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Dysregulated production of leukemia inhibitory factor in immune cells of relapsing remitting multiple sclerosis patients☆

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ABSTRACT

Leukemia inhibitory factor (LIF) is known to potentiate the differentiation and survival of neuronal and oligodendrocyte precursors. Systemic therapy with LIF reportedly ameliorated the severity of experimental autoimmune encephalomyelitis and prevented oligodendrocyte death. We studied the secreted LIF levels from immune cells of relapsing remitting multiple sclerosis (RR-MS) patients compared to age- and gender-matched healthy controls (HCs). LIF was barely detected in the supernatants when the cells were not stimulated. After stimulation with anti-CD3/CD28 monoclonal antibody, LIF levels were up-regulated in both patients and controls, although to a significantly lower extent in RR-MS patients compared to HC. There were no significant differences between untreated patients and interferon-β1a treated patients. This is a heretofore unreported aspect of immune dysregulation in patients with RR-MS that may be related to insufficient remyelination and neurogenesis in MS lesions.
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1. Introduction

Multiple sclerosis (MS) is a chronic disease of the central nervous system with accumulating lesions of inflammatory infiltration that are associated with demyelination, oligodendrocyte death, axonal injury and neuronal loss (Ferguson et al., 1997; Barnett and Prineas, 2004; Prat and Antel, 2005; Trapp and Nave, 2008). Thus, the interplay between the immune and the nervous systems has a fundamental role in the process of the disease, and the understanding of those mechanisms would influence the development of therapeutic strategies for MS. The currently approved medications for MS exert their main effect on controlling and reducing the immune activity of the inflammatory processes. These compounds were found to reduce the relapse rate of relapsing disease, but they have a relatively mild effect on preventing the increasing disability that results from the disease and almost no effect on the progressive type of MS. There are no commercially available approved medications capable of inducing repair of the tissue damage. A better understanding of the ways to promote tissue recovery via the induction of neurogenesis and oligodendrogenesis processes is needed and motivates the search for such agents (Luc et al., 2004; Matute and Pérez-Cerdá, 2005).

Adult neuronal stem cells (NSCs) are present in neuroproliferative brain areas, i.e., the subventricular zone (SVZ) of the lateral ventricle and the subgranular zone (SGZ) of the hippocampus. Under normal conditions, adult NSCs obtain an ability to self-replicate and differentiate into multiple neural lineages, including neurons, astrocytes, and oligodendrocytes that promote remyelination (Van Praag et al., 2002; Conover and Notti, 2008). During pathological conditions, such as cerebral ischemia, apoptosis or autoimmune inflammatory demyelination, there is an increase in adult NSC proliferation and migration to the damaged tissue (Jin et al., 2003; Magavi et al., 2000; Picard-Riera et al., 2002). Despite the recruitment of neuronal precursor cells (NPCs) in MS lesions, their ability to differentiate into mature functioning oligodendrocyte or neuron cells is very poor (Back et al., 2005; Kotter et al., 2006; Kuhlmann et al., 2008).

A large number of extrinsic signals were found to modulate NSC differentiation and to affect their fate, among them, leukemia inhibitory

Abbreviations: LIF, leukemia inhibitory factor; MS, multiple sclerosis; RR-MS, relapsing remitting multiple sclerosis; HCs, healthy controls; NSCs, neuronal stem cells (NSCs); SVZ, the subventricular zone; SGZ, subgranular zone; IFN-β, interferon-β; PBMCs, peripheral blood mononuclear cells; mAb, monoclonal antibody; TNFα, tumor necrosis factor α; INF, interferon; gp, glycoprotein; GFAP, glial fibrillary acidic protein; EAE, experimental autoimmune encephalomyelitis; LPS, lipopolysaccharide; MACS, magnet-activated cell separation; IC, isotype controls; OPCs, oligodendrocyte precursor cells; NPC, neuronal precursor cells.

