Acta Derm Venereol (Stockh) 1994; 74: 364–367

Interleukin 1α (IL-1α) in Human Skin *In vivo*: Lack of Correlation to Markers of Collagen Metabolism

M. KYLMÄNIEMI[1], P. AUTIO[2] and A. OIKARINEN[2]

Departments of [1]Oral Surgery, Institute of Dentistry, and [2]Dermatology, University of Oulu, Oulu, Finland

Levels of interleukin 1α (IL-1α) were studied from blister fluids collected from 14 patients with various types of blistering diseases. In all the fluids, IL-1α could be detected, the concentrations varying from 5 to 1730 pg/ml. For comparison IL-1α was also assayed from suction blisters of 13 subjects; 8 atopic patients and 5 healthy controls. IL-1α was also present in suction blisters in measurable quantities, suggesting that during suction IL-1α is released into the blister cavity. Since IL-1α has been shown to have marked effects on collagen metabolism, the marker of collagen synthesis (carboxyterminal propeptide of type I procollagen (PICP)) and gelatinase were assayed from the same samples. There was no apparent correlation between the levels of IL-1α, PICP or gelatinase in blister fluids. The possible association of IL-1α and collagen metabolism was further studied in experimental conditions. Topical glucocorticoid markedly decreased the level of PICP in suction blisters but did not have any significant effect on IL-1α. UVB-radiation, on the other hand, caused increase in IL-1α but did not have any profound effect on collagen metabolism. During the re-epithelialization of the blister floor the level of IL-1α decreased markedly, and at the same time the expression of gelatinase was increased. The results indicate that IL-1α is released in large quantities into blister fluid when using the suction blister model. However, no apparent correlation could be observed in healthy or diseased skin between the levels of IL-1α, collagen synthesis marker or gelatinase. ***Key words: blister; collagen synthesis; gelatinases.***

(Accepted March 7, 1994.)

Acta Derm Venereol (Stockh) 1994; 74: 364–367.

A. Oikarinen, Department of Dermatology, University of Oulu, SF-90220 Oulu, Finland.

Interleukin-1 (IL-1) is originally characterized from monocytes and exhibits various important biological activities (1, 2). It can augment T-cell activation, stimulate synthesis of lymphokines and activate other cells such as endothelial cells and macrophages. IL-1 can increase both the synthesis rate of collagen and activate interstitial collagenase (3–5). Originally IL-1 was thought to be a product of inflammatory cells. However, recent studies have revealed that many other cells can produce IL-1, including keratinocytes (2, 6, 7). In keratinocytes IL-1 is present in large quantities. We were interested in investigating the relationship between the levels of IL-1 and markers of collagen metabolism *in vivo* in human skin.

MATERIALS AND METHODS

Patients

Blister fluids were collected from spontaneous blisters of 14 patients with various types of blistering diseases and immediately frozen and kept at −20°C. Suction blisters were induced on the healthy-looking abdominal skin of 8 patients with atopic eczema and on 5 control subjects of the same age (8).

The effects of steroids were studied in 3 subjects in which clobetasol-17-propionate cream was applied three times a day for 1, 2 and 4 days before induction of blisters. In order to study the effects of UVB-irradiation abdominal skin was irradiated with a Waldman lamp (output 1.90 mW/cm^2, irradiation time 120–180 s; 2 × MED) and blisters were induced at 4 and 24 h after irradiation and collected as described above.

In order to study the effect of re-epithelialization on IL-1α and gelatinase, blister fluid samples were taken immediately after blister formation and at 24 and 72 h post-blistering. In each experiment 2–3 intact blisters were emptied and pooled and the remaining blisters were protected by a cap until used.

Assays

IL-1α was assayed by an immunoassay (Quantikine immunoassay kit, R&D System) according to the instructions of the manufacturer. Carboxyterminal propeptide of type I procollagen (PICP) was assayed as described (9, 10) and gelatinase measured by a zymography method (11–13).

For statistical analyses, the Mann-Whitney rank sum test, Spearman rank order correlation and Student's *t*-test were used.

