The multifunctional role of leukaemia inhibitory factor in cutaneous biology

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SUMMARY

Leukaemia Inhibitory Factor (LIF) is a polyfunctional cytokine, that belongs to the family of haemopoietic growth factors. LIF plays a role in growth-promotion and differentiation, regulates calcium and bone metabolism, induces acute phase proteins and causes cachexia in organisms with neoplastic disorders. LIF is also to be found in normal skin, where it may be involved in the differentiation process of keratinocytes. In addition, recent data in medical literature indicates that LIF is engaged in the pathogenesis of some skin disorders as well. It has been clearly demonstrated that LIF may act as a proinflammatory cytokine. In allergic contact dermatitis, the expression of LIF mRNA is augmented to a significant degree, indicating that LIF may play a role in the early phase of allergic contact dermatitis. LIF also plays an important role in psoriatic lesions. As the mechanism is not yet fully understood, however, it is hypothesized that the LIF function in psoriatic processes is solely connected with IL-8, as it is known that LIF is able to induce the release of IL-8. Also, some reports have suggested that LIF may play a role in the carcinogenesis of the skin.

KEY WORDS

leukaemia inhibitory factor, skin cancer, contact dermatitis, psoriasis

Leukaemia inhibitory factor as a polyfunctional cytokine

Leukemia Inhibitory Factor (LIF) is a cytokine that belongs, together with interleukin 6 (IL-6), IL-11, GM-CSF (granulocyte/macrophage colony stimulating factor), G-CSF and M-CSF, to the family of haemopoietic growth factors (1). It was first isolated from fibroblasts in 1984 and is characterized by its ability to induce differentiation of M1 murine leukaemia cells. LIF is a variably glucosylated single chain polypeptide of Mr 38000 - 67000 Daltons (2). It is produced by various cells, including fibroblasts, endothelial cells, monocytes, macrophages, mast cells, T lymphocytes, astrocytes, synoviocytes, chondrocytes and osteoblasts (1).

LIF possesses a diverse range of activities. Its growth-promoting and differentiation activities depend on the type of the target cells. LIF stimulates proliferation of clonogenic haemopoietic cells, suppresses differentiation of embryonic stem cells, modulates the signalling of autonomic nerves, changing it from an adrenergic to a cholinergic signal, it induces acute phase protein synthesis by hepatocytes, inhibits lipoprotein li-

pase activity in adipocytes, causes cachexia in mice with implanted tumours and regulates calcium metabolism by influencing osteoclasts and osteoblasts (1,3,4). LIF has also been shown to be involved in the inflammatory process. It is able to stimulate release of proinflammatory cytokines, such as IL-1, IL-6 and IL-8 from different cells, including monocytes, synoviocytes, chondrocytes, neurons and endothelial cells, and in addition IL-1, IL-6 and tumor necrosis factor alpha (TNF-alpha) enhance the production of LIF itself (1,3,5). Moreover, LIF mediates septic shock in mice and biologically active LIF has been found in the sera of patients with both septic shock syndrome and giant cell arteritis (6), and in the synovial fluids of patients with rheumatoid arthritis (7,8). LIF has also been detected in the pleural fluid of patients suffering from pneumonia, in the peritoneal fluid of patients with peritonitis and in the cerebrospinal fluid of patients with meningitis (6).

As LIF is active in so many different areas, it is not surprising that it has been described more or less simultaneously in different branches of medicine and differently named depending on the discovered function. Today, the term Leukaemia Inhibitory Factor (LIF) is widely accepted. One commentator, Hilton, even suggests that the acronym LIF, because of its broad range of applications, could stand for "lots of interesting functions" (9).

Expression of leukaemia inhibitory factor in keratinocytes and normal skin

It has been demonstrated by reverse-transcription polymerase chain reaction (RT-PCR) that normal keratinocytes in culture contained mRNA for both LIF and LIF receptors (10). The expression of mRNA for the LIF receptor in normal human keratinocytes suggests the hypothesis that LIF plays an autocrine, juxtacrine and paracrine role in keratinocyte biology. The expression of LIF mRNA has also been demonstrated continuously in normal human skin (11). Our group, using immunohistochemical methods, demonstrated LIF immunoreactivity in normal human skin. The LIF immunostaining was cytoplasmic and was found in the epidermis and hair follicles. The lower layers of the epidermis, especially the basal layer, showed weaker staining than the upper (suprabasal) parts of the epidermis. There was no LIF-positive cell in the dermis (10-12). No difference in the LIF immunoreactivity pattern was seen in biopsies obtained from different areas of the skin, such as the scalp, breast, forearm, back and foreskin. The pattern remained consistent across tests with three different anti-LIF antibodies (12). LIF immunoreactivity in the epidermis and hair follicles has also been observed by French investigators (13). This data introduced LIF to dermatological research and pointed out for the first time that LIF may play a role in cutaneous biology.