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factor (LIF) (Butzkueven et al., 2002; Ferguson and Slack, 2003; Marriott et al., 2008). LIF is a member of the neuro-regulatory cytokines that affect both the immune and nervous systems (Patterson, 1994). In addition to classic hematopoietic and neuronal actions, LIF plays a critical role in several endocrine functions, including the utero-placental unit (Shellard et al., 1996; Stewart CL), the hypothalamo–pituitary–adrenal axis (Akita et al., 1996; Shimon et al., 1997), bone cell metabolism (Martin et al., 1992; Van Vlasselaer, 1992), energy homeostasis (Nonogaki et al., 1996; Sarraf et al., 1997), and hormonally responsive tumors (Auernhammer and Melmed, 2000). It was also found to bind to the LIFRβ/glycoprotein (gp) 130 complex. The activation and phosphorylation of this receptor complex lead to the cross-phosphorylation of JAK and STAT and to the further activation of particular genes (Marriott et al., 2008; Chollangi et al., 2009). LIF also promotes the differentiation of various cell types. In vitro studies have demonstrated that LIF potentiates the differentiation and survival of oligodendrocyte precursors (Kerr and Patterson, 2005). Similarly, LIF promotes the generation and survival of sensory neurons and motor neurons in vitro (Kurek et al., 1996). LIF induces, also in vitro, the differentiation of NSCs into glial fibrillary acidic protein (GFAP)-expressing astrocytes (Weible and Chan-Ling, 2007; Asano et al., 2009). Additionally, LIF directly stimulates neurite outgrowth (Leibinger et al., 2009), and exogenous LIF was shown to have pro-myelinating effects on mixed culture derived from embryonic mouse forebrain (Stankoff et al., 2002). In vivo administration and depletion of LIF have been studied in experimental autoimmune encephalomyelitis. Systemic LIF treatment induced a significant reduction in experimental autoimmune encephalomyelitis (EAE) symptoms and prevented oligodendrocyte death in this animal model (Butzkueven et al., 2002). Additionally, the induction of EAE in mice heterozygous for both the LIFRβ-null mutation and for a truncated form of gp130 resulted in rapidly worsened disease, with increased demyelination and oligodendrocyte loss. Neutralizing anti-LIF antibodies had similar effects in EAE, indicating that endogenous LIF may play a beneficial role during MS (Butzkueven et al., 2006). Several reports implicated the role of the immune system in neuroprotection, neurogenesis and remyelination processes (Moalem et al., 1999; Bomstein et al., 2003; Hauben et al., 2003; Mikami et al., 2004). In vivo studies demonstrated that mice deficient in T cells and macrophages exhibited impaired remyelination after a demyelinating injury (Kotter et al., 2001; Bieber et al., 2003). Moreover, the co-culture of microglia (stimulated with interleukin 4 [IL-4] and interferon γ [IFN-γ]) and NSC resulted in neurogenesis and oligodendrogenesis (Butovsky et al., 2006).

We recently reported defective and dysregulated production of two neurogenetic factors, noggin and follistatin, in immune cells of patients with MS (Urshansky et al., 2011a, 2011b). We now studied the secreted LIF protein levels of immune cells of patients with relapsing remitting (RR)-MS compared to those levels of age- and sex-matched healthy controls (HCs).

2. Materials and methods

2.1. Study population

Patients with RR-MS attending the Neuroimmunology Clinic at the Tel-Aviv Sourasky Medical Center were included in the study. This investigation was approved by the local ethical (Helsinki) committee. After the participants had given their informed consent, blood samples were drawn from 40 patients with RR-MS during clinical remission, of whom 16 were untreated and 24 were treated with IFN-β1a. Blood samples were also drawn from 21 age- and gender-matched apparently healthy volunteers (HC group) (Table 1). The sample size was considered prior to the study to be able to reject the null hypothesis that the population means of the experimental and control groups are equal with probability (power) of 0.9. The Type I error probability associated with the test of null hypothesis was 0.05.

2.2. Experimental cells and culture conditions

Peripheral blood mononuclear cells (PBMCs) were isolated from heparinized venous blood by centrifugation over Ficoll-Paque (Amersham Biosciences, Uppsala, Sweden). The cells were resuspended (2.5×10^6 cells/ml) in complete culture medium comprised of RPMI 1640 medium supplemented with 10% fetal bovine serum, 4 mM L-glutamine, 25 mM Hepes buffer, penicillin 50 units/ml, and streptomycin 50 μg/ml (Biological Industries, Kibbutz Bet Haemek, Israel).

