The LIF cytokine: towards adulthood

Marina Trouillas, Claire Saucourt, Bertrand Guillotin, Xavier Gauthereau, Jean-Luc Taupin, Jean-François Moreau, Hélène Bœuf

Université de Bordeaux, CNRS-UMR-5164-CIRID, 33076 Bordeaux, France

Correspondence: H. Bœuf, CNRS-UMR-5164-CIRID, Université Bordeaux-2, bâtiment 1B, BP 14, 146, rue Léo-Saignat, 33076 Bordeaux, France <helene.boeuf@u-bordeaux2.fr>

Accepted for publication March 20, 2009

ABSTRACT. The aim of this article is to recapitulate the key features of leukaemia inhibitory factor cytokine (LIF), to review its numerous physiological effects and to comment on the most recent data. We will also present results of transcriptome analyses, which have highlighted different categories of LIF targets, identified in murine embryonic stem (ES) cells and early derivatives. We hope to stimulate new research fields on this puzzling cytokine, which, forty years after its discovery, has still not disclosed all its secrets.

Keywords: LIF, IL-6, ES cells, pleiotropy, chromatin, p53

LIF discovery was performed independently by several research groups in the 1970-80's, and was based on the different biological effects characterized in distinct cellular models, and was hence given different names. The name LIF has remained the most commonly used despite it being the least appropriate [1, 2]. Pleiotropy is the property of a protein to display various and sometimes opposing effects. In this regard, LIF could sustain proliferation or differentiation depending upon cell maturity or type [1-3]. While the physiological, non-redundant function of LIF stands in its effect during blastocyst implantation, an essential role of LIF has also been reported for mammary gland involution after the lactation [4, 5]. Unregulated LIF secretion is also associated with pathological conditions such as cancer [6-9].

A BRIEF HISTORY OF LIF

LIF activity was first reported in 1969 by Ichikawa, who described a biological activity associated with embryo-conditioned medium, which was able to inhibit proliferation and to induce macrophage differentiation of the M1 leukemic myeloid cell line [15]. This biological activity, then called D-factor (for differentiation factor), was partially purified from embryos and Krebs ascites cell-conditioned medium [16], followed by purification to homogeneity of the active protein. At the same time, this protein was characterized from cell line-conditioned media: fibroblasts [17-19], heart cells [20], human alloreactive T-cells stimulated by alloantigens [21] and mitogen-triggered spleen cells [17]. These studies led to the molecular cloning of LIF in many species including human [19, 22-25].

THE LIF LIGAND

Main features

Human LIF is a 180-amino acid glycoprotein and belongs to the IL-6 sub-family of the larger “four-helix superfamily” of cytokines, comprising the following six members: ciliary neurotrophic factor (CNTF), interleukin 11 (IL-11), oncostatin M (OSM), cardiotrophin-1 (CT-1), CLC/CLF (cardiotrophin-like cytokine-cytokine-like factor (CNTF II ligand [26]), and IL-27 [27-30]. Purified human LIF has a MW of 37 to 62 kDa depending on its degree of glycosylation [31, 32]. The glycosylation
sites of LIF can hold mannose-6 phosphate residues, creating a ligand site for the mannose-6 phosphate receptor (CIMPR, calcium-independent mannose-6 phosphate receptor), which participates in the cellular recycling of the LIF ligand [33, 34]. LIF exists in at least three isoforms, i.e. a soluble form called LIF-D, an intracellular form found in the nucleus, called LIF-i or LIF-T, and a third form localized in the extracellular matrix, termed LIF-M [35-37]. LIF-M and LIF-D are translated from alternative transcripts differing in the first exon, which encodes partially for different signal peptides, which target the mature protein to different locations. The LIF-T isoform is a truncated protein because of the absence of the ATG codon in the first exon, leading to the initiation of translation at the first ATG codon of exon 2. This isoform has pro-apoptotic activity in the COS and 293T cell lines [37], but the function of this internal form of LIF has not been investigated in other cell systems or tissues. Expression of these three isoforms is regulated in an independent way during embryogenesis and adult life, whereas other isoforms have also been identified in liver [38, 39], but their functions await further clarification.

