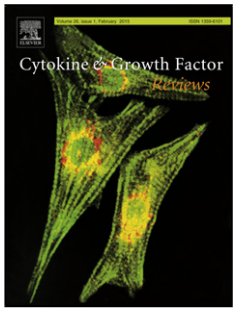

LEUKEMIA INHIBITORY FACTOR: RECENT ADVANCES AND IMPLICATIONS IN BIOTECHNOLOGY

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LEUKEMIA INHIBITORY FACTOR: RECENT ADVANCES AND IMPLICATIONS IN BIOTECHNOLOGY

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Highlights

- LIF is a pleiotropic cytokine, triggering different functions in health and disease with implications for the treatment of serious pathologies such as stroke and multiple sclerosis.
- Much of the LIF related research has been directed towards its role in the reproduction, despite the potential of LIF as therapeutic agent in the treatment of neurological disorders
- LIF is currently being tested in a limited number of clinical trials, mainly investigating issues related to fertility.
- The combination of biotechnology and nanotechnology holds promise in maximizing the therapeutic and diagnostic applications of LIF.

Abstract

Leukemia inhibitory factor (LIF) is a pleiotropic cytokine with several functions in health and disease ranging from inflammation to cancer. LIF is also a potential target and/or therapeutic agent for diseases such as multiple sclerosis, stroke and even psychological disorders, where the function of LIF as a neurotrophic factor has only recently been explored. In recent years, a limited number of LIF clinical trials have been completed, which partially explains the shortage of effective applications as a therapeutic agent. With the increasing interest from biotechnology companies producing recombinant LIF, this status quo will certainly change, and the potential impact of LIF in terms of disease diagnosis, treatment and management will be realized.

Keywords: Cytokine, LIF, Multiple sclerosis, Astrocytes, Brain injury, Myelination, Stroke

1. INTRODUCTION

The interleukin-6 (IL-6) cytokine family is characterized for their multifunction roles in health and disease and being involved in diverse biological processes [1]. This family consists of nine members (IL-6, IL-11, IL-27, IL-31, ciliary neurotrophic protein factor, cardiotrophin-1, cardiotrophin-like cytokine factor 1, oncostatin M and leukemia inhibitory factor (LIF)), all of which that interact with the membrane glycoprotein 130 (gp130) as part of their signaling process [2–4] . LIF is a cytokine of 180 amino acids in its mature form, which exerts its functions upon to the LIF Receptor (LIFR) complex [1,4,5]. LIF acts in diverse organs such as liver [6,7] kidney [8], skeletal muscle [9,10], bone [11], heart [12], pancreas [13], brain [5,14,15], uterus [16], and lung [17]. Its pleiotropic behavior implies that the effects depend on cell type [8,12,13], and on a complex system of promoters/inhibitors of the signaling pathway[8,12,14].

The LIFR complex is composed of both LIFR and gp130 which, upon LIF binding, promotes phosphorylation of the cytoplasmatic domains of Janus Kinase (JAK) receptors. This phosphorylation process results in the activation of several signaling pathways (Figure 1), namely: (i) phosphorylation of signal transducers, STAT, that homodimerize and translocate to the nucleus, binding to DNA; (ii) activation of phosphoinositide-3-Kinase (PI3K) pathway; and (iii) mitogen-activated protein kinase kinase / extracellular signal-regulated kinase (MEK/ERK) pathway.

Regarding its structure and phylogeny, LIF is characterized by a four α-helix bundle topology stabilized by three disulfide bridges, highly conserved between mouse and humans [19,20]. This conserved topology is important for the known cross-reactivity of human and murine LIFs – human LIF is active in both human and murine LIFRs, although murine LIF is only capable of eliciting the receptor of the same species [21,22]. LIF also possesses six sites for N-glycosylation that, in studies with recombinant proteins, proved to be secondary for activity and more relevant for protein stability [21,23].

Several studies explored LIF multifunctionality to understand its potentialities for both *in vivo* and *in vitro* applications. Furthermore, advances in recombinant DNA technology have improved the number of patents related to its biotechnological production. Nevertheless, although several studies focusing on LIF emerged in recent years, most of them relate to the reproductive apparatus with poorly explored information regarding its activity in other organs or cell types, such as the nervous system. With that in mind, the aim of this systematic review is to explore and organize recent articles, published in the last five years, to uncover the role of LIF in neurology, and to depict a general portray of the areas of interest and possible biotechnological applications.

