Interleukin 1 Binds to Specific Receptors on Human Keratinocytes and Induces Granulocyte Macrophage Colony-stimulating Factor mRNA and Protein

A Potential Autocrine Role for Interleukin 1 in Epidermis

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Abstract

Cultured human keratinocytes have been shown to produce IL-1 alpha and beta mRNA and protein. IL-1 biological activity has been identified in normal human epidermis; in vitro, most biologically active IL-1 resides in a cell-associated compartment. The potential for autocrine effects of IL-1 on human keratinocytes was assessed by measurement of keratinocyte IL-1 receptors. Both high- and low-affinity cell surface receptors that bound recombinant (r) IL-1 alpha and beta with comparable affinities could be identified on cultured human keratinocytes, using ^{125}I-labeled rIL-1. Chemical crosslinking experiments identified a cell surface molecule of roughly 72,500 M_r that bound ^{125}I-labeled IL-1, similar to the molecular weight of previously described IL-1 receptors on fibroblasts, B cells, and T cells. To assess the biological consequences of keratinocyte IL-1 binding, granulocyte-macrophage colony-stimulating factor (GM-CSF) gene expression was measured. The addition of exogenous rIL-1 alpha led to a dose-dependent increase in the accumulation of GM-CSF mRNA, as measured by a sensitive and specific S1 nuclease assay. This increase in mRNA was reflected in a marked increase in GM-CSF biological activity as measured by proliferation of blast cells from chronic myelogenous leukemia patients. The biological activity was completely inhibitable by an antibody to human rGM-CSF. GM-CSF activates mature neutrophils and macrophages and appears to enhance the efficiency of Langerhans cell antigen presentation to T cells. Release of IL-1 from injured or activated keratinocytes may lead to enhanced epidermal GM-CSF gene expression via an autocrine mechanism, thus enhancing local host defense.

Introduction

Epidermal keratinocytes and their terminally differentiated progeny make up the principal physical and cellular barrier between the environment and the internal milieu of the host. By their pattern of differentiation and their production of lipids and structural proteins, keratinocytes are responsible for the physical barrier function of skin. The potential for interactions between keratinocytes and cells of lymphoid and myeloid lineages has been appreciated only recently (reviewed in reference 1). Through their production of cytokines, keratinocytes can also be viewed as cells that signal the immune and inflammatory systems that penetration or disruption of the integument has occurred (1, 2).

The study of keratinocyte cytokine production and its regulation has been greatly enhanced by the development of conditions under which keratinocytes can be grown in virtually pure cultures in vitro (3, 4). Cultured human keratinocytes produce detectable IL-1 alpha and beta in the absence of identifiable stimulation (5). Most of this in vitro IL-1 remains within the cell or associated with the cell membrane, and relatively little is released into the culture medium (2, 6). There is evidence that IL-1 is also made normally by keratinocytes in vivo, since significant IL-1 activity can be demonstrated in stratum corneum and normal epidermis (7, 8). These observations have led to speculation that the epidermis is a reservoir of IL-1, and that injury to the skin results in release of preformed IL-1 which has important effects on immune and inflammatory cells.

Recent evidence indicates that IL-1 is mitogenic for keratinocytes, implying that keratinocytes express receptors for this molecule (9, 10). Other studies have indicated that IL-1 has important effects on cells that normally reside in dermis (e.g., fibroblasts and endothelial cells), including the induction of cytokine gene expression (11, 12). We have recently shown that normal murine keratinocytes and murine keratinocyte cell lines produce granulocyte-macrophage colony-stimulating factor (GM-CSF),[1] and that mRNA from Pam 212 cells, a murine keratinocyte line (13), protects murine T cell GM-CSF cDNA from S1 nuclease-mediated digestion (14). In the present study, we show that human keratinocytes bear significant numbers of IL-1 receptors in vitro. Furthermore, we demonstrate that cultured human keratinocytes produce GM-CSF and that the production of GM-CSF can be increased significantly by recombinant (r)IL-1 alpha. The concurrent production of IL-1, its maintenance in a cell-associated compartment, and expression of IL-1 receptors may represent a biological system poised to respond to environmental injury.

Methods

Cytokines. For GM-CSF induction studies, rIL-1 alpha was provided by Dr. Peter Lomedico (Hoffmann-LaRoche, Nutley, NJ). Human rGM-CSF was produced and purified as previously described (15).

Cytokine assays. An assay for GM-CSF involving [^{3}H]thymidine incorporation by myeloblasts from peripheral blood of chronic my-

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1. *Abbreviations used in this paper:* CML, chronic myelogenous leukemia; DSS, disuccinimidyl suberate; GM-CSF, granulocyte-macrophage colony-stimulating factor.

elogenous leukemia (CML) patients, and using a specific neutralizing antibody for human rGM-CSF, has been described previously (15).