Role of leukaemia inhibitory factor in keratinocyte differentiation

The incubation of keratinocytes in media containing a high concentration of calcium causes terminal differentiation of keratinocytes within 3 to 4 days (11). A dramatic increase in expression of LIF mRNA was observed during the incubation of keratinocytes with a high calcium (1.5 mmol/l) medium. In the latter case, increase of LIF mRNA expression was time dependent. After 24 hours, a 2.5-fold increase in LIF mRNA expression was observed, followed by 3.8-fold increase at 48 hours and an 8.5 fold (maximal) increase at 72 hours, as compared to basal levels. LIF protein in a low calcium medium of incubated keratinocytes showed barely detectable levels, although its expression increased 155-fold after 72 hours in keratinocyte culture with high concentrations of calcium (11). This may suggest that differentiated keratinocytes express high levels of LIF and that LIF could be involved in the differentiating process of keratinocytes.

Role of leukaemia inhibitory factor in cutaneous inflammation

The role of LIF in cutaneous inflammation was suggested on the basis that LIF induced release of proinflammatory cytokines from keratinocytes. Twenty-four hours after incubation of keratinocytes in culture with 10 ng/ml recombinant LIF, a 2.4 fold increase in IL-1 alpha and 2.2-fold increase in IL-8 protein was observed. No stimulatory effect of LIF on a synthesis of IL-6 and TNF-alpha was observed. Moreover, incubation of keratinocytes with 10 ng/ml of IL-1 alpha and IL-8, but not with IL-6 and TNF-alpha, significantly induced secretion of LIF protein (11).

The proinflammatory activity of LIF in the skin was also studied in animal models (14). The injection of 100 ng LIF into a mouse ear caused significant increase in ear swelling 12 hours and 24 hours after injection. This phenomenon was associated with an accumulation of infiltrating leukocytes. At 12 hours a 12-fold increase was observed in the number of leukocytes, that had increased 17-fold at 24 hours. The histology of the ear injected with LIF revealed identical features to a parallel example that was injected with IL-1, a well-known proinflammatory cytokine (14). The suggestion of the

proinflammatory role of LIF in the skin was also confirmed *in vivo*, as LIF mRNA was found to be enhanced in some of the inflammatory skin diseases (see below).

Role of leukaemia inhibitory factor in selected cutaneous diseases

Allergic contact dermatitis

Allergic contact dermatitis is a typical example of acute inflammatory skin disorder. Recently it has been shown that, in previously proven contact dermatitis patients, the expression of LIF mRNA was significantly augmented in the skin 24 hours after nickel challenge, when compared to both vehicle-treated skin and non-treated skin (15). A LIF protein study after nickel application revealed features in the majority of patients that were almost identical with vehicle-tested and non-tested skin. However, in some of biopsies observed after nickel challenge, LIF immunoreactivity was more dispersed and equally distributed throughout the whole epidermis. All these observations indicate that LIF may play a role in the early phase of allergic contact dermatitis, although such a role can only be hypothesized for now (15).

LIF may act directly, but if so, the mechanism is unknown. It cannot be discounted that LIF may act via IL-1, which is known to be an important cytokine in allergic contact dermatitis. Previous studies of our group have shown clearly that LIF is able to induce release of IL-1 from keratinocytes (11). If LIF acts via IL-1, it may influence all the IL-1 dependent processes that are critical in the pathogenesis of allergic contact dermatitis. It may directly enhance Langerhans cell function, induce expression of ICAM-1, VCAM-1, E-selectin on endothelial cells and LFA-3 on lymphocytes, enhance vascular permeability and induce release of other proinflammatory cytokines (16).