Injuries to the central nervous system result in the activation of the immune system to act against such lesions. For a correct balance, several cytokines are activated, not only for stimulation of the immune response, but also to regulate the initiated mechanisms that could result in damage to the neural systems. In the case of LIF, this cytokine is undetectable in the nervous system under physiological conditions. However, during the first hours after lesion, the protein can initiate a pro- and an anti-inflammatory response [24,25]. In fact, LIF influences the responses in several neurological pathologies that are further discussed in this review.

2. The effect of LIF and the cell environment in demyelinating pathologies: Multiple sclerosis

Multiple sclerosis (MS) is a well-known, non-curable demyelinating disease, characterized by focal areas of inflammation with defacement of myelin and myelin-producing cells (oligodendrocytes and astrocytes). As a response, there is an increase in macrophages, T cells [26] [27], oxidative stress enzymes [27] and tumor necrosis factor (TNF) [28]. The result is a loss of cognitive, emotional and motility functions, affecting the quality of life of the affected person. Existing therapies focus on immune system modulation, normally based on antiviral substances and their capacity to modulate myelin production, with possible long-term side effects [29].

In a murine model of MS, it has been demonstrated that treatment with LIF prevents demyelination [30] through enhancement of hippocampal remyelination and stimulation of oligodendrocyte proliferation [31]. Due to the involvement of LIF in inflammatory responses, there are reports of elevated levels of LIF expression in activated immune cells (*e.g.* T cells and monocytes). According to Levy and co-workers, T cells are major producers of LIF upon CD3 and CD28-mediated activation. Nevertheless, LIF is produced in lower levels compared to stimulated T cells from healthy controls, not promoting neurogenesis or oligodendrogenesis. This finding demonstrated an unknown aspect of dysregulation in MS [32]. *In vitro* studies have further substantiated the important role of LIF concentration demonstrating its relation with increased expression of the erythropoietin receptor [33], the ability to enhance astrocytes interaction with oligodendrocytes [28], and how the use of enhancers for LIF secretion may help in the treatment of multiple sclerosis [34].

Due to the complexity of the *in vivo* studies, researchers are forced to use different multiple sclerosis models. For instance, the introduction of cuprizone in mice diet is one of the most used inducible animal models and has been used to study the effect of LIF in oligodendrocyte response [28], and in LIF-stimulated alterations of the disease process [27]. Another example relates to experimental autoimmune encephalomyelitis, in which the isolation and culturing of peripheral blood mononuclear cells (PBMC) from whole blood enabled the evaluation of cellular responses to LIF [24,32], and to study the expression of LIF after stimulation of the immune system with anti-human CD3 and CD28 monoclonal antibodies, corresponding isotype controls (IC) and lipopolysaccharides [32].

The initial response to multiple sclerosis is remyelination, guaranteed by the proliferation and maturation of oligodendrocyte progenitor cells (OPCs) [33,35,36]. Studies with mice restricted to a cuprizone diet demonstrated the central role of LIF in this process, promoting the differentiation of these cells in different brain regions (cerebellum and corpus callosum) [35,36]. In the cerebral cortex, LIF is responsible for inducing the expression of opalin and myelin oligodendrocyte glycoprotein (MOG) in oligodendrocytes (Figure 2A). Consequently, OPCs differentiate into myelin-producing oligodendrocytes [36]. In the corpus callosum, LIF mediates myelin protection against cuprizone toxicity in mice by inducing galanin secretion in OPCs. Galanin, in turn, induces ERK 1/2 phosphorylation by binding to GalR2 receptor in some cells, resulting in myelin production (Figure 2A) [35].

LIF-induced processes are dependent on the cytokine microenvironment and are mediated by the presence of erythropoietin (EPO), which has been found to display neuroprotective

functions and emerged as a possible therapy for neurodegenerative diseases [37]. *In vitro* studies using OPCs from rats overexpressing EPO receptors, demonstrated the importance of LIF concentration in regulating EPO-induced myelin gene expression in oligodendrocytes (ODs) [33]. In the presence of EPO, high concentrations of LIF (> 10 ng/mL) activate SOCS3, a factor responsible for blocking the EPO receptor, resulting in a decrease of MOG expression and leading to the inhibition of OD maturation (Figure 2B). On the other hand, low concentrations of LIF (< 0.2 ng/mL) do not increase SOCS3 expression, allowing the normal response of the EPO receptor to increase MOG expression, promoting OD maturation. When LIF ranges between 0.2 and 10 ng/mL, MOG expression allows the maturation of ODs to occur, but this begins to subside as the concentration of LIF increases up to 10 ng/mL. At this point, SOCS3, which acts downstream of STAT3, is produced and inhibits the EPO receptor response, inhibiting OD maturation (Figure 2B). Considering the EPO microenvironment, researchers also demonstrated the occurrence of different responses depending on the maturity level of the oligodendrocytes. In differentiated oligodendrocytes, the TLR (toll-like receptors) pathway is activated (TLR2) in a concentration-independent manner, blocking OD maturation. LIF's role in undifferentiated cells such as OPCs, and in the presence of EPO, is inhibitory, because EPO-activated PTPRE (protein tyrosine phosphatase receptor type E) is blocked and does not facilitate the differentiation of OPCs (Figure 2C). This mechanism is important to LIF-mediated activation of the ERK pathway and further stimulation of OPCs proliferation [33].

