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Leukemia inhibitory factor (LIF) and its biological activity

Leukemia Inhibitory Factor (LIF) is a pleiotropic cytokine that was initially described by its ability to induce differentiation and to inhibit the proliferation of the murine myeloid leukemic cells (1,12). It belongs to the family of functionally and structurally related cytokines, that include interleukin-6 (IL-6), interleukin-11 (IL-11), oncostatin M (OSM), cardiotrophin-1 (CT-1) and ciliary neurotrophic factor (CNTF) (2, 10). Being a member of the haemopoietic family of growth factors, it exerts a diverse range of biological activities on various target cells (7,12,14). These activities include the capacity of the growth promotion and activation of haemopoietic, hepatic, osteogenic, and neuronal cells; stimulation of the acute phase protein synthesis in hepatocytes, inhibition of lipoprotein lipase activity, and regulation of the bone metabolism (1, 2, 7, 10-14).

In human beings, LIF can be produced by a variety of mesenchymal and immune system cells, including fibroblasts, monocytes/macrophages, activated T-lymphocytes and endothelial cells, astrocytes, thymic cells, keratinocytes, human marrow stromal cells, chondrocytes, synoviocytes, osteoblasts (1, 4, 5, 7, 12, 13, 15). LIF is synthesized spontaneously or can be induced in response to other cytokines, especially TNF-α, IL-1, endotoxin and other proinflammatory stimuli (1, 4, 5, 7, 12, 13, 15). LIF exerts its biological activities through a specific cell surface receptor, which consists of two components, LIFR alfa-chain (LIFRα) that binds LIF with low affinity and gp130 that can convert receptor into a high affinity receptor complex and lead to intracellular signalling (14). LIF shares the signal transducing subunit gp130 with IL-6, IL-11, OSM, CT-1, CNTF, which may explain some common properties of this family of cytokines (1, 2, 10, 12, 14). LIFRα is a transmembrane signalling subunit with a long cytoplasmic tail (14). After LIF binding to the cell-surface receptor, LIFRα dimerizes with gp130 which results in tyrosine phosphorylation and activation of the tyrosine kinase signal transduction pathway (8, 14). LIF receptors have been found on macrophages, monocytes and their precursors and are expressed on the structural cells of lung (12,15). It has been also demonstrated that both normal human keratinocytes in culture and normal human epidermis are capable of expressing mRNA for the LIF receptor (7).

One of the most vital biological roles of LIF is its profound effect on the proliferation and maturation of haemopoietic progenitor cells (3, 5, 12, 13, 15). LIF has been shown to stimulate the bone marrow production of blasts and megacaryocytes, to induce macrophage differentiation, to augment the differentiation of the myeloid leukemic cells into macrophage lineage, to suppress the differentiation of the normal pluripotent embryonic stem cells, (10, 15). LIF has been detected in supernatants from unstimulated marrow stromal cells (5). It has appreared that the LIF production by the marrow cells is enhanced by IL-1, IL-6, IL-8 and TNF-α (5). Taking into consideration that LIF upregulates these cytokines' synthesis, the increased release of LIF by marrow stromal cells may be of interest thus

suggesting potential regulatory loop mechanism of production between all these inflammatory cytokines in the marrow (5). LIF is also capable to regulate the maturation of T-lymphocytes and balance between the B and T-lymphocytes compartments, possibly through IL-4 (3, 8).

Biological effects of cytokines are, at least in part, connected with their contribution in the regulation of the other cytokine production. LIF is integrated in the cytokine network through its induction of other potent proinflammatory cytokines, such as IL-1 and TNF-α (12). What is more, this protein shares with IL-1 and TNF-α the ability to stimulate cytokine production, including IL-1, IL-6, IL-8, monocyte chemoattractant protein-1 (MCP-1) in different cell types (1, 4, 12). Among the cells, that can respond to the LIF stimulation are human chondrocytes, synoviocytes, blood monocytes, epithelial and neuronal cells (8, 12). Through the induction of these proinflammatory and mitogenic cytokines in diverse cell types LIF is capable (similarly as IL-1 and TNF-α) to initiate and propagate inflammatory and immunological responses (4, 5, 12). It is worth to stress a special relation existing between LIF and IL-1 since both cytokines can induce each others' expression (12).

