

Decreased Levels of IL-1 α and β in Psoriatic Lesional Skin

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TAKEMATSU, H., OHMOTO, Y. and TAGAMI, H. *Decreased Levels of IL-1 α and β in Psoriatic Lesional Skin.* Tohoku J. Exp. Med., 1990, **161** (3), 159-169 — Interleukin 1 (IL-1), which mediates a wide range of biological activities, is thought to play an important role in many inflammatory and immunologic diseases. Normal human epidermal keratinocytes constitutively produce IL-1. Based on our previous data indicating decreased IL-1 activity in psoriatic scale extracts, in the present study, we measured immunoreactive IL-1 α and β levels in the suction blister fluids as well as in the psoriatic scale extracts using enzyme immunoassay for IL-1 α and β . The results showed that although similarly low levels of IL-1 α were detectable in the suction blister fluid from normal and psoriatic lesional skin, and that no IL-1 β was found in most of the blister fluids, indicating that IL-1 α is major IL-1 species produced by human skin. As compared to those in the blister fluids, IL-1 α levels in the horny tissue extracts were found to be much higher, and they were significantly higher in the orthokeratotic stratum corneum extracts than in the psoriatic scale extracts. However, gel filtration of the orthokeratotic horny tissue extracts demonstrated that constituents for immunoreactive IL-1 α and β were quite variable depending upon the source of the horny tissues. The present study has confirmed that IL-1 levels in the psoriatic scale extracts are decreased when compared with those in the orthokeratotic horny tissue possibly due to an increased epidermal proliferation activity associated with its high turnover rate. The role of IL-1 psoriatic lesions remains unknown. ——— psoriasis; suction blister; horny tissue; interleukin 1; EIA

Interleukin 1 (IL-1), which mediates a wide range of biological reactions, is thought to play an important role in many inflammatory and immunologic diseases (Oppenheim et al. 1986). On the basis of isoelectric points, two major forms of IL-1 have been identified: IL-1 α (pI 5.0) and β (7.0) (Auron et al. 1984; March et al. 1985; Gubler et al. 1986). Biological analyses of purified native and recombinant human IL-1 α and β have shown that these proteins possess very similar, if not identical, bioactivities in a broad variety of bioassays (Wood et al.

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1985), presumably mediated by binding to the same receptor (Dower et al. 1986; Kilian et al. 1986). The similarities in functional activity of IL-1 α and β , however, are not reflected in primary sequences; IL-1 α and β demonstrate only 35% nucleotide identity and only 23% amino acid identity (Auron et al. 1984; March et al. 1985).

In the normal human epidermis, keratinocytes constitutively produce IL-1 (Luger et al. 1983; Hauser et al. 1986), and a large quantity of IL-1 is contained within the human stratum corneum (Gahring et al. 1985; Camp et al. 1986; Takematsu et al. 1986). mRNAs for IL-1 has also been found in keratinocytes (Kupper et al. 1986; Bell et al. 1987). Keratinocytes possess receptors for IL-1 (Kupper et al. 1988; Blanton et al. 1989), which can elicit a variety of responses in keratinocytes in culture, including chemoattraction (Martinet et al. 1988), and induction of various cytokines (Kupper et al. 1987).

Previously, we showed that IL-1 activity in psoriatic scale extracts in decreased when compared with that in the orthokeratotic horny tissue extracts (Takematsu et al. 1986). The decreased IL-1 activity in the psoriatic scales could be due to a decreased IL-1 synthesis, inactive form of produced IL-1, the presence of IL-1 inhibitor (Hammerberg et al. 1989), or due to rapid epidermal turnover with a decreased trapping of IL-1 in the stratum corneum.