Differentiation and proliferation of oligodendrocytes and their progenitors are impaired in multiple sclerosis, which leads to remyelination failure. In this regard, many therapies focus on these targets. Exogenous LIF administration is a promising strategy, and administration of LIF enhancers also demonstrated to be efficient, as stated by Vela and coworkers [34]. Oligodendrocytes and their progenitor cells are also stimulated by astrocytes to contribute to remyelination, a process where LIF is a key cytokine. This stimulation is mediated by tumor necrosis factor (TNF). Activation of TNF receptor 2 triggers the PIK3-PKB/Akt pathway and subsequently leads to the secretion of LIF by astrocytes that will, in turn, promote OPCs differentiation (Figure 3) [28].

The studies referred earlier focused on specific neuronal cells and how manipulation of the microenvironment could be used in therapy. Multiple sclerosis is however characterized by a highly activated immune system, with growing number of macrophages and T cells infiltrating the central nervous system. Activated T cells are major producers of LIF in healthy control cells but in relapsing-remitting MS (RR-MS), Levy and coworkers [32] demonstrated a defective regulation of LIF. These data support the hypothesis that in RR-MS there is an imbalance in LIF secretion needed to stimulate neurogenesis and oligodendrogenesis [32]. Furthermore, mice in cuprizone diet treated with recombinant LIF partially recovered from the damage to the oligodendrocytes and neurons (in both cortex and cerebellum) [27]. The effect remained for two months due to a decreased content of immune cells and reactive oxidative species [27].

Another important feature of LIF is its balance with IL-6 (interleukin 6) that showed to be essential to modulate the number of regulatory T cells (Treg). LIF increases the number of Treg cells, but IL-6 decreases the number of LIFR, creating a balanced response (Figure 4) [24]. In multiple sclerosis, LIFR is highly expressed, thus increasing the number of Treg cells and resulting in the characteristic highly activated immune system [24].

In summary, cells of the immune system, oligodendrocytes and its progenitors, are potential targets to treat multiple sclerosis. However, it should be considered that the response triggered is context dependent. Despite all the targets mentioned in the aforementioned studies, the

complexity of the human body limits the implementation of novel LIF-based therapies to treat multiple sclerosis. In fact, although treatment with recombinant LIF is a very promising approach, more studies are necessary to understand the side effects of LIF administration. Other strategies, such as gene therapy, may be a different therapeutic approach for multiple sclerosis. As demonstrated *in vitro*, the introduction of genes for important cytokines, such as LIF, allowed the target cells to produce the therapeutic agent themselves [38].

3. Therapeutic potential of LIF in brain recovery after vessel occlusion diseases

Occlusion of brain vessels is one of the deadliest types of acute ischemic strokes and a major cause of adult disability [39]. Vessel occlusion is associated with energy failure, low oxygen levels, neural cells damage and neuroinflammation [40]. The standard FDA-approved drug for treating this stroke type is the tissue plasminogen activator (tPA), although the application of stents has become an option for severe cases [41,42].

Using mice models for middle cerebral artery occlusions, researchers studied the relationship between LIF and strokes considering four types of this disease: intracerebral hemorrhage (IC) [43], emergent large vessel occlusion (ELVO) [25], focal cerebral ischemia (FCI) [15] and hypoxia-ischemia (H-I) [44] (Figure 5).

