Leukaemia Inhibitory Factor (LIF) Expression in Skin from Amyotrophic Lateral Sclerosis Patients Compared with Skin from Normal Individuals: What is the Function of LIF in the Skin?

To the Editor

We read with interest the recent paper by Hu et al (2000) describing leukaemia inhibitory factor (LIF) expression in the skin of patients with amyotrophic lateral sclerosis (ALS). To our surprise, however, the authors recorded no or negligible LIF immunoreactivity in their “disease control” skin (Fig 1, Hu et al, 2000) We also note that they used the skin of patients suffering from other neurodegenerative disorders as controls for the ALS patient skin. We do not think these are appropriate controls, principally because they give the impression that normal undiseased skin expresses little or no LIF.

Our group has conducted several studies recently on LIF expression in normal skin and in a variety of inflammatory disorders, using skin from normal individual donors as controls. We always observed LIF immunoreactivity in normal skin, using the standard avidin-biotin complex immunohistochemical method. We have sampled skin from normal individuals both from surgical patients receiving elective surgery and from punch biopsy of normal volunteers. In the latter case, samples were dropped immediately into formalin. In both cases, the results were the same. A typical result is shown in Fig 1A, using Fast Red B as chromogen. Essentially the pattern of LIF staining in normal skin is the same as reported by Hu et al for ALS skin. The immunostaining for LIF is always cytoplasmic and was present in the epidermis only. The lower layers of the epidermis, the stratum corneum, showed weaker staining compared with the suprabasal epidermis and stratum corneum layers. Similarly to Hu et al we found no LIF immunostaining in the dermis (Fig 1A).

These results on normal skin are consistent and reproducible, not only in our hands, but French investigators using both polyclonal and monoclonal antibodies were also able to demonstrate a similar pattern and intensity of LIF immunoreactivity in normal skin to that which we had originally recorded.1

Furthermore we have used three different antibodies: rabbit polyclonal IgG (R303 antibody from the Genetics Institute, Cambridge, MA); rabbit polyclonal IgG (R&D Systems, Minneapolis, MN), and rabbit polyclonal IgG (UBI, Lake Placid, NY) with the same results (Paglia et al, 1996; Szepietowski et al, 1997). Our reverse transcriptase–polymerase chain reaction analysis of normal skin shows constitutive expression of LIF mRNA, both in vivo and in cultured human keratinocytes (Paglia et al, 1996; Szepietowski et al, 1997). The constitutive expression of LIF in normal skin is unusual, since studies are in progress. Conventional chromosome analyses of one tumor revealed, in addition to the structurally abnormal Y-chromosome, a clonal 1; 6 translocation t(1; 6)(q25;q25). Coupling analyses in the family have not been performed thus far because of lack of cooperation.

In summary, the presented pedigree is unique as it shows an exclusive male-to-male transmission of hereditary glomus tumors. Our findings also suggest a causal involvement of a structurally abnormal Y-chromosome.
Despite our reservations about the choice of control tissue by Hu et al, we find their results most interesting, particular the fact that they find that LIF induces a hyperproliferative state in keratinocytes. We and others have recorded high levels of LIF mRNA expression in other hyperproliferative diseases such as psoriasis (Bonifati et al., 1998) and basal and squamous carcinomas (Szepietowski et al., 2001). We found higher levels of LIF mRNA expression in lesional (but not nonlesional) volar skin from psoriatic patients than in normal skin. We agree that the results of Hu et al show hyperproliferative effects of LIF in ALS epidermis and higher expression of LIF compared with skin from patients suffering other neurologic disorders. The hyperproliferative effects of LIF on keratinocytes demonstrated by Hu et al may also have relevance to the growth of keratinocyte stem cells in the outermost layer of the hair follicle (Akiyama et al., 2000). We noted high levels of LIF expression in a single cell layer of the outermost part of the hair follicle (Paglia et al., 1996). Moreover, LIF has been characterized as a stem cell growth factor in other cell systems (reviewed in Gearng, 1993).

Regarding points made by Hu et al about LIF and inflammation, we feel that it should be pointed out that in the majority of studies, LIF was found to have pro-inflammatory activity. For example, LIF injection into C3H/HeN mouse ear stimulates neutrophil influx, probably due to secondary induction of pro-inflammatory cytokines (McKenzie et al., 1996). LIF treatment of cell cultures induces IL-1, IL-6, and IL-8; cachexia is dramatically increased in mice bearing LIF-expressing tumor; and septic shock and cancer patients have high systemic circulating levels of LIF (reviewed with other studies demonstrating pro-inflammatory effects of LIF in Alexander et al., 1994). These effects are consistent with the reported role of LIF as a stimulator of the acute phase factor (reviewed in Baumann and Gaullide, 1994). Banner et al. (1998) is the only study (in a mouse knock-out, not in normal skin) that purports to show anti-inflammatory effects for LIF in the skin. We feel the majority of data are consistent with a pro-inflammatory/growth promotion function for LIF in the skin.

REFERENCES


Figure 1. Immunostaining for LIF in normal skin. (A) LIF antibody and ABC-HRP conjugate incubated with Fast Red B as substrate and counterstained with hematoxylin; (B) negative control. Scale bar: 100 µM. Normally cytokines are only made in traumatized tissue or on cell stimulation. A notable exception is interleukin (IL)-1, which is expressed in suprabasal layers of normal human epidermis (Didierjean et al., 1989). We propose that LIF expression in normal skin may serve as an early alarm system in the skin that IL-1 appears to do. Damage to the skin would release preformed LIF that could then stimulate IL-1 and IL-8 release from keratinocytes (Paglia et al., 1996), bringing in host leukocytes to deal with infection.

We suggest an explanation as to why Hu et al did not find LIF immunostaining in their disease controls. The formalin fixation may have masked the LIF antigens to a greater degree in the thinner disease control specimens. Did the authors try microwave or protease antigen retrieval on the disease controls?

We believe that the mild proliferation (maximum, 40% over controls) observed by Hu et al could be consistent with paracrine stimulation due to IL-8 release from adjacent keratinocytes; IL-8 has been demonstrated to induce proliferation of keratinocytes (Renekampf et al., 2000) and LIF receptor expression has been demonstrated in normal human keratinocytes (Paglia et al., 1996), suggesting an autocrine stimulatory cytokine network. We have shown that stimulation of keratinocytes with 10 ng per ml of LIF protein induces a 4-fold increase in IL-8 (Paglia et al., 1996). None the less, their results do suggest abnormal LIF expression in neurologic disorders, which is consistent with our model (Paglia et al., 1996) that the LIF protein may be an interface between the immunologic and neural systems in the skin. Of interest in this respect is that LIF expression is elevated in ALS epidermis, a disease marked by loss of the glutamate transporter function (Rothstein et al., 1995). Do defects in the metabolism of this excitatory neurotransmitter induce LIF expression?