For the analyses of proinflammatory chemical mediators, we used stratum corneum extracts, assuming that they contain those produced or released in preceding events in the epidermis (Takematsu et al. 1986), and suction blister fluids for those involved in ongoing inflammatory reactions in the lesional skin (Takematsu and Tagami 1990a, b). The activity of IL-1 has traditionally been measured by the ability to augment the proliferative response of murine thymocytes to suboptimal concentrations of T cell mitogens (Mizel et al. 1978). In the present study, we measured immunoreactive IL-1 α and β levels in the suction blister fluids as well as in the horny layer extracts from orthokeratotic and psoriatic skin using specific enzyme immunoassay (EIA) for IL-1 α and β (Tanaka et al. 1987). In addition to the demonstration of a predominance of IL-1 α over IL-1 β in the human epidermis, our present studies have confirmed our previous finding that IL-1 is decreased in the psoriatic scale extracts when compared with those in the orthokeratotic horny tissue extracts.

MATERIALS AND METHODS

Suction blister formation

Eighteen psoriatic patients (12 males and 6 females ranging in age from 18 to 63 years with a mean of 34 years) and 18 male healthy volunteers ranging in age from 10 to 23 years with a mean age of 21 years were studied. None of the psoriatic patients had received any kind of treatment for at least two weeks before examination. Blisters were produced on the skin of the abdomen or forearm with attachment devices using hollow syringes, whose broad and flat ends were placed on the skin, and a negative pressure of 200–300 mmHg was applied for one to two hours. Some of the blister fluids thus raised on the psoriatic involved skin

were contaminated with blood. The blister fluids were collected, and supernatant was obtained after centrifugation at $1,660 \times g$ for 15 min. Only blister fluids without much blood contamination were stored at -70°C before use (Takematsu and Tagami 1990a).

Extracts of horny tissues

Psoriatic scales were collected from 17 patients and orthokeratotic horny tissues were from 7 patients with plantar callus, 1 with ichthyosis vulgaris, a plantar hyperkeratotic horny tissue of pityriasis rubra pilaris (PRP), and a sheet of normal stratum corneum from the amputated thigh of a 17-year-old woman. Water-soluble components were extracted from the stratum corneum in phosphate-buffered saline, pH 7.4 containing streptomycin 100 $\mu\text{g}/\text{ml}$, as reported previously (Takematsu and Tagami 1990a).

Molecular sieve chromatography

TSK-G2000SW exclusion high performance liquid chromatography column (Tosoh, Tokyo; 7.5×30 cm) was equilibrated with phosphate-buffered saline (PBS) and calibrated with dextran blue (2,000 kDa), γ -globulin (158 kDa), bovine serum albumin (67 kDa), ovalbumin (44 kDa), and carbonic anhydrase (29 kDa). After injecting 0.5 ml of suction blister fluids or horny tissue extracts into the column, the column was eluted with the buffer, and fractions of 0.5 ml were collected. PBS was used as the eluent. The protein content was estimated by the measurement of absorbance at 280 nm. The fractions were assayed for IL- 1α and β .

EIA for IL- 1α and β

IL- 1α and β were measured by sandwich EIA (Tanaka et al. 1987).

Protein measurement

The amount of protein in the horny tissue extracts was measured using Bio-Rad protein assay (Bio-Rad, Richmond, CA, USA).

Statistical analyses

Levels of IL- 1α or IL- 1β were given in terms of mean \pm S.D. Differences between mean values were evaluated by Wilcoxon rank-sum test and considered to be significant when a p value was less than 0.05.

RESULTS

IL- 1α and β in suction blister fluids

The levels of IL- 1α were low in the suction blister fluids from normal (90.0 ± 43.8), psoriatic uninvolved (89.4 ± 35.4), and involved skin (88.8 ± 37.8 pg/ml) (Fig. 1A). There were no significant difference between them and even between the psoriatic uninvolved and involved skin from the same individuals. Except for two samples (one from normal and the other from psoriatic involved skin), the levels of IL- 1β in the suction blister fluids were lower than the detection limit of 120 pg/ml (Fig. 1B).