LIF is secreted by many cell types such as fibroblasts, activated T-cells, spleen or macrophage cells, chondrocytes, bone marrow stromal cells, mesenchymal stem cells, endothelial cells, astrocytes and also tumor cells [17, 40, 41]. In vivo, LIF production is associated with inflammation and autoimmune diseases [42-44]. It also promotes N-cadherin-mediated cell adhesion via the STAT3/ Wnt5 pathway in cardiomyocytes [45]. LIF is also naturally produced by the uterine decidua under the control of the estradiol peak, before implantation [14, 46-48] and thereafter is sustained by progesterone, presumably to induce a tolerant environment for the foetus during gestation [49]. Indeed, in endometrium, LIF directly induces the production of the class I non-classical HLA-G protein, whose function is essential for immune tolerance at the maternal-fetal interface during the establishment of gestation [50]. A high level of active LIF is also associated with transplantation tolerance, with direct regulation of Treg cell function in which the expression of the Foxp3 gene seems to be controlled, at least in part, by LIF [11, 51]. Recent studies, conducted with KO model mice, have also revealed that LIF is a direct transcriptional target of the Fox-related protein Fra2, involved in the regulation of osteoclast functions [52]. A deleterious immunosuppressive function of LIF, which allows tumor cells to escape the immune system, has also been described in a model of ovarian cancer. Indeed, it was shown that LIF was a tumor-secreted factor that induces differentiation of monocytes to highly immunosuppressive TAM cells (tumor associated macrophages), [9].

**Excess of LIF**

The effect of an excess of LIF has been investigated in mice injected with the FDCP1 hematopoetic stem cells engineered to constitutively produce biologically active, murine LIF. These cells were engrafted normally into bone marrow, spleen and lymph nodes. After two to three months, engrafted animals lost weight, became hyperactive, developed osteopetrosis of long bones, calcifications in liver, heart, pancreas and skeletal muscles as well as abnormalities in the adrenal cortex and ovarian corpora lutea [53]. Studies of excess of LIF in the thymus of transgenic mice have led to the conclusion that LIF is important for maintaining a functional thymic epithelium that will support proper T cell maturation, a property also shared by oncostatin M [54, 55]. In embryos, excess of LIF-M blocks gastrulation by inhibiting the inner-cell mass differentiation into primitive ectoderm, a result also observed in *in vitro* with embryoid bodies whose differentiation was blocked in the presence of an excess of LIF [56, 57]. These pioneering experiments, along with those showing that LIF maintains the pluripotency of
mouse embryonic stem (ES) cells in vitro [23, 58-61], have led to numerous studies aimed at elucidating the mechanisms of action of LIF in stemness.

**LIF and pluripotency**

Knowledge of LIF signaling in the mouse ES cell system, along with years of expertise in manipulating mouse embryos, have led to the understanding of the species-dependent requirement of LIF for the maintenance of ES cell pluripotency. At first glance, it was puzzling to find that while mouse ES cells (mES) required LIF for maintenance of pluripotency, human ES cells (hES) did not need LIF, but rather have to be grown under the influence of activin and FGF factors in order to maintain their undifferentiated state [62-65]. A pioneering study, conducted using the mouse ES cell system, has already depicted a LIF-independent paracrine activity (named ESRF), secreted by differentiated cells, which allows the maintenance of mES cell pluripotency [66]. However, this activity has not been characterized further and its potential activity on human ES cells has not been evaluated. Since these first observations, transcriptome studies have led to the characterization of similarities and differences in expression profiles of genes in mES and hES cells, suggesting that these cells are probably not derived from equivalent parts of the early embryo [67, 68]. More recently, key results from two independent laboratories have been obtained which demonstrated that hES cells were probably not LIF-dependent because of their derivation from late rather than early epiblast [69-71]. These studies point to the fact that there is a short time frame for LIF dependency, and for maintenance of pluripotency in early embryos, which can be different between species and which deserves careful exploration (see also paragraph “LIF signaling: oldies and novel-...”). However, the potential requirement of LIF for hES cell derivation and maintenance, which could depend on the maturity states of the embryos used to derive hESs, might explain why some laboratories have used LIF in their culture medium, for growing hES cells [72].

