cells were cultured in differentiation media containing D-MEM/F12, B27, 30 ng/ml T3, 30 ng/ml T4, 10 ng/ml NT-3, 2 mmol/L L-Glutamine, 100 I.U./ml penicillin and 100 μ g/ml Streptomycin.

For isolation, purification and culture of astrocytes and microglia primary cells, the whole brain of mice embryos (E16) was harvested and dissociated with the Neural Tissue Dissociation Kit (Miltenyi Biotec Inc., Auburn, CA) following the manufacturer's instructions. For astrocyte purification, the dissociated cells were centrifuged at 300 g for 10 min, then resuspended with astrocyte culture media, and seeded on poly-lysine-coated 60-mm dishes at a density of 1×10^6 /dish. The astrocyte culture media contained D-MEM (Mediatech, Inc.), 10% fetal bovine serum (Invitrogen), 2 mmol/L L-Glutamine, 100 I.U./ml penicillin and 100 μ g/ml Streptomycin. For astrocyte passage, after 7 days' culture, the remaining cultures were trypsinized and replanted in Petri dishes.

Cultures that had been passaged twice were used as astrocytes. Microglia cells were purified with anti-CD11b microbeads (Miltenyi Biotec Inc., Auburn, CA) following the

manufacturer's MACS instructions. Purified microglia cells were centrifuged at 300 g for 10 min, then resuspended with D-MEM/10% FBS plus 5 ng/ml M-CSF (PeproTech), and seeded on 60-mm dishes at a density of 1×10^6 /dish. After 7 days, cultures were trypsinized and replated in Petri dishes. Cells from cultures that had been passaged once were used as microglia cells.

Cytokine concentrations used for all treatments were as follows: IL-9, 20 ng/ml; IL-17, 50 ng/ml; IL-1 β , 20 ng/ml; IFN- γ , 10 ng/ml; and TNF- α , 10 ng/ml. For chemokine expression in astrocytes, 1×10^6 cells/well, cells were treated with different combinations of cytokines for 12 h; cells were then harvested for RNA purification, cDNA synthesis and real-time PCR analysis. For proliferation assay, cells were treated with different cytokines for 3 days. For OPC differentiation experiments, cells were treated for 5 days.

2.5. Immunohistochemical staining

Immunohistochemical staining was performed as previously described (Yan et al., 2012), with some modifications. Briefly, spinal cords were carefully excised from the brain stem to the lumbar region and cryoprotected with 30% sucrose in PBS. The lumbar enlargement was identified and then transected at the exact midpoint of the lumbar enlargement to standardize a site along the longitudinal axis of the cord, ensuring that the same lumbar spinal cord regions were analyzed for all conditions. Transverse sections of the brain and spinal cord were cut, and immunohistochemistry was performed using different antibodies. Immunofluorescence controls were routinely generated with irrelevant IgGs as the first antibody. Finally, slides were covered with Vectashield Mounting Medium (Vector Laboratories, Burlingame, CA), containing 1 μ M DAPI. Results were visualized by fluorescent microscopy (Eclipse 800, Nikon). The staining procedure for cells was similar to that for tissue slides; cells were incubated on coverslips pretreated with poly-lysine and were then stained following the same procedures mentioned above.

2.6. Real-time quantitative RT-PCR

Total RNA was isolated from tissues or cultured cells with TRIzol (Life Technologies, Gaithersburg, MD). For RT-PCR, 2 μ g of total RNA was transcribed into cDNA. Then, quantitative real-time PCR was performed using the Applied Biosystems 7000 Real-time PCR system and SYBR Green detection. PCR reactions were performed (50 °C 2 min, 95 °C 10 min, followed by 40 cycles on 95 °C, 15 s; 60 °C, 1 min), after which melting curve reaction was performed to verify the specificity of amplification using the Power SYBR Green PCR master mix (Applied Biosystems). Transcript quantization was relative to HPRT standard. Error bars indicate SEM values calculated from – Ct values from triplicate PCR reactions, according to the Applied Biosystems protocols. PCR primer pairs were listed as follows: HPRT: 5'-GTAATGATCAGTCAACGGGGGAC-3' and 5'-CCAGCAAGCTTGCAACCTTAACCA-3'; CXCL1: 5'-CTTGCCTTGACCCTGAAGCTC-3' and 5'-AGCAGTCTGTCTTCTTTCTCCGT-3'; CXCL2: 5'-CCCCCTGGTTCAGAAAATCA-3' and 5'-GCTCCTCCTTCCAGGTCAGT-3'; CCL20: 5'-GTGGCAAGCGTCTGCTCT-3' and 5'-TGTACGAGAGGCAACAGTCG-3'; CXCL9: 5'-TGCACGATGCTCCTGCA-3' and 5'-AGGTCTTTGAGGGATTGTGTA GTGG-3'; CXCL10: 5'-CTCATCCTGCTGGGTCTGAG-3' and 5'-CCTATGGC

