Effects of Recombinant Interleukin 1 and Interleukin 2 on Human Keratinocytes

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The effects of recombinant interleukin 1 alpha and beta, as well as recombinant interleukin 2, on human keratinocyte proliferation were studied in serum-containing as well as defined media. Both interleukin 1 preparations did not stimulate keratinocyte growth; interleukin 2 also did not stimulate keratinocyte growth. To determine whether interleukin 1 beta binds to keratinocytes, a cell membrane assay was developed for these cells. Iodinated interleukin 1 beta binds to keratinocytes with a kD of 6.2 nm and 2500 receptors per cell. To determine the effects of interleukin 1 beta on protein synthesis, the molecular patterns of radiolabeled cell extracts of interleukin 1 beta-treated and nontreated keratinocytes were compared using two-dimensional polyacrylamide gel electrophoresis. No significant changes in the molecular pattern of newly synthesized proteins were detected. Finally, none of these lymphokines induced HLA-DR expression by keratinocytes. *J Invest Dermatol 93:121–126, 1989*

Both murine and human keratinocytes produce an interleukin 1 (IL-1)-like substance termed epidermal cell-derived thymocyte activating factor (ETAF) [1,2]. Whether this substance is identical to IL-1 is still not clear, but ETAF appears to have functions similar to those of IL-1 [3,4]. Other potential sources of IL-1 in the human skin include macrophages and Langerhans cells (LC) [5,6]. In the presence of a foreign antigen, IL-1 activates T cells and, in conjunction with interleukin 2 (IL-2), causes proliferation of these cells [7,8]. An IL-1-like substance has been documented in the skin in psoriasis [9]. Indeed, several investigators have suggested that an IL-1-like substance enhances murine as well as human keratinocyte proliferation in vitro [10,11]. By contrast, when injected into the skin in vivo, IL-1 does not cause acanthosis [12].

An IL-2-like substance, termed keratinocyte-derived T-cell growth factor, has recently been described in human skin [13,14]. Interleukin 2 is the actual proliferative signal for T lymphocytes and induces IL-2 receptors on these cells [8].

Because products, such as gamma interferon, of immune competent cells, influence the growth of keratinocytes, as well as inducing the synthesis of new proteins [15,16], we examined the effects of two other cytokines, IL-1 and IL-2, on human keratinocytes.

MATERIALS AND METHODS

Lymphokines, Labeling with Monoclonal Antibodies (mAb) and Fluorescence Microscopy The recombinant IL-1 (rIL-1) alpha and beta were a gift of Dr. A. Allison (Syntex Corp. Palo Alto, CA) and were biologically active as measured in the thymocyte proliferation assay and the EL-4 conversion assay (E. Eugui, personal communication). The recombinant IL-2 (rIL-2) was obtained from both Cetus, Inc., Emeryville, CA, and Cellular Products, Inc., Buffalo, NY. The latter preparation comes diluted in 2% fetal calf serum (FCS). Both rIL-2 products demonstrated biological activity in that they stimulated human peripheral blood lymphocyte proliferation. The antiHLA-DR mAb (L243) was obtained from Becton Dickinson, Mountain View, CA, as was the isotype control mAb, anti-Leu-2b. The endotoxin concentration in the Cetus rIL-2 preparation was less than 0.1 ng/ml.

One million epidermal cells were stained for 30 min with the murine mAb diluted in 5% heat-inactivated FCS in phosphate-buffered saline (PBS) containing 0.02% sodium azide (AZ), as described previously [17]. The cells were washed with 5% FCS/PBS/AZ, stained with fluorescein isothiocyanate-conjugated rabbit anti-mouse IgG (R/M-FITC] (ICN Immunobiologicals, Lisle, IL) for 30 min, washed with and then resuspended in 5% FCS/PBS/AZ. The number of fluorescent cells was determined by fluorescence microscopy or fluorescent-activated cell sorter (FACS) analysis [17].