RESULTS

Levels of IL-1α, PICP and gelatinase in blister fluids

IL-1α could be detected in blister fluids of various blistering diseases. The lowest levels were observed in blisters induced by liquid nitrogen (Table I). In further studies IL-1α was also measured from suction blisters of patients with atopic eczema

Table I. *The levels of interleukin 1α (IL-1α), carboxyterminal propeptide of type I procollagen (PICP) and gelatinase in various blisters*

BP = bullous pemphigoid; Ery = erysipelas; EM = erythema multiforme; RA = rheumatoid arthritis; ND = not determined.

Patient number	Diagnosis	IL-1α (pg/ml)	PICP μ/l	Gelatinase (densitometric units, DU)
1	BP	25	1570	380
2	BP	65	950	710
3	BP	100	1585	660
4	BP	25	670	460
5	Pompholyx	20	1535	640
6	Pompholyx	35	505	350
7	Ery	40	2615	335
8	Ery	25	170	370
9	Ery	120	2245	480
10	EM	20	2395	845
11	RA	100	ND	830
12	RA	1730	ND	1005
13	Cryoblister	10	70	400
14	Cryoblister	5	130	530

Table II. *The levels of interleukin 1α (IL-1α), carboxyterminal propeptide of type I procollagen (PICP) and gelatinase in suction blisters of control subjects and patients with atopic eczema (AE)*

Patient number	Diagnosis	IL-1α (pg/ml)	PICP ng/l	Gelatinase (densitometric units, DU)
1	AE	85	1030	260
2	AE	1050	895	200
3	AE	135	310	160
4	AE	480	60	900
5	AE	75	40	240
6	AE	160	20	630
7	AE	370	215	1210
8	AE	380	425	360
9	control	230	1285	675
10	control	385	1200	720
11	control	500	70	855
12	control	305	475	615
13	control	175	420	445

and healthy controls (Table II). IL-1α could be determined from all suction blisters; the mean IL-1α level was 340 pg/ml in atopic eczema patients and 320 pg/ml in controls. The levels of PICP were 380 μg/l in atopic eczema patients and 690 μg/l in controls, and gelatinase 490 densitometric units (DU) in atopic patients and 660 DU in healthy controls. There was no statistically significant difference between IL-1α, PICP and gelatinase levels in controls and atopic eczema patients. The levels of IL-1α in spontaneous blisters or suction blisters did not correlate with the corresponding levels of PICP or gelatinase (Tables I and II). The level of IL-1α was also studied during healing of blisters up to 72 h, and the levels decreased markedly as shown in Fig. 1A. At the same time gelatinase expression was considerably increased (Fig. 1B). In one subject IL-1α was also measured from blisters induced on four different sites: abdomen, upper arm, lower leg and back. The individual levels of IL-1α were 230, 330, 100 and 400 pg/ml, respectively.

Effects of topical glucocorticoid and UVB-irradiation

Topical clobetasol-17-propionate reduced the level of PICP in suction blister fluids ($p<0.005$) but did not have any significant effect on IL-1α (Fig. 2).

The effect of UVB on IL-1α was studied by irradiating skin with 2 × MED UVB and by inducing suction blisters 4 and 24 h after irradiation (Fig. 3). The levels of IL-1α were 1.5-fold higher at 4 h than in control blisters. However, the increase was not statistically significant. The levels of PICP and gelatinase did not change in irradiated skin.

DISCUSSION

The levels of IL-1α found in spontaneous blisters of various blistering diseases were relatively low, but still higher than serum levels found in general. Part of the IL-1α could be derived from inflammatory cells, which are abundantly present in various blistering diseases. Surprisingly higher levels of IL-1α were present in suction blisters induced on abdominal skin of healthy controls and patients with atopic eczema. The levels of IL-1α found in blister fluids were within the same range as those found in other studies using a different method (14). Since, usually, no apparent inflammation is present in healthy skin, IL-1α could not be derived from inflammatory cells. Instead it is plausible that most of IL-1α in suction blisters is derived from epidermis, since as many previous studies have shown, epidermal cells contain IL-1α. It is possible that the local trauma to epidermis during suction releases abundantly preformed IL-1α from disrupted keratinocytes into the blister cavity. This would be an explanation for the wide variation in the levels of IL-1α in blister fluids. During healing of the blister floor, the levels of IL-1α decreased markedly, and in fact the levels were close to those found in spontaneous blisters. This indicates that during regeneration of epidermis, most of the released IL-1α is gradually degraded or bound to receptors of fibroblasts (15).

