

# Leukaemia inhibitory factor: induction in the early phase of allergic contact dermatitis

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It has been suggested that leukaemia inhibitory factor (LIF), may be involved in the pathogenesis of cutaneous inflammation. In 5 patients with previously proven contact allergy to nickel, LIF mRNA and protein expression were assessed by reverse transcription – polymerase chain reaction and immunohistochemistry in 5% nickel sulfate patch test biopsies 24 h after application of the patch. Control specimens were obtained from non-tested and vehicle-tested skin from the same individuals. LIF mRNA expression was significantly increased in nickel-tested skin compared with both vehicle-tested ( $p=0.045$ ) and non-tested skin ( $p=0.041$ ). All biopsies showed similar patterns of LIF immunoreactivity, with no significant differences between nickel-tested, vehicle-tested and non-tested skin. Immunostaining was cytoplasmic and was present in the epidermis and hair follicles. No dermal staining was observed. This study suggests that LIF may play a role in the early phase of allergic contact dermatitis.

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Leukaemia inhibitory factor (LIF) is a polyfunctional cytokine, belonging to the haemopoietic family of growth factors, with a wide range of activities (1–3). Despite its role in growth-promotion and cell differentiation, it was suggested that LIF might also be involved in the inflammatory process (4). LIF mediates septic shock in mice (5) and biologically active LIF was demonstrated both in the serum of children with septic shock syndrome (6) and in synovial fluid of patients with rheumatoid arthritis (7). Moreover, LIF was detected in the cerebrospinal fluid of patients with bacterial or viral meningitis, in pleural fluid of patients with pneumonia and tuberculosis, and in peritoneal fluid of patients with peritonitis (6).

LIF has been found both in normal human skin and in keratinocyte cultures (8,9), but its role in normal skin and cutaneous inflammation remains uncertain. Recently it has been shown that injection of human recombinant LIF into mouse ear promotes ear swelling and leukocyte infiltration of the dermis (10). This suggests that LIF may be a mediator of cutaneous inflammation. The present study was undertaken to determine whether con-

tact sensitization reactions to nickel affected the expression of LIF mRNA and protein.

## Materials and Methods

### *Patients and biopsies*

5 patients (4 female and 1 male, age range 19–53 years; mean 35.4 years) were studied after obtaining informed consent. All patients had previously proven allergy to nickel. 6 mm punch biopsies were taken from patches tested with 5% nickel sulfate 24 h previously. Control specimens were obtained from the same individuals. These were non-tested skin and vehicle-tested skin (after 24 h). Biopsies were divided into 2 parts; 1 was placed in 4M guanidinium thiocyanate containing 0.72% 2-mercaptoethanol solution (solution D) (11) and stored at  $-70^{\circ}\text{C}$  for reverse transcription – polymerase chain reaction (RT-PCR); the 2nd was snap frozen in liquid nitrogen for immunohistochemistry.

### *RNA extraction*

Skin specimens were homogenised in 650  $\mu\text{l}$  of solution D, using an Ultra-Turrax T8, homogeniser

(IKA Labortechnik, Staufen, Germany. To prevent cross-contamination between samples, the homogenizer head was soaked in a 1% virkon solution (Autec International Ltd, Sudbury, Suffolk UK). Total RNA was extracted according to the method of Chomczynski and Sacchi (11) (acid guanidinium thiocyanate – phenol – chloroform extraction). Quantification of RNA was performed by spectrophotometry (Philips PU8625 UV/VIS).

#### *Reverse transcription (RT) – polymerase chain reaction (PCR)*

RNA was reverse transcribed using oligo (dT)<sub>12–18</sub> primers (Pharmacia Biotech, St Albans, Herts, UK), as described before (12). The PCRs were carried out using labelled primers with gamma [<sup>32</sup>P] – ATP (13). Human LIF primers (Dalton Chemicals, North York, ON, Canada) were as follows: 5'-CACCCATGTCACAACAACC, 3'-ACACTTCAAGACCTCCTGC (expected product size – 515 bp). A cDNA template for LIF (kindly provided by Dr G Wong, Genetics Institute Inc. MA, USA) was used as a positive control. To control for differences in RNA abundance, samples were screened for expression of the “housekeeping” gene glyceraldehyde-3-phosphate dehydrogenase (G3PDH). G3PDH primers were synthesized by Pharmacia Biotech. Primer sequences for G3PDH were as follows: 5'-TGAAGGTCGGAGTCAACG GATTGGT, 3' CATGTGGGCCATGAGGT CCACCAC (expected product size – 983 bp). Samples were placed in a Techne Gene E Thermal Cycler (Techne Ltd., Cambridge, UK) and subjected to the following cycles: denaturation at 94°C

for 1 min, annealing at 60°C for 1 min and extension at 72°C for 1 min. A 10 min extension at 72°C was performed for the last cycle. In preliminary experiments using combined samples, the linearity of signal intensity with increasing PCR cycle numbers was assessed to gain optimal signal strength and to permit semi-quantitative analysis (12).

