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A Novel Endogenous Mediator of Cutaneous Inflammation: Leukemia Inhibitory Factor

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Keratinocytes produce a variety of cytokines, including leukemia inhibitory factor. We hypothesised that this cytokine may play a pro-inflammatory role in the skin and tested this hypothesis by injecting recombinant leukemia inhibitory factor (1–100 ng) into the ear pinnae of C3H/HeJ mice. To other groups of animals, we injected boiled leukemia inhibitory factor or phosphate-buffered saline (negative control) or 0.4 ng human interleukin-1α as a positive control. Following injection of 100 ng leukemia inhibitory factor, ear thickness, measured by micrometer, increased 66% over controls at 12 h and 100% at 24 h (overall $p=0.041$ by analysis of variance). Injection of 0.4 ng interleukin-1α caused greater ear swelling. Compared with controls, swelling increased by 67% at 6 h, 100% at 12 h and 340% after 24 h (overall $p \leq 0.00001$). Leukemia inhibitory factor (100 ng only) stimulated a 3.5-fold increase in leukocytes after 6 h. After 12 h, a 14-fold increase was seen in ears injected with 10 ng leukemia inhibitory factor and a 12-fold increase with 100 ng leukemia inhibitory factor, which remained elevated (17-fold) at 24 h (overall $p=0.0001$). Injection of interleukin-1α led to a 3.4-fold increase in leukocytes (mean per 20 high-power fields) after 6 h, a 14-fold increase at 12 h and a 25-fold increase at 24 h (overall $p \leq 0.00001$). These results demonstrate that leukemia inhibitory factor appears to be a mediator of cutaneous inflammation. ***Key words: leukocytes; skin; cytokines.***

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Leukemia inhibitory factor (LIF) is a polyfunctional cytokine with a diverse range of activities (for ref, see 1–3). The glycoprotein LIF is a growth and differentiation regulator; the distinction between growth-promoting and differentiation-inducing activities is largely determined by the target cell type. LIF proved to be identical to the so-called human interleukin for DA cells (HILDA). This is a haematopoietic growth factor, that suppresses the spontaneous differentiation of murine embryonic stem cells *in vitro* and facilitates their long-term growth in a totipotent state. Also known previously as hepatocyte stimulating factor III, LIF also has the ability to induce acute phase proteins in the liver (for ref see 4), and LIF is also able to stimulate cachexia in mice implanted with tumours overexpressing LIF (5).

An inflammatory role for LIF is indicated by the findings that biologically active LIF mediates septic shock in mice and is found in the synovial fluid of patients with rheumatoid arthritis and in body fluids of patients with a variety of acute and chronic inflammatory conditions (for ref see 6).

Recently LIF has been reported to be produced by murine mast cells and the murine keratinocyte cell line PAM (7), as well as by a human keratinocyte line (8) and human melanoma cell lines (9). The function of LIF in the skin is presently unknown. This study suggests that at least one function of LIF in the skin is to initiate inflammation.

MATERIALS AND METHODS

Animal treatments

Eight-week-old female C57BL/6 and C3H/HeJ mice were obtained from Charles River Laboratories (Que., Canada) and were anaesthetised by i.p. injection of 200 µl of a 2.5 mg/ml solution of sodium pentobarbital in phosphate-buffered saline (PBS). Ear thickness was measured on each animal using a spring-loaded micrometer (Ozaki Co., Tokyo, Japan), prior to injection. The ear pinnae were injected with cytokines dissolved in PBS containing 0.01% bovine serum albumin. Ear swelling was measured at 6, 12 and 24 h post-injection. At these time points, ear measurements were made prior to termination of the mice by cervical dislocation. The ears were removed and samples fixed in 10% buffered formalin for routine histological staining with haematoxylin-eosin (H & E). All procedures involving animals had been previously approved by the local animal care committee.