Cell Culture Conditions Single cell suspensions of healthy skin were prepared from skin obtained at surgery [18]. Trimmed skin

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Abbreviations:
AZ: sodium azide
BPE: bovine pituitary extract
BSA: bovine serum albumin
DMEM: Dulbecco's modified Eagle's medium
EDTA: ethylenediaminetetraacetic acid
ETAF: epidermal cell-derived thymocyte activating factor
FACS: fluorescent activated cell sorter
FCS: fetal calf serum
IEF: isoelectric focusing
IL-1: interleukin 1
IL-2: interleukin 2
KDM: keratinocyte defined medium
KGM: keratinocyte growth medium
LC: Langerhans cell
1D-PAGE: one dimensional polyacrylamide gel electrophoresis
PBS: phosphate-buffered saline
rIL-1: recombinant interleukin 1
rIL-2: recombinant interleukin 2
R/M-FITC: fluorescein isothiocyanate conjugated rabbit anti-mouse IgG
SDS: sodium dodecyl sulfate
2D-PAGE: two-dimensional polyacrylamide gel electrophoresis
2-ME: 2-mercaptoethanol

was cut into 1 × 5 cm strips and split-cut with a Castroviejo keratotome set at 0.1 mm. The resulting slices were treated for 35 min at 37°C with 0.3% trypsin plus 0.1% ethylenediaminetetraacetic acid (EDTA) in GNK (0.8% NaCl, 0.04% KCL, 0.1% glucose, 0.084% $NaHCO_3$, pH 7.3). Other keratinocyte cultures, derived from breast skin, were obtained from Clonetics Corp., San Diego, CA. Keratinocytes were grown using three different methods of culture. In the first, dispersed cells were suspended in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% heat-inactivated FCS, 50 μg/ml gentamicin and 2 mM L-glutamine (complete medium), and seeded at $1.8-2 \times 10^6$ cells/3.5 cm collagen-coated Petri dish (Lux, Miles Scientific, Naperville, IL) [18]. For culture in serum-free medium, the method described by Ham and Boyce was used [19,20]. The cells were trypsinized and seeded at 3×10^4 cells/3.5-cm Petri dish in keratinocyte growth medium (KGM) (Clonetics) for use in the experiments. Using the third method, adult keratinocytes were grown in a fully defined medium (keratinocyte defined medium [KDM], Clonetics), without bovine pituitary extract (BPE). Both KGM and KDM have a short shelf-life and were used within 2 weeks of arrival as recommended by the manufacturer. In both the complete medium and KGM the cell viability was above 95%, whereas in KDM the cell viability was lower and varied. Unless otherwise indicated, the lymphokine(s) was added 24 h after seeding the cells and readded biweekly with each medium change.

Cell Harvesting and Cell Counts At the times indicated, cultures were washed once with PBS, 1 ml of 0.3% trypsin/0.1% EDTA in GNK added, and the plates incubated for 10 min at 37°C. The detached cells were transferred to tubes, the plates rinsed once with complete medium to remove adherent cells, and this rinse combined with the 1-ml aliquots already in the tubes. Using a hemacytometer, the cells were counted immediately to determine total number of cells per plate, and an aliquot was diluted with trypan blue to document the numbers of viable cells.