#### *Analysis of PCR products*

The PCR products were size-fractionated by electrophoresis for 1 hour at 150V on 12% sodium dodecylsulphate-polyacrylamide gels. Gels were exposed to Kodak XAR-5 film which was developed to visualize the PCR products. Semi-quantification was done by image analysis (Seescan, Cambridge, UK). Data shown in the figure represent a typical result from 2 independent experiments. Statistical analysis was performed using the Student's *t* test; *p* < 0.05 was considered significant.

#### *Immunohistochemistry*

5 µm sections were cut on a cryostat and were mounted on poly-L-lysine (Sigma, Poole, Dorset, UK) coated slides. They were air-dried (3 h at room temperature) and fixed in acetone for 5 min. After 5 min washing in Tris-buffered saline (TBS, pH 7.6) a standard avidin-biotin complex (ABC) method was used. Non-specific binding was blocked with a 20% normal swine serum (Dako, High Wycombe, Bucks., UK) for 10 min. The primary rabbit polyclonal antibody anti-LIF R303 (Genetics Institute Inc., Cambridge, MA, USA) diluted 1:200, was applied for 1 h. After washing in TBS (2× for 5 min), the specimens were incubated with biotinylated secondary antibodies to rabbit (Dako; 1:400 dilution) for 30 min. The slides were then again washed in TBS (2× for 5 min) and ABC solution (Dako) was applied for 30 min. After washing in TBS (2× for 5 min) the bound complexes were visualized with Fast Red TR (Dako) substrate (pink/red staining). Finally, slides were lightly counterstained with Harris' haematoxylin and mounted using Crystal Mount (Biomedica Corp., Foster City, CA, USA). Specificity of the anti-LIF antibody was checked previously (8), so in this study the negative control was performed by omitting the primary antibody.

## Results

#### *PCR data*

Strong signals for LIF mRNA were achieved with 35 PCR cycles. G3PDH mRNA required 38 cycles. Densitometric measurements of LIF mRNA signal were compared and normalized to those for

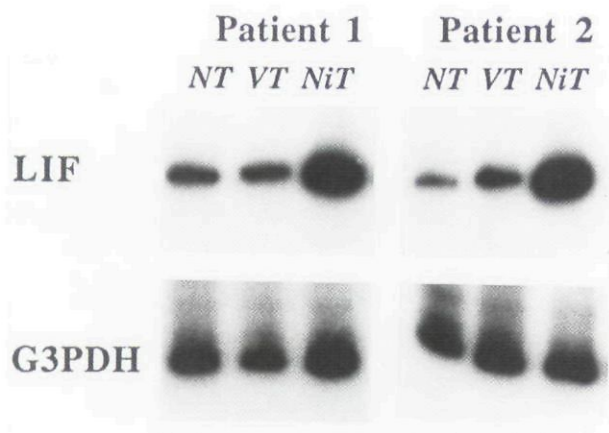


Fig. 1. Induction of LIF mRNA 24 h after nickel challenge. Total mRNA was extracted from skin biopsies, then reverse transcribed and amplified using specific primers, as described in Materials and Methods. The PCR products were analyzed by electrophoresis/autoradiography. NT: non-tested skin; VT: vehicle-tested skin; NiT: nickel-tested skin.

G3PDH. The upregulation of LIF after nickel challenge was evident in all 5 studied patients (Fig. 1). Net results are presented in Fig 2. Expression of LIF mRNA in vehicle-tested skin did not significantly differ from those taken in non-tested skin. However, statistically significant enhanced expression of LIF mRNA was found in 24 h nickel patch test biopsies when compared both to vehicle-tested ( $p=0.045$ ) and non-tested skin ( $p=0.041$ ).

#### Immunohistochemical data

Generally, all biopsies showed similar pattern of immunostaining (Fig. 3). There was no difference in the intensity of LIF immunoreactivity between non-tested (Fig. 3b) and vehicle-tested skin (Fig. 3c). Immunostaining was cytoplasmic and was present in the epidermis and in the hair follicles. The lower layers of epidermis, especially the basal layer, showed weaker staining compared with the upper parts of the epidermis. There was no LIF immunoreactivity seen in the dermis. Similar observations were made in nickel-tested skin. However, in 2 of 5 studied patients immunostaining after nickel challenge seemed to be more dispersed and equally distributed throughout the whole epidermis (Fig. 3d).