For obtaining LIF-secreted protein, the PBMCs were either stimulated or not stimulated with 1 μg/ml mouse anti-human CD3 monoclonal antibody (mAb) (BD Biosciences Pharmingen, San Diego, CA, USA) and 5 μg/ml mouse anti-human CD28 (R&D Systems, Minneapolis, MN, USA) or corresponding isotype controls (IC), or with 100 ng lipopolysaccharide (LPS, Sigma-Aldrich, St. Louis, MO, USA) for 24 h. Culture supernatants were collected after 24 h of incubation. Both stimulated and unstimulated cells were incubated in 14-ml polypropylene sterile culture tubes (Greiner bio-one, Germany) at 37 °C, 5% CO_2 humidified atmosphere. The protein level was determined by ELISA (R&D Systems).

The optimal time for the detection of LIF secretion levels was determined after time scale experiments at 24 h, 48 h and 72 h after the incubation of HC with CD3/CD28 mAbs or the relevant IC or LPS or without stimulation. We detected the maximal secretion of LIF in stimulated PBMCs of HC with anti-CD3/CD28 mAbs after 24 h, while LPS did not change the secreted LIF protein level (Fig. 1).

2.3. ELISA

The LIF secretion level was measured with an ELISA kit (R&D Systems) according to the manufacturer's instructions. The sensitivity of this assay was 7.8 pg/ml. Briefly, round-bottom 96-well plates

Table 1
Clinical characteristics of the precipitants.

*P value a. untx vs. HC, b. untx vs. IFN-β-Tx	Healthy controls	IFN-β-treated patients	Untreated patients	
	21	24	16	Number
a. 0.60	9:12	11:13	6:10	Male:Female
b. 0.74				
a. 0.19	34.67 ± 8.17	42.3 ± 12.8	40.9 ± 9.1	Age (mean ± SD) years
b. 0.65				
	22–55	23–64	22–51	Range, years
b. 0.62		1.79 ± 1.52	1.67 ± 1.8	EDSS (mean ± SD)
		0–6	0–6.5	EDSS range
b. 0.58		8.9 ± 3.7	8.6 ± 3.4	Disease duration, years
		1–19	1–17	Range, years

* Untx — untreated patients, IFN-β-Tx–IFN-β-treated patients, HC — healthy controls.

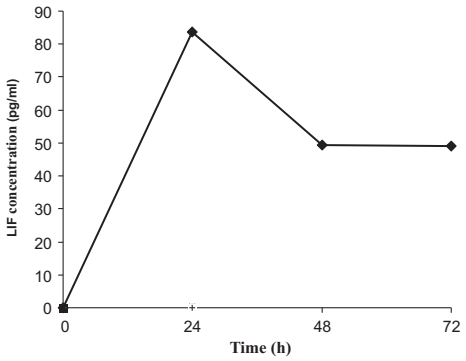

Fig. 1. LIF secretion levels from PBMCs were measured at different times (0, 24 h, 48 h and 72 h) after stimulation with anti-CD3/CD28 mAb in five healthy individuals. The higher LIF levels were detected 24 h after the stimulation, thus the 24 h secreted LIF protein levels were used for studying our study groups.

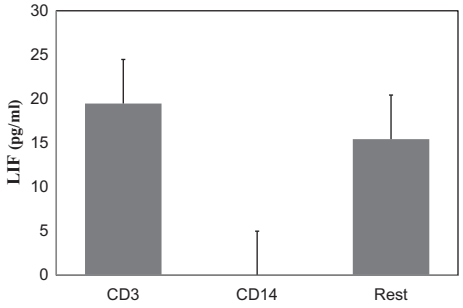

Fig. 2. T cells ($CD3^+$), monocytes ($CD14^+$), and the remaining $CD3^-CD14^-$ cells (Rest) were purified from PBMCs of nine healthy controls (HCs) using MACS. Cell purity was determined by flow cytometry as being >95%. Separated cells were incubated for 24 h without stimulation, after which secreted LIF protein levels in supernatants were measured by ELISA. LIF was significantly more secreted from T cells and non-T cell non-monocytes than from the monocytes.

(Greiner bio-one) were coated with monoclonal anti-human LIF. Captured LIF was detected with secondary biotinylated monoclonal anti-human LIF, followed by the addition of streptavidin conjugated horseradish-peroxidase. The LIF contents were quantified by a Termo Max ELISA reader (Molecular Devices microplate reader, USA).