CCTCATTCTCAC-3'; CXCL12: 5'-AACCAGTCAGCCTGAGCTACC-3' and 5'-CTGAAGGGCACAGTTTGGAG-3'; MMP3: 5'-GTTGGAGATGACAGGGAA GC-3' and 5'-CGAACCTGGGAAGGTACTGA-3'; MMP9: 5'-CCAGATGATG GGAGAGAAGC-3' and 5'-GGCCTTTAGTGTCTGGCTGT-3'.

2.7. Microglia and OPC proliferation assay

Microglia and OPC proliferation were assayed by ³H-thymidine incorporation. Briefly, triplicate aliquots of 1×10^4 of cells in 96-well plates were stimulated with different combinations of cytokines in 200 μ l of culture medium. After 60 h of incubation, cells were pulsed with 1 μ Ci ³H-thymidine/well for 16 h and were then harvested on fiberglass filters. Thymidine incorporation was determined using a Wallac 1450 MicroBeta TriLux scintillation counter (PerkinElmer, Turku, Finland).

2.8. Statistical analysis

Data are presented as mean $\pm$ SE. Differences between groups were analyzed by unpaired, two-tailed Student's *t*-test. A value of *P* < 0.05 was considered statistically significant.

3. Results

3.1. IL-9 receptor expression in CNS-resident cells

A spinal cord section was used to analyze the expression of IL-9R in different CNS-resident cells. Co-staining of the IL-9R and CNS cell-specific marker showed that IL-9R was expressed by GFAP positive astrocytes, A2B5 positive OPCs, OSP positive oligodendrocytes and CD11b positive microglia. NeuN positive neurons did not express IL-9R (Fig. 1A). In vitro-cultured primary cells were then used to confirm the above results, IL-9R was expressed in primary astrocytes, OPCs, oligodendrocytes and microglia, but not in neurons (Fig. 2B). In addition, IL-9R was highly induced in both brain and spinal cord during EAE. IL-9R expression in the CNS was induced at disease onset and reached its highest level at disease peak, after which IL-9 expression decreased but still remained at a high level (Fig. 1C). The results suggest that IL-9R is expressed in diverse CNS-resident cells and that its expression in CNS tissues is induced during EAE.

3.2. IL-9 induced chemokine expression in primary astrocytes

Astrocytes play a role in CNS autoimmunity in part due to their expression and secretion of chemokines to chemoattract inflammatory cell infiltration (Ding et al., 2015; Yan et al., 2012). Our results showed that IL-9 stimulation induced four- to six-fold expression of CCL20 and CXCL9, and MMP-3 expression was also significantly induced by IL-9 treatment (Fig. 2). The results suggest that IL-9 treatment induced the expression of several chemokines in astrocytes, resulting in infiltration of inflammatory cells that contribute to disease development.

3.3. IL-9 did not alter microglia proliferation

Microglia are CNS resident immune cells whose activation and proliferation are involved in CNS autoimmune diseases. We used different combinations of inflammatory cytokines to

treat microglial cells and found that of all the cytokines we tested, TNF- α promoted while IFN- γ inhibited microglial proliferation. Although IL-9R was highly expressed in microglial cells, IL-9 itself or in combination with other proinflammatory cytokines had no effect on microglial proliferation (Fig. 3).

3.4. Diverse roles of IL-9 in OPC proliferation and differentiation

OPC proliferation and differentiation are very important for demyelination or remyelination in CNS autoimmune disease. It has been shown that IL-9 itself does not affect OPC differentiation, but once combined with other inflammatory cytokines, it has a synergetic effect on OPC differentiation. Interestingly, IL-9 plus IFN- γ synergistically promoted OPC differentiation, while IL-9 plus IL-17, TNF- α or IL-1 β synergistically inhibited the differentiation of OPCs (Fig. 4A, B). In addition, in all the cytokines we tested, only IL-1 β could promote OPC proliferation. Although IL-9 has no effect on proliferation of OPCs, IL-9 plus IFN- γ treatment significantly induced their proliferation, while IL-9 plus TNF- α or IL-1 β effectively inhibited OPC proliferation (Fig. 4C).

4. Discussion

Here we demonstrate that the IL-9 receptor is highly expressed in astrocytes, microglia, oligodendrocyte progenitor cells and oligodendrocytes and that its expression is induced in the brain and spinal cord during EAE development. In vitro experiments showed that IL-9 induced expression of chemokines in astrocytes, and, in combination with different inflammatory cytokines, promoted or inhibited oligodendrocyte progenitor cell proliferation and/or differentiation. The results suggest a possible correlation of CNS-resident cell specific IL-9 signaling with CNS autoimmunity.