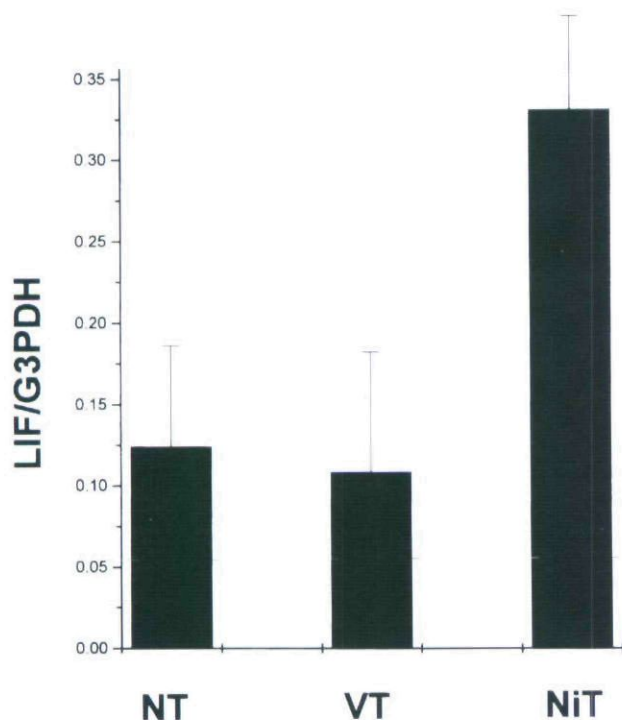


Fig. 2. Semi-quantification of LIF mRNA autoradiographs in non-tested (NT), vehicle-tested (VT) and nickel-tested skin (NiT). Semi-quantification was by image analysis. The densitometric value of LIF mRNA was normalized to that of G3PDH mRNA.  $*p=0.045$  relative to VT skin.

#### Discussion

Our results indicate that LIF may be involved in the early phase of the contact dermatitis reaction, as LIF mRNA expression was significantly increased 24 h after nickel challenge compared with both non-tested and vehicle-tested skin. LIF is an epidermal cytokine (8), which was confirmed by our immunohistochemical data. Murine (14) and human keratinocytes (8) and keratinocyte lines (15) were reported to produce LIF mRNA. LIF was also found to be a product of antigenically-activated T cells (16), and contact dermatitis is regarded as a form of delayed-type hypersensitivity reaction, which is mediated by antigen-specific T lymphocytes (17). However, our 24 h nickel patch tests revealed only a scanty dermal infiltrate in 2 of 5 studied subjects (data not presented), and the infiltrating cells did not show immunostaining for LIF. This suggests that increased expression of LIF mRNA in our patients after nickel challenge could be due to the enhanced expression by keratinocytes. This is difficult to prove by immunohistochemistry, as LIF protein is constitutively expressed in the epidermis (8). The intensity of the staining is not a good marker, as it can differ with even small variations of section thickness and for other technical reasons.

It is well-known that inflammatory epidermal cytokines play an important role in allergic contact dermatitis reactions (reviewed in (18)). We believe that LIF belongs to this group of cytokines. Maybe LIF acts directly, but this remains unclear. Recently, significant induction of IL-1  $\alpha$  and IL-8 in cultured human keratinocytes by LIF was demonstrated (8). So, it is possible that LIF stimulates IL-1 release from keratinocytes and may act via IL-1. IL-1 is known to enhance Langerhans cell function (reviewed in (18)). In addition, IL-1 also plays a role in induction of adhesion molecules (ICAM-1, VCAM-1, E-selectin on endothelial cells, LFA-3 on lymphocytes), and the expression of these adhesion molecules is related to the recruitment of inflammatory cells in the site of reaction. Moreover, IL-1 enhances vascular permeability by activating phospholipase and activates helper T cells to secrete IL-2. Finally, IL-1 promotes the release of other cytokines (IL-6, IL-8, GM-CSF) (reviewed in (18)). All these activities appear important in promoting contact dermatitis.

