Original contribution

Archives of Dermatological Research

Arch Dermatol Res (1992) 284:71–76

Psoriatic skin reveals the in vivo presence of an epidermal IL-1 inhibitor

N.-I. Kim[1,*], K. D. Cooper[2], G. J. Fisher[1], O. Baadsgaard[1,], J. J. Voorhees[1], and C. Hammerberg[1]**

[1] Department of Dermatology, University of Michigan Medical School and
[2] The Veterans Administration Hospital, Ann Arbor, Mich., USA

Received April 27, 1991

Summary. Production of inhibitor(s) of IL-1 activity can be induced in keratinocytes by exposure to UVB. We describe in this study the characterization of an endogenous constitutively expressed IL-1 inhibitor which is present in extracts of human psoriatic epidermal keratome biopsies. Size-fractionated extracts of normal human epidermis did not reveal IL-1 inhibitory factor(s) activity in normal epidermis. Psoriatic epidermal extracts, however, contained virtually no IL-1 bioactivity and inhibited the activity of recombinant human IL-1β. This IL-1 inhibitor has a molecular weight of approximately 30 kDa and a pI of 5.3, as revealed by fast protein liquid chromatography size fractionation and chromatofocusing of psoriatic epidermal extracts. IL-1 inhibitory activity was not blocked by neutralizing anti-TGFβ monoclonal antibody. It did not have any inhibitory effect upon normal cellular proliferation but could block the IL-1 induction of IL-2 production by LBRM.33 cells as late as 4 h after exposure of LBRM.33 cells to IL-1. Thus, in vivo human psoriatic epidermis expresses an IL-1 inhibitor that specifically inhibits IL-1 activity but which appears distinct from previously described UV-induced epidermal IL-1 inhibitory activity or TGFβ.

Key words: Psoriasis – Epidermis – IL-1 – IL-1 inhibitor

The epidermis is often the first point of contact between the immune system and the environment. Numerous cytokines are produced by cells located within the epidermis for the purpose of modulating the immune response. Among the cytokines produced by keratinocytes are: IL-1α and β [26], murine IL-3 [31], IL-6 [28], GM-CSF [27], IL-8 [29], TNFα [38] and TGFα [14, 18] and β [15]. The epidermis can also produce inhibitors of cytokines, in particular IL-1. TGFβ can block some effects of IL-1 [46], and accounts, at least in part, for the inhibitory activity of keratinocyte lymphocyte inhibitory factor [37]. An epidermal inhibitor of IL-1 activity that is inducible by UVB radiation has also been described. This IL-1 inhibitor has a molecular weight of 40 kDa and an isoelectric point of 8.8 [40]. Additional IL-1 inhibitors have been described in other systems. IL-1 inhibitors have been detected in the urine of patients with fever [30, 41] and monocytic leukemia [3], and natural antagonists of the IL-1 receptor have been described [5, 21].

We have previously found that IL-1 activity is markedly decreased in psoriatic epidermis [9]. This was due to a decrease in IL-1α protein and non-functionality of increased IL-1β protein levels. However, the clear absence of IL-1 activity in many patient samples, despite the presence of detectable IL-1α (albeit at reduced levels), suggested that the reduced functional activity may also be influenced by the presence of an IL-1 inhibitor. We found that, indeed, psoriatic cytosolic extracts from involved skin, but not uninvolved skin, inhibited IL-1 activity. The epidermal IL-1 inhibitor detected in psoriatic epidermis was functionally and physicochemically characterized. Based upon these results the molecule was identified as being different from such epidermal IL-1 inhibitors as TGFβ and the UVB-inducible keratinocyte IL-1 inhibitor.

Materials and methods

Materials

Polyethylene glycol (PEG, MW 8000), 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT), Tween 20, and phenylmethylsulfonyl fluoride (PMSF) were obtained from Sigma Chemical Company, St. Louis, Mo., USA. A fast protein liquid chromatography (FPLC) apparatus, Mono P anion exchange

* *Present address:* Department of Dermatology, Kyung Hee Medical Center, Seoul, Korea
** *Present address:* Department of Dermatology, Gentofte Hospital, Hellerup, Denmark
Correspondence to: Kevin D. Cooper, M.D., The University of Michigan, Immunodermatology Unit, Kresge I, Room 5548, Ann Arbor, MI 48109-0530, USA

column and Polybuffer 74 were purchased from Pharmacia, Piscataway, N. J., USA. A TSK G2000SW molecular sizing column was from Toyo Sodo, Tokyo, Japan. Human recombinant rIL-1β was a gift from Dr. R. C. Newton (DuPont Glenolden Lab., Glenolden, Pa., USA). Peroxidase-conjugated goat anti-rabbit IgG was from Tago, Inc. (Burlingame, Calif., USA). The monoclonal antibody to TGFβ2 (1D11.16), which crossreacts with TGFβ1 [11], was obtained from Collagen Corp. (Palo Alto, Calif., USA).