Intracerebral hemorrhage is characterized by increased astrogliosis and the formation of a glial scar at the lesion site. Zhou and co-workers confirmed that LIF has a role in reactive astrogliosis since LIFR showed to be upregulated three days after injury, inducing the phosphorylation of p-STAT3 dimer, and resulting in increased GFAP mRNA expression (a marker in reactive astrocytes) [43,45]. In hypoxia-ischemic injuries, the Notch pathway plays an important role with direct correlation to LIF stimulation. Notch signaling is important to regulate proliferation, maintenance and differentiation of neural stem and progenitor cells, crucial to conservation of the neural progenitor pool (NSPs). Following hypoxia-ischemic injuries, a regenerative response from the neural stem/progenitors (NSPs) of the subventricular zone (SVZ) is induced. Astrocytes in SVZ are stimulated to secrete LIF, which in turn triggers the Notch-DSL signaling pathway to expand NSPs and boost support cells in their niche [44].

Focal cerebral ischemia strokes are characterized by increased levels of pro-oxidant molecules [46–49], membrane depolarization [50,51], and calcium influx [52]. These pro-oxidant molecules generate reactive oxidative species (ROS) that cause neuronal death. In turn, LIF induces the production of superoxide dismutase (SOD) enzymes, such as SOD3, that are used to degrade ROS [15]. This phenomenon is triggered after LIF binds to its receptor in neurons, activating the Akt pathway, increasing SOD3 enzyme expression and protecting neurons from the oxidative environment. This has also been confirmed *in vivo* [15]. Likewise, human umbilical cord blood cell therapy has been studied in relation to oxidative stress and focal cerebral ischemia strokes. In this therapy, these same cells produce soluble factors, such as LIF, that activate the Akt pathway leading to the expression of antioxidant proteins, and contributing to functional recovery, reduction of white matter injury and infarct volume [53].

Emergent large vessel occlusion strokes are characterized by immune system activation and immune cells recruitment to the brain. Davis and co-workers found a correlation between this type of stroke and the presence of T-cells in the spleen after LIF treatment [25]. IP-10, a chemokine that facilitates pro-inflammatory mechanism from CD4+ T cells, and IL-12, a pro-inflammatory cytokine produced by microglia/macrophages, are detectable at high levels after

stroke. Along with IP-10, IFN-ɤ promotes migration of T cells to the brain after stroke. However, after LIF treatment, the IL-12/IFN-ɤ/IP-10 pathway was downregulated: IP-10 expression in the spleen decreases, preventing the maturation of CD8+ T cells and preventing the migration of immune cells to the brain after stroke. Despite that, a small population of CD11b+ cells levels were observed leaving the spleen, meaning that chemotaxis in the brain decreases with LIF treatment [25].

While LIF is a potential target for the treatment of vessel occlusion diseases, there is the need of further studies in order to understand its influence in the cell's microenvironment. Furthermore, age proved to be an important factor in LIF therapeutic capacity, as aged mouse model of MCAO (middle cerebral artery occlusion) showed low response to LIF treatment due to lack of LIFR expression [54].

4. LIF mediated neuroprotection in traumatic injuries

Traumatic injury resulting from a concussion is a very common pathology and symptoms include loss of conscience, confusion and disorientation. Activation of several signaling cascades occur after trauma, as well as a glial response characterized by astrocytes activation and immune cells recruitment, that can be favorable or detrimental [55]. A highly activated immune response can lead to cell death, due to the increasing levels of ROS and intracellular calcium. In this regard, LIF acts as an important microglial modulator and astrocyte stimulator, preventing secondary neurogenerative responses [56]. The neuroprotective effect of LIF has also been demonstrated after severe traumatic injuries. While most published studies focus on the brain, spinal cord injuries are also prone to trauma with severe consequences. Li and co-workers performed spinal cord lesions in mice and administrated LIF to evaluate its potential to treat this type of lesions [57]. The study found that LIF increased the number of neural precursor cells (NPCs), suggesting activation and regeneration of neurons, resulting in an improved locomotion capacity of mice with spinal cord injury [57].

In summary, LIF clearly has important neuroprotective functions, in both severe and simple injuries.

5. Neurological disfunction caused or potentially prevented by LIF

Many pathologies, such as obesity and infertility, may be associated with some type of neurological disfunction. Obesity-associated inflammation involves the recruitment of IL-1β, IL-6 and TNF-α. As an IL-6 family member, LIF is overexpressed in the hypothalamus, suppressing food intake. An *in vivo* study using high fat diet (HFD) models with induced brain inflammation demonstrated that LIF exerts an anorexic effect, modulating neuronal excitability in the brain stem [58]. Using the same model, LIF demonstrated to repress gene expression of gonadotropin releasing-hormone (GnRH), a reproduction-related hormone. Increasing the concentration of LIF lead to an increase in cFOS production, a marker for recent neuronal activity that binds to specific sites in GnRH-producing neurons, decreasing its expression and causing fertility problems [59,60]. In this case, LIF is not an adjuvant but a damaging cytokine [60]. Another example of the disadvantageous effects of LIF is the promotion of aggressiveness of chordoma cancer cells after LIF treatment, which exacerbates the migration, invasion and chemoresistance of these cells [61].