Labeling of Cells with ^{35}S-Methionine and One- and Two-Dimensional Polyacrylamide Gel Electrophoresis (1 and 2D-PAGE) Keratinocytes were isolated and cultured in complete medium as described above. The cultures were incubated with [^{35}S]methionine (10 μCi/ml) for 18 h in methionin-free DMEM and harvested using trypsin. After addition of serum to stop trypsin activity, the cells were collected by centrifugation, washed once with PBS, resuspended in 90 μl PBS containing 10 μg/ml DNase I, 10 μg/ml RNase, 1 mM phenyl methane sulphonyl fluoride, 1 mM *N*-methylmaleimide, 5 mM Mg_2SO_4, and 10 μl 10% Triton x-100 in PBS was added with mixing. After centrifugation, the supernatants were carefully removed as the Triton-soluble fraction. The pellet was reextracted successively with 50 mM citrate buffer, pH 2.65 (prekeratin fraction), in the cold and 2% sodium dodecyl sulfate (SDS), 2.5% 2-mercaptoethanol (2-ME) in Tris, pH 6.8 (sample buffer), at 100°C for 10 min. Where necessary, protein fractions were brought to 2% SDS, 2.5% 2-ME by addition of an equal volume of twice the sample buffer and boiled for 10 min. One-D-PAGE was carried out using the discontinuous system of Laemmli [21] at a constant current density of 200 mA/cm^2 until the tracking dye (bromophenol blue) reached the bottom of the gel. Lane loads were adjusted to the same total radioactivity. Two-D-PAGE was performed using isoelectric focusing (IEF) with pH 5–7 ampholines (LKB, Broma, Sweden) in the first dimension and the discontinuous SDS buffer system in the second [22]. All gels were fixed in 50% methanol, 10% acetic acid water, soaked in EnHance, washed for 30 min in water, dried, juxtaposed to Kodak X-Omat film, and stored in the dark at −70°C for 6–72 h, depending on the radioactivity in the gel, and the films developed. Newly synthesized proteins in the IL-1 treated cultures were compared visually with those in mock treated cultures.

Keratinocyte Particulate Preparation Cultured human keratinocytes were removed from 3.5-cm Petri dishes and homogenized, using a Polytron (Brinkman, Westbury, NY) (setting #4 for 30 sec), to a 5% homogenate (wt/vol) in 0.05 M phosphate buffer (81 mM Na^+, 9 mM K^+, 0.09 mM Ca^{2+}, 0.5 mM Mg^+, pH 7.43 at 4°C) [23]. Each homogenate was washed by diluting it with 20 ml of phosphate buffer and centrifuging it at 50,000 *g* for 15 min in a Beckman J2-21 centrifuge (Beckman Instruments, Inc., Fullerton, CA). The cell homogenates were washed by dilution and centrifugation to remove any endogenous substances that might interfere with the [^{125}I]rIL-1 binding assay. The supernatant was discarded and the pellet resuspended in the appropriate volume of phosphate buffer. A 0.1-ml aliquot was saved for analysis of protein content by the method of Lowry et al [24].

Receptor Binding Studies For the standard cell-membrane assays, cells were cultured either in serum-containing medium or in KGM and then switched to KDM 2 days before harvesting. At confluence, the cells were scraped off, and aliquots of the washed tissue suspension were added to two sets of duplicate 2-ml plastic centrifuge tubes that were pretreated with 1% bovine serum albumin (BSA) and allowed to air dry for 12 h before the experiment [23]. One set (total binding) contained varying [^{125}I]rIL-1 beta concentrations. The other set (nonspecific binding) contained varying [^{125}I]rIL-1 beta concentrations and 100 nm unlabeled rIL-1 beta in 1 ml phosphate buffer. The binding of [^{125}I]rIL-1 beta was studied between 0.025 and 10 nm. Both sets of tubes (total and nonspecific binding) were routinely incubated at 4°C for 60 min to ensure steady-state conditions. The suspensions were diluted with 4 ml of cold buffer and filtered immediately through glass fiber filters (Whatman GF/B, 2.5 cm). Each of the tubes was rinsed again with 4 ml ice-cold buffer, which was poured over the appropriate filter in a 30-well filter box (Steed Engineering Co., Palo Alto, CA). All filters were washed an additional two times with 4 ml of ice-cold buffer. The filters were air-dried and the membrane-bound radioactivity estimated by gamma spectrometry (Beckman Instruments, Inc., Fullerton, CA) with a counting efficiency of 43%. Specific [^{125}I]rIL-1 binding for each substrate concentration (expressed as nmol [^{125}I]rIL-1/mg protein) was calculated by subtracting the nonspecific from the total binding.