Preparation of keratome biopsy cytosols

Keratome biopsies were taken from untreated psoriatic plaques or from normal skin of the hips or buttocks. Biopsies were taken using a Castro Viejo keratome set at 0.2–0.3 mm for normal skin and 0.5 mm for psoriatic skin. Keratome strips were immediately snap frozen in liquid nitrogen.

Keratome biopsies were pulverized under liquid nitrogen, then Dulbecco's phosphate buffered saline (DPBS, Ca^{2+}- and Mg^{2+}-free) with 1 m*M* PMSF and 0.03% PEG was added to obtain a solution consisting of 100 mg wet sample per millilitre of buffer. After homogenization with a glass homogenizer, cytosols were obtained by ultracentrifugation ($100000 \times g$ for 1 h), then sterile filtered and stored at −70°C.

Fast protein liquid chromatography (FPLC)

The biopsy cytosol (2 ml) was applied to a TSK G2000SW column equilibrated with DPBS plus 0.03% PEG. The flow rate was 1.0 ml/min and 2.0 ml fractions were collected. The column was calibrated with bovine serum albumin (Sigma, 66000 MW), carbonic anhydrase (Sigma, 29000 MW), cytochrome C (Sigma 12400 MW) and aprotinin (Sigma, 6500 MW). Each fraction was sterilized with a 0.2 µm millipore filter and assayed for the presence of IL-1 inhibitory activity.

TSK G2000SW fractions with inhibitory activity were pooled, dialyzed against 0.025 *M* Bis-Tris (pH 6.3) and loaded into a Mono P column (0.5 × 20 cm) equilibrated with 0.025 *M* Bis-Tris (pH 6.3). The column was eluted with 10% polybuffer 74 (pH 4.0) at a rate of 0.5 ml/min and 1 ml fractions were collected. Fractions were dialyzed against DPBS using dialysis membrane with a 12000–14000 MW cut-off (Spectra, Por, Los Angeles, Calif., USA). Fractions were then sterile filtered and assayed for IL-1 inhibitory activity.

IL-1 inhibitory assay

Thymocyte proliferation. The presence of IL-1 inhibitory activity in biopsy cytosols was detected by inhibiting the co-proliferation signal of IL-1 upon mouse thymocytes stimulated with PHA. C3H/HeN mouse thymocyte cells (50 µl, 3×10^7/ml), 50 µl PHA (4 µg/ml), 50 µl rIL-1β (1.4 U/ml) and 50 µl biopsy cytosol at various dilutions were added per well of 96-well flat-bottomed plates. Each dilution and controls were done in triplicate. After a 3-day incubation period at 37°C, thymocytes were pulsed with 1 µCi ^{3}H-thymidine and incubated for another 6 h before harvesting and counting on a liquid scintillation counter.

LBRM.33 IL-2 production. Alternatively, IL-1 inhibitory activity was measured by blocking the production of IL-2 by LBRM.33 cells in response to IL-1. Fractions from the TSK G2000SW column were tested for IL-1 inhibitory activity. Each undiluted fraction (50 µl) was added to LBRM.33 cells (50 µl, 10^5 cells/well), PHA (50 µl, 10 µg/well) and rIL-1β (50 µl, 1 U/well) in flat-bottomed 96-well microtiter tissue culture plates and the mixture incubated for 24 h at 37°C. Supernatants from stimulated LBRM.33 cells were serially diluted, and 100 µl of each dilution was then transferred into flat-bottomed 96-well microtiter plates containing 10000 HT-2 (20 µl) cells, an IL-2 addictive murine T-cell clone. After a 48 h incubation at 37°C, viability of the HT-2 cells was tested by the degree of MTT reduced by live cells. The OD of reduced MTT was measured by an ELISA reader using wavelengths of 570 and 620 nm as described by Mosmann [35]. Quantifitation of the amount of IL-2 produced by LBRM.33 cells was made in relation to an IL-2 standard curve that had 250 U/ml of IL-2 activity (rat TCGF). Comparison of the 50% of maximal response of the LBRM.33 sample was made with the standard to obtain the amount of IL-2 U/ml.

Neutralization assay using anti-TGFβ1 serum

Monoclonal antibody (40 µl, 150 ng/well) against TGFβ1 (1D11.16) was added to 96-well flat-bottomed tissue culture plates and preincubated with 40 µl TGFβ1 (0.5 ng/well) or undiluted TSK G2000SW fraction pools containing IL-1 inhibitory activity for 2 h at 37°C. Then 40 µl of each of the following were added to achieve the indicated concentrations: rIL-1β (1 U/well), PHA (2 µg/well), and LBRM.33 cells (10^5 cells/well). The cultures were then incubated for 24 h at 37°C. The IL-1 inhibitor assay was then continued as described above.

