

Interleukin-1 Receptor Antagonist in Normal and Psoriatic Epidermis

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Abstract

The objective of these studies was to characterize the IL-1 inhibitory activity present in normal and psoriatic epidermis from clinically stable lesions. Fractionation of normal epidermal cytosol on a molecular sizing column failed to reveal the presence of IL-1 inhibitory bioactivity. However, specific ELISAs indicated that both the IL-1 receptor antagonist (IL-1ra) and IL-1 α were present in overlapping peaks. Further fractionation of the normal epidermal cytosol by anion exchange chromatography separated these two molecules, revealing the IL-1 inhibitory bioactivity of the IL-1ra molecule. Similar studies on psoriatic epidermal cytosol indicated the presence of IL-1 inhibitory bioactivity and IL-1ra protein. The IL-1 inhibitory bioactivity of both normal and psoriatic cytosol was neutralized by a mAb specific for IL-1ra. The ratio of IL-1ra to IL-1 α proteins was significantly increased in involved psoriatic skin compared with normal skin. By Western blot analysis this IL-1ra was \approx 20 kD, slightly larger than monocyte-derived IL-1ra and equivalent to an intracellular variant of IL-1ra expressed by keratinocytes. Polymerase chain reaction indicated the presence of mRNA for both forms of IL-1ra in normal epidermis, with both forms increased in psoriatic-involved skin. Immunofluorescence studies revealed the IL-1ra protein to be concentrated in the stratum granulosum of normal skin and in the basal-midbasal layers of psoriatic epidermis. These results suggest that the balance between intracellular IL-1ra and IL-1 α may be an important influence on keratinocyte growth and/or differentiation, as well as on the inflammatory potential of IL-1 in injured skin. (*J. Clin. Invest.* 1992. 90:571-583.) Key words: IL-1 α • IL-1 β • homeostasis • differentiation • keratinocyte

Introduction

The skin is a major reservoir of IL-1 (1, 2) because of constitutive production by keratinocytes (3, 4) and sweat gland epithelium (5). IL-1 is capable of inducing cutaneous inflammation

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upon injection into human skin (6) and is released from the epidermal cells after ultraviolet (UV) radiation (7-9). Epidermal IL-1 release not only recruits an inflammatory response, but also potentiates T cell subset activation and the nature of the subsequent inflammatory response (10). The mechanisms by which the inflammatory potential of the large amounts of IL-1 contained in the epidermis are restrained may be due in part to the inability of keratinocytes to effectively proteolyse inactive IL-1 β precursor molecules (11), such that human epidermis contains inactive IL-1 β that has been processed into novel forms of the molecule (12). IL-1 α , which is active in vivo in both the precursor and processed forms, remains intracellular in unstimulated keratinocytes (13). Thus, the role of IL-1 in skin and its regulation in cutaneous homeostasis in vivo remain poorly understood.

A skin condition that may provide clues to the involvement of IL-1 in skin biology is psoriasis. IL-1 has the ability to cause cutaneous inflammation (6), potentiate T cell activation (14), stimulate eicosanoid metabolism (15), induce fibroblast proliferation (16), upregulate endothelial cell adhesion molecule expression (17, 18), and potentially stimulate keratinocyte proliferation (19, 20). Because each of these elements may be present in psoriatic skin (21-28), overexpression of IL-1 has been postulated as a possible trigger for this disease process. However, the growth-promoting capacity of IL-1 for human keratinocytes has been called into question (29) and IL-1 activity is markedly reduced in psoriatic skin (30). Despite existing in a processed form, epidermal IL-1 β was incapable of activating a murine T cell line expressing the type I IL-1 receptor or of activating murine thymocytes (30). The nonfunctionality of the IL-1 β in concert with a 14-fold decrease in IL-1 α levels are consistent with the reduced levels of IL-1 activity in psoriatic keratome extracts (30). Further complexity was added to the situation on finding that an inhibitor of IL-1 activity also contributed to the markedly reduced bioactivity of IL-1 in psoriatic lesions (30). This inhibitor was not secreted constitutively, had a molecular mass of \sim 30 kD and an isoelectric point of 5.3 and was distinct from transforming growth factor β (TGF β)¹ (31).

Recently, a specific human IL-1 receptor antagonist (IL-1ra) has been purified from the supernatants of IgG-stimulated monocytes (32) and from the myelomonocytic leukemia cell line U937 after induction of differentiation (33). The IL-1ra molecule was sequenced and is a single peptide of 152 amino acids that exists in both 17-kD nonglycosylated and 22-25-kD

1. Abbreviations used in this paper: DPBs, Dulbecco's modified PBS; FPLC, fast protein liquid chromatography; IL-1ra, interleukin-1 receptor antagonist; PCR, polymerase chain reaction; TGF β , transforming growth factor β .

glycosylated forms with a pI of 5.0 (32, 33). IL-1ra has 30% amino acid sequence homology to IL-1 β and 19% homology to IL-1 α . Complementary DNAs have been cloned and expressed in *Escherichia coli* with production of recombinant 17-kD IL-1ra molecules that retain full biological activity (32–34). IL-1ra appears to be a true receptor antagonist in that it competitively binds to the type I IL-1 receptor on T cells (32, 33), endothelial cells (33), fibroblasts (32), synovial cells, and chondrocytes (35) and the human type II IL-1 receptor on B cells and polymorphonuclear leukocytes (36, 37) without inducing detectable cellular responses.

The mRNA for a structural variant of monocyte-derived IL-1ra has recently been described in cultured keratinocytes and other epithelial cells (38). The cDNA clones derived from both monocytes and cultured keratinocytes predict that this mRNA encodes for a protein that is identical to mature 17-kD monocyte IL-1ra except for the presence of an additional seven NH₂-terminal amino acids and the lack of a leader peptide for secretion. Cultured keratinocytes produce a large quantity of IL-1ra that remains entirely intracellular (icIL-1ra) and is slightly larger in size than 17-kD monocyte IL-1ra protein (sIL-1ra) (38, 39). IL-1ra production by cultured keratinocytes increases with cell differentiation (39).

The purpose of the present studies was to examine freshly obtained human skin from normal subjects to determine whether IL-1ra is produced in vivo by epidermal keratinocytes. Furthermore, we wanted to determine if the IL-1 inhibitory activity that dominates in psoriatic epidermis could be attributed to expression of IL-1ra. Cytosolic extracts of keratome biopsies were fractionated by both gel filtration and ion exchange chromatography. The fractions were analyzed for IL-1 inhibitor bioactivity, both IL-1ra and IL-1 α proteins by specific ELISAs, and neutralization of functional activity with specific antibodies. In addition, IL-1ra was localized in intact skin by immunofluorescence techniques and the presence of mRNA transcripts within the epidermis were identified by polymerase chain reaction (PCR). Our results raise the possibility that IL-1ra is important for cutaneous homeostasis not only by limiting the inflammatory effects of extracellular IL-1 in injured skin, but also by limiting putative intracellular actions of IL-1.

Methods

Materials. Polyethylene glycol (PEG, mol wt 8,000), sucrose, Tris, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, MOPC21, Tween 20, and PMSF were obtained from Sigma Chemical Co. (St. Louis, MO). PHA was obtained from Wellcome Diagnostics (Dartford, England). DTT was purchased from Bio-Rad Laboratories (Rockville Center, NY). The Fast Protein Liquid Chromatography (FPLC) apparatus and Mono Q anion exchange column were purchased from Pharmacia Inc. (Piscataway, NJ). A TSK G2000SW molecular sizing column was obtained from Toyo Soda (Tokyo, Japan). A rabbit antiserum and a mAb to IL-1 α used in the ELISA and human recombinant IL-1 α were gifts from Dainippon Pharmaceutical Corp. (Osaka, Japan). Human recombinant IL-1 β was purchased from Cistron Corp. (Pine Brook, NJ). Rabbit polyclonal antiserum to IL-1ra was prepared and characterized as previously described (32, 40). Hybridomas secreting mAbs to the IL-1ra protein were derived using described techniques (41). The spleen cell donors were CAF₁ mice that had been injected four times with 5–20 μ g purified or recombinant IL-1ra. None of the resulting mAbs, I.4, I.5 and I.10, reacted with human IL-1 α or IL-1 β in ELISAs. These mAbs and the human recombinant IL-1ra protein were provided by The Upjohn Company. The mAb to TGF β 1 was obtained from Collagen Corp. (Palo Alto, CA).

Peroxidase-conjugated goat anti-rabbit IgG antibodies, goat anti-mouse IgG-biotin conjugate and goat anti-mouse IgG rhodamine-B conjugate were obtained from Tago Inc. (Burlingame, CA). Streptavidin-horseradish peroxidase conjugate was from Bethesda Research Laboratories (Gaithersburg, MD). Keratinocyte culture medium (modified MCDB 153) was formulated as described (42).