RESULTS

Effect of rIL-1 Alpha and Beta as well as rIL-2 on Keratinocyte Growth The effects of rIL-1 alpha and beta on keratinocyte growth were tested using various media. In medium-containing serum, all concentrations of IL-1 beta tested (1–100 ng/ml) inhibited growth in a dose-dependent fashion (Fig 1). One hundred

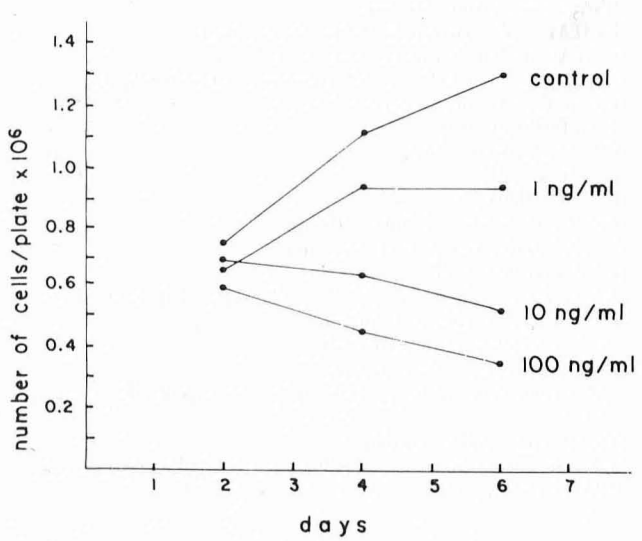


Figure 1. The effect of nanogram concentrations of rIL-1 beta on keratinocyte growth in serum-containing medium. The doses of rIL-1 beta used were 1–100 ng/ml. The lymphokine was added 48 h after seeding the cells. The IL-1 beta significantly inhibits keratinocyte growth at 10–100 ng/ml. The SEM was less than 20% for each time point. A similar pattern of growth inhibition was seen in a second, separate experiment.

ng/ml rIL-1 beta inhibited cell proliferation about threefold by day 6 after addition of the cytokine.

In KGM, the rIL-1 beta at low concentrations (2.5 ng/ml) did not significantly affect cell numbers/Petri dish of logarithmically growing keratinocytes over a 7-day period (not shown). At higher concentrations (100 ng/ml), however, rIL-1 beta again markedly inhibited keratinocyte growth (not shown). The rIL-1 alpha at 10 ng/ml also did not affect keratinocyte growth under these conditions (not shown).

Because keratinocytes grown in serum-containing medium or in KGM may already be proliferating maximally, the effect of the lymphokines on cells grown in KDM also was examined. Keratinocytes grown in KGM were switched to KDM for 2 days, and then reseeded in KDM at 3×10^4 cells/plate. Twenty-four hours later, 10 ng/ml rIL-1 alpha or beta was added (Fig 2). At the various time-points indicated, total numbers of cells/plate, as well as viable cells/plate, were determined. As can be seen, rIL-1 alpha and beta did not significantly affect either the total number of cells/plate or the number of viable cells/plate.

To determine if picogram quantities of IL-1 alpha or IL-1 beta would stimulate growth of keratinocytes, the cells were incubated with 1, 5, 25, and 125 pg/ml doses of these lymphokines in KDM. Cell numbers were determined on the days indicated after addition of the proteins (Table I). No stimulation by either lymphokine could be documented.

The rIL-2 preparation from Cellular Products comes diluted in 2% FCS and we routinely dilute the stock rIL-2 from Cetus in human serum to prevent binding to the plastic. Therefore, we determined the effects of low concentrations of protein (e.g., serum, BSA) in this system of culture using KDM (Table I). Low concentrations of either human serum alone or BSA alone stimulated keratinocyte growth under these conditions of culture.

To determine the effect of rIL-2 on keratinocyte proliferation, the cells grown in either KDM or KGM were incubated with 12.5, 125, and 625 pg/ml rIL-2. No stimulation of growth could be demonstrated vis-a-vis the controls cultured in the appropriate amounts of protein (Table II). In a separate experiment, even higher doses of rIL-2 (up to 1.25 ng/ml) were used and again demonstrated no effect on keratinocyte growth (not shown).

Finally, keratinocytes were grown in KGM in the presence of between 12.5–625 pg/ml rIL-2. These cells also showed no difference in their growth rates as compared with controls (not shown).