IL- 1α and β in horny tissue extracts

The levels of IL- 1α and β in the horny tissue extracts were much higher than those in the suction blister fluids (Fig. 2). The levels of IL- 1α in the horny tissue extracts of the orthokeratotic skin (10.53 ± 4.32) were significantly higher than

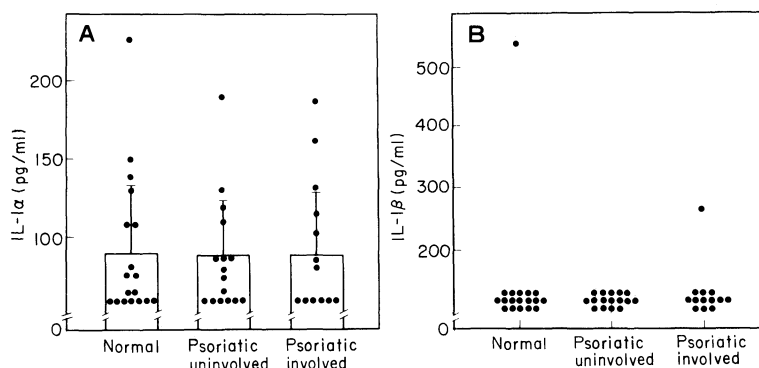


Fig. 1. Levels of IL-1 α (A) and β (B) in the suction blister fluids from normal, psoriatic uninvolved, and involved skin. The data are represented as means \pm S.D.

those in the psoriatic scale extracts (4.54 ± 3.03 ng/ml) ($p < 0.01$) (Fig. 2A). Although the mean of the levels of IL-1 β in the orthokeratotic horny tissue extracts (5.52 ± 5.02) was higher than that in the psoriatic scale extracts (2.32 ± 2.59 ng/ml), there was no significant difference between its levels between the orthokeratotic and psoriatic horny tissue extracts (Fig. 2B).

When the levels of IL-1 α and β were expressed per milligram protein, the IL-1 α levels in the horny tissue extracts of the orthokeratotic skin (10.40 ± 5.39) were significantly higher than those in the psoriatic scale extracts (1.65 ± 2.19 ng/ml) ($p < 0.01$) (Fig. 3A), and the levels of IL-1 β in the orthokeratotic horny tissue

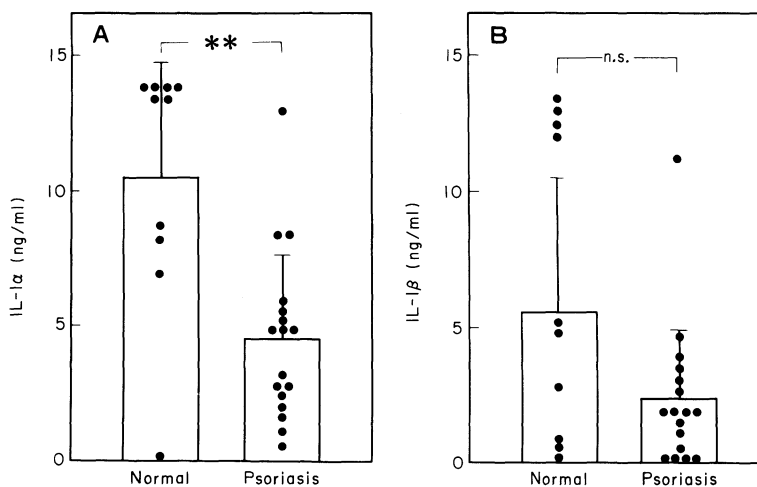


Fig. 2. Levels of IL-1 α (A) and β (B) expressed per milliliter in the horny tissue extracts of orthokeratotic (left) and psoriatic skin (right column). The data are shown as means \pm S.D. The levels of IL-1 α in the horny tissue extracts of orthokeratotic skin were significantly higher than those in the psoriatic scale extracts (** $p < 0.01$). n.s., not significant.