Astrocytes are the most abundant cell type in the mammalian CNS. During CNS inflammation, astrocytes secrete proinflammatory cytokines, activate autoreactive T cells (Dong and Benveniste, 2001) and are the major producers of chemokines, thus promoting secondary waves of immune cell infiltration into the CNS (Kang et al., 2010). In our report, IL-9 signaling induced expression of CCL20, CXCL9 and MMP3, which are important for attracting immune cells, in particular, neutrophils and monocytes, to the site of inflammation (Chang and Dong, 2011), suggesting that IL-9 signaling in astrocytes may contribute to inflammatory cell infiltration during EAE.

MS/EAE is a CNS autoimmune demyelinating disease. The myelin repair process, including repopulation of oligodendrocytes and remyelination, is as important as regulation of immune response in MS treatment (Kuhlmann et al., 2008). Since oligodendrocytes are not mitotically active, their repopulation mainly depends on OPC proliferation, migration, and differentiation into oligodendrocytes (Horner et al., 2000; Kuhlmann et al., 2008). In our experiments, we found that although IL-9 itself had no effect on proliferation and differentiation of OPCs, it might promote or inhibit OPC proliferation and differentiation in combination with different cytokines. As there are different cytokines enriched in MS/EAE lesions, IL-9 may still synergistically affect demyelination/remyelination processes during disease development and repair. As opposite effects of IL-9 plus different cytokines on OPCs were observed, the net effect of IL-9 signaling in OPCs need further exploration.

In conclusion, as a proinflammatory cytokine in MS/EAE, IL-9 may also interact with its receptor that is expressed on different CNS-resident cells to affect inflammatory cell infiltration or demyelination/remyelination. Our results suggest a possible CNS cell-specific IL-9 signaling that contributes to MS/EAE development. Of course, this in vitro observation needs in vivo experiments for validation, and the complex role of IL-9 signaling in OPC proliferation and differentiation need further investigation in future work.

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References

- Chang HC, et al., 2010 The transcription factor PU.1 is required for the development of IL-9-producing T cells and allergic inflammation. *Nat. Immunol* 11, 527–534. [PubMed: 20431622]
- Chang SH, Dong C, 2011 Signaling of interleukin-17 family cytokines in immunity and inflammation. *Cell. Signal* 23, 1069–1075. [PubMed: 21130872]
- Dardalhon V, et al., 2008 IL-4 inhibits TGF-beta-induced Foxp3+ T cells and, together with TGF-beta, generates IL-9+ IL-10+ Foxp3(-) effector T cells. *Nat. Immunol* 9, 1347–1355. [PubMed: 18997793]
- Ding X, et al., 2015 Silencing IFN-gamma binding/signaling in astrocytes versus microglia leads to opposite effects on central nervous system autoimmunity. *J. Immunol* 194, 4251–4264. [PubMed: 25795755]
- Dong Y, Benveniste EN, 2001 Immune function of astrocytes. *Glia* 36, 180–190. [PubMed: 11596126]
- Gerlach K, et al., 2014 TH9 cells that express the transcription factor PU.1 drive T cell-mediated colitis via IL-9 receptor signaling in intestinal epithelial cells. *Nat. Immunol* 15, 676–686. [PubMed: 24908389]
- Horner PJ, et al., 2000 Proliferation and differentiation of progenitor cells throughout the intact adult rat spinal cord. *J. Neurosci* 20, 2218–2228. [PubMed: 10704497]
- Kang Z, et al., 2010 Astrocyte-restricted ablation of interleukin-17-induced Act1-mediated signaling ameliorates autoimmune encephalomyelitis. *Immunity* 32, 414–425. [PubMed: 20303295]
- Knoops L, et al., 2005 IL-9 promotes but is not necessary for systemic anaphylaxis. *J. Immunol* 175, 335–341. [PubMed: 15972666]
- Kuhlmann T, et al., 2008 Differentiation block of oligodendroglial progenitor cells as a cause for remyelination failure in chronic multiple sclerosis. *Brain* 131, 1749–1758. [PubMed: 18515322]
- Li H, et al., 2010 Neutralization of IL-9 ameliorates experimental autoimmune encephalomyelitis by decreasing the effector T cell population. *J. Immunol* 185, 4095–4100. [PubMed: 20805418]
- Li H, et al., 2011 IL-9 is important for T-cell activation and differentiation in autoimmune inflammation of the central nervous system. *Eur. J. Immunol* 41, 2197–2206. [PubMed: 21674475]
- Li H, Rostami A, 2010 IL-9: basic biology, signaling pathways in CD4+ T cells and implications for autoimmunity. *J. Neuroimmune Pharmacol* 5, 198–209. [PubMed: 20020328]
- Monteyne P, et al., 1997 IL-4-independent regulation of in vivo IL-9 expression. *J. Immunol* 159, 2616–2623. [PubMed: 9300680]
- Nalleweg N, et al., 2014 IL-9 and its receptor are predominantly involved in the pathogenesis of UC. *Gut*
- Nicolaides NC, et al., 1997 Interleukin 9: a candidate gene for asthma. *Proc. Natl. Acad. Sci. U. S. A* 94, 13175–13180. [PubMed: 9371819]
- Noelle RJ, Nowak EC, 2010 Cellular sources and immune functions of interleukin-9. *Nat. Rev. Immunol* 10, 683–687. [PubMed: 20847745]
- Ouyang H, et al., 2013 Increased interleukin9 and CD4+IL-9+ T cells in patients with systemic lupus erythematosus. *Mol. Med. Rep* 7, 1031–1037. [PubMed: 23291628]