**LIF knock-out models**

If homozygous LIF<sup>−/−</sup> mice are viable, they nevertheless present many defects, including a decrease in the survival of hematopoietic stem cells and of their primordial germ cell pools [73], a profound loss of motor neurons [74] and of glial cells, along with an alteration in oligodendrocyte function [75]. In addition, maturation of olfactory neurons is impaired [76]. Recent studies have also shown that LIF represses hypoxia-induced VEGF expression, resulting in regulated capillary network formation [77]. Of major importance, LIF<sup>−/−</sup> females are fertile, but their blastocysts cannot implant, leading to the definition of LIF as being the “nidation hormone” [73]. This implantation defect is due to the absence of LIF production by the uterus. LIF production is normally induced by estradiol via the TP53 loop. Indeed, there are functional TP53 binding sites in the LIF promoter [13, 78-80]. Of note, p53<sup>−/−</sup> mice display the same implantation defect phenotype as LIF<sup>−/−</sup> mice [13]. Interestingly, LIF<sup>−/−</sup> or p53<sup>−/−</sup> blastocysts develop normally if implanted in a wild-type female or upon direct injection of LIF into the vagina of TP53<sup>−/−</sup> or LIF<sup>−/−</sup> mutated mice. None of the other IL6-related ligands has this specific effect on implantation, as deduced from the knock-out phenotypes reported for these ligands (table 1).

**THE LIF RECEPTOR**

Members of the IL-6 subfamily activate preformed, heteromeric receptors, which do not have any intrinsic catalytic activity and which all share the gp130 subunit [27, 28, 81-83]. The association of the ubiquitous gp130 (also called “subunit α”) with subunits specific for one or a few sub-family members (the so-called “β subunit” (e.g. gp190/ LIFRβ; CNTFRβ; gp80/IL-6Rβ; OSMRβ; IL-11Rβ), and whose expression is more or less restrained, is responsible for both the diversity and the redundancy of the cell responses in this sub-family [84]. The functional LIF receptor is an heteromer comprising LIF low affinity binder gp190 together with the high affinity-converter gp130, both being transmembrane subunits with signalling ability. Preformed, inactive receptors are most probably present at the cell surface in the absence of ligand, as shown by co-precipitation of the heterodimeric complex without ligand, with anti-gp130 or anti-gp190 antibodies. However LIF binding increases interaction between subunits, and is essential for cellular responses [85-87]. The stoichiometry of the functional ligand/receptor complex is not known, but recently crystallographic analysis has demonstrated that this complex is a tetramer consisting of two LIF/gp190 molecules, [88, 89]. The intracellular part of both gp130 and gp190 associates with kinases of the JAK family, recruiting transcription factors of the STAT family, especially STAT3, as well as attenuators of LIF signaling such as the SHP2 tyrosine phosphatase and SOCS3 [90-92].

Mice knocked-out for the gp190 subunit, which also has low affinity binding for CT-1, display reduction of bone volume, severe osteopenia, metabolic liver disorder and reduction in astrocyte and motor neuron numbers in cerebral trunk and spinal chord [93-95]. They die perinatally, in part due to feeding difficulties because of their lack of development of sucking muscles. The placenta is also profoundly affected.

Gp130<sup>−/−</sup> mice have also been generated in several laboratories, but the phenotypes reported are discrepant. Mutant mice produced by Yoshida et al. die between days 12 to 16 of embryogenesis with cardiac, hematopoetic and neuronal disorders [96], whereas those generated by Nichols et al. showed a critical function of gp130 in the completion of embryogenesis, only after diapause induction. This phenotype suggests a specialized function of gp130 as a response to environmental stress [97]. The reasons for these discrepancies have not been further investigated. Conditional gp130 deficient mouse mutants have also been derived in which gp130 was deleted in specific tissues or organs such as liver, lung or heart. These studies revealed a key role for gp130 signals in counterbalancing stress-dependent apoptotic signals [98].
HUMAN PATHOLOGY ASSOCIATED WITH LIF DEFICIENCY

LIF and fertility

In humans, as demonstrated in mice, LIF is expressed by the decidua following the estradiol peak and seems to be involved in blastocyst implantation. Gp190 is expressed in the luminal epithelium during the proliferative and secretory phases of the uterine cycle [99]. In addition, significant differences in the level of the soluble form of the gp190 protein (sgp190) have been found in the serum of pregnant versus non-pregnant women, suggesting a role for this cytokine system throughout pregnancy, and more specifically in the functioning of the placenta [100]. LIF deficiency may be associated with a subset of female infertility due to an implantation defect. However, the direct effect of LIF is still a matter of debate since sterility has been associated with a decrease in LIF expression [101, 102] or to mutations in the LIF gene [103, 104], while other studies have shown that a low LIF level was a good implantation predictor [105]. However, in sterile women with endometriosis, LIF is not detected in the endometrium at the implantation stage [106].