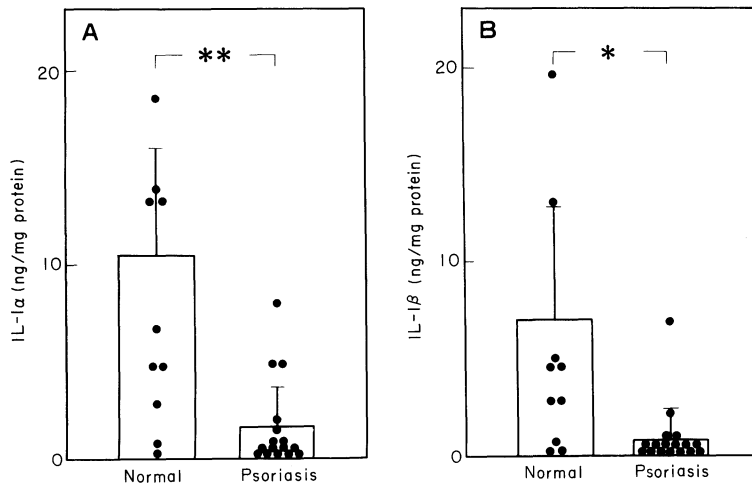


Fig. 3. Levels of IL- 1α (A) and β (B) expressed per milligram protein in the horny tissue extracts from orthokeratotic (left) and psoriatic skin (right column). The data are shown as means \pm S.D. The levels of IL- 1α and β in the horny tissue extracts of orthokeratotic skin were significantly higher than those in the psoriatic scale extracts (* $p < 0.05$ and ** $p < 0.01$, respectively).

extracts (5.44 ± 5.95) were also significantly higher than those in the psoriatic scale extracts (0.83 ± 1.61 ng/ml) ($p < 0.05$) (Fig. 3B).

Among the orthokeratotic horny tissue extracts, 6 out of 7 samples of plantar callus showed high IL- 1α as well as β levels, and the remaining 1 revealed low levels both in IL- 1α and β . Ichthyotic scale and normal stratum corneum skin extracts showed high IL- 1α but very low IL- β levels (13.31 vs. 0.42, 13.75 vs. 0.75

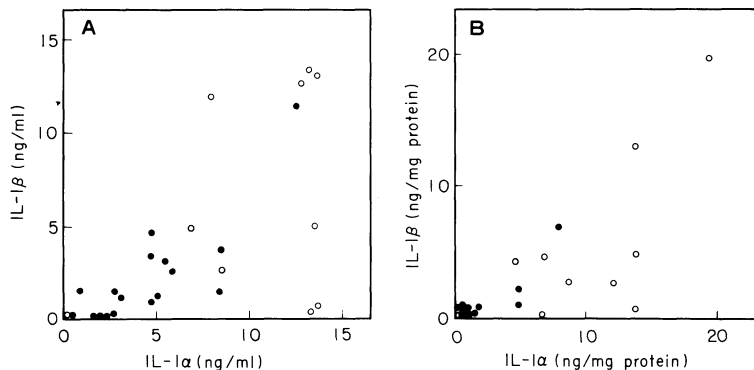


Fig. 4. Relationship between the levels of IL- 1α and β expressed per milliliter (A) and per milligram protein (B) in the horny tissue extracts of orthokeratotic (\circ) and psoriatic skin (\bullet). There was a significant relationship between IL- 1α and β levels when expressed per milliliter ($R = 0.58$, $p < 0.01$) (A) or per milligram protein ($R = 0.74$, $p < 0.01$) (B). Due to overlapping of the points, points less than the actual number are shown in (B).

ng/ml, respectively), while the plantar horny tissue extract of PRP showed both high IL-1 α and β levels (13.71 and 13.32 ng/ml, respectively).

Relationship between IL-1 α and β levels

There was a significant relationship between the levels of IL-1 α and β in the horny tissue extracts when their levels were expressed per milliliter ($R=0.58$, $p<0.01$) (Fig. 4A) or when expressed per milligram protein ($R=0.74$, $p<0.01$) (Fig. 4B). The levels of IL-1 α were significantly higher than those of IL-1 β .