Preparation of keratome and keratinocyte cytosols. Keratome biopsies were obtained from lesional psoriatic plaques, clinically uninvolved psoriatic skin, and skin of normal subjects. Patients with psoriasis in this study had clinically stable plaques of sufficient size to enable two 10-cm² strips to be biopsied. The patients were off active therapy (except for bland emollients) for \geq 1 wk before the biopsy. The biopsies from both normal and psoriatic skin were performed on the hips or buttocks using a Castro Viejo keratome set (Storz Co., St. Louis, MO) at 0.2–0.3 mm. Keratome strips were immediately snap frozen in liquid nitrogen. Keratomes were pulverized under liquid nitrogen. Dulbecco's PBS (DPBS, Ca⁺⁺- and Mg⁺⁺-free) with 1 mM PMSF and 0.03% PEG was added at 100 mg keratome wet wt/ml buffer for samples to be examined by FPLC; DPBS plus 5 mM DTT were added if cytosolic IL-1ra and IL-1 α levels were to be measured directly in an ELISA. After homogenization with a glass homogenizer, cytosols were obtained by ultracentrifugation (100,000 g, 60 min) and the supernatants were sterile filtered and stored at –70°C.

Cytosols were prepared from cultured adult human keratinocytes grown from keratomes in modified MCDB 153 by washing the monolayer three times with HBSS (Ca⁺⁺- and Mg⁺⁺-free) and then scraping the cells off in the presence of DPBS with 1 mM PMSF and 0.03% PEG. After three cycles of freeze thawing, cells were sonicated, ultracentrifuged (100,000 g, 60 min), and the supernatant stored at –70°C. Protein concentrations of keratomes and cytosols were determined by a protein assay kit (Bio-Rad Laboratories).

FPLC. 2 ml of keratome cytosol were applied to a TSK G2000SW column equilibrated with DPBS plus 0.03% PEG. The flow rate was 3.0 ml/min and 2.0-ml fractions were collected. Each fraction was sterilized by passage through a 0.2- μ m millipore filter and assayed for the presence of IL-1 α protein, IL-1 inhibitory activity, and IL-1ra protein.

Keratome cytosols were dialyzed against 0.025 M Tris (pH 7.6) with 0.1% sucrose before application to a Mono Q column equilibrated with 0.025 M Tris (pH 7.6) plus 0.1% sucrose. Samples were eluted with a NaCl gradient ranging from 0 to 250 mM NaCl at a rate of 0.5 ml/min and 1-ml fractions were collected. Fractions were dialyzed against DPBS, then sterile filtered, and assayed for the presence of IL-1 α protein, IL-1 inhibitory activity, and IL-1ra protein.

IL-1 α ELISA. Immunoreactive IL-1 α in samples was quantified by using an ELISA as previously described (30). Standard curves of recombinant human IL-1 α ranging from 20 to 1,000 pg/ml were run for each assay. The sensitivity of the IL-1 α ELISA was 10 pg/ml.

IL-1 inhibitor assay. IL-1 inhibitory activity was measured by blocking the production of IL-2 by LBRM.33 cells in response to IL-1 and PHA as previously described (31). Keratome cytosols or fractions from either the TSK G2000SW or Mono Q columns were tested for IL-1 inhibitory activity. Quantitation of the amount of IL-1 produced by LBRM.33 cells was made in relation to an IL-2 standard curve that covered a range of 2–250 U/ml of activity. Maximal response of the LBRM.33 sample was compared with the standard curve to obtain the amount of IL-2 units per milliliter.

IL-1ra ELISA. IL-1ra protein was measured using a sandwich ELISA recently described in detail (40). The primary antibody in this ELISA was affinity-purified polyclonal rabbit antibodies specific for IL-1ra. The secondary antibody was the IgG fraction of this antiserum coupled to biotin, followed by streptavidin peroxidase conjugate and 2,2'-azino-bis(3-ethylbenzthiazoline-6-sulfonic)acid (ABTS) solution. The sensitivity of this ELISA was < 200 pg/ml and the ELISA failed to recognize up to 100 ng/ml of IL-1 α or IL-1 β . The recombinant human IL-1ra standard was provided by Dr. Robert C. Thompson, Synergen Inc., Boulder, CO.

IL-1ra indirect immunofluorescence. mAbs to IL-1ra (I.4 and I.10 ascites) or isotype control MOPC21 ascites were diluted 1:100 or 1:200

and reacted with acetone-fixed 6- μ m sections of human skin-punch biopsies frozen in O.C.T. compound (Miles Inc., Elkhart, IN) as previously described (43). To test whether soluble recombinant IL-1ra protein would inhibit the binding of mAbs to the skin sections, a 1:200 dilution of antibody I.4 was preincubated with recombinant IL-1ra (10 μ g) at 37°C for 1 h. The antibody-antigen mixture was then centrifuged in a microfuge at 4°C for 15 min and the supernatants were used to react with the skin sections as described above.

IL-1ra neutralization assay. 50 μ l of mAb ascites against IL-1ra (I.10, murine IgG₁ isotype) was added to 96-well flat-bottom tissue culture plates and preincubated with 50 μ l of TSK G2000SW or Mono Q IL-1 inhibitory fractions for 2 h at 37°C. As an isotype control, 1D.116, an IgG₁ murine mAb against TGF β 1 (44), was added at the same dilutions as mAb I.10.25 μ l of PHA (2 μ g/well), LBRM.33 cells (10⁵ cells/well) and 50 μ l of rIL-1 β (0.25 or 2.5 U/well) were then added and incubated for 24 h at 37°C. The IL-1 inhibitor assay was then continued as described above.

Immunoblotting. Human epidermis or keratinocyte cytosol were concentrated 5–10-fold with a Centricon 3 system (3,000 mol wt cut off) (Amicon Division, W. R. Grace Co., Beverly, MA) and samples were electrophoresed on a 12% SDS-PAGE gel and Western blotted as described previously (43). After reaction with a mAb to IL-1ra (I.5) or a myeloma isotype ascites control (MOPC 21), the nitrocellulose membrane was reacted with a goat anti-mouse IgG biotin conjugate followed by a streptavidin-horseradish peroxidase conjugate. The presence of the immune complex was detected using chemiluminescence (ECL Western blotting detection system and Hyperfilm; Amersham International, Arlington Heights, IL).

IL-1ra mRNA in normal and psoriatic skin. PCR was used to determine the presence of mRNA for sIL-1ra, icIL-1ra, and IL-1 β in normal and psoriatic skin. Total RNA was prepared from normal and clinically stable psoriatic keratomes by using RNazol (Tel-Test, Inc., Friendswood, TX). Total RNA from monocytes was prepared as previously described (38). RNA was reverse-transcribed into cDNA using random hexamers as primers and PCR performed essentially as reported (45). PCR primer pairs specific for icIL-1ra and sIL-1ra were those used by Haskill et al. (38) and those for IL-1 β were described by Wang et al. (46). A standard curve, which ranged from 14 to 0.014 pg/ μ l, was constructed for each PCR product using plasmid containing a cDNA insert of the appropriate protein. PCR products were determined at 24, 26, and 28 cycles, but only data from 28 cycles are shown. That the PCR products are from the correct sequence was confirmed by repeating PCR upon the PCR products with primer pairs internal to the original sequence. Ethidium bromide-stained gels were photographed on an UV light box with Polaroid type 55 positive/negative film. The negative was rinsed in water, dried, and analyzed with a Laser Densitometer (LKB Instruments, Gaithersburg, MD).

Statistical analysis. After log transformation of the ratio of IL-1ra and IL-1 α protein levels in the different samples, the mean of the individual ratios of the involved psoriatic epidermis was compared with the mean of the ratios of uninvolved psoriatic or normal epidermis by univariate one-way analysis of variance.