A large number of studies have demonstrated an essential role of LIF in inflammation (15). One of the most important contributions of LIF to the propagation of inflammation is its ability to trigger the acute phase proteins synthesis (1, 5, 13). LIF belongs to the set of cytokines being the acute-phase inducers (TNF-α, IL-1 and IL-6) that are released during sepsis in response to endotoxin and that are involved in the pathogenesis of fever and septic shock (1, 2, 10, 12, 13). LIF has the ability to affect also the other important inflammatory events, since it induces monocytes to produce chemotactic factors for neutrophils and monocytes and upregulates the recruitment of these cells to the site of tissue damage and inflammation (12, 15). It takes part in the recruitment of inflammatory cells by stimulating synthesis of two potent chemoattractants: IL-8 and MCP-1 (3). So, many findings support the belief that in the immune system LIF can not only directly and indirectly initiate the inflammatory events but is also engaged in their further propagation (3, 5, 12).

It has been found recently that LIF may also be implicated as a bridge between the neuroendocrine and immune systems participating in neuroendocrine stress responses (7, 10, 11, 15). This cytokine is capable not only to promote the generation of sensory and cholinergic neurons, but also to promote the neuronal differentiation, including the conversion of sympathetic neurons from the adrenergic to cholinergic phenotype, as well (7, 10, 14). LIF is believed to be a part of the regulatory mechanism developed to control the inflammatory process and limit the tissue damage responsible for the clinical symptoms of inflammation (11). Exposure of neural tissue to proinflammatory cytokines, such as IL-1β or injury increases the synthesis and release of LIF, which in turn increases substance P mRNA and protein together with its receptor (7, 12, 15). Similarly LIF can also induce neuropeptide synthesis and release in neurons that do not normally produce neuropeptides (15). So, LIF affects the neural system by increase in neuronal substance P expression, and possibly can also indirectly influence the biological events mediated by substance P (12). Therefore, it is suggested that LIF release may bind together and integrate the activities of the neural-immune network (7). Moreover, in explants of the respiratory system it has been found that LIF released by the smooth muscle cells and fibroblasts in response to inflammatory stimuli can enhance the contractile reactions to tachykinins (substance P, neurokinin-A) (15).

The profound influence of LIF on the bone metabolism has been also recognized (1, 3, 5, 10, 12, 15). This pleiotropic cytokine was shown to stimulate bone remodelling by regulating both the calcification and bone destruction (1, 5). LIF expressed by synovial tissue cells can promote connective tissue metabolism (12), but on the other hand it can induce expression of collagenase and stromelysin by human articular chondrocytes, but it does not stimulate the expression of tissue inhibitor of metalloproteinases (12). So, it has appeared that LIF can directly stimulate cartilage destruction through the induction of proteinases by chondrocytes and indirectly through the upregulating of IL-1 (3, 12).

LIF takes part in the tissue metabolism by the inhibition of lipoprotein lipase activity, which can lead to cachexia (1, 5, 12).

LIF is known to play an important role in the blastocyst implantation and maintenance of pregnancy (3, 8). This glycoprotein is constitutively expressed throughout normal pregnancy in the placenta, but not in the peripheral blood of pregnant women (8). Taking into consideration that LIF has proved to be a potent inhibitor of HIV-1 (and possibly of other viral agents), the placenta may have an innate ability to inhibit HIV-1 infection (8). The HIV-1 inhibitory activity of LIF has been evidenced in three distinct tissue compartments: placenta, peripheral blood mononuclear cells and thymus (8). On the other hand, some authors believe that LIF can also stimulate HIV replication in mononuclear phagocytes (12).

Proinflammatory properties of LIF, its relation with other potent inflamatory cytokines suggest the possible contribution of LIF in the development of various inflammatory diseases. Especially, its ability to induce IL-1, IL-6, MCP-1 and IL-8 mRNA expression by chondrocytes and IL-6 secretion by monocytes, and participation in both the bone formation and resorption directed special interest to the inflammatory process in arthritis (1). What is more, high levels of LIF have previously been detected in synovial fluid from patients with various types of inflammatory arthritides (1). It is believed that LIF can be implicated as a potent mediator in the local and systemic inflammatory process leading to joint destruction in arthritis (1). Moreover, synoviocytes and osteoblasts have been identified as the intraarticular sources of LIF (1). LIF levels have already been studied in various body fluids (2). Majority of the healthy individuals have no detectable amounts of LIF and other cytokines of the IL-6 family in their sera (9). Elevated concentrations of LIF have been detected in serum/plasma, cerebrospinal, peritoneal, and pleural fluids from patients with various infectious diseases (2, 13). High LIF levels have also been found in synovial fluid from patients with rheumatoid arthritis and other inflammatory arthritides (1, 2). Substantial concentrations of circulating LIF have been evidenced in some inflammatory diseases, such as the giant-cell arteritis, rheumatoid arthritis, systemic *lupus erythematosus*, acute allograft rejection and septic shock (3, 4, 9, 10, 13). What is more, elevated plasma concentrations of LIF correlated with the disease severity in patients with septic shock due to *Neisseria meningitidis* infection (3, 13). High levels of LIF are reported in pleural effusions and bronchoalveolar lavage fluid of patients with various inflammatory pulmonal diseases, including the acute respiratory distress syndrome (ARDS) (3, 4, 5, 15). These results indicate that LIF can be a member of the inflammatory cytokine cascade in ARDS. Possibility of LIF being involved in the pathogenic process in bronchial asthma is of special interest, taking into consideration that human eosinophils express LIFR mRNA and store and release abundant amounts of LIF protein (15). In accordance with this belief, the elevated serum LIF levels in atopic asthmatic patients has been found (15).