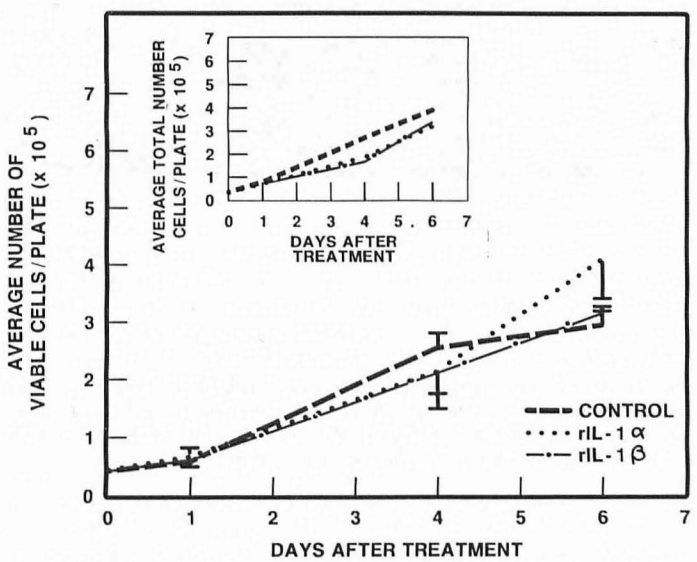


Figure 2. The effect of rIL-1 alpha (10 ng/ml) and beta (10 ng/ml) on keratinocyte growth. The *insert* documents average total number of cells/plate. The *error bars* indicate standard error of duplicate experiments. This data is representative of a pattern seen in three separate experiments.

Table I. Effect of Various Proteins on Proliferation of Keratinocytes Grown in KDM

Days in Culture	Addition	Cell Number (% Control)[a]
5	0.01% HS[b]	142[c]
10	0.01% HS	123
5	0.004% BSA	187[c]
10	0.004% BSA	255[c]
5	1 pg/ml IL-1 alpha	116
5	5 pg/ml rIL-1 alpha	108
5	25 pg/ml rIL-1 alpha	108
5	125 pg/ml rIL-1alpha	116
6	1 pg/ml IL-1 beta	93
6	5 pg/ml IL-1 beta	100
6	25 pg/ml IL-1 beta	87
6	125 pg/ml IL-1 beta	106

[a] The n = 4 for each value.
[b] HS = human serum.
[c] These numbers are statistically different from the controls with a p value less than 0.005.

Binding of [^{125}I]rIL-1 Beta to Cultured Keratinocytes Because the absence of a growth stimulatory effect of rIL-1 beta on keratinocytes could be explained by a lack of cell-surface receptors for this protein, we studied specific binding of rIL-1 beta to keratinocytes. The rIL-1 beta was labeled with ^{125}I, using Bolton Hunter reagent according to the method described previously, and then used to measure rIL-1 beta binding to cultured, human keratinocyte cell membranes [23]. Saturation of the rIL-1 binding sites was measured at [^{125}I]rIL-1 beta concentrations between 0.025–10 nm. Unlabeled rIL-1 beta was used to define nonspecific binding and maximally inhibited [^{125}I]rIL-1 beta binding at a concentration of 100 nm. Saturable, specific [^{125}I]rIL-1 beta binding was approximately 70% of the total binding at 10 nm and approximately 80% of the total binding at 0.025 nm [^{125}I]rIL-1 beta. Analysis of these saturation isotherms gave a K_d of 6.2 nm and a B_{max} of 1.0 pmole/mg protein for the cell-membrane preparation (Fig 3). Approximately the same number of receptors/cell were demonstrated regardless of whether the cells were cultured in KGM/KDM or in serum-containing medium.