2.4. T cells and monocytes separation

Purification of monocytes ($CD14^+$) and T cells ($CD3^+$ cells) from PBMCs was performed by a positive selection of $CD14^+$ cells following an additional positive selection of $CD3^+$ cells using a magnet-activated cell separation (MACS) extraction kit (Miltenyi Biotec Inc., Bergisch Gladbach, Germany). Cell purity was determined by flow cytometry using PE-conjugated mouse monoclonal antibody (mAb) anti-human CD3, FITC-conjugated mouse mAb anti-human CD14 and their corresponding isotype controls (all from BD Biosciences Pharmingen, San Diego, CA, USA), and it was >95% for each cell population. LIF secreted level was determined in isolated unstimulated $CD3^+$, $CD14^+$ and the remaining $CD3^-CD14^-$ subsets, that were separately incubated in 14-ml polypropylene sterile culture tubes (Greiner bio-one) at 37 °C, 5% CO_2 humidified atmosphere, and the culture supernatants were collected after 24 h of incubation. The secreted LIF protein levels were determined by ELISA.

2.5. Statistics

The differences in LIF levels between the study and control groups were evaluated by a Student *t* test. Non-parametric parameters were compared by the Fisher Exact test. Differences were considered significant when p values were <0.05

3. Results

3.1. T cells and $CD3^-CD14^-$ cells secrete higher LIF levels than monocytes

In order to detect differences in LIF secretion by different cell types that comprise the PBMCs, we performed a cellular analysis for LIF secretion among cell subtypes of the PBMCs from nine HCs. Those subtypes were separated into T cells ($CD3^+$), monocytes ($CD14^+$) and non-T and non-monocyte cells ($CD3^-CD14^-$) using MACS. The purity was above 95% for each subgroup. The separated cells were incubated for 24 h without specific stimulation, and the LIF levels were determined in the supernatant by ELISA. LIF was detected mainly in T cells in six of those nine HC donors and in none of the monocyte supernatants ($p = 0.009$). As in the case of the T cells, the same amounts of detectable LIF levels were found for $CD3^-CD14^-$ cells. The average LIF level from T cells was 19.5 ± 5.2 pg/ml, and the average LIF level from non-T cell non-monocytes was 15.4 ± 5.6 pg/ml (Fig. 2).

3.2. Stimulated immune cells via CD3/CD28 of RR-MS patients secreted lower LIF levels

The supernatant LIF levels from unstimulated PBMCs of the study and HC groups were lower than detectable levels. Similarly, after stimulation with LPS, the LIF levels in the supernatants were mostly undetected. However, the stimulation of the PBMCs with anti CD3/CD28 mAb significantly increased the secreted LIF protein levels to detectable levels compared to incubation with IC (Fig. 3a) in all the participants. Specifically, the mean LIF level after PBMC stimulation with anti-CD3/CD28 mAb was 97.0 ± 23.7 pg/ml for the untreated RR-MS patients vs. 22.1 ± 5.1 pg/ml after incubation with IC of those with detectable levels, $p = 0.007$, in the supernatants of stimulated PBMCs of IFN-β treated RR-MS patients (91.3 ± 14.3 pg/ml vs. 27.9 ± 3.7 pg/ml, $p < 0.001$, respectively), and in the supernatants of HC (170.6 ± 16.2 pg/ml vs. 14.7 ± 1.2 pg/ml, respectively, $p < 0.001$). Moreover, the levels in the supernatants from anti-CD3/CD28-stimulated PBMCs of the RR-MS patients were significantly lower than in the supernatants of the anti-CD3/CD28-stimulated PBMCs of the HC ($p = 0.021$ for untreated RR-MS patients vs. HC and $p < 0.001$ for IFB-β-treated RR-MS patients vs. HC). There were no differences between the two patient subgroups (Fig. 3b). We further calculated the stimulation ratios of LIF (i.e., the ratio of LIF secretion from PBMCs that were stimulated with anti-CD3/CD28 mAbs and the LIF secretion in the presence of IC in each of the participants). The LIF stimulation ratios were significantly elevated in the HC (33.2 ± 5.1) compared to the untreated RR-MS patients (8.3 ± 2.3, $p = 0.001$) and to the IFN-β RR-MS patients (10.3 ± 3.1, $p = 0.001$). Again, no significant differences were found between the two patient subgroups (Fig. 3c).