Keratinocyte cultures. Human keratinocytes from foreskins or from patients undergoing reconstructive surgery were obtained as described previously (2, 6) and grown either in DME with 20% FCS on mitomycin C-treated 3T3 fibroblasts by the method of Rheinwald and Green (3), or in MCDB 153 medium (Clonetics Corporation, San Diego, CA) supplemented as described by Boyce and Ham (4). Cells grown in DME were trypsinized and replated on tissue culture plastic after primary cultures reached confluence; these "secondary" cultures were used for all subsequent studies. MCBD 153 cultures were split at 70–80% confluence and were always used no later than the third passage.

IL-1 binding assays. rIL-1 alpha purified as described elsewhere (16) was radiolabeled by a modified chloramine-T method (17). For binding assays, a stock solution of 3×10^{-8} M ^{125}I-rIL-1 alpha was prepared in binding medium (RPMI 1640, 1% BSA, 0.1% Na azide, and 20 mM Hepes, pH 7.2). For binding assays, keratinocytes grown in 24-well trays (Costar, Cambridge, MA) (see above) were washed once with binding medium, which was then replaced with 0.5 ml of the appropriate dilution of the radiolabeled IL-1 with or without 1×10^{-7} M unlabeled IL-1 alpha. For inhibition assays mixtures of ^{125}I-rIL-1 alpha and unlabeled IL-1 alpha or IL-1 beta were added to the washed cultures. All binding data points were generated from duplicate cultures, and two wells on each plate were exposed only to binding medium and counted for cell number at the end of the assay. Plates were subsequently incubated on a shaker platform in a cold room for 2 h. At the end of the incubation 60 μl of supernatant was withdrawn from each well for determination of unbound ^{125}I-rIL-1 alpha concentrations and the remaining supernatant aspirated. Subsequently, plates were washed once with binding medium (3 ml/well) and three times with PBS (pH 7.2). After the final wash the plates were inverted and drained for 5 min and 0.5 ml of trypsin-EDTA was added to each well. Plates were incubated 15–30 min at 37°C on a shaker platform to detach cells, and 0.4 ml of the contents of each well was withdrawn to determine cell-bound IL-1 by gamma counting. Binding data were collected online to a VAX 11/780 computer and converted from counts per minute to concentrations in molars (free ^{125}I-rIL-1 alpha) or in molecules/cell (bound ^{125}I-rIL-1 alpha) using the RPL program BINDINGASSAY. Formatted data were analyzed by nonlinear least squares fitting using RS/1 (Bolt, Beranak, and Newman, Cambridge, MA) to obtain site numbers and affinity constants, as described previously (18).

Affinity crosslinking and SDS-PAGE. Crosslinking experiments were performed essentially as previously described (18). Briefly, 10^7 cells were incubated with 5×10^{-10} M ^{125}I-IL-1 in 150 μl of binding medium for 2 h at 8°C. Subsequently, the cells were washed twice by centrifugation with 1 ml PBS, pH 7.4, and resuspended in 100 μl PBS. 2 μl disuccinimidyl suberate (DSS) (50 mg/ml) in DMSO was added to give a final concentration of 1 mg/ml, and the mixture was incubated for 1 h at 8°C. The cells were then washed twice with PBS and finally resuspended in 50 μl PBS containing 1% Triton X-100 and 2 mM PMSF. The detergent extraction mixture was incubated for 5 min on ice and then centrifuged for 15 min at 8°C in a microfuge to remove nuclei and other cell debris. 10 μl of supernatant was removed for gamma counting and 40 μl was removed and frozen at −20°C until SDS-PAGE analysis. To each of these samples 2 μl of 10% SDS was added and the sample dried under vacuum. The dried aliquots were resuspended in 60 μl of sample buffer (0.06 M Tris-HCl, pH 6.8, 2% SDS, 10% glycerol), boiled for 3 min, and then subjected to electrophoresis in 8% PAGE gels using the stacking gel procedure of Laemmli as described elsewhere (18).

S1 nuclease protection analysis. Total cellular RNA was extracted from the cells by using the guanidinium thiocyanate method (19). The human GM-CSF probe was a 1.2-kb NcO I DNA fragment isolated from pcD-hGM-CSF cDNA (~ 10^6 cpm/μg). 5×10^4 cpm was hybridized with 100 μg of total RNA isolated from human keratinocytes for 16 h at 50°C, then digested with S1 nuclease under conditions described previously (20). The S1-resistant hybrids were denatured and separated on a 5% polyacrylamide gel containing 7 M urea. 5 μg of poly A+ RNA from the human bladder carcinoma line 5637 was used as a positive control for GM-CSF mRNA.

Results

Recently, the cellular receptor for IL-1 present on T cells, B cells, and fibroblasts was identified (8, 18, 21). We investigated whether keratinocytes expressed receptors for IL-1 in vitro. A representative experiment is shown in Fig. 1. In this experiment, human keratinocytes were grown to 75% confluence in MCDB 153 medium and tested for IL-1 binding as described in Methods. *A* shows a simple binding isotherm. Total bound counts were compared with counts bound nonspecifically in the presence of a 100-fold excess of unlabeled IL-1 alpha (*open circles* and *squares,* respectively). Specific binding, the difference between total and nonspecific binding, ostensibly represents binding of IL-1 to a specific cell-surface receptor. A curve extrapolated from these data is consistent with saturable binding.