Cell proliferation inhibition assays

A squamous cell carcinoma cell line (SCC 38) (100 µl) provided by Dr. T. E. Carey (Department of Otorhinolaryngology, University of Michigan Medical School) [22] or low passage human dermal fibroblasts (10^5 cells/ml) were added to each well of flat-bottomed 96-well tissue culture plates and incubated overnight at 37°C. Medium was removed and 50 µl fresh medium and 50 µl undiluted fractions or DPBS were added to each well. Cells were then incubated for 48 h, pulsed with 1 µCi ^{3}H-thymidine per well for 6 h, harvested using a PHD cell harvester and counted in the presence of Bio-Safe liquid scintillation fluid.

HT-2 cellular proliferation was measured by the degree of MTT reduced. HT-2 cells (20 µl, 5×10^5/ml) were added to 50 µl 5% Rat TCGF and 50 µl undiluted fractions. After a 48 h incubation period at 37°C, 10 µl MTT (5 mg/ml) was added and incubated for another 4 h at 37°C. The OD of reduced MTT was measured by an ELISA reader using wavelengths of 570 and 670 nm as described by Mosmann [35]. The amount of suppression of HT-2 growth was calculated by dividing the delta absorbance obtained in the presence of fractions by that obtained with cells grown without fractions but in the presence of 5% rat TCGF.

Results

Psoriatic cytosolic extracts of involved, but not uninvolved, skin block IL-1 activity in the thymocyte proliferation assay

The thymocyte co-stimulation assay assesses the co-stimulatory activity of IL-1 with suboptimal doses of PHA to activate murine thymocytes. When skin cytosols were added to murine thymocytes stimulated with PHA and a known amount of IL-1β, psoriatic cytosols from involved skin, but not cytosols from uninvolved skin, blocked the IL-1 stimulation of thymocyte proliferation

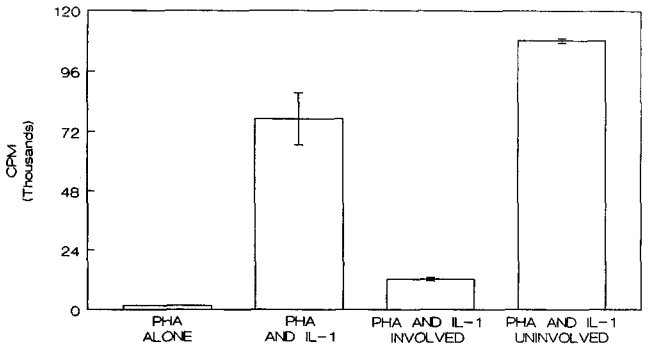

Fig. 1. Psoriatic involved skin, but not uninvolved skin, cytosol inhibits the IL-1 co-mitogenic signal to PHA-stimulated murine thymocytes. Proliferative response of mouse thymocytes to PHA (1 μg/ml) alone or PHA (1 μg/ml) and IL-1β (0.35 U/ml) is indicated by the *first* and *second bars*, respectively. The effect of the addition of involved or uninvolved skin cytosol to mouse thymocytes stimulated with IL-1β and PHA is indicated by the *third* and *fourth bars*, respectively. Data is expressed as mean cpm ± SEM of triplicates from one experiment

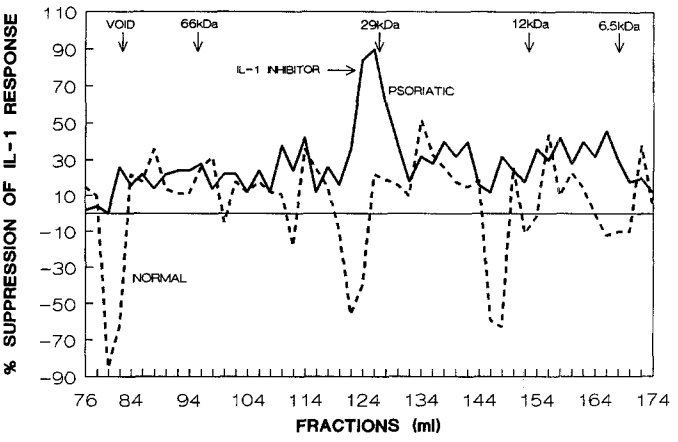

Fig. 2. Normal and psoriatic keratome biopsy cytosols of equivalent protein concentration were fractionated by FPLC on a TSK G2000SW column. Fractions were then assayed for IL-1 inhibitory activity. Levels in normal keratome biopsy cytosols are indicated by a *broken line* and by a *solid line* for psoriatic cytosol fractions. Data is representative of six and three independent fractionations of psoriatic and normal keratome biopsy cytosols, respectively

(Fig. 1). Cytosols from lesional psoriatic skin were virtually devoid of IL-1 activity in the mouse thymocyte assay (mean ± SEM, 3900 ± 1290 cpm; $n = 11$), whereas, uninvolved psoriatic skin (mean ± SEM, 15300 ± 3522 cpm; $n = 10$) and control skin (mean ± SEM, 20948 ± 7030 cpm; $n = 6$) contained substantial activity, similar to our previous findings utilizing the LBRM.33 cell line [9].