Results

IL-1ra in normal epidermal cytosol. To determine whether IL-1 inhibitory activity was present in *in vivo* epidermis of normal skin, keratome cytosols were assayed for inhibition of IL-1 β stimulation of LBRM.33 cells. At a cytosol concentration of 3 μ g/ml, IL-1 activity was detected (Fig. 1). However, at a higher cytosol concentration (13 μ g/ml), IL-1 activity disappeared. This result suggested the presence of an IL-1 inhibitor. Indeed, when a suboptimal concentration of recombinant IL-1 β was added to LBRM.33 cells in the presence of human keratome cytosols, additive IL-1 bioactivity was observed at 0.4 and 3 μ g/ml. However, inhibition of the IL-1-induced pro-

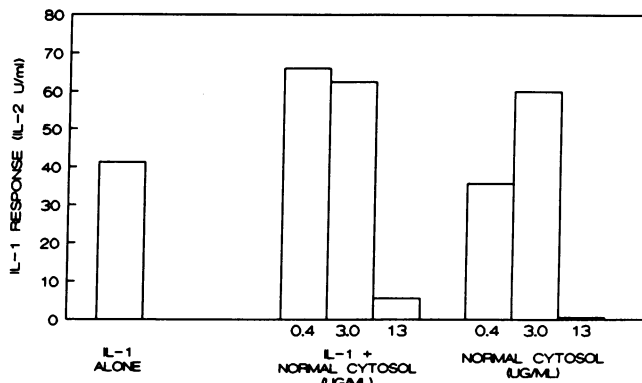


Figure 1. Both IL-1 and IL-1 inhibitory activities are present in keratome cytosol from normal human skin. The data are expressed as U/ml of IL-2 produced by LBRM.33 cells stimulated with normal keratome cytosol in the presence or absence of a suboptimal concentration of IL-1 β . The first bar indicates IL-1 activity of 0.25 U/ml IL-1 β alone. Bars 2–4 show the detected levels of IL-1 activity where 0.25 U/ml IL-1 β was added to each of the indicated amounts of keratome cytosol. Bars 5–7 indicate the levels of IL-1 activity present in the normal keratome cytosol alone.

duction of IL-2 occurred at a keratome cytosol protein concentration of 13 μ g/ml (Fig. 1). This finding suggests that either a critical concentration of an IL-1-specific inhibitor must be achieved to detect its function or that the keratome cytosols contain a complex mixture of nonspecific and IL-1-specific inhibitory and costimulatory factors.

To ascertain whether IL-1ra was present in the epidermal cytosol, we used FPLC fractionation on a TSK G2000SW molecular sizing column to separate nonspecific inhibition from IL-1-specific inhibitors. Although partial inhibition by a variety of fractions was found, the presence of IL-1 inhibitory activity that could completely suppress the IL-1 induction of IL-2 production by LBRM.33 cells was not revealed (Fig. 2 A). However, when an ELISA specific for IL-1ra was performed on the same fractions, IL-1ra protein was detected in fractions coeluting at \sim 30 kD (Fig. 2 B). Elution profiles of ELISA-detectable precursor and processed forms of IL-1 α demonstrated that both partially overlapped the IL-1ra peak, although they eluted slightly before and after the IL-1ra protein, respectively (Fig. 2 C). However, LBRM.33 cells are relatively insensitive to the antagonist effect of the IL-1ra protein, a ratio in excess of 500:1 (IL-1ra:IL-1 α) is required to obtain complete suppression of IL-1 induction of IL-2 production (data not shown) (33). Thus, the apparent absence of IL-1 inhibitory biological activity in the TSK G2000SW fractions may be due to the presence of IL-1 α molecules, which were also present in the same fractions containing IL-1 inhibitory activity (Fig. 2 C).

Because the presence of IL-1 α in the same fractions as the ELISA-detectable IL-1ra protein on gel-filtration chromatography might mask IL-1 inhibitory activity, cytosol from normal epidermis was fractionated on an anion exchange Mono Q column (Fig. 3). This fractionation now clearly revealed the presence of IL-1 inhibitory activity that eluted between 85 and 95 mM NaCl (Fig. 3 A). The peak of IL-1 inhibitory activity coeluted with ELISA-detectable IL-1ra protein (Fig. 3 B). Additional ELISA-detectable IL-1ra protein was present in later fractions without any corresponding IL-1 inhibitory activity. This result could be explained by the presence of small

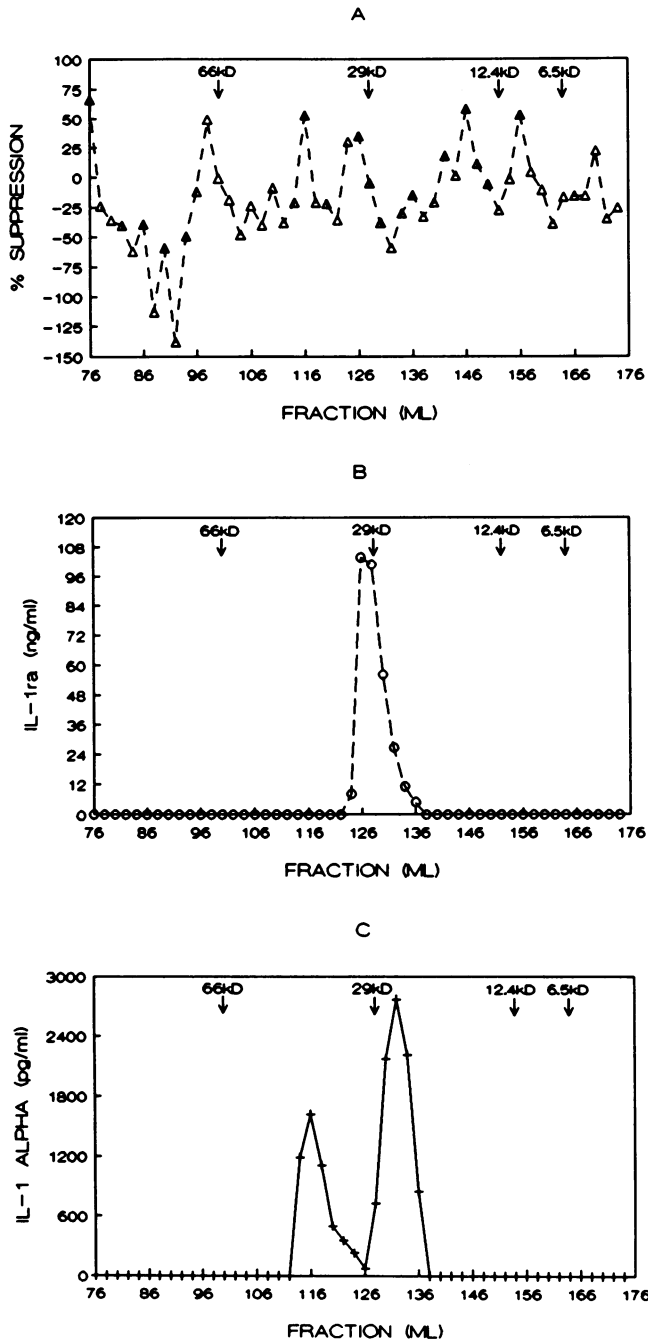


Figure 2. IL-1 α and IL-1ra proteins are both present in normal keratome cytosol. The cytosol was fractionated by a FPLC TSK G2000SW sizing column. Fractions were assayed for (A) IL-1 inhibitor bioactivity (percent suppression of IL-1 effects on LBRM.33 cells), (B) IL-1ra protein (ng/ml by ELISA), and (C) IL-1 α protein (pg/ml by ELISA).

amounts of IL-1 α in these fractions eluting at high salt concentrations (Fig. 3 C). However, most of the IL-1 α detectable by ELISA eluted at NaCl concentrations (65–80 mM NaCl) lower than those required for elution of the IL-1ra. Only when the IL-1 α levels dropped below the limit of detection was IL-1 inhibitory activity observed in fractions that contained ELISA-detectable IL-1ra protein. The amount of IL-1 α (Fig. 3 C) in