The Stüve-Wiedemann syndrome

The Stüve-Wiedemann syndrome (SWS) or type 2 Schwartz-Jampel syndrome is a severe, autosomal recessive condition characterized by bowing of the long bones, respiratory distress, feeding difficulties, and hyperthermic episodes responsible for early lethality [107]. The molecular defect has been identified as null mutations in the gene encoding for the gp190 subunit of the LIF receptor. Functional studies have indicated that these mutations alter the stability of gp190 transcripts, resulting in the absence of this LIF receptor protein subunit and in the impairment of the JAK/STAT3 signaling pathway in patient cells. Of note, the phenotype of the gp190−/− mice mimicked the complex phenotype of SWS patients, especially regarding the bone and nerve defects and early death [94].

REDUNDANCY AND PLEIOTROPY

LIF induces differentiation of the M1 cell line and apoptosis of normal mammary gland cells, after the lactating period. LIF is a pro-differentiative cytokine for adipocytes, [108], for cardiac muscle cells in a dose-dependent way and in synergy with BMP2 [109], and it induces differentiation of cardiac stem cells into endothelial cells [110]. Conversely, LIF maintains self-renewal of murine ES cells in synergy with serum or BMP4 or Wnt family members (Wnt3A and Wnt5) [111-113]. In addition, LIF regulates the differentiation process of stromal cells from bone marrow. Indeed, LIF secretion by human mesenchymal cells decreases when cells differentiate towards the adipogenic or osteoblast lineages. The LIF level, which is controlled by specific miRNA, is an essential, regulated parameter for proper differentiation of these cells [114]. LIF also favors the proliferation and survival of germ cells, hematopoietic progenitors, megakaryocytes, myoblasts and neural cells [1-3]. Some of these effects have been reported with other IL-6 family members [84]. Indeed, OSM, CNTF, CT1 and IL6 (in the presence of its α receptor subunit soluble gp80, which is not expressed by ES cells) are all endowed with the ability to maintain murine ES cell pluripotency [115, 116]. Additionally, OSM, CT1 and IL-6 induce macrophage differentiation of the M1 cell line [117, 118]. Gene ablation of LIF, CT-1 or CNTF or of their receptors (tables 1, 2), demonstrate their involvement in motor neuron survival [93, 119-121]. Double Lif−/−/Cntf−/− and triple Lif−/−/Cntf−/−/Ct-1−/− knock-outs prove that these three cytokines have distinct functions for motor neuron survival and that LIF has an important role in post-natal maintenance of distal axons and motor neuron junctions [74].