Detection of IL-1 inhibitory activity in size-fractionated psoriatic epidermal cytosols

To determine whether IL-1 inhibitory activity in psoriatic skin was similar in size to previously described IL-1 inhibitors, cytosols were size-fractionated by FPLC. The fractions were then tested for suppression of IL-1 stimulation of LBRM.33 cells, an assay that is more specific for IL-1 than the thymocyte proliferation assay. Psoriatic keratome biopsy cytosols, when fractionated by FPLC on a TSK G2000SW column, revealed the presence of a molecule of about 30 kDa with IL-1 inhibitory activity (Fig. 2, *solid line*). When normal keratome biopsy cytosol of equivalent protein concentration was fractionated in a similar manner, no IL-1 inhibitory activity was observed at this molecular weight (Fig. 2, *dashed line*).

Epidermal IL-1 inhibitor pI differs from EC-contra-IL-1

Because of the similarity in molecular weight with the UV-inducible epidermal-contra-IL-1 molecule, the isoelectric point of the psoriatic epidermal IL-1 inhibitor was determined. The 30 kDa fractions from the TSK G2000SW column with IL-1 inhibitory activity were pooled and chromatofocused by FPLC on a Mono P column. IL-1 inhibitory activity eluted at pH 5.3 (Fig. 3). This is much more acidic than the pI 8.8 reported for EC-contra-IL-1 [40].

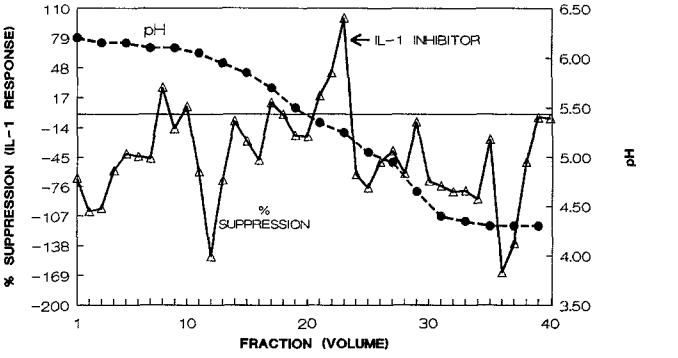

Fig. 3. Fractions from the TSK G2000SW column containing IL-1 inhibitory activity were pooled and loaded into a Mono P column. Both the fractions and the Mono P column were equilibrated with 0.025 *M* Bis-Tris buffer (pH 6.3) before the fractions were loaded. The sample was eluted with Polybuffer 74 (pH 4.0). pH gradient is indicated by *closed circles*. Percent suppression of IL-1 response is indicated by *open triangles*

Epidermal IL-1 inhibitor is not TGFβ1

The homodimer form of TGFβ1 (MW, 25 kDa) is a known inhibitor of IL-1 action [46] and is also similar in molecular weight to the epidermal IL-1 inhibitor (MW, 30 kDa). We, therefore, determined whether the IL-1 inhibitory activity could be neutralized by a neutralizing anti-TGFβ1 monoclonal antibody. TGFβ1 was indeed capable of inhibiting the response of LBRM.33 cells to IL-1 (Fig. 4, *second bar*). A monoclonal antibody against TGFβ1 (1D1.116) was able to neutralize the TGFβ1 inhibitory activity (Fig. 4, *third bar*). However, when this monoclonal antibody was added to the epidermal IL-1 inhibitor no effect was observed on the IL-1 inhibitory activity (Fig. 4, *fifth bar*). Even when the monoclonal antibody concentration was utilized at a concentration 100 times that used in Fig. 4, no neutralization of the

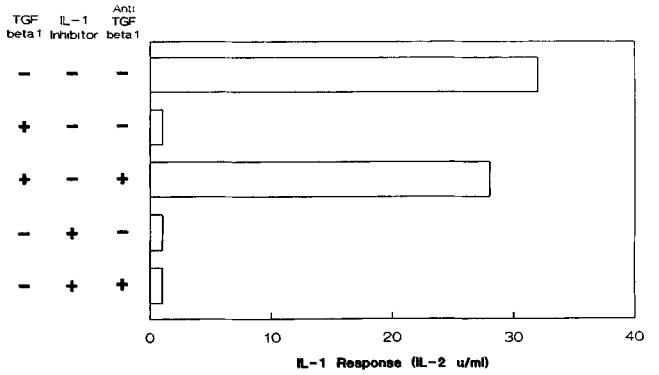

Fig. 4. Fractions from the TSK G2000SW column containing IL-1 inhibitory activity of 30 kDa molecular weight were pooled and used in these experiments. LBRM.33 cells were exposed to TGFβ1 (*second row*), TGFβ1 and anti-TGFβ1 (*third row*), IL-1 inhibitor (*fourth row*), or IL-1 inhibitor and anti-TGFβ1 (fifth row). The IL-1 response is given in terms of the amount of IL-2 produced by the stimulated LBRM.33 cells