Cytokines

Recombinant human interleukin-1α (hIL-1), (kindly provided by Dr. R. Chizzonite, Hoffmann-La Roche, New Jersey), was used as a positive control (0.4 ng (50 U) per ear). This amount had previously been shown to stimulate leukocyte infiltration and footpad swelling in mice (11). To autoclaved PBS, bovine serum albumin (BSA) was added to a final concentration of 0.01% w/v. This was filtered asceptically and used to dissolve the cytokines. The PBS/BSA carrier was prepared fresh for each experiment. Recombinant murine LIF, produced in bacteria, was obtained from Upstate Biologicals Inc., Lake Placid, NY, and was dissolved and injected at doses of 1, 10 or 100 ng/ear. Total volume of injected liquid was 40 µl. As a negative control, ears were either injected with PBS or LIF which had been boiled (BL) for 45 min (100 ng/ ear).

Leukocyte counts

Sections stained with H & E were assessed by a naive observer, using water immersion (magnification ×625). The mean number of leukocytes per high-power field (HPF) from at least 20 random HPF was determined.

Statistical analysis

The statistical significance of the overall differences in means for ear swelling and leukocyte counts between treatments was assessed by analysis of variance (ANOVA). Values of $p<0.05$ were considered to be significant.

RESULTS

Effect of LIF injection on the ear histology, swelling and leukocytic infiltration

The injection of 100 ng LIF significantly increased ear swelling overall at 12 h and 24 h after injection ($p=0.041$), compared to the boiled LIF control (BL) (Fig. 1). Effects of the injection of LIF and IL-1 on ear swelling are shown as the increase over swelling in control (BL-treated ears). There was also a

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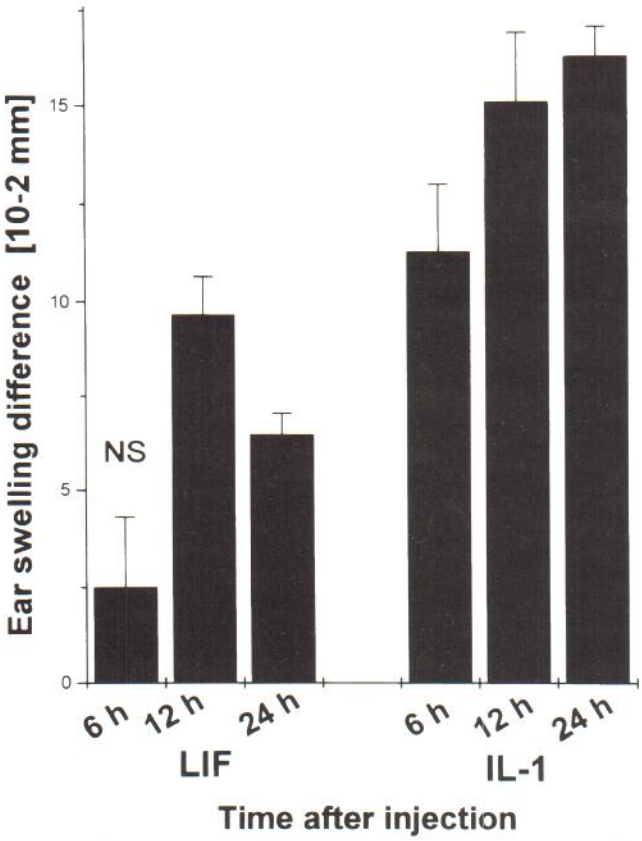

Fig. 1. Difference in ear swelling induced by recombinant murine LIF (100 ng/ear) or recombinant hIL-1α (0.4 ng/ear) from BL control-treated ears. The cytokines were injected into the ear pinnae of C3H/HeJ mice (6 ears per group), and ear thickness was measured by micrometer at the times shown after injection. Representative data from one experiment of three are shown. Overall difference in mean ear swelling of 100 ng LIF-treated ears from BL-treated control ears, $p=0.041$ by ANOVA. Overall difference in mean ear swelling of IL-1-treated ears from control-treated ears, $p=0.00001$, NS = not significant.