Table 1

Physiological effects of IL-6 cytokine family members revealed by KO mice models

<table>
<thead>
<tr>
<th>Cytokines</th>
<th>Physiological effects</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>LIF</td>
<td>Survival of lif− mouse, but decreased numbers of stem cells in spleen and bone marrow</td>
<td>[73]</td>
</tr>
<tr>
<td></td>
<td>No implantation of blastocysts in mouse lacking LIF gene. Implantation and development to term of the blastocysts when transferred to wild-type pseudopregnant recipients or by injection of LIF</td>
<td>[46, 78]</td>
</tr>
<tr>
<td></td>
<td>Role in survival and differentiation of glial cells and oligodendrocytes of the hippocampus. Alteration of olfactory receptor neuron maturation</td>
<td>[75, 190]</td>
</tr>
<tr>
<td>IL-6</td>
<td>Normal development of IL-6-deficient mice. Impairment of the T-cell-dependent antibody response against vesicular stomatitis virus and the inflammatory acute-phase response after tissue damage or infection</td>
<td>[191, 192]</td>
</tr>
<tr>
<td></td>
<td>Hematopoietic stem cell regulation</td>
<td></td>
</tr>
<tr>
<td>OSM</td>
<td>Role in survival of neurons in the adult trigeminal and dorsal root ganglia and in the development of a subtype of nociceptive neurons</td>
<td>[193]</td>
</tr>
<tr>
<td>CNTF</td>
<td>Implicated in survival of motor neurons in adult mice</td>
<td>[120, 194]</td>
</tr>
<tr>
<td>CT-1</td>
<td>Implicated in survival of motor neurons in spinal cord and brainstem nuclei of mice during a period between embryonic day 14 and the first postnatal week</td>
<td>[121]</td>
</tr>
<tr>
<td>LIF/CNTF</td>
<td>Important implication of LIF and CNTF in survival, and in the function of motor neurons in the postnatal period. Co-operation of the two cytokines in the maintenance of motor neurons after lesion in deficient mice of four weeks</td>
<td>[120]</td>
</tr>
<tr>
<td>LIF/CNTF/CT-1</td>
<td>Different functions of CNTF, LIF and CT-1 for the survival and the function of motor neurons. A more important role of LIF for postnatal maintenance of distal axons and motor endplates</td>
<td>[74]</td>
</tr>
</tbody>
</table>
naling are the activated JAK tyrosine kinases [129, 130].
JAK1 is essential for LIF signaling in mES cells [131,
132] and in trophoblast differentiation [133], and JAK2
seems to play a critical role in LIF-dependent muscle
satellite cell proliferation [134]. The third obligatory
component of LIF signaling is the feedback control regu-
lation [129, 130]. JAK1 is essential for LIF signaling in mES cells [131,
132] and for the involution of the mammary gland
at the end of the lactation period [147, 148]. SOCS3 is
also essential for regulation of the immuno-tolerant func-
tivity [142-144], for trophoblast differentiation [133,
145, 146] and for the involution of the mammary gland
at the end of the lactation period [147, 148]. SOCS3 is
effect in M1 or ES cells, see tables 3 and 4 [142, 150, 151].
However, so far, the mechanism of LIF pleiotropy has not
been elucidated and both transcriptomic and proteomic/
phosphoproteomic approaches should help to resolve this
complex issue. Several cell-type specific targets of LIF
such as hepatocyte growth factor (Hgf) induced in
SEK1 cells or the insulin growth factor binding protein
3 (Igfbp3), amphiregulin and the immune response gene
1 (Irg1), identified in endometrium cells, also indicate
that different end point transcriptional targets might
explain pleiotropy [152, 153]. In mouse ES cells, LIF also induces
the PI3Kinase pathway, which maintains cell pluripotency [154-156] while
activating the ERK/ RSK/ CREB pathways which, along
with the FGF pathway induce pro-differentiative programs [157-159]. Thus, several LIF induced pathways
are concomitantly required to maintain ES cell plasticity,
a peculiar property of stem cells.
Recent transcriptomic analyses performed in mouse ES
cells and early derivatives, treated for short period
(30 min) with LIF, have allowed identification of com-
mon and cell-specific LIF-regulated genes (summary in

Table 2
Physiological effects of IL-6 subunit receptor members revealed by KO mice models

<table>
<thead>
<tr>
<th>Cytokines</th>
<th>Physiological effects</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gp130</td>
<td>Death of embryos homozygous for the gp130 mutation between 12.5 days postcoitum and term. Crucial role in myocardial development and hematopoiisis during embryogenesis</td>
<td>[96]</td>
</tr>
<tr>
<td>LIFRβ/gp190</td>
<td>Disruption of normal placental development in mutant mice leading to poor intrauterine nutrition, but foetuses reach term. Alterations in fetal liver metabolism and formation of bone. Role in the survival of astrocytes and motor neurones in the spinal cord and brain stem</td>
<td>[93, 94]</td>
</tr>
<tr>
<td>IL-11Rβ</td>
<td>Infertility in female mice due to a defective decidualization</td>
<td>[195, 196]</td>
</tr>
<tr>
<td>OSMRβ</td>
<td>Role in regulation of hematopoiisis. Key role in liver regeneration</td>
<td>[197, 198]</td>
</tr>
<tr>
<td>CNTFRβ</td>
<td>Mutant death perinatally. Role of CNTFR in motor neuron survival. Critical effects of CNTFR on the developing nervous system</td>
<td>[119]</td>
</tr>
</tbody>
</table>