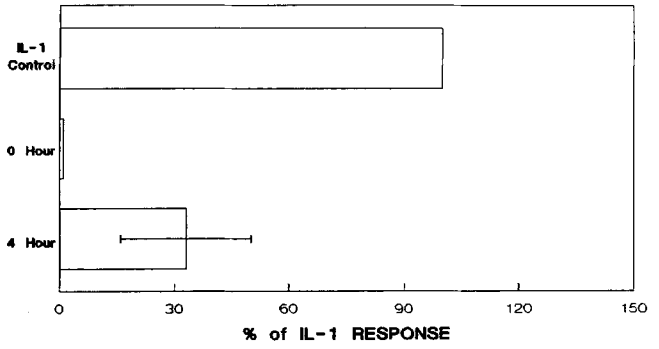

Fig. 5. TSK G2000SW fractions containing the 30 kDa IL-1 inhibitor were assayed for an inhibitory effect after IL-1 receptor binding and stimulation had already been initiated. LBRM.33 cells were stimulated with rIL-1β and PHA and the IL-1 inhibitor was added at the initiation of the stimulant (*middle row*, 0 h) or 4 h later (*bottom row*, 4 h). Results are expressed as the percent of the IL-1-induced response (IL-2 production) and are the mean $\pm$ SD of three experiments. Addition of IL-1 inhibitor at 0 h (*middle row*) always resulted in no IL-1 response, thus no error bars

epidermal IL-1 inhibitor activity was observed (not shown).

Epidermal IL-1 inhibitor has a delayed inhibitory effect

A recently cloned IL-1 receptor antagonist encodes for a protein of 17–25 kDa MW that inhibits IL-1 activity by competing for binding to the IL-1 receptor [5, 21]. If the psoriatic epidermal IL-1 inhibitor was acting by blocking the IL-1 receptor, then addition of the IL-1 inhibitor 4 h after exposure of the LBRM.33 cells to IL-1 should result in no inhibition. Binding of IL-1 molecules to IL-1 receptors expressed on LBRM.33 cells is rapid, with equilibrium being reached within 1 h [13]. When the 30 kDa IL-1 inhibitor was given to LBRM.33 cells 4 h after IL-1 stimulation, only one-third of the normal IL-1 response was observed (Fig. 5). The degree of delayed inhibition of LBRM.33 IL-2 production by inhibitor ranged from

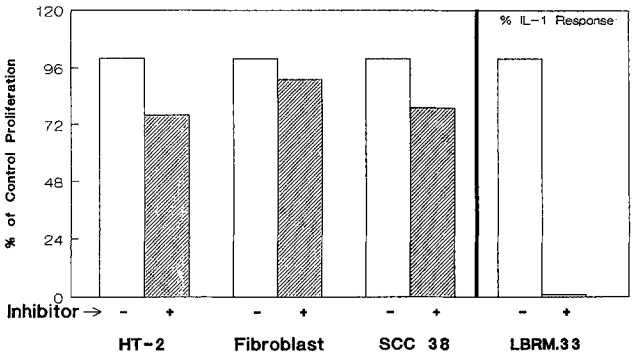

Fig. 6. The TSK G2000SW purified IL-1 inhibitor was added to various cell types to determine the effect upon cell proliferation. HT-2 cell growth (*first two bars*) was measured by MTT reduction. ^{3}H-thymidine incorporation was used to measure cell proliferation for fibroblast (*second pair of bars*) and SCC 38 cells (*third pair of bars*). The *last pair of bars* represents the effect of the IL-1 inhibitor upon the LBRM.33 response to IL-1. *Unhatched bars* are cells cultured without IL-1 inhibitor and *hatched bars* represents cells cultured in the present of the IL-1 inhibitor. Results are expressed as percent of cell growth in the absence of IL-1 inhibitor

45% to 100% in three experiments, indicating an action on LBRM.33 cells that is beyond pure receptor antagonism.

Epidermal IL-1 inhibitor does not non-specifically inhibit cell growth

The 30 kDa epidermal IL-1 inhibitor was added to other cell types to determine if it affects the growth response of the HT-2 T-cell clone to IL-2 or has antiproliferative effects upon other cells such as a squamous carcinoma cell line (SCC38) or dermal fibroblasts (Fig. 6). When the epidermal IL-1 inhibitor was added directly to HT-2 cells stimulated with IL-2, no effect upon HT-2 proliferation was observed (Fig. 6). No effect of the inhibitor was observed on proliferation of dermal fibroblasts or the SCC 38 cell line, as measured by ^{3}H-thymidine uptake (Fig. 6). By contrast, the same semi-purified 30 kDa inhibitor used in the above experiments completely suppressed the IL-1 response of LBRM.33 cells (Fig. 6).