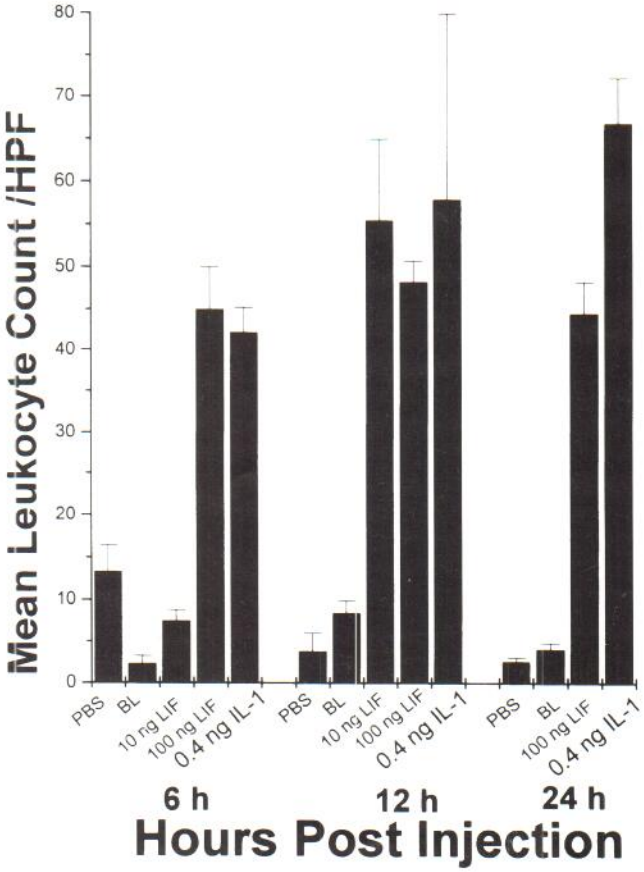

Fig. 2. Effect of LIF and IL-1 on leukocyte infiltration. Total leukocytes were counted in formalin-fixed/H&E stained sections from mouse ears injected with cytokine and removed at 6, 12 and 24 h after injection. Total leukocytes were counted in 20 high-power fields (HPF) under water immersion lens (magnification X625). Figures presented are the mean counts ± SEM per HPF. Four sections were examined from each of 6 ears. With 1 ng LIF, no increase in leukocytes was seen at any time point, so these counts have been omitted for clarity. Overall difference in mean counts of 100 ng LIF-treated ears from PBS control = $p \leq 0.0001$ by ANOVA. Overall difference in mean counts of IL-1 treated ears from PBS = $p \leq 0.00001$.

highly significant increase in overall leukocyte infiltration ($p=0.0001$ compared with PBS control) following injection of 100 ng LIF at all of these timepoints (Fig. 2). The positive control, IL-1-injected ears, showed a highly significant overall increase in swelling from 6–24 h after injection ($p=0.00001$). The ear swelling due to LIF injection was significantly less overall than that seen with IL-1 ($p=0.0034$).

At 6 h after injection with 100 ng LIF, there was a small (14%) increase in ear swelling over BL which failed to reach significance (Fig. 1). This is in contrast to IL-1 (0.4 ng), which induced a 67% increase in ear swelling over the BL control. Earlier experiments had shown that the BL-treated ear swelling was the same as that seen in ears treated with PBS. Lower doses (1 and 10 ng) had no effect (data not shown).

Injection of 100 ng stimulated a 3.4-fold increase in leukocyte numbers relative to the PBS control. A 3.1-fold increase in leukocyte counts was seen relative to the PBS control in the IL-1 injected ears (Fig. 2).

At 12 h, 100 ng LIF was the only concentration which gave consistent increase in ear swelling. Ear swelling in the 100 ng LIF-treated mice was 66% greater than the BL control (Fig. 1) — but was less than the swelling seen with IL-1 injection — and LIF-treated ears showed blood vessels with a considerable number of leukocytes either in the vessel or extravasating into the reticular and papillary dermis. Very few of these cells were seen to reach the epidermis and most of the cells appeared to be polymorphonuclear cells. Very few monocytes and still fewer lymphocytes could be seen. There also appeared to be some elongated elliptically shaped cells which could be tissue macrophages. The histopathology of the LIF-injected skin was qualitatively indistinguishable from that seen following IL-1 injection, although generally the IL-1 injected ears showed a greater degree of infiltration, oedema and vasodilation.