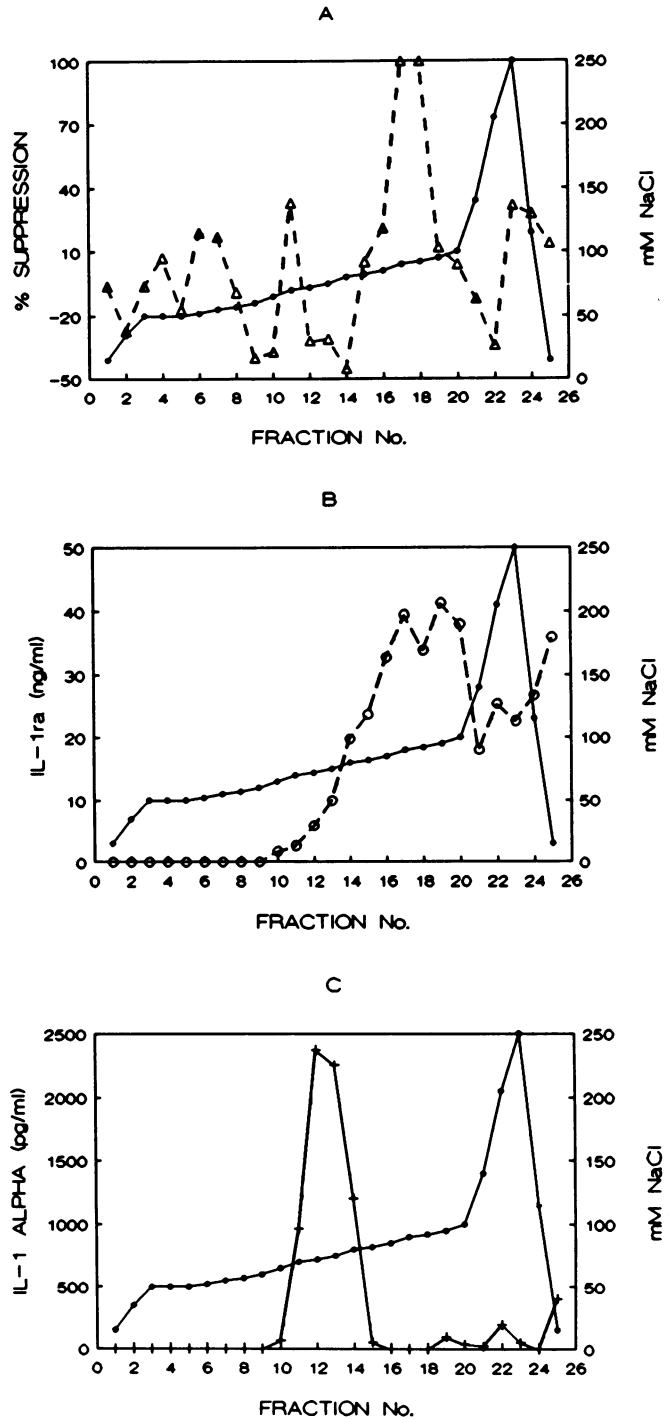


Figure 3. Fractionation of normal keratome cytosol by FPLC ion-exchange chromatography on a Mono Q column. A–C are described in the Fig. 2 legend. The NaCl gradient (mM) is indicated on the right ordinate.

fractions 19–25 would appear to be too high to allow detection of IL-1 inhibitory activity (Fig. 3 A) by the IL-1ra protein shown to be present in these fractions (Fig. 3 B).

IL-1ra in psoriatic epidermal cytosol. Unfractionated cytosol from clinically stable psoriatic lesions displays a pattern of IL-1 inhibitory activity that differs from the pattern observed in unfractionated cytosol from normal epidermis (Fig. 4). Un-

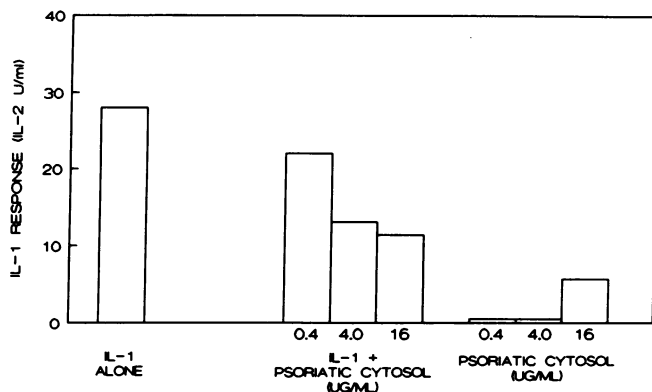


Figure 4. IL-1 and IL-1 inhibitory activities are present in keratome cytosol from psoriatic-involved skin. The first bar indicates activity of 0.25 U/ml IL-1 beta alone. Bars 2-4 indicate detected IL-1 activity where 0.25 U/ml IL-1 β was mixed with the indicated concentrations of psoriatic keratome cytosol. Bars 5-7 show the endogenous IL-1 activity in the keratome cytosol in the absence of added IL-1 β .

like normal cytosol, IL-1 activity was observed in psoriatic cytosol only at concentrations > 4 μ g/ml. In addition, the IL-1 inhibitory activity at these higher concentrations was not complete. These results again point toward a complex set of interactions among cytokines with synergistic stimulatory action and inhibitory action in crude unfractionated cytosols.

To determine whether IL-1ra is present in clinically stable involved psoriatic epidermal cytosol, fractionations were performed similar to those described for cytosols from normal skin. In contrast to normal skin, size fractionation alone revealed the presence of IL-1 inhibitory activity (Fig. 5 A). Indeed, as detected by ELISA, IL-1ra protein coeluted with IL-1 inhibitory activity in a major peak of 30 kD (Fig. 5 B). IL-1 α protein was only detected in a single fraction (Fig. 5 C), confirming that little IL-1 α was present in the psoriatic keratome cytosol (30). The relative lack of IL-1 α in the psoriatic fractions (200 pg/ml in a single fraction) as compared to the abundant amounts of IL-1 α in the normal fractions (400-2,800 pg/ml in nine fractions) likely accounts for the ease of detection of IL-1 inhibitory activity in psoriatic cytosols.

To further characterize the IL-1 inhibitors in psoriatic skin, keratome cytosol from lesional epidermis was separated by anion exchange chromatography. A large peak of IL-1 inhibitory activity eluted at a similar NaCl concentration (Fig. 6) as was observed with normal skin (Fig. 3). The volume of ion-exchange fractions from the psoriatic skin cytosols was not sufficient to permit performance of ELISAs for IL-1ra and IL-1 α proteins.

Epidermal IL-1 inhibitory activity is neutralizable by an anti-IL-1ra protein mAb. To determine whether the IL-1 inhibitory activity that coeluted with the IL-1ra protein from normal skin is indeed due to a functional IL-1ra molecule, antibody neutralization of the Mono Q fractions (17 and 18) containing IL-1 inhibitory activity and ELISA-detectable IL-1ra protein was performed (Table I). Addition of 0.25 U/ml of IL-1 to LBRM.33 cells resulted in release of 8.9 U/ml of IL-2. Addition of normal keratome cytosol fractions containing IL-1 inhibitory activity (Mono Q fractions 17 and 18) resulted in 100% inhibition of LBRM.33 stimulation by IL-1 (0 U/ml IL-2 release). Addition of a neutralizing mAb against IL-1ra

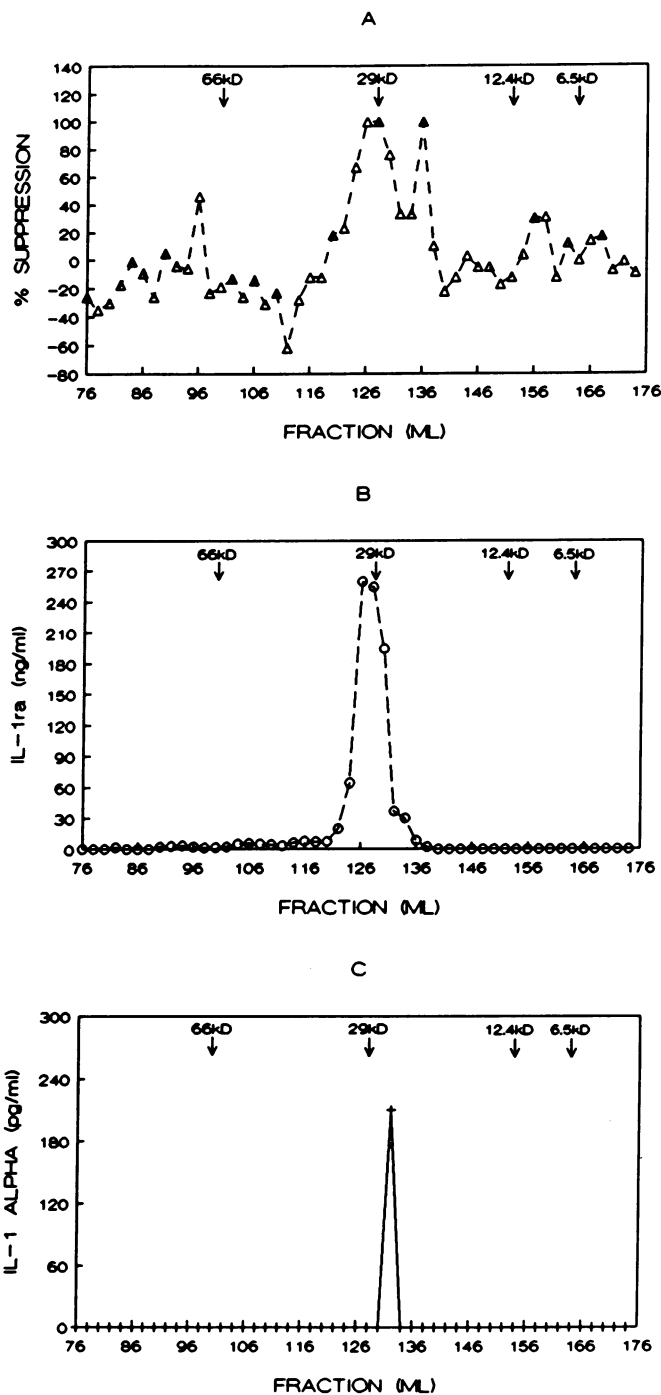


Figure 5. IL-1 inhibitor bioactivity and IL-1ra protein are present in involved psoriatic keratome cytosol. This cytosol was fractionated by a TSK G2000SW sizing column and fractions analyzed for (A) IL-1 inhibitor bioactivity, (B) IL-1ra protein, and (C) IL-1 α protein.

protein at dilutions of 1:25 and 1:50 resulted in complete neutralization of the IL-1 inhibitory activity as evidenced by the release of 10.5 and 9.4 U/ml of IL-2, respectively. A mAb isotype control, 1D.116, which is capable of neutralizing TGF β activity, did not affect the IL-1 inhibitory activity in these fractions (0 units IL-2 release).