Although most of the studied diseases are related to a physical pathology, there are also psychological disorders associated to LIF expression. Some psychiatric disorders rely on alterations of the maternal-fetal LIF pathway. Two examples of specific psychiatric disorders associated with maternal immune activation (MIA) by viral infection are schizophrenia and autism. The mechanism begins with LIF inducing adrenocorticotropic hormone from the placenta, which stimulates fetal red blood cells to secrete LIF thus promoting neurogenesis. To simulate a viral infection *in vivo*, researchers used mice as model and injected polyriboinosic-polyribocytidylic acid, a synthetic analog of double-stranded RNA that causes MIA. This infection demonstrated to reduce the levels of LIF in the fetal cerebrospinal fluid, by inducing SOCS3, a factor responsible for blocking EPO receptor in ODs, resulting in reduced neurogenesis in the cerebrum [62].

With the available data, we can conclude that neurological disorders lead to the appearance of other pathologies and LIF can be a target for therapy, either through reduction of its expression, for example in chordoma cancer, or by increasing it for treatment of psychiatric disorders caused by viral infections.

6. Biotechnological implications of LIF therapeutic promise

Playing so many roles in both health and disease, LIF is a promising target candidate for novel therapies. However, a search in the main clinical trials databases (isrctn.com, Clinicaltrials.gov, clinicaltrialsregister.eu and ICTRP) reveals only a limited number of trials (n=5) involving LIF, and all directed for applications related with the reproductive apparatus and fertility [63–67]. An exception is the study with Emfilermin, the commercial name for recombinant human LIF, which has been used in a clinical trial to prevent chemotherapy-induced peripheral neuropathy in cancer patients, although without positive feedback [68]. In this study, while patients showed no improvement in their diagnosis after treatment with LIF, they also did not show any side effects. This is a very promising prognosis for systemic treatment of several pathologies using LIF and should be further explored. In fact, Merck KGaA has filled and concluded two clinical trials related to the use of LIF for improvement of embryo implantation [69,70], demonstrating and reinforcing the potential use of LIF as a therapeutic agent.

For many of these studies and trials to occur, researchers recur to the use of recombinant forms of LIF. Taking as example the final dataset of this study, more than 70% used recombinant LIF to conduct research studies [15,24,25,27,28,33–36,38,43,44,53,54,57,58,60–62]. Several ways to express recombinant LIF are used, being the most common *Escherichia coli* [71], although other hosts may include plants [72–74] and mammalian COS-1 cells [75,76]. Using different production hosts results in a set of different advantages or disadvantages, mainly regarding production levels, post-translational modifications and the production of virulent factors. For instance, bacteria such as *E. coli*, are highly used because they are simple organisms with low maintenance. Also, it is possible to obtain high expression levels of recombinant proteins in a short period of time. The main disadvantage is the lack of many post-translational modifications. Plants and mammalian cells, on the other hand, hold the necessary mechanisms to promote post-translational modifications with many variants available, but at the cost of time efficiency when compared with bacteria. Considering the information available for the recombinant production of LIF, the use of a bacterial expression system presents advantages and is overall more efficient. In fact, the use and optimization of bacterial expression systems

resulted in several patent submissions filled in the last 30 years, most of them related to the production of recombinant LIF, its receptor and agonist/antagonist molecules [77–82].

The heterologous expression of LIF refers mostly to recombinant human or mouse LIF but other sources are being studied. Researchers were able to successfully produce recombinant LIF from *Trichosurus vulpecula*, the common brushtail possum, in *E. coli* [83]. Interestingly, the sequence showed to be highly conserved to other mammals, demonstrating once more the important functions of LIF in mammals [83]. In 2015, feline LIF was also produced for the first time in an active form in *E.coli*, showing high similarity to the human (91%) and mouse (81%) variants. Despite the high similarity, it has been demonstrated that hLIF is unable to bind the feline LIFR due to specific differences in amino acid sequence and secondary structure [84]. More recently, works from Kaur *et al*. (2017) and Ali *et al*. (2018) describe the recombinant production of buffalo LIF (rBuLIF) in COS-1 cells, demonstrating its *in vitro* activity on bovine stem cell culture [75].