The number of infiltrating leukocytes did not dramatically increase from 6 h-24 h following injection of 100 ng of LIF. At 12 h post injection, the effects were similar to those observed with IL-1 injection. There were vasodilation and leukocytic infiltration (Fig. 3a) but not with the boiled LIF control (Fig. 3b). Oedema, vasodilation and erythema were detected at 12 h in both the LIF- and the IL-1-treated mice. No effects were seen in either the BL- or PBS-injected control animals.

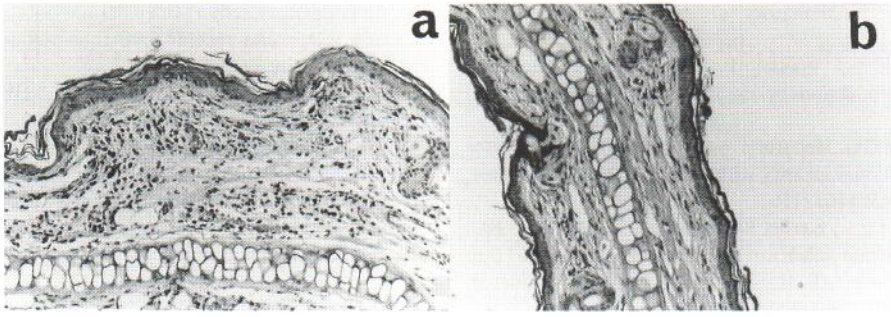

Fig. 3. Effect of cytokines on ear histology. Sections shown were made from formalin-fixed ears removed at 12 h after injection with 100 ng LIF injection (*a*), and boiled 100 ng LIF (*b*). Magnification X250.

At 12 h after injection, 10 ng LIF stimulated a 14-fold increase in leukocytes, 100 ng increased leukocyte counts by 12-fold, but 1 ng was ineffective. The infiltration induced by 10 and 100 ng LIF was similar in degree to that induced by IL-1 (Fig. 2). These effects were seen in three different experiments, one of which was carried out with a different mouse strain, viz. C57BL/6.

At 24 h after injection, the ears treated with 100 ng LIF remained swollen (100% increase over control ear thickness), although less so than the IL-1-treated ears (340% increase over control). The leukocyte counts in the LIF-injected ears also remained elevated (17-fold relative to PBS-injected) and the IL-1-treated ears had 25-fold more leukocytes than those treated with PBS.

No difference was observed in the numbers of mast cells visualised by toluidine blue staining, in either the LIF- or IL-1-treated animals, suggesting that the inflammation is not mediated by mast cell degranulation.

DISCUSSION

The production of LIF mRNA by murine (7) and human keratinocyte cell lines (8) has been described. The function of LIF in the skin is still unknown. Injection of recombinant LIF protein into mouse ear promoted leukocytic infiltration 12 h after injection; the histology observed was consistent with what was seen following injection of IL-1α into mouse footpads (11) or into human skin (12) and following injection of IL-1 into mouse ear in this study. Our results suggest that LIF may mediate inflammation and leukocyte infiltration of the skin following damage. We also confirmed, in the mouse ear, earlier observations in C3H/HeJ mouse footpads that IL-1 stimulates leukocytic infiltration (10). In other organs, there is ample evidence to show that LIF can mediate both acute and chronic inflammation and cachexia (for ref see 6).

We found that injection of LIF stimulated ear swelling and leukocyte infiltration of the dermis. We were anxious to rule out the possibility that the inflammation was a result of endotoxin contamination of the LIF preparation. Two pieces of evidence suggest that this is an unlikely possibility. First, we show in Figs. 1–3 that boiled LIF was devoid of inflammatory activity, which one would not expect to be the effect of endotoxin, which is known to be heat stable. Secondly, we performed the experiment on the C3H/HeJ strain in which leukocyte infiltration is not significantly stimulated by LPS (11).