LIF expression in both human keratinocytes in culture and normal human skin has been found (6, 7). LIF immunoreactivity in normal skin, has been observed using the immunohistochemical method (6). The immunostaining of LIF is always found intracellularly being present solely in the epidermis (6, 7). Also hair follicle root sheaths exibit positive immunostaining for LIF (7). It is of interest, that in normal human keratinocytes LIF expression is regulated not only by proinflammatory stimuli but also by differentiation (7). The immunohistochemical data show weaker staining in the lower layers of epidermis, especially in the basal layer, compared with the suprabasal epidermis and cornified layers (6). Similarly *in vitro*, in differentiating keratinocytes the expression of mRNA and protein for LIF is dramatically upregulated (7). Contrary to the data of other authors, P a g l i a et al. (7) have not found, however, any evidence of influence of LIF or anti-LIF antibodies on the keratinocyte proliferation (7). The biological function of LIF in keratinocytes is still unknown (7). The expression of mRNA for LIF receptor in normal human keratinocytes suggests that LIF may play an autocrine or paracrine role in the epidermal biology (6, 7). Some authors believe that the biological role of constitutive LIF expression in the normal skin may be similar to the role of IL-1 (6). It is possible that LIF may be

the element of an early alarm system, being activated in traumatized tissue (6). Damage to the skin would release preformed LIF that could then stimulate potent proinflammatory cytokines (IL-1 and IL-8) and their release from keratinocytes (6). It has been demonstrated that stimulation of keratinocytes with LIF protein induces a fourfold increase in IL-8 (6). LIF protein in skin is believed to be a link between the immunologic and neural systems, like in other organs (6). Although physiologic function of LIF in skin is unclear, dysregulation of LIF expression may be important in a variety of inflammatory skin diseases (7). Literature data indicate that LIF is capable to induce a hyperproliferative state in keratinocytes (6). High levels of LIF mRNA expression have been reported in some hyperproliferative diseases, such as psoriasis, basal and squamous carcinomas (6). It is believed that LIF has a proinflammatory growth promotion function in the skin (6).

Diverge range of biological activities enables LIF to contribute to various physiological and pathological phenomena and suggests that the exact biological role of this potent cytokine has not been fully recognized yet.

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SUMMARY

Leukemia inhibitory factor (LIF) is a pleiotropic glycoprotein belonging to the IL-6 family of cytokines. It shows a wide range of biologic activities that include the growth promotion and cell differentiation of different types of target cells, influence on bone metabolism, cachexia, neural development, embryogenesis and inflammation. LIF has potent proinflammatory property, being the inducer of the acute phase protein synthesis and affecting the cell recruitment into the area of damage or inflammation. LIF is also one of the cytokines that are capable to regulate the differentiation of embryonic stem cells, hematopoietic and neuronal cells. Due to its polyfunctional activities, LIF is involved in the pathogenic events and development of many diseases of various origin.

Czynnik hamujący białaczkę (leukemia inhibitory factor – LIF) i jego rola biologiczna

Czynnik hamujący białaczkę (leukemia inhibitory factor – LIF) jest wielofunkcyjną glikoproteiną należącą do rodziny cytokin związanych z interleukiną-6. Wykazuje możliwości oddziaływania na ważne procesy biologiczne, do których należy pobudzanie wzrostu i różnicowania różnych typów komórek docelowych, wpływ na metabolizm kości, powstawanie wyniszczenia, rozwój tkanki nerwowej, embriogenezę i stan zapalny. LIF posiada silne właściwości prozapalne, ma zdolność pobudzania syntezy białek ostrej fazy, a także rekrutacji komórek do miejsca uszkodzenia lub stanu zapalnego. LIF należy do cytokin, które wywierają regulacyjny wpływ na różnicowanie embrionalnych komórek pnia, komórek hematopoetycznych i nerwowych. Posiadając szerokie spektrum oddziaływania biologicznego, LIF uczestniczy w wielu zjawiskach patologicznych i rozwoju chorób różnego pochodzenia.