Table II. Effect of rIL-2 on the Proliferation of Keratinocytes Grown in KDM and KGM

Days in Culture	Concentration of rIL-2 (pg/ml)	Medium	Number of Cells/Plate
	Control[a]	KDM	$2.2 \pm 0.14^b \times 10^4$
5	12.5[c]		$2.2 \pm 0.35 \times 10^4$
	125		$1.7 \pm 0.1 \times 10^4$
	625		$1.9 \pm 0 \times 10^4$
	Control		$1.2 \pm 0 \times 10^4$
10	12.5		$1.6 \pm 0.14 \times 10^4$
	125		$1.5 \pm 0.14 \times 10^4$
	625		$1.3 \pm 0.2 \times 10^4$
	Control[a]	KGM	$7.3 \pm 0.7 \times 10^4$
5	12.5		$5.6 \pm 0.6 \times 10^4$
	125		$6.1 \pm 0.35 \times 10^4$
	625		$6.2 \pm 1.0 \times 10^4$
	Control		$2.4 \pm 0.14 \times 10^5$
10	12.5		$2.6 \pm 0.14 \times 10^5$
	125		$2.5 \pm 0.14 \times 10^5$
	625		$2.3 \pm 0.1 \times 10^5$

[a] The control cells received as much human serum (0.5×10^{-4}%) as was present in the highest concentration of rIL-2 added to the lymphokine-treated cultures.
[b] These counts represent the average of duplicate plates ± SEM. The numbers of cells per plate did not show a significantly different trend between control and rIL-2-treated cultures.
[c] 12.5 pg/ml rIL-2 = 0.1 U/ml.

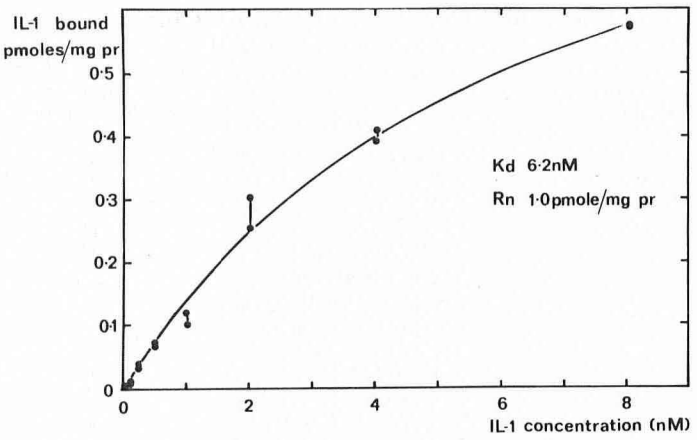


Figure 3. Binding of rIL-1beta to the particulate fraction prepared from cultured keratinocytes. Each point represents an independent measurement. K_d is the dissociation constant of the binding reaction and Rn is the concentration of IL-1 binding sites in the particulate fraction. The points were fitted directly to the binding equation by the method of least squares using a simplex algorithm iterative procedure [37]. Equal weight was given to each experimental value. The ordinate plots IL-1 bound as pmole/mg protein.