Discussion

We have demonstrated the presence of an epidermal IL-1 inhibitor in psoriatic lesions. The expression of this inhibitor, in concert with previously described reduced IL-1α levels, accounts for the virtual absence of IL-1 activity in the intraepidermal compartment of psoriatic lesions. This IL-1 inhibitor has a molecular weight of approximately 30 kDa by FPLC on a silica gel column and a pI of 5.3. It also specifically inhibits IL-1-stimulated responses without affecting fibroblast or squamous cell carcinoma cellular growth or the response of a T-cell line, HT-2, to IL-2. These properties would suggest that it is not like other known epidermal inhibitors of IL-1-inducible responses.

TGFβ1 is produced by keratinocytes [15] and is capable of inhibiting the response of T cells to IL-1 [46]. Thus, the 30 kDa epidermal IL-1 inhibitory activity could be attributed to the TGFβ1 produced by keratinocytes. Three lines of evidence suggest that this molecule is not TGFβ1. First, the IL-1 inhibitory molecule did not alter fibroblast proliferation, either by stimulating or inhibiting growth, known properties of TGFβ1 [43]. Much stronger evidence is provided by the inability of a neutralizing monoclonal antibody to TGFβ1 to neutralize the 30 kDa epidermal IL-1 inhibitory activity. Finally, the pI of TGFβ1, which ranges between 6.8 and 7.4 [39], is distinct from that of the inhibitor detected in psoriasis.

The other known IL-1 inhibitor present in the epidermis is inducible by UVB radiation. This IL-1 inhibitor, also known as EC-contra-IL-1, has a molecular weight of 40 kDa and a pI of 8.8 [40]. The smaller size of the IL-1 inhibitor described in this study, its much more acidic pI and its lack of induction in keratinocytes by UVB (data not shown) appear to distinguish it from EC-contra-IL-1.

The size (17 – 25 kDa) and pI (4.5 – 5.3) ranges of the IL-1 receptor antagonist produced by peripheral blood mononuclear cells are similar to the physicochemical characteristics of the inhibitor detected in psoriatic cytosols, albeit somewhat smaller in size [24, 42]. The ability of the epidermal IL-1 inhibitor activity to act 4 h after IL-1 stimulation would suggest that the epidermal protein's activity is not limited to IL-1 receptor antagonism [5, 21]. It has recently been demonstrated that a natural IL-1 inhibitor which acts as a receptor antagonist has to be added within 20 min of IL-1 in order to be effective [33]. This question will finally be clarified by the determination of the amino-acid sequence of the epidermal IL-1 inhibitor contained in psoriatic epidermis; however, we have not yet been able to obtain amino-acid analysis of our epidermal material due to insufficient purity.

Other potentially related inhibitory activities produced by the epidermis might include products of arachidonic acid metabolism [4, 45], chalone-like growth inhibitors [6, 7, 32], growth-inhibitory pentapeptides [16] and proteins [23]. The small size of these molecules and their generalized effects on cell lines and bioassays tends to make them unlikely candidates.

Our failure to detect the epidermal IL-1 inhibitor in normal epidermis could be due to either the restriction of expression of the molecule to psoriatic lesions or the high levels of IL-1α in normal epidermis compared with psoriatic lesions. Psoriatic fractions from the TSK G2000SW column containing IL-1 inhibitory activity also lacked significant levels of IL-1α. However, equivalent fractions in the region where IL-1 inhibitory activity is observed in psoriatic extracts from fractionated normal keratome biopsy cytosol possessed high levels of IL-1α (not shown), suggesting that the lack of IL-1 inhibitory activity in the normal fractions may be due to the presence of endogenous IL-1α molecules which overwhelm the IL-1 inhibitory activity. However, the cellular source of this IL-1 inhibitor is not clear due to the large number of inflammatory cells present in psoriatic lesions, such as monocytes, that could be producing IL-1 inhibitors [21]. This may explain the presence of this IL-1 inhibitor in psoriatic skin and its apparent absence in normal epidermis.

The occurrence of constitutively produced IL-1α and β molecules in the epidermis would predict the presence of a balancing IL-1 inhibitor which could specifically regulate IL-1 responses in the skin. Such an IL-1 inhibitor may be needed where the release of large amounts of IL-1 by the keratinocytes would affect a wide variety of cells such as fibroblasts, endothelial cells, Langerhans cells, T cells and even keratinocytes [12]. Because IL-1 drives activated T cells to differentiate toward a TH_2 phenotype (high IL-4, low γ-interferon), low IL-1 activity due to the combination of an IL-1 inhibitor and low IL-1α [9, 44] might explain the tendency of psoriatic skin to harbor CsA-sensitive T cells that produce γ-interferon [1, 2, 8, 19, 20, 34], that is a TH_1 phenotype [36], and to Koebnerize with γ-interferon [17, 25]. Because of the diversity of cells affected by epidermal IL-1 molecules, an IL-1 inhibitor that would specifically modulate the IL-1 response and not affect other cellular functions might play a critical role. The IL-1 inhibitor isolated from psoriatic epidermis, and possibly normal epidermis, could thus be involved in the down-regulation of IL-1-mediated responses in psoriatic skin [10].