4. Discussion

The role of the immune response in MS appears to be highly complex. On the one hand, inflammation has a detrimental effect on the CNS tissue (Lassman, 2010), while, on the other hand, it has a neuroprotective role (Schwartz and Cohen, 2000; Hohlfeld et al., 2000). One of the major obstacles for the successful remyelination in MS plaque is the lack of maturation-inducing factors of oligodendrocyte precursor cells (OPCs) and the presence of inhibitory factors in OPC (Franklin and Ffrench-Constant, 2008). These inhibitory factors may include pro-inflammatory cytokines, such as INFγ, IL-17, TNFα and others, as well as a group of modulating growth factors, such as bone morphogenic proteins (Mausner-Fainberg et al., 2013), while the pro-remyelinating factors, such as noggin and follistatin, are down-regulated in the immune cells of patients with MS (Urshansky et al., 2011a, 2011b). It

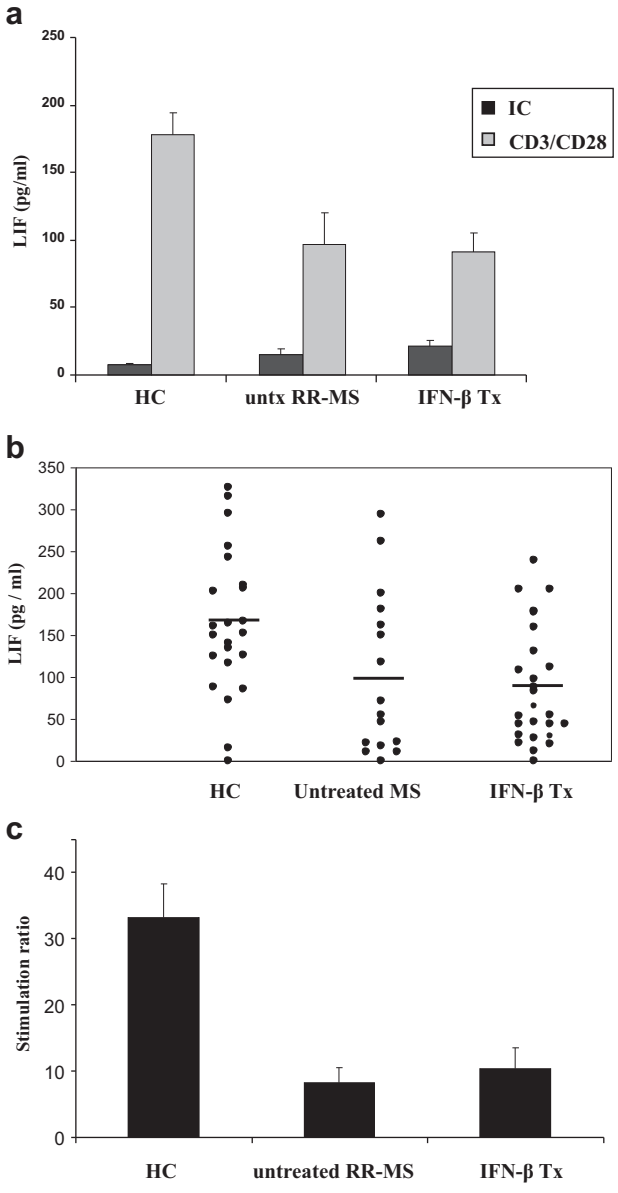

Fig. 3. a) PBMCs were isolated from 16 untreated RR-MS patients (Untx RR-MS), 24 IFN-β1a-treated RR-MS patients (IFN-β Tx) and 21 matched healthy controls (HCs). The 24 h secreted LIF protein levels were compared between incubation with anti-CD3/CD28 mAb and incubation with their isotype controls (IC). LIF secretion levels were significantly up-regulated after stimulation with anti-CD3/CD28 mAb in all study groups. b) Stimulated PBMCs of HC secreted significantly higher levels of LIF than those of untreated patients (Untx RR-MS) and IFN-β1a-tx patients. c) The stimulatory ratio of LIF, which was calculated for each participant by dividing the levels after stimulation with anti-CD3/CD28 mAb by the levels after incubation with the IC, was significantly higher in HC compared to the two patient subgroups (Untx andTx).