Aside from recombinant production and evaluation of the therapeutic effects of its free form, LIF can also be used as an active agent in the formulation of delivery platforms for neurological applications. The incorporation of LIF into nanoformulations provides advantages such as prolonged stability and activity, limiting the rate of release to some extent, thus allowing the delivery of the bioactive agent in controlled amounts. For instance, LIF-loaded nanoparticles have been used for neuroprotection purposes aiming to prolong the neuroprotective and anti-inflammatory effect of the cytokine and to target specific targets [14,85]. Rittchen *et al* [14] used poly(lactide-co-glycolide) (PLGA) nanoparticles coated with avidin, and Davis *et al* [85] used a constitution of poly(lactic acid) coated with polyethylene glycol and with CD11b antibody. The former study reports an increase in myelin and potent enhancement of remyelination in MS and OPCs function [14]. Davis *et al*, delivered LIF into murine myeloid leukemia (M1) cells, resulting in decreased cell proliferation compared to free LIF. Both studies concluded that the controlled release of LIF is important to the extended treatment of a brain and, inclusively, LIF demonstrated to be more stable inside the nanoparticles allowing for a sustained treatment for remyelination or to reduce brain inflammation. This is especially important when using the non-glycosylated recombinant LIF, which is more susceptible to proteolysis.

New discoveries lead to intellectual property protection and while some related patents are focused on the maintenance of stem cells pluripotency and even on embryo implantation, no patents were found describing the use of LIF for neuronal disorders treatment. The controversial results, with LIF being able to treat or exacerbate neurologic results, are probably threatening investments in this area. More research is therefore necessary before unraveling the full therapeutic potential of LIF for neurologic disorders. Nevertheless, the research presented here reveals roles of LIF that were not previously known, with regard to treatment of multiple sclerosis, strokes or even psychological disorders.

We believe that improvements on the recombinant production of LIF will greatly contribute to make this process faster, providing easier access to recombinant LIF.

7. Final remarks

The study demonstrated a shortage of research in fields other than the reproductive apparatus. In fact, neurology-based papers represent only a limited fraction of the non-duplicate sample.

Regarding multiple sclerosis, LIF seems to stimulate remyelination, oligodendrocyte maturation and differentiation. However, its effect involves not only the protein by itself, but is also dependent on concentration and other molecules such as IL-6 and EPO. In the studies depicted here, it was shown that LIF is an interesting therapeutic target for treatment of multiple sclerosis. In vessel occlusion diseases, LIF demonstrated to have an effect in four different stroke types. Its impact is characterized, in general, at the levels of astrocytes and NSP proliferation. In traumatic injuries, LIF modulates the inflammation process and triggers two different effects: prevention of secondary neurodegenerative responses and increase of NPCs, allowing the recovery of normal brain function. The importance of LIF in psychological and hormonal disorders, such as obesity, fertility, schizophrenia and autism, is also referred.

This review also aims to draw attention to the utilization of LIF for different biotechnological applications. LIF is already being applied in clinical trials, but in a limited number and mostly related to fertility issues. In a high percentage of the described studies, researchers used recombinant LIF, showing the importance of this industry to this type of biomedical studies. In fact, most of the patents related to LIF are associated with its recombinant expression.

As a conclusion, we can state that LIF is an interesting target for treatment of neurologic disorders, but controversial results in some studies need to be fully elucidated in order to effectively potentiate its use in the clinical practice.

CRediT author statement

Vanessa Pinho: Investigation, Methodology, Formal Analysis, Writing – Original draft preparation. **Mário Fernandes**: Visualization, Writing- Original draft preparation. **André da Costa**: Conceptualization, Writing- Reviewing and Editing. ***Raúl Machado****:* Writing- Reviewing and Editing, Funding acquisition.**: Andreia C Gomes**: Conceptualization, Writing- Reviewing and Editing, Funding acquisition, Project administration, Supervision.

Author Agreement

All authors have seen and approved the final version of the manuscript being submitted. The article is the authors' original work, hasn't received prior publication and isn't under consideration for publication elsewhere.

Declaration of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Figure 1. LIF functions and pathways: A) LIFR signaling is responsible for the activation of MEK/ERK, MAPK and PI3K pathways. Depending on the type of microenvironment in which LIF is present, activation of the different pathways could lead to proliferation, differentiation or survival functions. B) After LIF binding to the receptor, phosphorylation of the JAK domains occurs and a cascade of events starts. The MEK/ERK pathway is activated, initiating with GRB2 phosphorylation, followed by RAS activation, and MEK and ERK activation by phosphorylation. PI3K pathway is activated leading to PIP3 formation, allowing AKT phosphorylation. Furthermore, STAT dimer formation could occur as result of LIF binding to the receptor. Adapted from [3] and [18].