Acknowledgement. This work was supported in part by Kyung Hee University (N-IK), the Dermatology Foundation (CH), NIH grant no. 86-2502-J1-1K08/AM01770-01 (KDC), the VA Merit Review Board (KDC), NIH grant no. AR39691-02 (GJF), and the Babcock Foundation.

References

1. Baadsgaard O, Gupta AK, Taylor RS, Ellis CN, Voorhees JJ, Cooper KD (1989) Psoriatic epidermal cells demonstrate increased numbers and function of non-Langerhans antigen-presenting cells. J Invest Dermatol 92:190 – 195
2. Baadsgaard O, Tong P, Elder JT et al. (1990) UM4D4 + (CDw60) T-cells are compartmentalized into psoriatic skin and release lymphokines that induce a keratinocyte phenotype expressed in psoriatic lesions. J Invest Dermatol 95:275 – 282
3. Balavoine J-F, de Rochemonteix B, Williamson K, Seckinger P, Cruchaud A, Dayer J-M (1986) Prostaglandin E2 and collagenase production by fibroblasts and synovial cells is regulated by urine-derived human interleukin 1 and inhibitor(s). J Clin Invest 78:1120 – 1124
4. Burrall B, Ziboh VA (1986) Increased biosynthesis of lipoxygenase products by UVB-irradiated guinea pig epidermis: evidence of a cyclooxygenase inhibitor. J Invest Dermatol 86:643 – 648
5. Carter DB, Deibel MR Jr, Dunn CJ et al. (1990) Purification, cloning, expression and biological characterization of an interleukin-1 receptor antagonist protein. Nature 344:633 – 638
6. Chopra DP, Flaxman BA (1973) Brief Communication: Mitotic inhibition of epidermal cells from psoriasis lesions in vitro by extracts from normal human skin. J Natl Cancer Inst 50:281 – 283
7. Chopra DP, Yu RJ, Flaxman BA (1972) Demonstration of a tissue specific inhibitor of mitosis of human epidermal cells in vitro. J Invest Dermatol 59:207 – 210
8. Cooper KD, Baadsgaard O, Gupta A et al. (1987) Phenotype and function of Cyclosporine A-sensitive epidermal immuno competent cells in psoriasis. Clin Res 35:387A