Molecular sieve chromatography

No IL-1 α or β were detected after gel column fractionation of the suction blister fluids. The horny tissue extract of ichthyosis vulgaris showed a 31 kDa

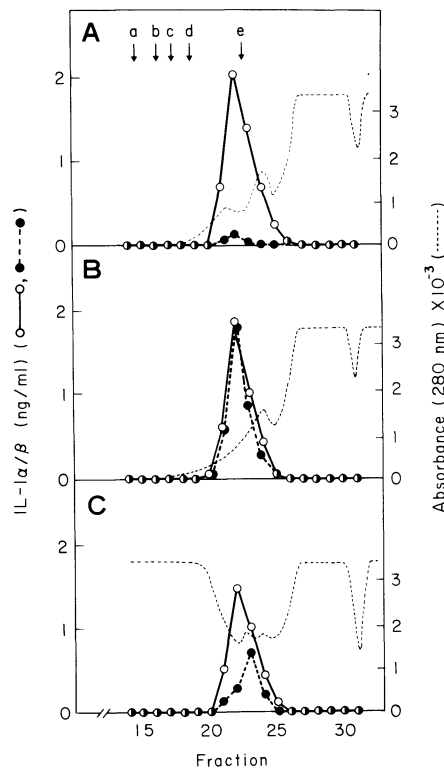


Fig. 5. Molecular sieve chromatography of horny tissue extracts. After injecting 0.5 ml of horny tissue extract of ichthyosis vulgaris (A), plantar callus (B), or psoriasis (C) were applied to TSK-G2000SW exclusion high performance liquid chromatography column, the column was eluted with phosphate-buffered saline, and fractions of 0.5 ml were collected. The fractions were assayed for IL-1 α (○—○) and β (●—●). The column was calibrated with dextran blue (2,000 kDa; a), γ -globulin (158 kDa; b), bovine serum albumin (67 kDa; c), ovalbumin (44 kDa; d), and carbonic anhydrase (29 kDa; e).

peak from IL-1 α and a very low peak from IL-1 β (Fig. 5A). The extract of a plantar callus revealed a coexistence of high peaks from IL-1 α and IL-1 β at 31 kDa (Fig. 5B). The scale extract of psoriasis showed a high 31 kDa peak from IL-1 α and a low peak from IL-1 β at 23 kDa (Fig. 5C).

DISCUSSION

The role of IL-1, which is constitutively produced by normal human epidermal keratinocytes (Gahring et al. 1985; Hauser et al. 1986; Bell et al. 1987), in psoriasis has been a great concern of many investigators (Camp et al. 1986; Takematsu et al. 1986; Gruaz et al. 1989; Romero et al. 1989). It has also not been settled whether the decreased IL-1 activity is due to an actual decrease or the presence of inhibitor of IL-1, and whether the biologic activity in the stratum corneum is due to IL-1 α , β , or both. We employed specific EIAs for IL-1 α and β in the present study, whose detection limits are 60 and 120 pg/ml, respectively (Tanaka et al. 1987); the assays well correlate with those of bioassay (Ohmoto et al. 1988). The present study revealed the presence of comparably low levels of IL-1 α in the suction blister fluids from normal, psoriatic uninvolved, and involved skin. In contrast, IL-1 β was detected only in two samples, one from normal and another from psoriatic involved skin, indicating that IL-1 α is a major IL-1 species produced in the human skin. Together with the results of the previous studies showing the predominant production of IL-1 α by cultured keratinocytes as identified on Northern blots (Kupper et al. 1986) and the strong staining for IL-1 α in human skin (Romero et al. 1989), we think that the keratinocyte-derived IL-1 constitute a major component in it.

In contrast to rather low levels of IL-1 α and β in the suction blister fluids, significantly high amounts of IL-1 α and β were detected in the horny tissue extracts. Accumulation of IL-1 within the horny tissue was also shown by its strongest reaction with anti-IL-1 antibodies in an immunohistochemical study (Romero et al. 1989). In the assessment of the levels of chemical mediators in suction blisters, therefore, a modification of the technique seems to be required in the future, e.g., collecting blister fluids after much longer skin contact.