The question remains as to whether the induction of LIF message is specific for the contact sensitization reaction, or whether it is a non-specific inflammatory response to skin damage. While the LIF transcript was induced by irradiation of human keratinocytes cell lines with ultraviolet B (19), preliminary studies on the effects of non-anoic

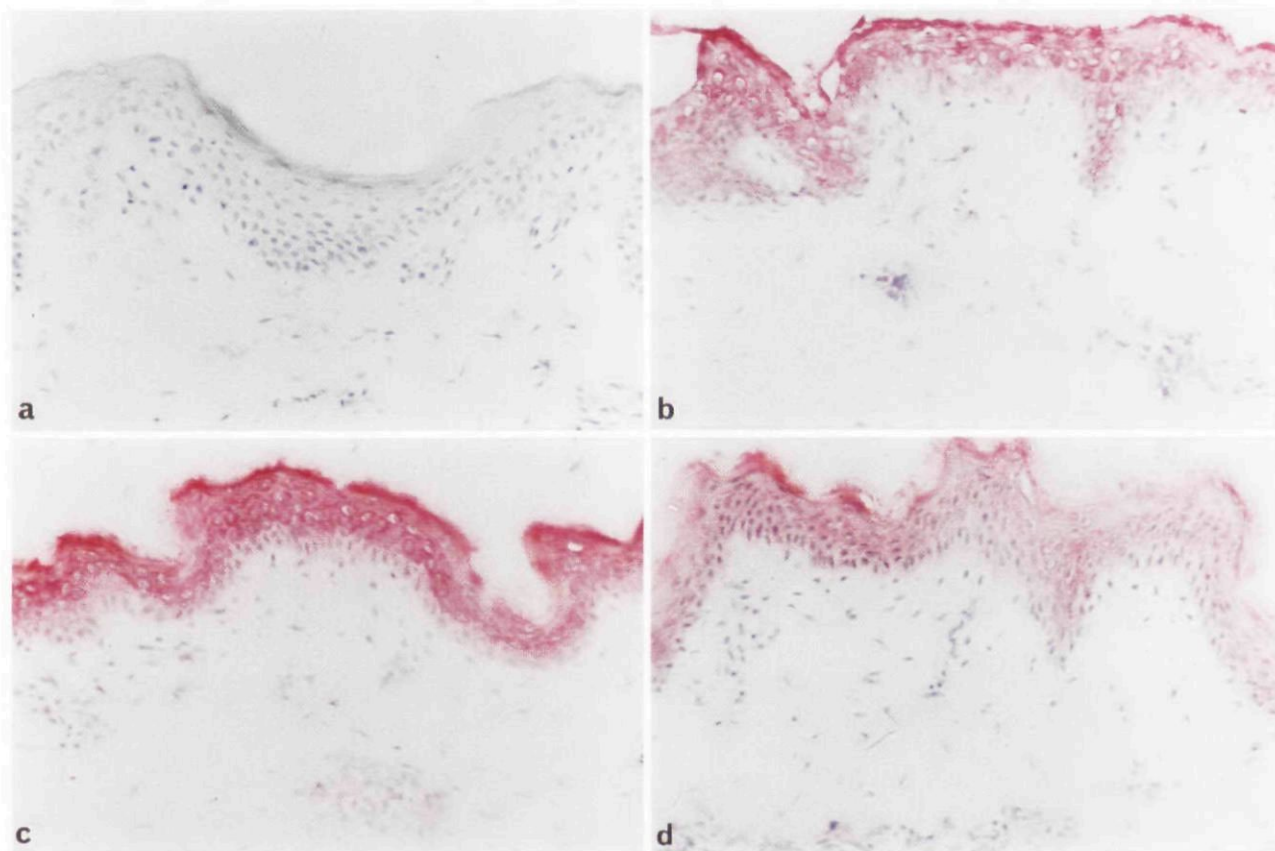


Fig. 3 Immunohistochemical staining of LIF in skin. Negative control (a), non-tested (b), vehicle-tested (c) and nickel-tested skin (d). All biopsies were from the same subject. Immunohistochemistry was performed by standard avidin-biotin complex (ABC) technique, as described in Materials and Methods (original magnification  $\times 250$ ).

acid on LIF mRNA expression in keratinocyte lines and skin organ cultures in vitro suggest that LIF expression is unaltered by irritants (data not shown). In a clinical study of 98 patients with pleural effusions, it was possible to distinguish 3 diagnostic groups on the basis of LIF levels (20), suggesting that in those patients, there is some specificity in LIF expression. Further studies, however, are necessary to determine whether LIF upregulation is a specific indicator of contact sensitization reactions.

In conclusion, this study demonstrates increased LIF mRNA expression 24 h after nickel challenge and points, for the 1st time, to a role for LIF in the early phase of allergic contact dermatitis. This is just the 2nd study to indicate that LIF is involved in the pathogenesis of a cutaneous inflammatory reaction, and further work is necessary to clarify its exact role.

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