appears that the immune cells that are present in abundance near the MS plaques fail to provide a favorable microenvironment for NPC and OPC maturation. Therefore, we studied the expression of LIF, a pro-myelinating and neurogenetic factor, in the immune cells of patients with RR-MS compared to LIF expression in HC. Our findings demonstrated that immune cell activation via CD3/CD28 expresses and secretes LIF. We report here, for what we believe to be the first time, that activated PBMCs of patients with RR-MS secrete lower levels of LIF than activated PBMCs of HC, demonstrating a defective regulation of secreted LIF protein levels in the PBMCs from the T cells of patients with RR-MS. These data support our hypothesis that immune cells of patients with RR-MS do not induce enough support for neurogenesis and oligodendrogenesis. We found that among the PBMCs, T cells are a major producer of LIF and, therefore, the induced LIF secretion upon the activation of T cells probably occurs directly by the activated T cells and not indirectly by the interaction of T cells with monocytes. We assume that the lower production of LIF as well as the production of noggin and follistatin from immune cells together with the elevated secretions of bone morphogenic proteins 2,4,5 from these cells of patients with MS contribute to the insufficient neurogenesis and remyelination in the MS plaques. Taking into account that LIF plays a role in the inhibition of Th17 cell differentiation (Cao et al., 2011), our results of a lower LIF production from immune cells suggest an additional and heretofore unreported aspect of immune dysregulation in MS. Finally, we did not find any significant effect of IFN-β therapy on LIF secretion.

References

Akita, S., Conn, P.M., Melmed, S., 1996. Leukemia inhibitory factor (LIF) induces acute adrenocorticotrophic hormone (ACTH) secretion in fetal rhesus macaque primates: a novel dynamic test of pituitary function. J. Clin. Endocrinol. Metab. 81, 4170–4178.

Asano, H., Aonuma, M., Sanosaka, T., Kohyama, J., Namihira, M., Nakashima, K., 2009. Astrocyte differentiation of neural precursor cells is enhanced by retinoic acid through a change in epigenetic modification. Stem Cells 27, 2744–2752.

Auernhammer, C.J., Melmed, S., 2000. Leukemia-inhibitory factor—neuroimmune modulator of endocrine function. Endocr. Rev. 21, 313–345.

Back, S.A., Tuohy, T.M., Chen, H., Wallingford, N., Craig, A., Struve, J., Luo, N.L., Banine, F., Liu, Y., Chang, A., Trapp, B.D., Bebo Jr., B.F., Rao, M.S., Sherman, L.S., 2005. Hyaluronan accumulates in demyelinated lesions and inhibits oligodendrocyte progenitor maturation. Nat. Med. 11, 966–972.

Barnett, M.H., Prineas, J.W., 2004. Relapsing and remitting multiple sclerosis: pathology of the newly forming lesion. Ann. Neurol. 55, 458–468.

Bieber, A.J., Kerr, S., Rodriquez, M., 2003. Efficient central nervous system remyelination requires T cells. Ann. Neurol. 53, 680–684.

Bomstein, Y., Marder, J.B., Vitner, K., Smirnov, I., Lisaey, G., Butovsky, O., Fulga, V., Yoles, E., 2003. Features of skin-coincubated macrophages that promote recovery from spinal cord injury. J. Neuroimmunol. 142, 10–16.

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

Butzkueven, H., Zhang, J.G., Soilu-Hanninen, M., Hochrein, H., Chionh, F., Shipham, K.A., Emery, B., Turnley, A.M., Petratos, S., Ernst, M., Bartlett, P.F., Kilpatrick, T.J., 2002. LIF receptor signaling limits immune mediated demyelination by enhancing oligodendrocyte survival. Nat. Med. 8, 613–619.

Butzkueven, H., Emery, B., Cipriani, T., Marriott, M.P., Kilpatrick, T.J., 2006. Endogenous leukemia inhibitory factor production limits autoimmune demyelination and oligodendrocyte loss. Glia 53, 696–703.

Cao, W., Yang, Y., Wang, Z., Liu, A., Fang, L., Wu, F., Hong, J., Shi, Y., Leung, S., Dong, C., Zhang, J.Z., 2011. Leukemia inhibitory factor inhibits T helper 17 cell differentiation and confers treatment effects of neural progenitor cell therapy in autoimmune disease. Immunity 35, 273–284.

Chollangi, S., Wang, J., Martin, A., Quinn, J., Ash, J.D., 2009. Preconditioning-induced protection from oxidative injury is mediated by leukemia inhibitory factor receptor (LIFR) and its ligands in the retina. Neurobiol. Dis. 34, 535–544.

Conover, J.C., Notti, R.Q., 2008. The neural stem cell niche. Cell Tissue Res. 331, 211–224.