Since IL-1α has been shown to modulate various aspects of collagen metabolism, we also compared the levels of IL-1α to

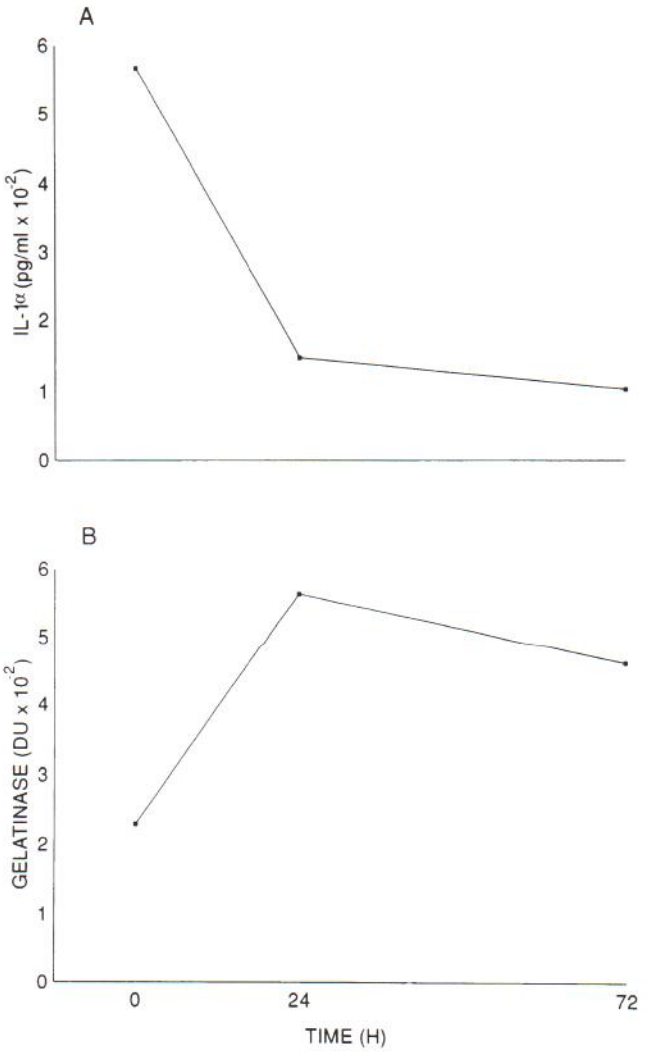

Fig. 1. The levels of IL-1α (A) and gelatinase (B) in healing suction blisters. Suction blisters were induced at time 0, and blister fluid samples were collected at 0, 24 and 72 h post-blistering and used for the assay of IL-1α (pg/ml) and gelatinase (densitometric units, DU). The values are the means of duplicate assays.