The LIF protein was not as potent an inflammatory agent as IL-1α. Effects were not consistently seen at less than 100 ng of LIF protein and 100 ng of LIF did not induce greater swelling or infiltration than 0.4 ng of IL-1. The LIF-induced swelling was not marked at 6 h and did not reach a maximum until 12 h. Ear swelling induced by IL-1 was already at its maximum by 6 h. The time delay for LIF- induced swelling to appear suggests that LIF may be acting by inducing some other chemokine, such as IL-1 or IL-8 (11–14). Consistent with this view, we have shown that in culture, human keratinocytes produce both message and protein for IL-1α and IL-8 but not IL-6 or TNF-α, 12–24 h after stimulation with LIF (Paglia et al., Br J Dermatol, in press). Furthermore, a reciprocal regulation of LIF mRNA by IL-1 has been recorded previously by Jonakait (15). It is possible that the skin releases LIF in response to damage, as has been seen for skeletal muscle (16).

In conclusion, injection of LIF, an endogenous keratinocyte cytokine, mediates ear swelling and leukocyte infiltration in the mouse ear *in vivo*. It is possible that LIF is involved in the pathogenesis of inflammatory dermatoses; this hypothesis is currently being tested on material from patients.

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REFERENCES

1. Gearing, D. The leukemia inhibitory factor and its receptor. Adv Immunol 1993; 53: 51–58.
2. Metcalf D. Leukemia inhibitory factor–a puzzling polyfunctional regulator. Growth Factors 1992; 7: 169–173.
3. Gough NM. Molecular genetics of leukemia inhibitory factor (LIF) and its receptor. Growth Factors 1992; 7: 175–179.
4. Ryffel B. Pathology induced by leukemia inhibitory factor. Int Rev Exp Pathol 1993; 34pt B: 69–72.
5. Mori M, Yamaguchi K, Honda S, Nagasaki K,Veda M, Abe O, Abe K. Cancer cachexia syndrome developed in nude mice bearing melanoma cells producing leukemia inhibitory factor. Cancer Res 1991; 51: 6656–6659.
6. Alexander H R, Billingsley KG, Block MI, Fraker DL. D-factor/leukemia inhibitory factor: evidence for its role as a mediator in

acute and chronic inflammatory disease. Cytokine 1994; 6: 589–596.
7. Marshall JS, Gauldie J, Nielsen L, Bienenstock J. Leukemia inhibitory factor production by rat mast cells. Eur J Immunol 1993; 23: 21116–21120.
8. Baumann H, Wong GG. Hepatocyte-stimulating factor III shares structural and functional identity with leukemia inhibitory factor. J Immunol 1989; 143: 1163–1167.
9. Paglia D, Oran A, Lu C, Kerbel RS, Sauder DN, Mckenzie RC. Expression of leukemia inhibitory factor and interleukin-11 in human melanoma lines: LIF, IL-6 and IL-11 are not co-regulated. J Interferon Cytokine Res 1995; 14: 455–460.
10. Kondo S, Kono T, Brown WR, Pastore S, Mckenzie RC, Sauder DN. Lymphocyte function-associated antigen-1 is required for maximum elicitation of allergic contact dermatitis. Br J Dermatol 1994; 131: 354–359.
11. Granstein RD, Margolis R, Mizel SB, Sauder DN. *In vivo* inflammatory activity of epidermal cell-derived thymocyte activating factor and recombinant interleukin-1 in the mouse. J Clin Invest 1986; 77: 1020–1027.
12. Dowd P, Camp RDR, Greaves MW. Human recombinant interleukin-1α is proinflammatory in normal human skin. Skin Pharmacol 1988; 1: 30–37.
13. Larsen, CG, Anderson, AO, Appella, E, Oppenheim JJ, Matsushima, K. The neutrophil-activating protein (NAP-1) is also chemotactic for T-lymphocytes. Science 1989; 243: 1464–1466.
14. Miossec P, Yu CL, Ziff M. Lymphocyte chemotactic activity of interleukin-1. J Immunol 1983; 133: 2007–2011.
15. Jonakait G M. Neural-immune interactions in sympathetic ganglia. Trends Neurosci 1993; 16: 419–423.
16. Barnard W, Bower J, Brown MA, Murphy M, Austin L. Leukemia inhibitory factor (LIF) infusion stimulates skeletal muscle regeneration after injury: injured muscle expresses LIF mRNA. J Neuro Sci 1994; 123: 108–113.