Ferguson, K.L., Slack, R.S., 2003. Growth factors: can they promote neurogenesis? Trends Neurosci. 26, 283–285.

Ferguson, B., Matyszak, M.K., Esiri, M.M., Perry, V.H., 1997. Axonal damage in acute multiple sclerosis lesions. Brain 120, 393–399.

Franklin, R.J., Ffrench-Constant, C., 2008. Remyelination in the CNS: from biology to therapy. Nat. Rev. Neurosci. 9, 839–855.

Hauben, E., Gothilf, A., Cohen, A., Butovsky, O., Nevo, U., Smirnov, I., Yoles, E., Akselrod, S., Schwartz, M., 2003. Vaccination with dendritic cells pulsed with peptides of myelin basic protein promotes functional recovery from spinal cord injury. J. Neurosci. 23, 8808–8819.

Hohlfeld, R., Kerschensteiner, M., Stadelmann, C., Lassmann, H., Wekerle, H., 2000. The neuroprotective effect of inflammation: implications for the therapy of multiple sclerosis. J. Neuroimmunol. 107, 161–166.

Jin, K., Sun, Y., Xie, L., Peel, A., Mao, X.O., Batteur, S., Greenberg, D.A., 2003. Directed migration of neuronal precursors into the ischemic cerebral cortex and striatum. Mol Cell Neurosci 24, 171–189.

Kerr, B.J., Patterson, P.H., 2005. Leukemia inhibitory factor promotes oligodendrocyte survival after spinal cord injury. Glia 51, 73–79.

Kotter, M.R., Setzu, A., Sim, F.J., Van Rooijen, N., Franklin, R.J., 2001. Macrophage depletion impairs oligodendrocyte remyelination following lysolecithin-induced demyelination. Glia 35, 204–212.

Kotter, M.R., Li, W.W., Zhao, C., Franklin, R., 2006. Myelin impairs CNS remyelination by inhibiting oligodendrocyte precursor cell differentiation. J. Neurosci. 26, 328–332.

Kuhlmann, T., Miron, V., Cui, Q., Wegner, C., Antel, J., Brück, W., 2008. Differentiation block of oligodendroglial progenitor cells as a cause for remyelination failure in chronic multiple sclerosis. Brain 131, 1749–1758.