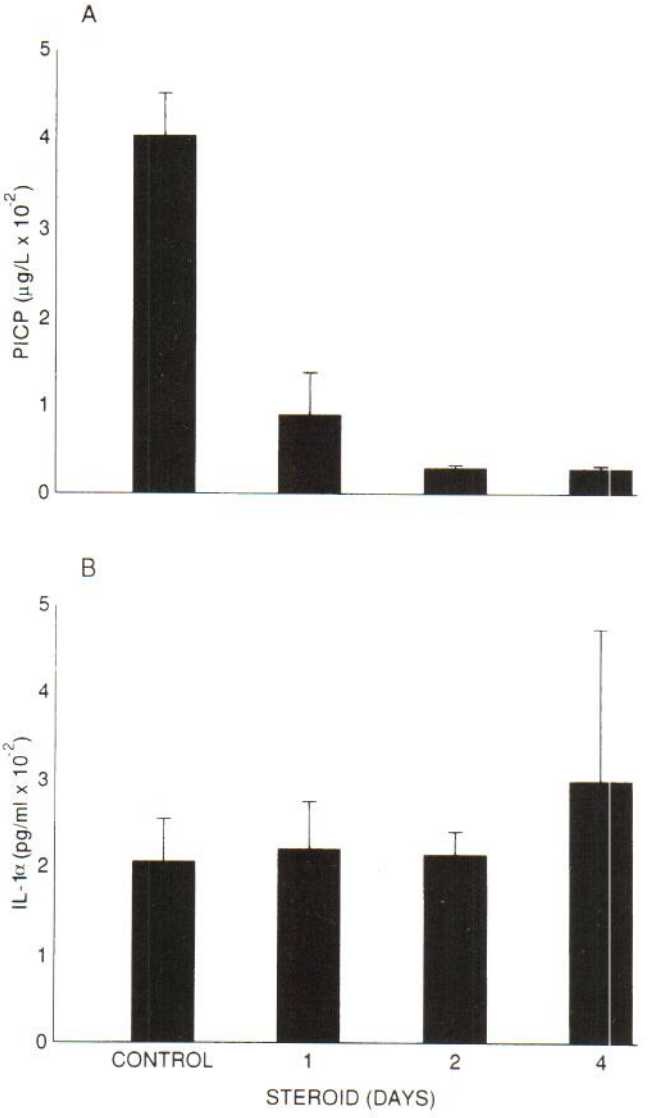

Fig. 2. Effect of topical clobetasol-17-propionate on the levels of PICP (A) and IL-1α (B) in suction blisters. Abdominal skin of three subjects was treated three times with clobetasol-17-propionate for 1, 2 or 4 days until the blisters were induced and blister fluid samples used for the assay of PICP and IL-1α. The values are the mean + SEM of triplicate assays.

markers of collagen synthesis and degradation. Collagen synthesis was monitored by a recently developed sensitive assay, which is based on the measurement of collagen propeptides from blister fluid (10). In order to measure degradation, gelatinase expression was measured by a zymography method. This method is based on lysis of gelatin by activated gelatinases. These enzymes have the ability to degrade gelatin (the denatured collagen), basement membrane collagen, elastin and some other collagen types such as collagen type V, VII and X (16–21). The origin of gelatinases varies and the 72 kDa is mostly derived from fibroblasts, whereas the 92 kDa stems from inflammatory cells and keratinocytes.

Even though numerous *in vitro* studies have shown that IL-1 can stimulate both collagen synthesis and degradation, we did not find an apparent correlation between IL-1α and collagen propeptides or gelatinases. This aspect was further studied in experimental situations. When topically potent glucocorticoid, was applied the level of PICP decreased by over 90 percent, but the level of IL-1α did not change markedly. This suggests that short-term glucocorticoid treatment in healthy skin does not significantly alter the endogenous production of IL-1α, or that during suction the same amount of IL-1α is released into the blister cavity even in steroid-treated skin. Similarly, in a recent study one-week treatment with topical potent glucocorticoid did not alter significantly the IL-1α levels in suction blisters (V.