Table 3
LIF transcriptional targets in different cell contexts

<table>
<thead>
<tr>
<th>Cell types</th>
<th>Transcriptional targets</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>ES cells</td>
<td>JunB; c-fos; Socs3; Zfp36; Stat3Loc; c-Myc; Cd9; Yip12; Ptcrl1; Dapp1</td>
<td>[116, 142, 161, 186, 199]</td>
</tr>
<tr>
<td>ES-derived, reversible committed state</td>
<td>JunB; c-fos; Socs3; Zfp36; stat3Loc; Egr1; Egr2; Ier2; Ier3; Klf4; Klf5; Ras1; Nfkbia; Dapp1; Yipel2; Dystonin; Pulp1; Etv6; Smo2; Ptcrl1</td>
<td>[161]</td>
</tr>
<tr>
<td>ES-derived, irreversible committed state</td>
<td>JunB; c-fos; Socs3; Zfp36; stat3Loc; Egr1; Egr2; Ier2; Ier3; Klf4</td>
<td></td>
</tr>
<tr>
<td>ES-derived, differentiated cells (10 days without LIF)</td>
<td>JunB; c-fos; Socs3; Zfp36; Egr1; Egr2; Ier3; Nfkbia; Expression of endogenous LIF and of LIFR subunits</td>
<td></td>
</tr>
<tr>
<td>Tumor cells</td>
<td>JunB; c-jun; JunD, Cis; Gp49B1; Socs1; Egr1</td>
<td>[151, 152, 172, 200, 201]</td>
</tr>
<tr>
<td>SEK1 (human melanoma)</td>
<td>Hgf</td>
<td>[202]</td>
</tr>
<tr>
<td>Normal tissues</td>
<td>Igfhp-3; Amphiregulin; Irg-1</td>
<td>[203]</td>
</tr>
<tr>
<td>Endoderm</td>
<td>Zfp36; Socs1; Socs2; Socs3</td>
<td>[153, 204]</td>
</tr>
<tr>
<td>Cardiomyocytes</td>
<td>Egr1; c-fos; Socs3</td>
<td>[205]</td>
</tr>
<tr>
<td>Fibroblasts</td>
<td>Socs2; Socs3</td>
<td>[206, 207]</td>
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</table>
future goal to unravel the functions of these LIF targets in pluripotent ES cells and at various stages of ES-derived cell maturation. STAT3 was also re-expressed in differentiated cells, and characterization of its interaction networks in stem cells versus differentiated cells remains a challenging issue for the further understanding of LIF pleiotropy. Novel Lifind genes, whose induction is restricted to ES-derived committed cells (24h of LIF withdrawal), have also been identified. This is the case of Yippee-like2, Strawberry Notch2 (Drosophila orthologs), Dystonin, Phospholipid scramblase1 and Dapp1 (a regulator of PI3K), whose functions in mouse are still poorly understood and which deserve further attention.

Mechanisms underlying LIF pleiotropy

STAT3/SOC3 are the common, obligatory, LIF-dependent effectors found in almost all LIF-sensitive cell lines. Surprisingly, it has been shown, in at least two mouse cell systems (ES cells and pituitary tumor cells), that cell type-specific effectors of LIF and STAT3 only partly overlap [161, 163, 165, 166]. Also, depending upon the cell context, STAT3 could be associated with various partners such as NANOG, as recently shown in certain cancer cells [167]. In addition, the proteins encoded by the primary response genes, which are activated in many LIF-sensitive cell types, are themselves part of various transcriptional complexes (e.g. API complexes), whose associations with different partners could lead to pleiotropic effects. Interestingly, different members of the Socs and Jun families are induced by LIF in M1 or ES cells (table 4). Egr1 is induced in both cell systems and is critical for the macrophage differentiation of M1 cells, but its effect in ES cells has not yet been investigated [168, 169].