A similar result was obtained with Mono Q fractions 14, 15, and 16 from involved psoriatic skin that contained IL-1 inhibi-

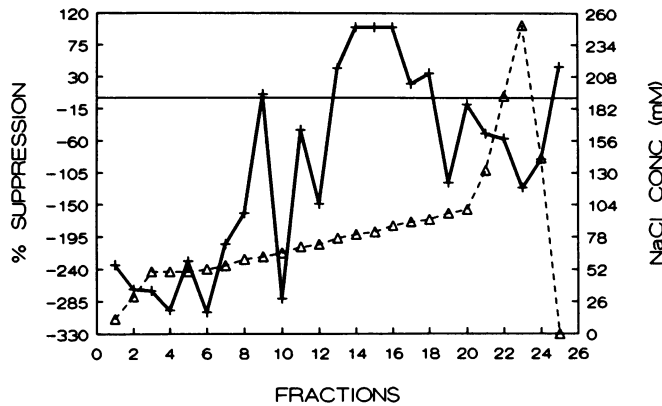


Figure 6. Fractionation of involved psoriatic keratome cytosol by FPLC ion exchange chromatography on a Mono Q column. The left ordinate depicts the percent suppression of IL-2 production by IL-1-induced LBRM.33 cells; the right ordinate shows the NaCl gradient in mM, and the abscissa represents the column fractions. The horizontal line indicates 0% suppression of IL-1 bioactivity; all data points above this line represent inhibition of IL-1 activity.

tory activity. In this experiment addition of 0.25 U/ml of rIL-1 resulted in the release of 16.9 U/ml IL-2 and this response was completely blocked by psoriatic cytosol (Table I). The IL-1 inhibitory activity of these fractions was completely neutral-

Table I. Monoclonal Antibody to IL-1ra Neutralizes IL-1 Inhibitory Activity in Pooled Mono Q Fractions

Addition to LBRM.33 cells and PHA	IL-2 released*	IL-1 inhibition [†]
	U/ml	% suppression
IL-1	8.9	—
IL-1 + normal keratome fractions	0	100%
IL-1 + normal cytosol + anti-IL-1ra (1:25) [§]	10.5	< 0
IL-1 + normal cytosol + anti-IL-1ra (1:50)	9.4	< 0
IL-1 + normal cytosol + anti-TGFβ (1:25)	0	100%
IL-1 + normal cytosol + anti-TGFβ (1:50)	0	100%
IL-1	16.9	—
IL-1 + psoriatic keratome fractions	0	100%
IL-1 + psoriatic cytosol + anti-IL-1ra (1:25)	28.4	< 0
IL-1 + psoriatic cytosol + anti-TGFβ (1:25)	0	100%

* Amount of IL-2 released as detected by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide reduction by LBRM.33 cells cultured with a suboptimal concentration of rIL-1 (0.25 U/ml) in the absence or presence of Mono Q fractions containing IL-1 inhibitory activity.

[†] IL-1 inhibition by cytosol fractions with or without antibodies is quantified as percent suppression of IL-2 release induced by 0.25 U/ml of rIL-1.

[§] Anti-IL-1ra ascites (I.10) was added to the mixture of IL-1, Mono Q fractions, PHA, and LBRM.33 cells at a dilution of 1:25.

^{||} Anti-TGFβ ascites (1D.116) was added to the mixture of IL-1, Mono Q fractions, PHA, and LBRM.33 cells at a dilution of 1:25.

ized by the mAb to IL-1ra (28.4 U/ml IL-2 release) but, again, unaffected by the neutralizing antibody to TGFβ. Further evidence that the IL-1 inhibitory activity from psoriatic skin was indeed due to IL-1ra was the complete neutralization of IL-1 inhibitory activity in the major size-fractionated TSK G2000SW peak (Fig. 4 A) in addition to unfractionated psoriatic cytosol by the anti-IL-1ra mAb (data not shown).

IL-1ra protein in skin. Although IL-1 inhibitory activity was much more easily demonstrable in psoriatic relative to normal skin, it was possible that this result might not be due to increased IL-1ra but to reduced IL-1α. The amounts of IL-1ra and IL-1α proteins in samples of unfractionated epidermal cytosols were therefore quantified by specific ELISAs and expressed per milligram of total protein. Normal skin extracts contained 73.9±15.6 ng IL-1ra/mg protein and 0.6±0.4 ng IL-1α/mg protein (Table II). Uninvolved psoriatic skin exhibited levels of IL-1ra, 78.6±14.6 ng/mg protein, and IL-1α, 1.3±1.2 ng/mg protein, that were similar to normal skin. However, the levels of both IL-1ra and IL-1α were lower in clinically stable involved psoriatic skin, with IL-1ra present at 53.8±7.2 ng/mg protein and IL-1α present at 0.05±0.08 ng/mg protein. If the relative amounts of IL-1ra to IL-1α are compared for normal (123), uninvolved (60.5), and involved (1,076) psoriatic skin, an approximate 10-fold increase in the amounts of IL-1ra to IL-1α occurred in involved psoriatic relative to normal or uninvolved psoriatic epidermis (Table II). If the individual IL-1ra/IL-1α ratios within the three groups are compared, the increase in the involved psoriatic IL-1ra/IL-1α ratio is statistically significant upon comparison to normal ($P < 0.0004$) or uninvolved ($P < 0.0007$) epidermis by univariate one-way analysis of variance of log-transformed data. However, by the same statistical analysis the individual IL-1ra/IL-1α ratios of normal and uninvolved psoriatic epidermis were not statistically significantly different ($P = 0.44$).

Epidermal IL-1ra is similar in molecular weight to icIL-1ra. Based upon size-fractionation chromatography of human keratome cytosol, the molecular mass of the epidermal IL-1ra

Table II. Elevated IL-1ra/IL-1α Ratios in Keratome Cytosols

Sample	IL-1ra (n)*	IL-1α (n)*	IL-1ra/IL-1α [‡]
	ng/mg protein		
Normal skin	73.9±15.6 (6)	0.6±0.4 (6)	123
Psoriatic skin, uninvolved	78.6±14.6 (7)	1.3±1.2 (4)	60.5
Psoriatic skin, involved	53.8±7.2 (6)	0.05±0.08 (6)	1076

* The concentrations of IL-1ra and IL-1α proteins in keratome cytosols from normal skin, uninvolved psoriatic skin, or involved psoriatic skin were determined by specific ELISAs. Data is expressed in terms of ng IL-1ra or IL-1α/mg protein. The same samples were analyzed for each cytokine, except for three samples of psoriatic uninvolved epidermis that were able to be analyzed for IL-1ra but were of insufficient volume to be simultaneously analyzed for IL-1α. Data represent mean±SD based upon the indicated sample numbers (n).

[‡] Ratio of IL-1ra to IL-1α based upon ng/mg protein of each protein in the keratome cytosol. Psoriatic involved vs normal, $P < 0.0004$; psoriatic involved vs uninvolved, $P < 0.0007$; psoriatic uninvolved vs normal $P = 0.44$ by univariate one-way analysis of variance of log-transformed data.

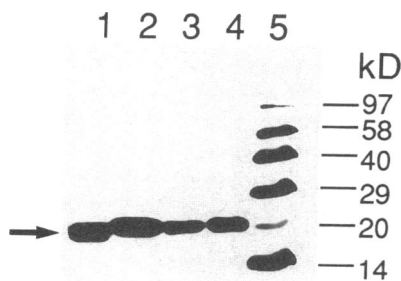


Figure 7. Western blot analysis of IL-1ra protein using mAb I.5. The arrow indicates the migration of recombinant monocyte IL-1ra in bar 1. Bar 2 contains cultured keratinocyte lysate, bar 3 involved psoriatic keratome cytosol, bar 4 normal keratome cytosol, and bar 5 mol wt markers.

would appear to be ~ 30 kD (Figs. 2 and 5). This is larger than the 17-kD (nonglycosylated) and 22–25-kD (glycosylated) sizes reported for the secreted IL-1ra protein obtained from activated monocytes or the 20-kD intracellular IL-1ra protein expressed by in vitro-cultured keratinocytes and lung epithelia (38). A comparison of the sizes of IL-1ra proteins from extracts of normal cultured keratinocytes and keratome cytosols was made to recombinant monocyte IL-1ra protein by immunoblotting after separation on SDS-PAGE (Fig. 7). The epidermal-derived IL-1ra from both normal and involved psoriatic skin migrated at 20 kD by SDS-PAGE, the same size as keratinocyte IL-1ra. Both the epidermal and keratinocyte IL-1ra protein migrated slightly larger than the recombinant IL-1ra protein derived from monocytes (secreted IL-1ra), suggesting that IL-1ra protein produced by epidermal keratinocytes in vivo represents the intracellular splice variant of IL-1ra.