In agreement with our previous study (Takematsu et al. 1986), IL-1 α levels in the orthokeratotic horny tissue extracts were significantly higher than those in the psoriatic scale extracts. Kupper et al. (1988) showed that keratinocytes allowed for in vitro differentiation release more IL-1 activity into the culture medium than do keratinocytes grown in MCDB153, a low calcium medium that prevents differentiation and stratification. Similar situation may occur in psoriatic lesional skin, although it may be argued that keratinocytes grown in the low calcium medium really represent those in psoriatic epidermis (Varani et al. 1989). It seems to be more plausible, however, that although IL-1 might be produced in psoriatic skin in amount comparable to that in normal skin, the amounts of IL-1 actually stored in psoriatic stratum corneum are lower than in normal stratum

corneum simply because of the rapid turnover of the psoriatic epidermis.

While high levels of IL-1 β were found in the horny tissue extracts of plantar callus, its levels were low in the horny tissue extracts from ichthyosis and normal skin. Didierjean et al. (1989a) also showed the presence of IL-1 α as well as β in the plantar calluses by EIA and immunoblot, and that the signal was more intense with IL-1 β than IL-1 α antibodies. This high levels of IL-1 β in the plantar callus could be derived from the permeated sweat. Hammerberg et al. (1989) showed that IL-1 β is increased in keratomed psoriatic skin specimens when compared with those from normal skin. This increase could partly be due to a contamination of the dermal papillae, which usually contain IL-1 β -producing macrophages.

The molecular cloning of IL-1 cDNA suggests that IL-1 is first synthesized without a conventional signal sequence as intracellular pro-hormones of 31 kDa molecule (Auron et al. 1984; March et al. 1985; Gray et al. 1986). IL-1 is processed by still undefined mechanism to a mature molecule of 17 kDa. IL-1 with an m.w. of 31 kDa is shown as cell-associated molecules (Brody and Durum 1989). The majority of IL-1 activity produced by keratinocytes was demonstrated to be cell-associated (Blanton et al. 1989). Gel column fractionation of the horny tissue extracts showed the presence of IL-1 α with as approximate m.w. of 31 kDa. Thus, this IL-1 with an m.w. of 31 kDa seem to represent the cell-associated molecule. The existence of membrane-associated IL-1 activity has been revealed in a variety of cells (Kurt-Jones et al. 1985; Nagelkerken and van Breda Vriesman 1986; Bakouche et al. 1987; Brody and Durum 1989). While major cell-associated IL-1 bioactivity is noted in free cytosolic molecules in monocytes (Matsushima et al. 1986; Fuhlbrigge et al. 1987), a significant component of the IL-1 activity was found to be associated with the cell surface in keratinocytes (Goldminz et al. 1987). Immunohistologically, IL-1 α is distributed predominantly intercellularly in normal skin (Oxholm et al. 1988; Didierjean et al. 1989b; Romero et al. 1989).

Molecular sieve chromatography of the IL-1-rich orthokeratotic horny tissue extracts also revealed that the constituents for immunoreactive IL-1 α and β to be variable depending upon the source of the horny tissue samples; the extract of ichthyosis vulgaris showed a 31 kDa peak from IL-1 α and a very low peak from IL-1 β , whereas the extract of callus showed a coexistence of peaks of IL-1 α and β , possibly due to contamination by sweat. Thus, we admit that most horny tissue samples used as orthokeratotic horny tissue in our study might not represent "normal" horny tissues. On the other hand, psoriatic scale extract showed a high peak from IL-1 α as well as a low peak from IL-1 β . As there are little sweating within psoriatic lesions, IL-1 β detected in the psoriatic scale extracts might have been produced by infiltrating macrophages.

The biologic implications of the IL-1 pools in the human epidermis are not understood. Although IL-1-like substance was shown to enhance keratinocyte

proliferation in vitro (Ristow 1987), recombinant IL-1 did not stimulate keratinocyte growth (Morhenn et al. 1989). IL-1 stimulates production of IL-6 (Kupper et al. 1989) by keratinocytes. Moreover, human epidermis overlying a type IV immune reaction contain increased amounts of IL-1 activity (Larsen et al. 1988), and ultraviolet light therapy for psoriasis increased plasma IL-1 level (Konnikov et al. 1989). Despite these pieces of evidence suggestive for its role in skin inflammation and immunological reactions, the roles of IL-1 in the pathogenesis of psoriasis remains to be clarified.

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