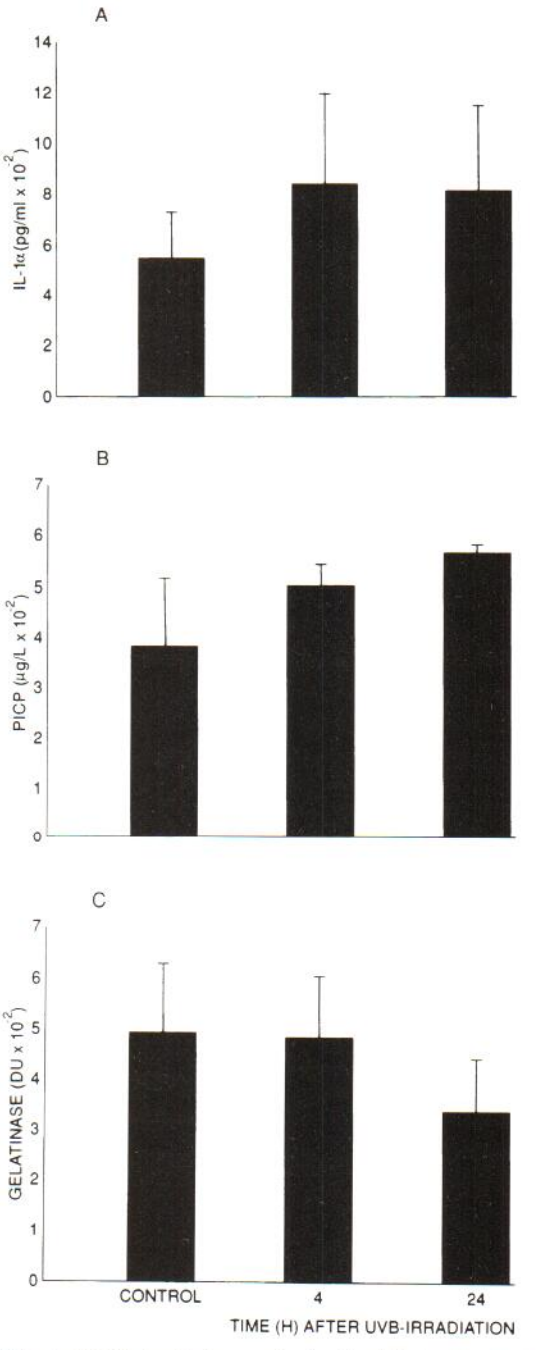

Fig. 3. Effect of UVB-irradiation on the levels of IL-1α (A), PICP (B) and gelatinase (C). Three subjects were irradiated by UVB (2 × MED) and suction blisters were induced at 4 and 24 h after irradiation and used for the assays. The values are the mean + SEM.

Koivukangas et al., unpublished). In inflammation, where IL-1α levels are upregulated, it is possible that topical glucocorticoid could downregulate IL-1α.

Interestingly the levels of gelatinases and IL-1α did not correlate in any of the patient groups studied. This aspect was further studied in UVB-irradiated skin. Numerous studies have shown that UV-irradiation can stimulate production of various interleukins (22, 23), and it has also been shown that UV-radiation increases interstitial collagenase (24, 25). Here UVB slightly enhanced the levels of IL-1α in suction blister fluids, but the levels of gelatinases were not altered. It should be noted that the follow-up after irradiation was only one day, and it is possible that during a longer follow-up gelatinase expression could be modulated.

When the possible association of IL-1α and collagen degradation was further studied during re-epithelialization of the blister floor, an opposite change in the levels of IL-1α and gelatinases was observed. Immediately after blister induction the level of IL-1α was high, but it was decreased markedly at 24 h post-blistering. In contrast, gelatinase was considerably increased at the same time. It is possible that the high level of IL-1α liberated into fresh blisters may induce gelatinase, as found at 24 h post-blistering.

ACKNOWLEDGEMENTS

This study was supported by grants from the Medical Research Council of the Academy of Finland, from the Yrjö Jahnsson Foundation and Cancer Society of Northern Finland.