Psoriasis

Psoriasis is regarded as a chronic inflammatory skin disease with disturbed keratinocyte proliferation and differentiation. The pathogenesis of the disease is still not fully understood, but it is clear that cytokine cascades play one of key roles in the development of psoriatic lesions (17). LIF mRNA expression was significantly increased (160%) in lesional psoriatic skin compared both to non-lesional skin of the same individuals and normal control skin (18). These observations of ours tally with protein studies performed by other investigators (19). Using the ELISA method they found enhanced expression of LIF mRNA in psoriatic plaques, that positively correlated with increased IL-8 mRNA expression. Negative significant correlation was also demonstrated between LIF mRNA expression and the duration of the last outbreak of psoriasis (19). Immunohistochemistry revealed the slight disappearance of LIF immunoreactivity in lesional psoriatic skin. LIF immunostaining was generally weaker in basal and suprabasal layers of the epidermis, although in the majority of lesional biopsies it was more concentrated in the stratum corneum (19). As several cytokines are expressed in psoriasis at local and systemic levels, we also investigated LIF levels in the sera of psoriatic patients. Using a commercially available ELISA kit, levels of LIF in the sera of all psoriatic patients and healthy volunteers were below the lowest detectable value (18).

Our hypothesis is that LIF function in psoriatic processes is strictly connected with IL-8, as it is known that LIF is able to induce release of IL-8 (1,11), and significant positive correlation between LIF and IL-8 mRNA in lesional psoriatic skin suggests a possible link between these two cytokines in the pathogenesis of psoriasis. IL-8 is involved in the pathogenetic process of psoriasis through stimulation of keratinocyte proliferation, induction of angiogenesis and chemotactic activity in leukocytes (17,20). LIF probably acts at the top of the cytokine cascade, suggesting that even a small increase in LIF expression may stimulate an increased release of IL-8. The negative correlation between LIF mRNA and the duration of the last exacerbation of psoriasis also confirms the role of LIF in the early stages of the inflammatory process (18). We believe that LIF in psoriasis is produced by keratinocytes, as all the infiltrating cells in the dermis do not show LIF immunoreactivity, but the fact that we were unable to demonstrate any relationship between LIF expression in lesional psoriatic skin with its accumulation in the stratum corneum may suggest that LIF is produced and quickly released from the skin in scales (18).

Cutaneous neoplasms

In 2001 our group demonstrated for the first time increased LIF mRNA expression in skin carcinomas (21). LIF mRNA expression was significantly elevated in squamous cell carcinomas (SCCs) compared to normal control skin. The expression of LIF mRNA in basal cell carcinomas (BCCs) was also augmented, but this increase did not reach levels of statistical significance (21). This could be explained by the fact that very frequently biopsies of BCCs are contaminated with unchanged skin, which may lower the expression of measured cytokines. In contrast to the psoriasis study there was no relationship between LIF and IL-8 in skin carcinomas, suggesting that no link exists between these two cytokines in the development of cutaneous neoplasms. To our surprise, immunohistochemical data showed the disappearance of LIF immunoreactivity in neoplastic cells of

both SCCs and BCCs (21). Probably LIF protein is produced and rapidly excreted from tumours, so the protein is no longer detectable. Enhanced expression of LIF mRNA and LIF protein was also found in human melanoma cell lines derived from primary and metastatic tumours (4). There was a trend toward higher levels of LIF mRNA expression in the advanced-stage cell lines, but this was not confirmed by protein study.

What could the role of LIF in cutaneous carcinogenesis be? It is possible that LIF acts via IL-1, as is hypothesized above in the case of allergic contact dermatitis (15). IL-1 may induce release of IL-8, an angiogenic factor, or may stimulate proliferation of fibroblasts and production of collagenase and hialuronic acid. These enzymes can degrade the extracellular matrix that contributes to the deeper invasion of tumours (22). It cannot be ruled out that LIF may be involved in the development and progression of cutaneous neoplasms by direct influence, as has been demonstrated in other types of neoplasms. It is known that LIF promotes proliferation of breast, kidney and prostate carcinoma cells (23,24). It is also of special interest that ultraviolet B - a well-known carcinogen – also enhances carcinogenesis by inducing release of LIF (25).

Conclusions

LIF is active in a number of diverse ways. Several studies have shown that LIF is involved in various processes of cutaneous biology. From the dermatological point of view, the proinflammatory role and regulation of proliferation and differentiation seem to be the primary functions. LIF has been detected in normal keratinocytes and normal human skin. The proinflammatory function of LIF in the skin has been well documented. Enhanced LIF expression is to be found in some inflammatory skin disorders as well as in cutaneous neoplasms. This clearly suggests that LIF is involved in the pathogenesis of various skin disorders although its precise pathogenic role can only be hypothesized for now. Further studies are necessary to clarify the exact role of LIF in the pathogenesis of dermatoses.

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