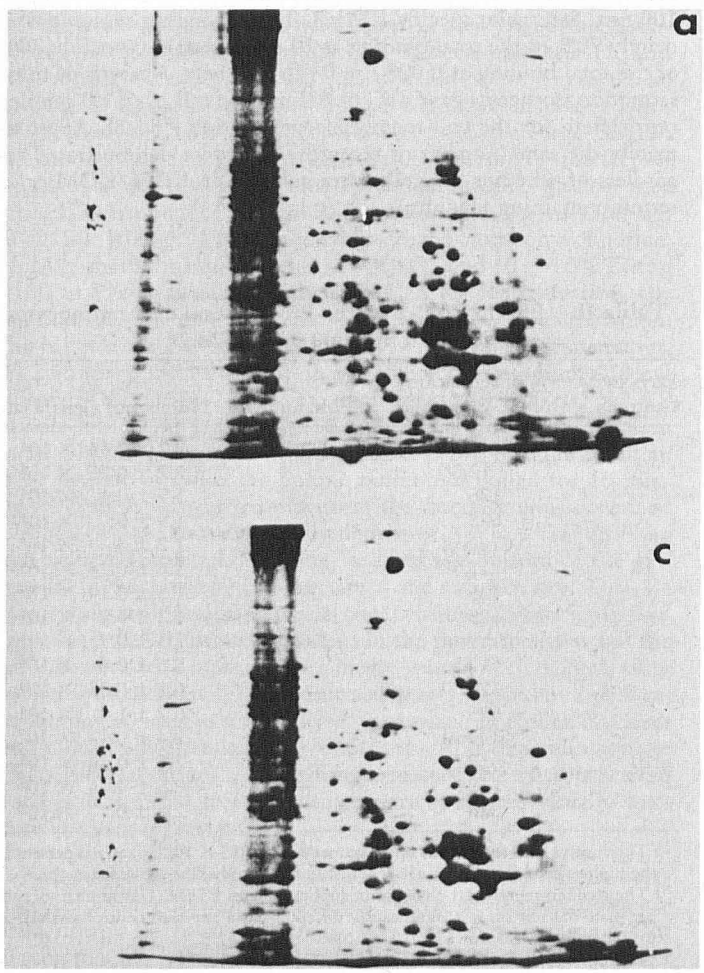
Molecular Pattern of the Proteins Synthesized by Keratinocytes After rIL-1 Beta Treatment To determine whether rIL-1 beta affected the protein synthesis of keratinocytes the cells were radiolabeled 3 days after the addition of lymphokine (0.5 and 5.0 ng/ml) and the cells harvested 18 hours later. Synthesis of total proteins was not affected significantly in rIL-1 beta-treated cultures at the doses tested. The [^{35}S]methionine-labeled cellular proteins synthesized after rIL-1 beta treatment were separated into Triton-soluble and Triton-insoluble fractions and analyzed using 1 and 2D-PAGE. Both Triton-soluble and Triton-insoluble proteins showed no major difference in their molecular patterns at either of the two concentrations of rIL-1 beta tested as compared with controls. The 2D-PAGE gels of the Triton-soluble proteins are shown (Fig 4).

Expression of HLA-DR Antigen by Keratinocytes After rIL-1 Alpha and Beta as well as rIL-2 Treatment Because gamma interferon induces the expression of HLA-DR antigen on keratinocytes, we examined whether rIL-1 alpha or beta had a similar effect. Cells were incubated with various concentrations of rIL-1 beta (1, 10, 100 ng/ml) in growth medium. On days 2, 4, and 6, representative cultures were harvested, and the expression of HLA-DR antigen was determined using immunofluorescence staining. No HLA-DR antigen expression could be detected on the keratinocytes at any of these time-points (not shown). In parallel cultures, all of these concentrations of rIL-1 beta inhibited keratinocyte growth in a dose-dependent fashion. The effect of rIL-1 alpha (10 ng/ml) on HLA-DR expression by keratinocytes was ascertained on day 8 after cytokine addition in KGM. The rIL-1 alpha also did not induce HLA-DR expression (not shown).

The capacity of rIL-2 (1.3 ng/ml; 10 U/ml) to induce the expression of HLA-DR was examined on day 10 after addition of the cytokine. No expression of DR antigen was found (not shown).

DISCUSSION

Isolated particulate matter from cultured human keratinocytes demonstrate specific, saturable [^{125}I]rIL-1 beta binding. This binding was both temperature- and time-dependent, and steady-state condi-



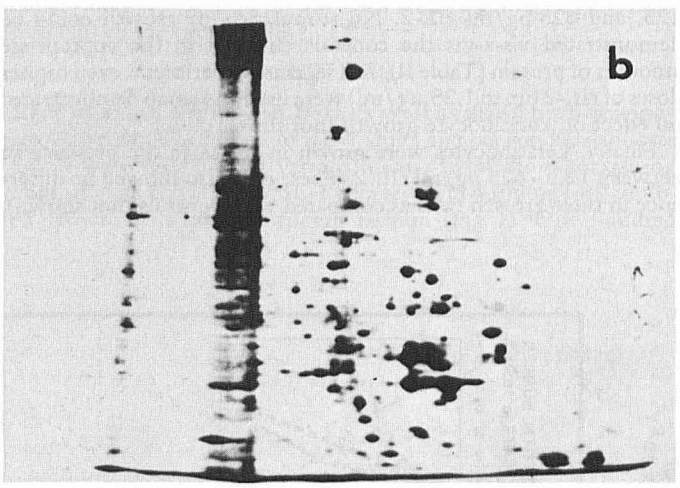


Figure 4. Two-dimensional polyacrylamide gels of labeled extracts of rIL-1 beta-treated keratinocytes show no major changes in the patterns of triton soluble protein(s). *a*: 0.5 ng/ml rIL-1 beta, *b*: 5 ng/ml rIL-1 beta. Gels from the control, untreated keratinocytes are depicted in *c*.