9. Cooper KD, Hammerberg C, Baadsgaard O et al. (1990) IL-1 activity is reduced in psoriatic skin: Decreased IL-1 alpha and increased non-functional IL-1 beta. J Immunol 144:4593–4603
10. Cooper KD, Hammerberg C, Baadsgaard O et al. (1990) Interleukin-1 in human skin: Dysregulation in psoriasis. J Invest Dermatol 95:24S–26S
11. Dasch JR, Pace DR, Waegell W, Inenaga D, Ellingsworth L (1989) Monoclonal antibodies recognizing transforming growth factor-beta. Bioactivity neutralization and transforming growth factor beta2 affinity purification. J Immunol 142:1536–1541
12. Dinarello CA (1989) Interleukin-1 and its biologically related cytokines. Adv Immunol 44:153–205
13. Dower SK, Kronheim SR, March CJ et al. (1985) Detection and characterization of high affinity plasma membrane receptors for human interleukin 1. J Exp Med 162:501–515
14. Elder JT, Fisher GJ, Lindquist PB et al. (1989) Overexpression of transforming growth factor alpha in psoriatic epidermis. Science 243:811–814
15. Elder JT, Ellingsworth LR, Fisher GJ, Voorhees JJ (1990) Transforming growth factor-beta in psoriasis. Pathogenesis and therapy. Ann NY Acad Sci 593:218–230
16. Elgjo K, Reichelt KL, Hennings H, Michael D, Yuspa SH (1986) Purified epidermal pentapeptide inhibits proliferation and enhances terminal differentiation in cultured mouse epidermal cells. J Invest Dermatol 87:555–558
17. Fierlbeck G, Rassner G, Muller C (1990) Psoriasis induced at the injection site of recombinant interferon gamma. Results of immunohistologic investigations. Arch Dermatol 126:351–355
18. Gottlieb AB, Chang CK, Posnett DN, Fanelli B, Tam JP (1988) Detection of transforming growth factor alpha in normal, malignant, and hyperproliferative human keratinocytes. J Exp Med 167:670–675
19. Gottlieb AB, Luster AD, Posnett DN, Carter DM (1988) Detection of a gamma interferon-induced protein IP-10 in psoriatic plaques. J Exp Med 168:941–948
20. Griffiths CEM, Voorhees JJ, Nickoloff BJ (1989) Gamma interferon induces different keratinocyte cellular patterns of expression of HLA-DR and DQ and intercellular adhesion molecule-I (ICAM-I) antigens. Br J Dermatol 120:1–7
21. Hannum CH, Wilcox CJ, Arend WP et al. (1990) Interleukin-1 receptor antagonist activity of a human interleukin-1 inhibitor. Nature 343:336–340
22. Hoffman HT, Subnani M, Cha M et al. (1990) Calcium regulation of antigen expression on normal and malignant human squamous cells in vitro. Arch Otolaryngol Head Neck Surg 116:299–303
23. Jensen PK, Bolund L (1986) Changes in proliferating cell subpopulations and mitotic activity in human epidermal cultures treated with epithelial growth inhibitors. J Invest Dermatol 86:46–50
24. Kligman LH (1989) Symposium on models for the study of human photoaging: American society for photobiology. Photochem Photobiol 50:903–905
25. Kowalzick L, Weyer U (1990) Psoriasis induced at the injection site of recombinant interferons. Arch Dermatol 126:1515–1516
26. Kupper TS, Ballard DW, Chua AO et al. (1986) Human keratinocytes contain mRNA indistinguishable from monocyte interleukin 1 alpha and beta mRNA. J Exp Med 164:2095–2100
27. Kupper TS, Lee F, Coleman D, Chodakewitz J, Flood P, Horowitz M (1988) Keratinocyte derived T-cell growth factor (KTGF) is identical to granulocyte macrophage colony stimulating factor (GM-CSF). J Invest Dermatol 91:185–188
28. Kupper TS, May L, Birchall N, Sehgal P (1988) Keratinocytes produce interleukin-6, a cytokine which can provide a 2nd signal in the activation of T cells (abstr). Clin Res 36:665A
29. Larsen CG, Anderson AO, Oppenheim JJ, Matsushima K (1989) Production of interleukin-8 by human dermal fibroblasts and keratinocytes in response to interleukin-1 or tumour necrosis factor. Immunology 68:31–36
30. Liao Z, Haimovitz A, Chen Y, Chan J, Rosenstreich DL (1985) Characterization of a human Interleukin 1 inhibitor. J Immunol 134:3882–3886
31. Luger TA, Wirth V, Kock A (1985) Epidermal cells synthesize a cytokine with interleukin 3-like properties. J Immunol 134:915–919
32. Marrs JM, Voorhees JJ (1971) A method for bioassay of an epidermal chalone-like inhibitor. J Invest Dermatol 56:174–181
33. Mazzei GJ, Seckinger PL, Dayer J-M, Shaw AR (1990) Purification and characterization of a 26-kDa competitive inhibitor of interleukin 1. Eur J Immunol 20:683–689
34. Morhenn VB, Abel EA, Mahrle G (1982) Expression of HLA-DR antigen in skin from patients with psoriasis. J Invest Dermatol 78:165–168
35. Mosmann T (1983) Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. J Immunol Methods 65:55–63
36. Mosmann TR, Cherwinski H, Bond MW, Giedlin MA, Coffman RL (1986) Two types of murine helper T cell alone. I. Definition according to profiles of lymphokine activities and secreted proteins. J Immunol 136:2348–2357
37. Nickoloff BJ (1988) Keratinocytes produce a lymphocyte inhibitory factor which is partially reversible by an antibody to transforming growth factor-beta. Ann NY Acad Sci 548:312–320
38. Oxholm A, Oxholm P, Staberg B, Bendtzen K (1989) Interleukin-6 in the epidermis of patients with psoriasis before and during PUVA treatment. Acta Derm Venereol (Stockh) 69:195–199
39. Roberts AB, Anzano MA, Lamb LC, Smith JM, Sporn MB (1981) New class of transforming growth factors potentiated by epidermal growth factor: Isolation from non-neoplastic tissues. Proc Natl Acad Sci USA 78:5339–5343
40. Schwarz T, Urbanska A, Gschnait F, Luger TA (1987) UV-irradiated epidermal cells produce a specific inhibitor of interleukin I activity. J Immunol 138:1457–1463
41. Seckinger P, Williamson K, Balavoine J-F et al. (1987) A urine inhibitor of Interleukin 1 activity affects both Interleukin 1alpha and 1beta but not tumor necrosis factor alpha. J Immunol 139:1541–1545
42. Segal R, Mozes E, Yaron M, Tartakovsky B (1989) The effects of methotrexate on the production and activity of interleukin-1. Arthritis Rheum 32:370–377
43. Sporn MB, Roberts AB, Wakefield LM, de Crombrugghe B (1987) Some recent advances in the chemistry and biology of transforming growth factor-beta. J Cell Biol 105:1039–1045
44. Takematsu H, Terui T, Ohkohchi K, Tagami H, Suzuki R, Kumagai K (1986) Interleukin-1-like activity in horny layer extracts: decreased activity in scale extracts of psoriasis and sterile pustular dermatoses. Dermatologica 172:236–240
45. Voorhees JJ (1983) Leukotrienes and other lipoxogenase products in the pathogenesis and therapy of psoriasis and other dermatoses. Arch Dermatol 119:541
46. Wahl SM, Hunt DA, Wong HL et al. (1988) Transforming growth factor-beta is a potent immunosuppressive agent that inhibits IL-1-dependent lymphocyte proliferation. J Immunol 140:3026–3032