IL-1ra mRNA in normal and psoriatic skin. To evaluate the relative expression of mRNAs for the secreted and intracellular forms of IL-1ra in normal and involved psoriatic skin, PCR analysis was performed. Both normal and clinically stable involved psoriatic skin expressed mRNA for the intracellular (Fig. 8 A) and secreted (Fig. 8 B) forms of IL-1ra. However,

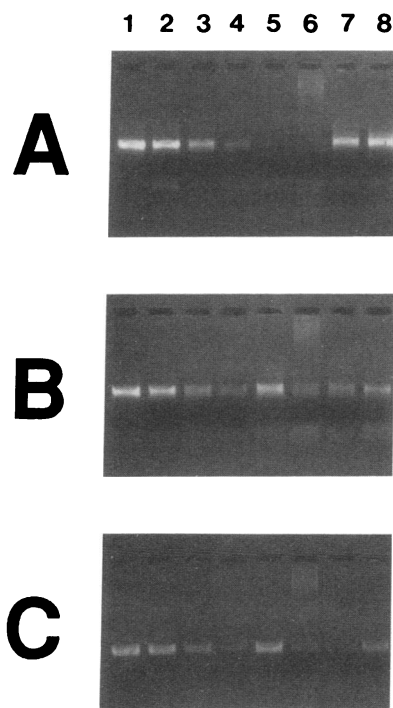


Figure 8. (A) Intracellular IL-1ra, (B) secreted IL-1ra, and (C) IL-1 β mRNAs as detected by PCR in normal and psoriatic keratomes. Bars 1–4 are the standard curve, ranging from 14 to 0.014 pg/ml. Bar 5 contains total RNA from 4-h adherent monocytes, bar 6 non-adherent monocytes, bar 7 normal keratome, and bar 8 psoriatic involved keratome.

only involved psoriatic skin had sufficient mRNA levels for IL-1 β as detected by PCR (Fig. 8 C). This is similar to our previous observation of increased IL-1 β mRNA levels in involved psoriatic skin relative to normal skin (30). Monocytes did not express detectable amounts of mRNA for the intracellular form of IL-1ra (Fig. 8 A), but contained mRNA for both secreted IL-1ra (Fig. 8 B) and IL-1 β (Fig. 8 C). Densitometer readings of these gels showed that mRNA levels in both normal and involved psoriatic skin for intracellular IL-1ra (Fig. 9 A) were ~ 10-fold greater than the mRNA levels for secreted IL-1ra (Fig. 9 B). These results also indicate that the mRNA levels for both IL-1ra forms were higher in psoriatic involved skin relative to normal skin. Of note, the mRNA levels of secreted IL-1ra in monocytes (Fig. 9 B) compared with IL-1 β (Fig. 9 C) were approximately equal.

Differential expression of the IL-1ra molecule in psoriatic and normal skin. Because our epidermal extracts of keratome biopsies represent an average value of all epidermal cells, we next determined whether IL-1ra was preferentially expressed in various epidermal compartments. Reaction of anti-IL-1ra

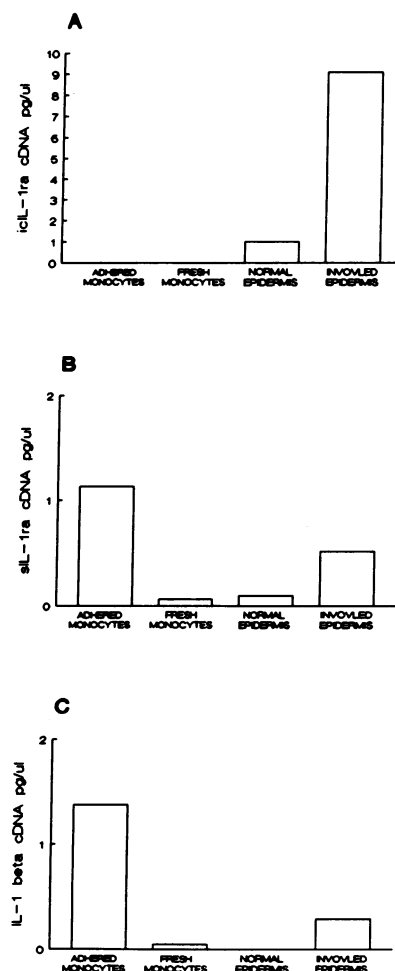


Figure 9. Amount of cDNA present in PCR products shown in Fig. 8. Densitometer readings were performed upon each sample and the appropriate cDNA standard curve. Based upon the standard curve the amount of cDNA in each reverse-transcribed sample was determined and expressed in pg/ μ l. Intracellular IL-1ra, secreted IL-1ra, and IL-1 β values are given in A, B, and C, respectively.

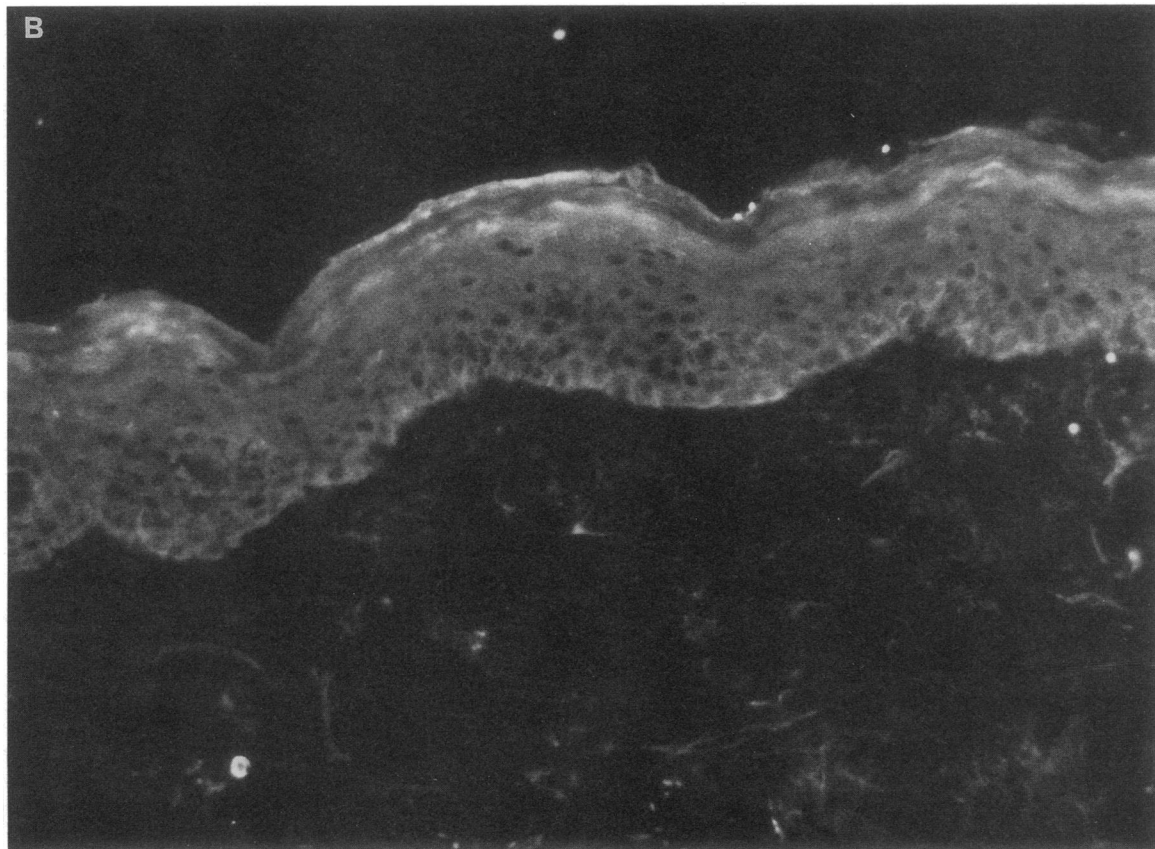
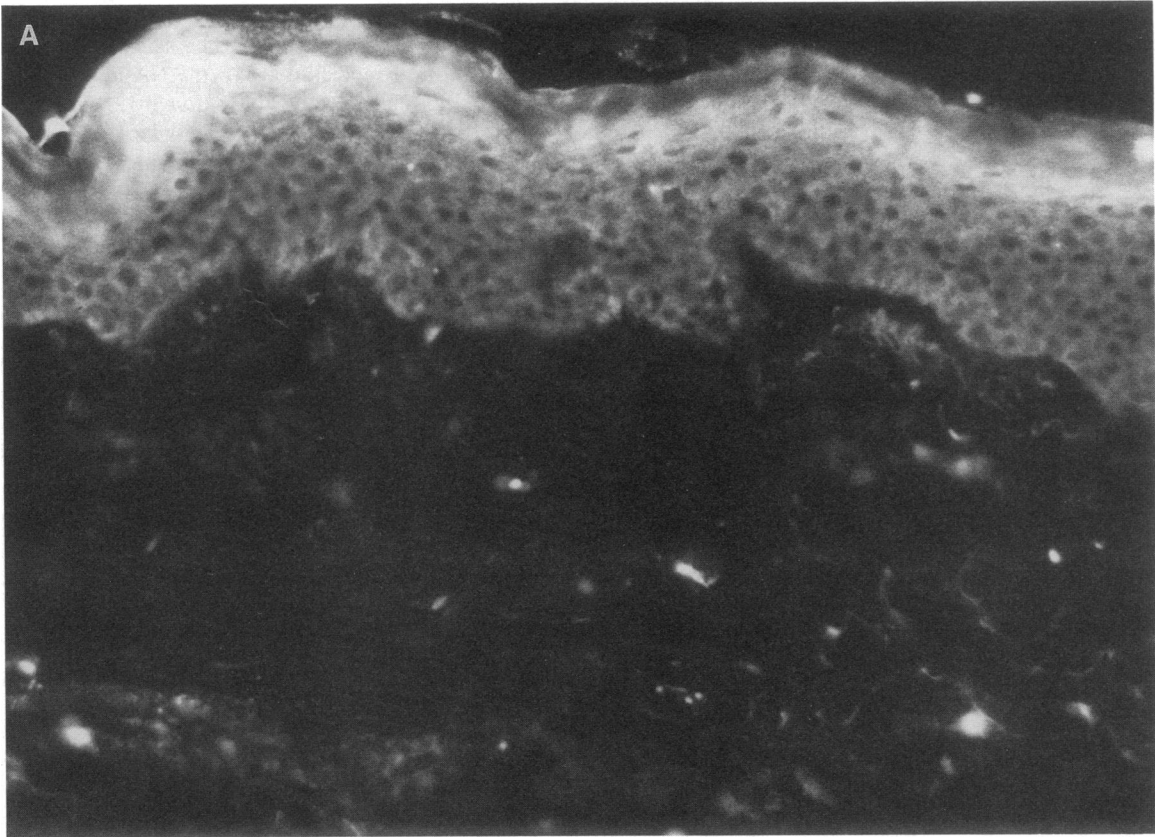