Kurek, J.B., Austin, L., Cheema, S.S., Bartlett, P.F., Murphy, M., 1996. Up regulation of leukaemia inhibitory factor and interleukin-6 in transected sciatic nerve and muscle following denervation. Neuromuscul. Disord. 6, 105–114.
Lassman, H., 2010. What drives disease in multiple sclerosis: inflammation or neurodegeneration? Clin. Exp. Neuroimmunol. 1, 2–11.
Leibinger, M., Müller, A., Andreadaki, A., Hauk, T.G., Kirsch, M., Fischer, D., 2009. Neuroprotective and axon growth-promoting effects following inflammatory stimulation on mature retinal ganglion cells in mice depend on ciliary neurotrophic factor and leukemia inhibitory factor. J. Neurosci. 29, 14334–14341.
Luc, D.C., Song, H., Colamarino, S.A., Ming, G.L., Gage, F.H., 2004. Neurogenesis in the adult brain: new strategies for central nervous system diseases. Annu. Rev. Pharmacol. Toxicol. 44, 399–421.
Magavi, S.S., Leavitt, B.R., Macklis, J.D., 2000. Induction of neurogenesis in the neocortex of adult mice. Nature 405, 951–955.
Marriott, M.P., Emery, B., Cate, H.S., Binder, M.D., Kemper, D., Wu, Q., Kolbe, S., Gordon, I.R., Wang, H., Egan, G., Murray, S., Butzkueven, H., Kilpatrick, T.J., 2008. Leukemia inhibitory factor signaling modulates both central nervous system demyelination and myelin repair. Glia 56, 686–698.
Martin, T.J., Allan, E.H., Evely, R.S., Reid, I.R., 1992. Leukaemia inhibitory factor and bone cell function. Ciba Found. Symp. 167, 141–155.
Matute, C., Pérez-Cerdá, F., 2005. Multiple sclerosis: novel perspectives on newly forming lesions. Trends Neurosci. 28, 173–175.
Mausner-Fainberg, K., Urshansky, N., Regev, K., Auriel, E., Karni, A., 2013. Elevated and dysregulated bone morphogenic proteins in immune cells of patients with relapsing–remitting multiple sclerosis. J. Neuroimmunol. 264, 91–99.
Mikami, Y., Okano, H., Sakaguchi, M., Nakamura, M., Shimazaki, T., Okano, H.J., Kawakami, Y., Toyama, Y., Toda, M., 2004. Implantation of dendritic cells in injured adult spinal cord results in activation of endogenous neural stem/progenitor cells leading to de novo neurogenesis and functional recovery. J. Neurosci. Res. 76, 453–465.
Moalem, G., Leibowitz-Amit, R., Yoles, E., Mor, F., Cohen, I.R., Schwartz, M., 1999. Autoimmune T cells protect neurons from secondary degeneration after central nervous system axotomy. Nat. Med. 5, 49–55.
Nonogaki, K., Pan, X.M., Moser, A.H., Shigenaga, J., Staprans, I., Sakamoto, N., Grunfeld, C., Feingold, K.R., 1996. LIF and CNTF, which share the gp130 transduction system, stimulate hepatic lipid metabolism in rats. Am. J. Physiol. 271, E521–E528.
Patterson, P.H., 1994. Leukemia inhibitory factor, a cytokine at the interface between neurobiology and immunology. Proc. Natl. Acad. Sci. U. S. A. 91, 7833–7835.
Picard-Riera, N., Decker, L., Delarasse, C., Goude, K., Nait-Oumesmar, B., Liblau, R., Pham-Dinh, D., Evercooren, A.B., 2002. Experimental autoimmune encephalomyelitis mobilizes neural progenitors from the subventricular zone to undergo oligodendrogenesis in adult mice. Proc. Natl. Acad. Sci. 99, 13211–13216.
Prat, A., Antel, J., 2005. Pathogenesis of multiple sclerosis. Curr. Opin. Neurol. 18, 225–230.
Sarraf, P., Frederich, R.C., Turner, E.M., Ma, G., Jaskowiak, N.T., Rivet III, D.J., Flier, J.S., Lowell, B.B., Fraker, D.L., Alexander, H.R., 1997. Multiple cytokines and acute inflammation raise mouse leptin levels: potential role in inflammatory anorexia. J. Exp. Med. 185, 171–175.
Schwartz, M., Cohen, I.R., 2000. Autoimmunity can benefit self-maintenance. Immunol Today. 21, 265–268.
Shellard, J., Perreau, J., Brulet, P., 1996. Role of leukemia inhibitory factor during mammalian development. Eur. Cytokine Netw. 7, 699–712.
Shimon, I., Yan, X., Ray, D.W., Melmed, S., 1997. Cytokine-dependent gp130 receptor subunit regulates human fetal pituitary adrenocorticotropin hormone and growth hormone secretion. J. Clin. Invest. 100, 357–363.
Stankoff, B., Aigrot, M.S., Noël, F., Wattilliaux, A., Zalc, B., Lubetzki, C., 2002. Ciliary neurotrophic factor (CNTF) enhances myelin formation: a novel role for CNTF and CNTF-related molecules. J. Neurosci. 22, 9221–9227.
Trapp, B.D., Nave, A., 2008. Multiple sclerosis: an immune or neurodegenerative disorder? Annu. Rev. Neurosci. 31, 247–269.
Urshansky, N., Mausner-Fainberg, K., Auriel, E., Regev, K., Bornstein, N.M., Karni, A., 2011a. Reduced production of noggin by immune cells of patients with relapsing–remitting multiple sclerosis. J. Neuroimmunol. 232, 171–178.
Urshansky, N., Mausner-Fainberg, K., Auriel, E., Regev, K., Bornstein, N.M., Karni, A., 2011b. Low and dysregulated production of follistatin in immune cells of relapsing–remitting multiple sclerosis patients. J. Neuroimmunol. 238, 96–103.
Van Praag, H., Schinder, A.F., Christie, B.R., Toni, N., Palmer, T.D., Gage, F.H., 2002. Functional neurogenesis in adult hippocampus. Nature 415, 1000–1034.
Van Vlasselaer, P., 1992. Leukemia inhibitory factor (LIF): a growth factor with pleiotropic effects on bone biology. Prog. Growth Factor Res. 4, 337–353.
Weible, M.W., Chan-Ling, T., 2007. Phenotypic characterization of neural stem cells from human fetal spinal cord: synergistic effect of LIF and BMP4 to generate astrocytes. Glia 55, 1156–1168.