REFERENCES

1. Dinarello CA. Interleukin-1. Rev Infect Dis 1984; 6: 51–95.
2. Dinarello CA, Mier SW. Lymphokines. N Engl J Med 1987; 317: 940–945.
3. Kähäri V-M, Heino J, Vuorio E. Interleukin-1 increases collagen production and mRNA levels in cultured skin fibroblasts. Biochim Biophys Acta 1987; 929: 142–147.
4. Postlethwaite AE, Raghow R, Stricklin GP, Poppleton H, Seyer JM, Kang AH. Modulation of fibroblast functions by interleukin 1: increased steady-state accumulation of type I procollagen messenger RNAs and stimulation of other functions but not chemotaxis by human recombinant interleukin 1 alpha and beta. J Cell Biol 1988; 106: 311–318.
5. Dayer J-M, de Rochemonteix B, Burrus B, Demczuk S, Dinarello CA. Human recombinant interleukin 1 stimulates collagenase and prostaglandin E2 production by human synovial cells. J Clin Invest 1986; 77: 645–648.
6. Sander DN, Carter C, Katz SI, Oppenheim JJ. Epidermal cell production of thymocyte activation factor (ETAF). J Invest Dermatol 1982; 70: 34–37.
7. Didierjean L, Salomon D, Merot Y, Siegenthaler G, Shaw A, Dayer JM, et al. Localization and characterization of the interleukin 1 immunoreactive pool (IL-1 alpha and beta forms) in normal human epidermis. J Invest Dermatol 1989; 92: 809–816.
8. Kiistala U. Suction blister device for separation of viable epidermis from dermis. J Invest Dermatol 1968; 50: 129–137.
9. Melkko J, Niemi S, Risteli L, Risteli J. Radioimmunoassay for the carboxy-terminal propeptide of human type I procollagen. Clin Chem 1990; 36: 1328–1332.
10. Oikarinen A, Autio P, Kiistala U, Risteli L, Risteli J. A new method to measure type I and III collagen synthesis in human skin in vivo: demonstration of decreased collagen synthesis after topical glucocorticoid treatment. J Invest Dermatol 1992; 98: 220–225.
11. Heussen C, Dowlde EB. Electrophoretic analysis of plasminogen activators in polyacrylamide gels containing sodium dodecyl sulfate and copolymerized substrates. Anal Biochem 1980; 102: 196–202.
12. O'Grady RL, Netherty A, Hunter N. A fluorescent screening assay for collagenase using collagen labeled with 2-methoxy-2,4-dipfenyl-3(2H)-furanone. Anal Biochem 1984; 140: 490–494.
13. Oikarinen A, Kylmäniemi M, Autio-Harmainen H, Autio P, Salo T. Demonstration of 72-kDa and 92-kDa forms of type IV collagenase in human skin: variable expression in various blistering diseases, induction during re-epithelialization, and decrease by topical glucocorticoids. J Invest Dermatol 1993; 101: 205–210.
14. Lawlor F, Bird C, Camp RDR, Barlow R, Barr RM, Kobza-Black A, et al. Increased interleukin 6, but reduced interleukin 1, in delayed pressure urticaria. Br J Dermatol 1993; 128: 500–503.
15. Boxman I, Löwik C, Aarden L, Ponec M. Modulation of IL-6 production and IL-1 activity by keratinocyte-fibroblast interaction. J Invest Dermatol 1993; 101: 316–324.
16. Murphy GJP, Murphy G, Reynolds JJ. The origin of matrix metalloproteinases and their familial relationships. FEBS 1991; 289: 4–7.
17. Senior RM, Griffin GL, Fliszar CJ, Shapiro SD, Goldberg GI, Welgus HG. Human 92- and 72-kilodalton type IV collagenases are elastases. J Biol Chem 1991; 266: 7870–7875.
18. Murphy G, McAlpine CG, Poll CT, Reynolds JJ. Purification and characterization of a bone metalloproteinase that degrades gelatin and types IV and V collagen. Biochim Biophys Acta 1985; 831: 49-58.
19. Welgus GW, Fliszar CJ, Seltzer JL, Schmid TM, Jeffrey JJ. Differential susceptibility of type X collagen to cleavage by two mammalian interstitial collagenases and 72-kDa type IV collagenase. J Biol Chem 1990; 265: 13521–13527.
20. Seltzer JL, Eisen AZ, Bauer EA, Morris NP, Glanville RW, Burgeson RE. Cleavage of type VII collagen by interstitial collagenase and type IV collagenase (gelatinase) derived from human skin. J Biol Chem 1989; 204: 3822–3826.
21. Nagase H, Ogata Y, Suzuki K, Enghild JJ, Salvesen G. Substrate specificities and activation mechanisms of matrix metalloproteinases. Biochem Soc Transact 1991; 19: 715–718.
22. Gahring L, Baltz M, Pepys MB, Daynes R. Effects of ultraviolet radiation on production of epidermal cell thymocyte-activating factor/ interleukin-1 in vivo and in vitro. Proc Natl Acad Sci USA 1984; 81: 1198–1199.
23. Granstein RD, Sauder DN. Whole-body exposure to ultraviolet radiation results in increased serum interleukin-1 activity in humans. Lymphokine Res 1987; 6: 187–193.
24. Scharffetter K, Wlaschenk M, Hogg A, Bolsen K, Schothorst A, Goerz G, et al. UVA irradiation induces collagenase in human dermal fibroblasts in vitro and in vivo. Arch Dermatol Res 1991; 283: 506–511.
25. Petersen MJ, Hansen C, Craig S. Ultraviolet A irradiation stimulates collagenase production in cultured human fibroblasts. J Invest Dermatol 1992; 99: 440–444.