Figure 10. Localization of IL-1ra protein in normal and psoriatic skin by indirect immunofluorescence. Normal skin reacted with (A) anti-IL-1ra mAb I.4 or (B) I.4 preincubated with soluble recombinant IL-1ra. Psoriatic involved skin stained with (C) I.4 or (D) I.4 preincubated with soluble recombinant IL-1ra. Psoriatic uninvolved skin stained with (E) I.4 or (F) I.4 preincubated with soluble recombinant IL-1ra (original magnification $\times 50$).

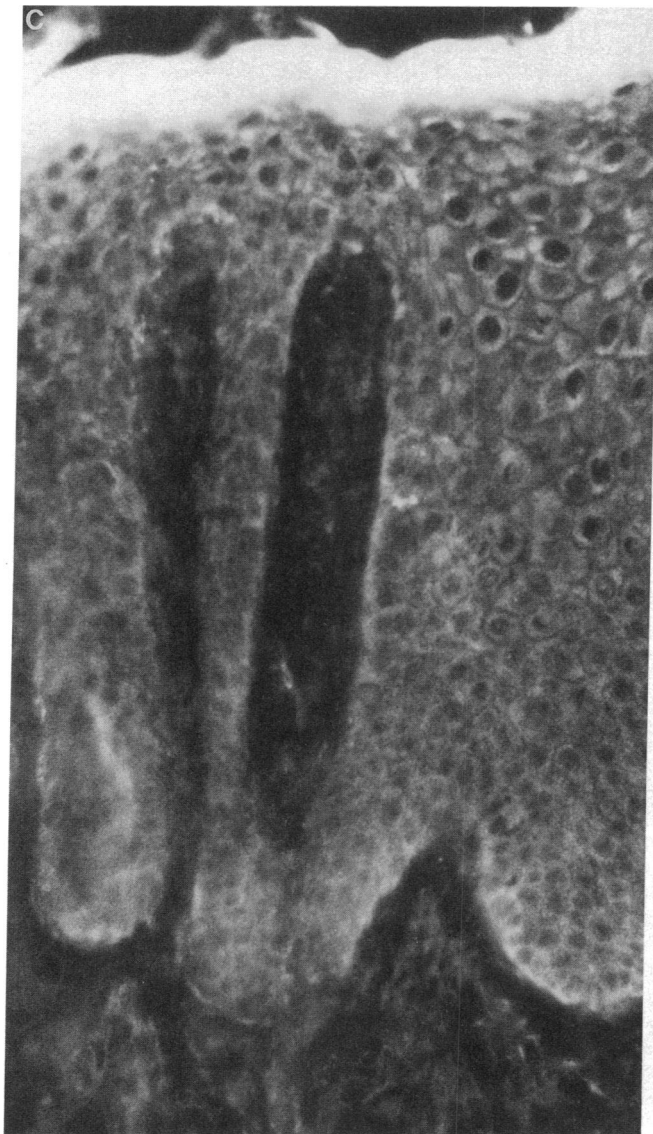


Figure 10 (Continued)

mAbs I.4 or I.10 against normal epidermis resulted in heavy staining of the cytoplasm of keratinocytes located in the stratum granulosum but in minimal staining of the basal and mid-epidermis (Fig. 10 A). This reactivity could be absorbed by preincubation with soluble recombinant IL-1ra (Fig. 10 B). This layer of the epidermis is associated with the expression of a number of genes associated with terminal differentiation of keratinocytes. In contrast to the stratum granulosum staining of normal skin, the greatest intensity of staining by I.4 against epidermis from clinically stable psoriatic lesions occurred in the basal-midbasal layers (Fig. 10 C). In lesional psoriatic skin, these compartments contain not only an increased population of cells in cell cycle, but also undergo accelerated differentiation as indicated by their expression of genes not normally expressed until the stratum granulosum (47, 48).

When the anti-IL-1ra mAb I.4 was reacted with epidermis from uninvolved psoriatic skin, a reactivity pattern intermediate between normal and involved psoriatic skin was observed (Fig. 10 E). Heavy staining was observed in both differenti-

ing mid-epidermal cells and basal cells, as well as in basal cells of the elongated rete pegs and papillary tips. As occurred in normal epidermis, preincubation of the mAb with soluble recombinant IL-1ra blocked the reactivity patterns observed in psoriatic involved (Fig. 10 D) or uninvolved (Fig. 10 F) epidermis. Nonspecific staining of the stratum corneum in psoriatic involved and uninvolved skin was revealed by the inability of soluble recombinant IL-1ra to block this staining (Fig. 10, D and F) and by visualization of an identical staining pattern using the MOPC 21 isotype control antibody (data not shown). Of additional note was the occurrence of unidentified dermal cells in lesional psoriatic skin that express IL-1ra (Fig. 10 C).

Discussion

The results of our studies indicate that IL-1ra is present in both normal and psoriatic epidermis. The epidermal IL-1ra had a molecular mass of 20 kD by SDS-PAGE and an apparent mo-