tions were reached in 40 min at 4°C. Saturation studies at steady-state conditions revealed a major population of binding sites with K_d in the nanometer range and an average of about 2500 binding sites/cell. The value of the K_d we have obtained is larger than that reported for murine T lymphocytes or fibroblasts [25,26].

Using three different types of media, we have been unable to verify that rIL-1 alpha or beta stimulates keratinocyte proliferation. The experiments by Gilchrest and Sauder [11] were not performed using recombinant material, and it is conceivable that other factors in their preparation were responsible for the increase in keratinocyte growth. Ristow [10], who also reported an increase in keratinocyte proliferation, actually only measured thymidine incorportion into DNA, rather than cell numbers. Thus, it is conceivable that rIL-1 activates uptake of [^{3}H]thymidine or initiates DNA synthesis without causing mitosis. Further in Ristow's system, murine keratinocytes were used, and it is possible that murine cells react differently to IL-1 than do human keratinocytes, as is the case for gamma interferon induction of HLA-DR antigen. Although gamma interferon induces HLA-DR antigen on normal human keratinocytes, this lymphokine does not induce Ia antigen expression on normal, muring keratinocytes in vitro (G. Krueger, personal communication, and V. Morhenn, unpublished data).

Because keratinocytes secrete ETAF, it is conceivable that this autocrine secretion masks the effects of the added, exogenous protein. This explanation is unlikely as the amount of ETAF secreted by keratinocytes would result in insignificant concentrations in the volume of medium used in these cultures. Moreover, the rIL-1 beta causes no major changes in the molecular pattern of Triton-soluble or Triton-insoluble keratinocyte proteins synthesized in vitro, suggesting that no major new protein is produced. It also seems unlikely that IL-1 induces keratinocytes to secrete a growth inhibitory factor that would counteract a putative mitogenic effect of the IL-1 itself. However, we cannot entirely rule out the possibility that a growth inhibitory substance, which is not a protein, is induced by IL-1. Finally, this study confirms an earlier report that IL-1 and IL-2 do not induce HLA-DR antigen expression and expands this finding to include both rIL-1 alpha and beta, as well as, rIL-2 [27].

In psoriasis, a disease of keratinocyte hyperproliferation, abnormalities in immunologic factors, such as cytokines, have been implicated [28–30]. Cyclosporine, a drug that has known immunomodulatory effects, decreases the secretion of IL-1 and IL-2 by lymphocytes and also produces a dramatic improvement in many cases of psoriasis [31,32]. Interestingly, this drug has no direct effect on the proliferation of normal, human keratinocytes in vitro, but does effect their growth when keratinocytes are in a hyperproliferative stage in murine skin [33,34]. Furthermore, cyclosporine does inhibit proliferation of normal murine keratinocytes as well as transformed murine and human keratinocytes in vitro [35].

Because rIL-1 does not stimulate proliferation of keratinocytes, but may activate DNA synthesis in these cells, we also determined the effect of rIL-2 on keratinocyte proliferation. Recombinant IL-2 also does not enhance keratinocyte growth in vitro. This in vitro documentation is consistent with the recent report that when rIL-2 is injected into humans, no acanthosis of the skin is observed [36]. Furthermore, these authors could not demonstrate IL-2 receptors on keratinocytes in situ. Possibly, the exacerbation of psoriasis after rIL-2 administration is due to an effect on the mononuclear leukocytes found in the dermal infiltrate or another dermal constituent (e.g., endothelial cells or fibroblasts) [36]. Based on our study, the exacerbating effect of rIL-2 in psoriasis does not appear to be due to a direct growth-promoting effect of rIL-2 on keratinocytes.

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