Another parameter, potentially involved in the pleiotropic effects of LIF, is the duration of the LIF signal. Indeed, while STAT3 activation is transient in ES cells (it starts decreasing after 1h of LIF stimulation), it is sustained in M1 cells where it lasts up to 36h [170-172]. Also and not yet tested in various LIF-sensitive cell types, epigenetic regulations could account for pleiotropic LIF effects. For example, in the ES cell system, a high level of phosphoacetylation of histone H3 has been reported as being a feature of ES-derived differentiated cells [173, 174]. In addition, repression of HDAC (histone deacetylase) activities by chemicals such as TSA (trichostatin A), in mouse ES cells grown with LIF, induces the expression of differentiation markers along with morphological changes similar to those observed upon LIF withdrawal [175, 176]. Furthermore, a specific epigenetic hallmark has been characterized, that is absent in differentiated cells: by mapping the histone methylation pattern in 2.5 % of mouse genome, it has been shown that a bivalent, tri-methylation mark at the histone H3 (H3K4 triMe / H3K27 triMe) was enriched in pluripotent cells only at differentiation gene loci, leading to silencing of these genes in undifferentiated cells. Upon cell differentiation, these bivalent modifications disappear and differentiation genes are expressed according to the remaining level of single H3K4 triMe (correlated with gene activation) or H3K27 triMe (correlated with gene repression). These crucial data led to the hypothesis that bivalent domains silence developmental genes in ES cells while keeping them poised for activation [177, 178]. It has also been demonstrated that Eed, the core component of the repressive polycomb complex, is a LIF-dependent STAT3 target in ES cells that is associated with high levels of H3K27 triMe. Eed could be the critical effector in silencing differentiation genes in pluripotent ES cells [179]. We would therefore propose the hypothesis that the absence of Eed or of a related repressive protein in M1 cells leads to a pro-differentiative effect of LIF, despite the presence of activated STAT3. It will be of great interest to determine the status of phosphorylation, acetylation and methylation at the histone H3 protein, a known chromatin regulator [182, 183], opens also new avenues for understanding the mechanisms underlying LIF pleiotropy.

Table 4

<table>
<thead>
<tr>
<th>Potential actors of the pleiotropic effects of LIF</th>
<th>LIF Proliferation</th>
<th>LIF Pluripotency</th>
<th>LIF Differentiation in macrophages</th>
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<tbody>
<tr>
<td></td>
<td>ES</td>
<td>M1</td>
<td></td>
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<tr>
<td>LIF −LIF</td>
<td>+LIF</td>
<td>−LIF</td>
<td>+LIF</td>
</tr>
<tr>
<td>c-Jun</td>
<td>−</td>
<td>++</td>
<td>−</td>
</tr>
<tr>
<td>JunB</td>
<td>++</td>
<td>++</td>
<td>−</td>
</tr>
<tr>
<td>JunD</td>
<td>++</td>
<td>++</td>
<td>−</td>
</tr>
<tr>
<td>Socs1</td>
<td>+</td>
<td>+</td>
<td>−</td>
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<tr>
<td>Socs2</td>
<td>−</td>
<td>−</td>
<td>−</td>
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<tr>
<td>Socs3</td>
<td>+</td>
<td>+</td>
<td>−</td>
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<tr>
<td>Egr1</td>
<td>−</td>
<td>+</td>
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<tr>
<td>Egr2</td>
<td>−</td>
<td>−</td>
<td>−</td>
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<tr>
<td>STAT3 +LIF</td>
<td>−</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Post-T.</td>
<td>T.</td>
<td>T.</td>
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</tbody>
</table>

The expression profiles of Jun, Socs and Egr family members (detected by northern blots or RT-PCR) and of STAT3 and Phospho-STAT3 (detected by western blots or RT-PCR) and of STAT3 and Phospho-STAT3 (detected by western blots) are: −: not expressed or not phosphorylated; +: expressed; ++: highly expressed or highly phosphorylated.

CONCLUSION

LIF orthologs have been characterized in many species, even in kangaroo in which gestation does not proceed through classical internal implantation [184]. Also intrigu-
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Acknowledgments. This work was funded by the European consortium FungenES (6th Framework, project n° LSHG-CT-2003-503494). CNRS. University of Bordeaux 2, the Ligue Nationale contre le Cancer, Comité de Gironde and Comité du Languedoc-Roussillon, the Région Aquitaine, the IFR 66 and the CHU, Bordeaux.

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