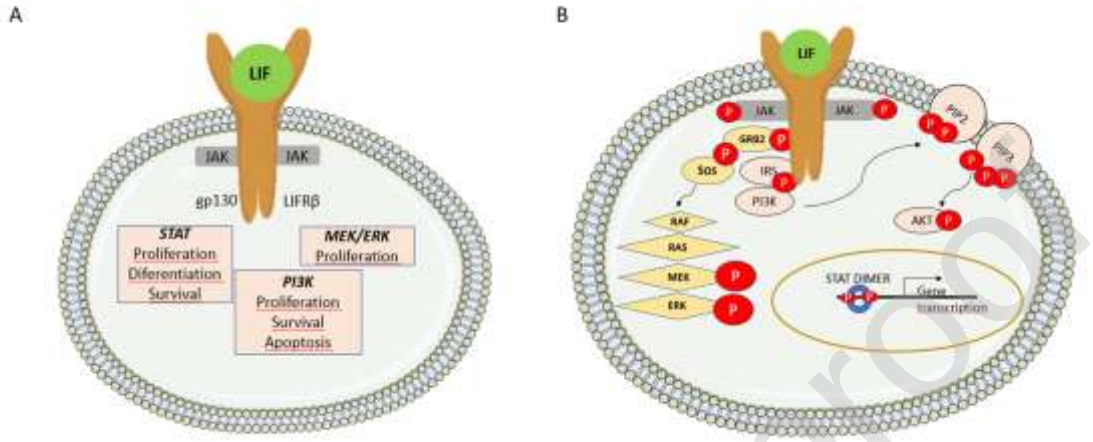

Figure 2. LIF influences oligodendrocyte (OD) maturation and proliferation. **A-** LIF promotes the expression of Galanin leading to increased myelin production. With a similar effect, LIF promotes Opalin expression, resulting in increased MOG expression and therefore OD maturation. **B-** OD maturation is dependent on LIF concentrations – when LIF concentration is between 0.2 and 10 ng/mL, STAT3 is not activated and MOG induced by EPO receptor increase it expression, allowing OD maturation. **C-** The effect of LIF is not only in differentiated but also in undifferentiated cells, such as ODs and OPCs, respectively. In OPCs, the ERK pathway is activated by LIF, resulting in OPC proliferation. In contrast, EPO activated PTPRE and inhibits LIF-induced signaling, leading to OPCs differentiation This mechanism is inhibited by EPO presence, activating PTPRE, starting OPCs differentiation. In ODs, TLR pathway is activated by LIF blocking ODs maturation.

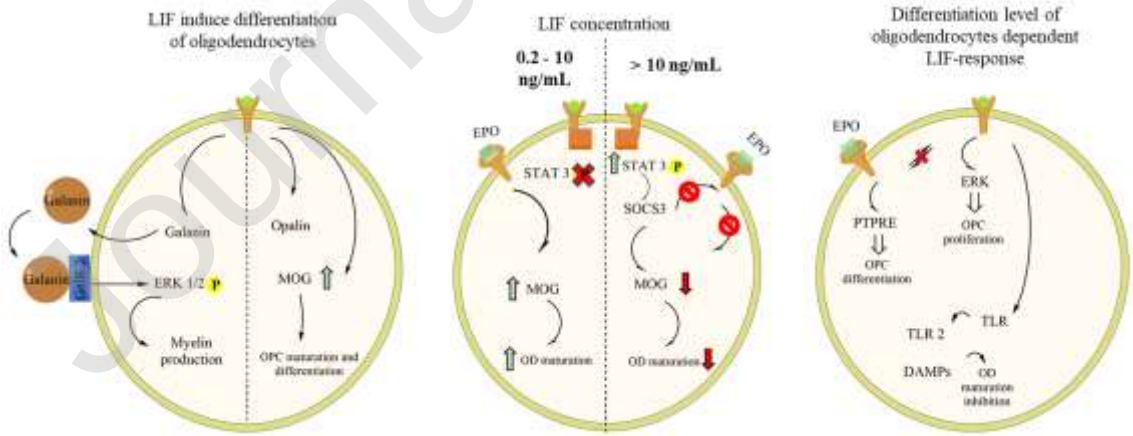

Figure 3. LIF influence in astrocyte-dependent remyelination of oligodendrocytes. Astrocytes are stimulated by TNF to produce LIF, by the activation of the PI3K-PKB/Akt pathway. LIF is then free to promote OPCs differentiation and subsequent remyelination.