- Renauld JC, et al., 1992 Expression cloning of the murine and human interleukin 9 receptor cDNAs. *Proc. Natl. Acad. Sci. U. S. A* 89, 5690–5694. [PubMed: 1376929]
- Sismanopoulos N, et al., 2012 IL-9 induces VEGF secretion from human mast cells and IL-9/IL-9 receptor genes are overexpressed in atopic dermatitis. *PLoS One* 7, e33271. [PubMed: 22413008]
- Staudt V, et al., 2010 Interferon-regulatory factor 4 is essential for the developmental program of T helper 9 cells. *Immunity* 33, 192–202. [PubMed: 20674401]
- Uyttenhove C, et al., 1988 Functional and structural characterization of P40, a mouse glycoprotein with T-cell growth factor activity. *Proc. Natl. Acad. Sci. U. S. A* 85, 6934–6938. [PubMed: 3137580]
- Veldhoen M, et al., 2008 Transforming growth factor-beta ‘reprograms’ the differentiation of T helper 2 cells and promotes an interleukin 9-producing subset. *Nat. Immunol* 9, 1341–1346. [PubMed: 18931678]
- Wilhelm C, et al., 2011 An IL-9 fate reporter demonstrates the induction of an innate IL-9 response in lung inflammation. *Nat. Immunol* 12, 1071–1077. [PubMed: 21983833]
- Yan Y, et al., 2012 CNS-specific therapy for ongoing EAE by silencing IL-17 pathway in astrocytes. *Mol. Ther* 20, 1338–1348. [PubMed: 22434134]

Fig. 1.

IL-9R expression in CNS resident cells in EAE. (A) Eighteen days after MOG₃₅₋₅₅ immunization, spinal cords were harvested after perfusion for immunohistochemical staining. The section from the L3 level was double stained by IL-9R and different CNS resident cell-specific markers. (B) Different types of primary CNS resident cells prepared at E16 were double stained by IL-9R and cell-specific markers (C) Real-time quantitative RT-PCR analysis of the expression of IL-9 receptor in the brain and spinal cord at different stages of EAE. Symbols represent mean $\pm$ SE (n = 5 each time point). *P < 0.05; **P < 0.01.

Fig. 2.

Expression of chemokines induced by IL-9 in astrocytes. Primary astrocytes prepared at E16 were treated with IL-9 (20 ng/ml) for 12 h; cells were then collected and analyzed by real-time quantitative RT-PCR for chemokine expression. Symbols represent mean $\pm$ SE of triplicate. A fold induction higher than 2 was considered statistically significant.

Fig. 3. Effects of cytokines on microglia proliferation. Primary microglial cells were stimulated in vitro with different cytokines for 3 days; microglial cell proliferation was measured by [^3H] thymidine deoxyribose incorporation (mean $\pm$ SE of triplicate). Different letters represent the statistically significant difference between groups.

Fig. 4.

Effects of cytokines on OPC proliferation and differentiation. A, OPCs were cultured in OPC differentiation medium for 5 days; the culture medium was replaced by fresh differentiation medium, and different cytokines were added into the differentiation medium. After 5 days' culture, cells were stained with mature oligodendrocyte-specific marker OSP; B, the ratio of OSP positive cells in DAPI positive cells was calculated (mean $\pm$ SE of triplicate); different letters represent the statistically significant difference between groups. C, primary OPCs were stimulated in vitro with different cytokines for 3 days; OPC proliferation was measured by [3 H] thymidine deoxyribose incorporation (mean $\pm$ SE of triplicate). Different letters represent the statistically significant difference between groups.