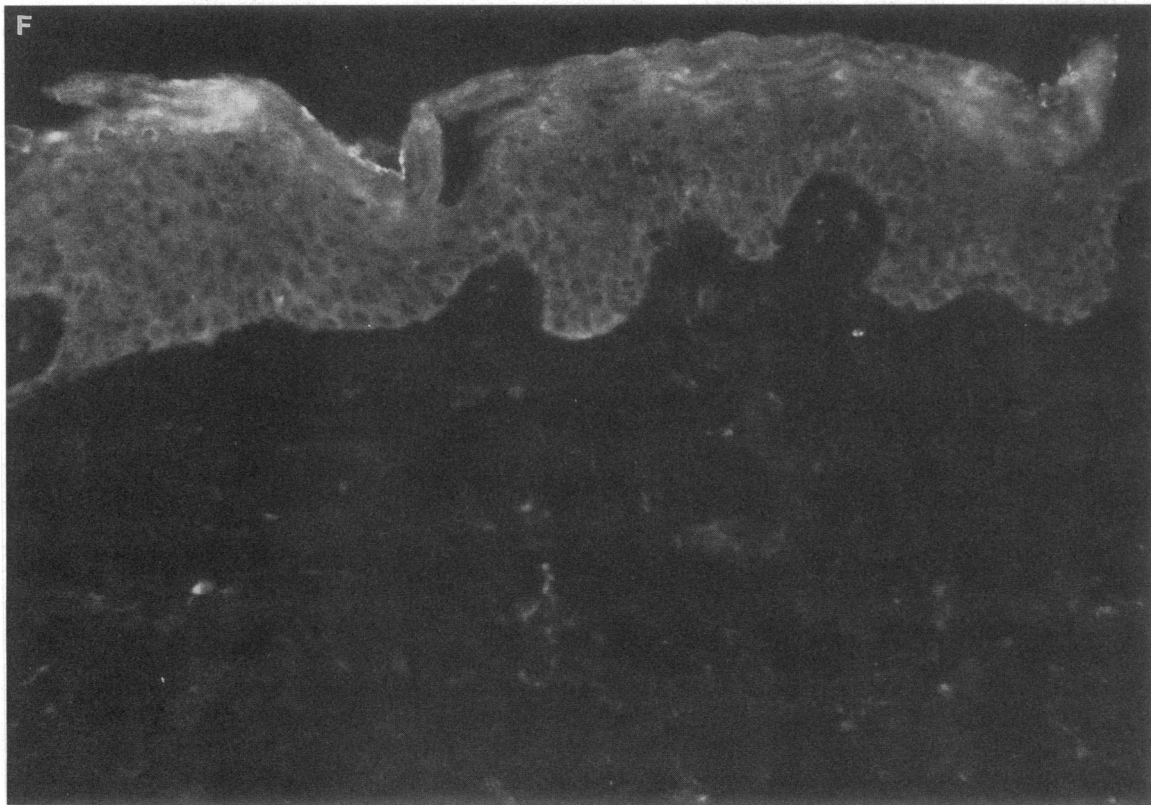


Figure 10 (Continued)

lecular mass of 30 kD by gel-filtration chromatography, likely because of the tendency of silica-based columns to retard movement of hydrophobic proteins such as IL-1. Although other keratinocyte-derived inhibitors of IL-1 activity have been described, (49, 50) their size (40 and 50 kD [49, 50], respectively) and more basic pI (8.8) (49) distinguish them from the IL-1ra activity described here. The size of the protein, as determined by SDS-PAGE, would indicate that epidermal IL-1ra is similar to the intracellular IL-1ra produced by cultured keratinocytes (38) and is slightly larger than the 17-kD nonglycosylated form of monocyte IL-1ra (32, 33, 38). However, extracellular monocyte-derived IL-1ra is primarily in the 22–25-kD glycosylated form (32, 33). The absence of this size form in keratinocyte lysates or conditioned media (39) or in epidermal keratome cytosols as shown in these studies suggests that epidermal IL-1ra is not glycosylated to any significant degree. Furthermore, the absence of the coding region for a leader peptide in the cDNA sequence for intracellular IL-1ra would predict that this molecule would remain inside the cells; indeed this molecule is not detected in the medium of cultured keratinocytes untreated or treated with UV light (100 mJ/cm²) (not shown).

PCR analysis revealed the presence in normal skin of mRNAs for both the monocyte and keratinocyte variants of IL-1ra. Both forms were also present in involved psoriatic epidermis, although in greatly increased amounts and with the mRNA for the keratinocyte variant of IL-1ra exceeding by a factor of 10 the mRNA for the monocyte form. However, given the possible problems with using PCR to quantitate mRNA these numbers should not be taken as absolute. Since cultured keratinocytes contain only the mRNA for the intracellular form of IL-1ra (38), the mRNA for the monocyte variant found in the epidermis by PCR may be derived from Langerhans or other cells. That the 17-kD monocyte-derived protein was not found in normal or psoriatic keratome cytosols by Western blot analysis may be due to the relative insensitivity of this technique. Alternatively, the mRNA for the monocyte variant of IL-1ra may be present in skin with little to no protein translated. The *in situ* immunofluorescence staining of IL-1ra protein did not resolve the differences in sIL-1ra expression obtained by Western blot analysis and PCR analysis, since the antibodies recognize a common sequence and cannot distinguish between the two forms.

The overall IL-1ra levels were lower in psoriatic epidermis from clinically stable plaques as compared with normal epidermis. However, the decrease in psoriatic IL-1ra levels was not as extreme as that observed for IL-1 α (30), resulting in a 10-fold increase in the amount of IL-1ra relative to IL-1 α in psoriatic lesions compared with normal skin ($P < 0.0004$). The epidermal IL-1ra levels may represent both the monocyte and keratinocyte variants of this molecule. Antisera are not yet available to discriminate between these two forms of IL-1ra. Another major difference between psoriatic and normal epidermis is the location of the IL-1ra protein as revealed by indirect immunofluorescence. IL-1ra protein in normal epidermis is localized predominantly in the stratum granulosum. In psoriatic epidermis from clinically stable plaques, the intense band of IL-1ra staining in the granular layer disappears and is replaced with a more homogeneous staining pattern in the squamous cell and granular layers. In addition, the psoriatic lesions showed increased presence of IL-1ra protein in the basal to

midbasal layers. This pattern resembles changes in differentiation that have been reported for involucrin and transglutaminase in epidermal cells *in vivo*. These molecules are normally expressed in the more differentiated cells of the stratum granulosum but in psoriasis are present in cells above the basal layer (47, 48, 51). IL-1 α , the IL-1 molecule that is present in the highest amount in the epidermis (30), has also been detected throughout the epidermis (52). However, IL-1 α may be compartmentalized to a region of normal epidermis reciprocal to IL-1ra, as polyclonal anti-IL-1 α antibodies preferentially react with the basal cell layer (53).

The increased expression of IL-1ra protein in the lower levels of lesional psoriatic epidermis may represent activation of IL-1ra in concert with other terminal differentiation-associated proteins. The signals that induce accelerated terminal differentiation and epidermal growth in clinically stable lesions of psoriasis are increasingly being attributed to cytokines released as a result of immune activation of cells (54–58). Increased numbers (22, 23, 59) and activity (55–58, 60) of skin-infiltrating T lymphocytes, antigen-presenting cells (24, 61), cytokines (62, 63), phospholipase C/protein kinase C-mediated cellular signals (64), and growth factors (54, 65) have been implicated in the maintenance of the characteristic features of this disease. In addition, these factors may influence the altered epidermal differentiation (47, 48, 66, 67) and the increased keratinocytic, endothelial, and dendritic cell proliferation characteristic of psoriatic lesions (25–27).

Alternatively, increased expression of the intracellular IL-1ra protein may be a driving force for altered keratinocyte differentiation in psoriasis. The intracellular nature of both the IL-1ra protein (38) and IL-1 α and IL-1 β (13) produced by epidermal cells suggests that the IL-1ra protein may play an intracellular role in regulating keratinocyte responses to cytoplasmic constitutively produced IL-1. This regulation may even be occurring in the nucleus because IL-1 has been detected in the nucleus of keratinocytes (30). Haskill et al. (38) proposed that the intracellular IL-1ra may antagonize IL-1 by binding to IL-1 receptors possibly present on the nuclear membrane (68).

The involvement of IL-1 in regulating keratinocyte growth is controversial, with either potentiating (20) or inhibitory effects (69) being observed when IL-1 is incubated with cultured human keratinocytes. The discrepancy between these observations could be due to differences in culture conditions. Ristow (29) showed that IL-1 alone is not directly mitogenic for keratinocytes, but, in the *in vivo* milieu of multiple cytokines and growth factors, extracellular IL-1 does appear to be a growth promoter. Varying the concentration of intracellular (keratinocyte) IL-1ra relative to IL-1 in keratinocytes may provide an additional level of control of keratinocyte growth. An imbalance in the icIL-1ra/IL-1 α ratio could be another exacerbating factor resulting in the maintenance of the hyperproliferative state or accelerated differentiation of keratinocytes in clinically stable psoriatic plaques.

In conclusion, we demonstrated that normal human epidermis constitutively produces an IL-1 inhibitor and that this inhibitor is identical to a mRNA splicing variant (38) of the monocyte-derived IL-1 receptor antagonist cloned by Eisenberg et al. (34) and Carter et al. (33). This variant lacks a leader sequence for secretion (38) and thereby resides intracellularly in unperturbed human epidermis, predominantly in cells un-

dergoing terminal differentiation in the stratum granulosum. Our initial finding that an IL-1 inhibitory bioactivity was elevated in clinically stable psoriatic epidermis can now be accounted for by the observations that this activity represents intracellular IL-1ra protein expressed aberrantly in lower layers of lesional epidermis in association with markedly decreased IL-1 α levels. Understanding the events leading to alterations in the ratio of IL-1ra and IL-1 in cells as well as the functional consequences of such alterations will yield important information on intracellular effects of such potent cytokines as IL-1.

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