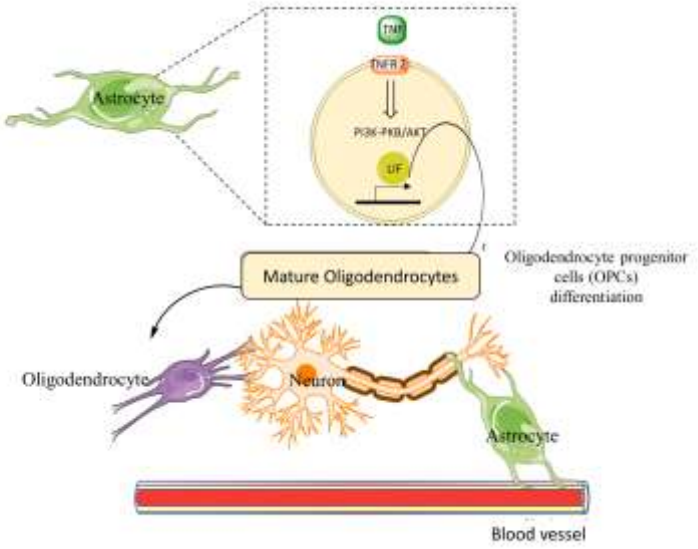

Figure 4. Balance between LIF and IL-6 in peripherical blood mononuclear cells (PBMCs). IL-6 is responsible for decreasing LIFR expression levels, which are elevated in MS, and LIF is responsible for boosting Treg cells, important in the therapeutic response to MS.

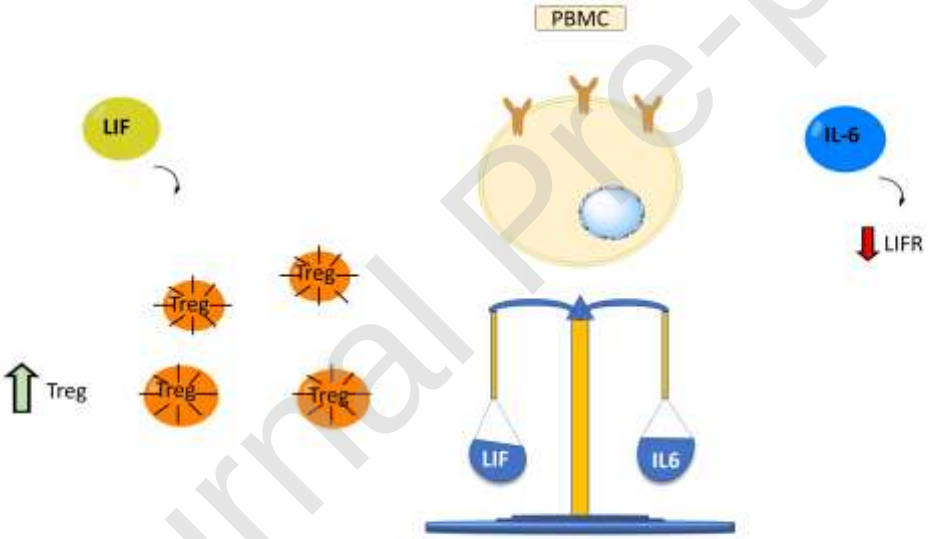

Figure 5. Four types of stroke and their relationship with LIF. The four types of strokes are IC (intracerebral hemorrhage), H-I (hypoxia-ischemia), ELVO (emergent large vessel occlusion) and FCI (focal cerebral ischemia). In IC astrocytes are more responsive to LIF because LIFR is upregulated. When LIF binds to LIFR, the JAK/STAT pathway is activated and p-STAT3 dimer is produced, binding to GFAP promoter and increasing its expression. GFAP is produced and allow astrocytes to become reactive and responsive to injury. In astrocytes, they are also stimulated to produce LIF in hypoxia-ischemic injuries. In this case, the Notch pathway is activated to promote proliferation of the NSPs. At a paracrine level, LIF is responsible for IL-12/IFN-α/IP-10 pathway inhibition, limiting the migration of immune cells from the spleen to the brain. The final type of stroke to be analyzed are FCI, where researchers attribute the decrease of the oxidative environment to LIF. In this mechanism, LIF activate Akt pathway and increase expression of SOD3, an enzyme responsible for degrading ROS.