Fig. 1 *B* shows a Scatchard analysis of the data presented in *A*. The curves, which superimpose upon the data, have been abstracted from the data by nonlinear least squares fitting of the equation: Bound = {[(R1)(K1)(C)]/[1 + (K1)(C)]} + {[(R2)(K2)(C)]/[1 + (K2)(C)]}, where the values R1 and R2 (sites per cell) and K1, K2 (1/M) were allowed to "float." This purely computational approach to the problem does not involve any graphical extrapolations. These data are consistent with the assumption of the presence of two classes of binding sites. The most prevalent species of site is present at 3.3 + 0.2 $\times 10^4$ sites/cell and has a K_a of 3.8 + 0.6 $\times 10^9$, while the other species of site is present at 843 + 259 sites/cell and has a K_a of 1 $\times 10^{12}$.

In the experiment shown in Fig. 1 *C,* varying amounts of unlabeled rIL-1 alpha and beta were added to a fixed amount of ^{125}I-labeled IL-1 alpha in keratinocyte cultures. The binding curves obtained indicate that both rIL-1 alpha and beta inhibited the binding of ^{125}I-IL-1 alpha to cultured keratinocytes in a comparable (but not identical) fashion. The K_a of the binding curve for IL-1 alpha was 2.9 + 0.7 $\times 10^{10}$, and the K_a of the

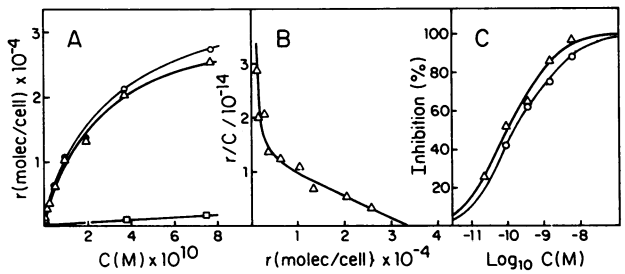

Figure 1. Binding of IL-1 to adult human keratinocyte monolayers. (*A*) Direct binding of ^{125}I-rIL-1 alpha. Total binding (○) has been corrected by subtraction for nonspecific binding (□) measured in the presence of 6×10^{-7} M unlabeled IL-1 alpha, yielding specific binding (△). The nonspecific binding parameter was 2.2×10^{12} molecules/cell/M. (*B*) Data from *A* transformed into the Scatchard coordinate system (see Results). (*C*) Inhibition of ^{125}I-rIL-1 alpha binding by (△) unlabeled rIL-1 alpha and (○) unlabeled rIL-1 beta. Free ^{125}I-IL-1 alpha concentration was 2×10^{-10} M; binding in the absence of inhibitor was $1.79 \times 10^4 \pm 70$ molecules/cell and in the presence of 5×10^{-7} M unlabeled rIL-1 alpha was 305 + 48 molecules/cell.

binding curve for IL-1 beta was $1.5 + 0.5 \times 10^{10}$. The reason that the K_as obtained from these experiments are higher than those of the predominant class of low affinity receptors is that the competition experiments performed in this fashion do not distinguish between high and low affinity binding sites. The significance of the roughly twofold difference in affinity for the two IL-1 species is unknown, but has been previously observed in other cell types (17).

The number of IL-1 receptors observed on cultured keratinocytes is higher than that previously reported for T cells, B cells, and fibroblasts. Similar numbers of receptors were observed in subconfluent cultures of keratinocytes grown in DME with 10% FCS (data not shown). The K_a of the more numerous sites is consistent with K_a observed for T cells and B cells. The second, less numerous class of receptors has a significantly higher K_a. Depending on conditions of growth, confluence, and medium constituents, receptor numbers can vary from >40,000 to 2–300/cell (Kupper, T., and S. Dower, unpublished observations).

Another approach to the determination of specific cell surface receptors for IL-1 on keratinocytes involves the use of chemical crosslinkers. Fig. 2 shows an SDS-PAGE analysis of detergent lysates from normal human keratinocytes incubated with ^{125}I-IL-1, washed, and exposed to the bivalent cross-linking agent DSS. A radiolabeled band at ~ 90,000 M_r is easily detected, yielding an estimated cell surface receptor of 72,500 M_r (rIL-1 alpha = 17,500 M_r). This value is consistent with previously reported molecular weight predictions for the IL-1 receptor on other cells, and is further consistent with the size of the human T cell IL-1 receptor extrapolated from cDNA cloning experiments (unpublished observations). The identity of the radioactive band at 205,000 M_r is unclear; however, similar bands have been detected in crosslinking experiments on human fibroblasts and T cells and appear to represent dimers of the IL-1/IL-1R complex (unpublished observations).

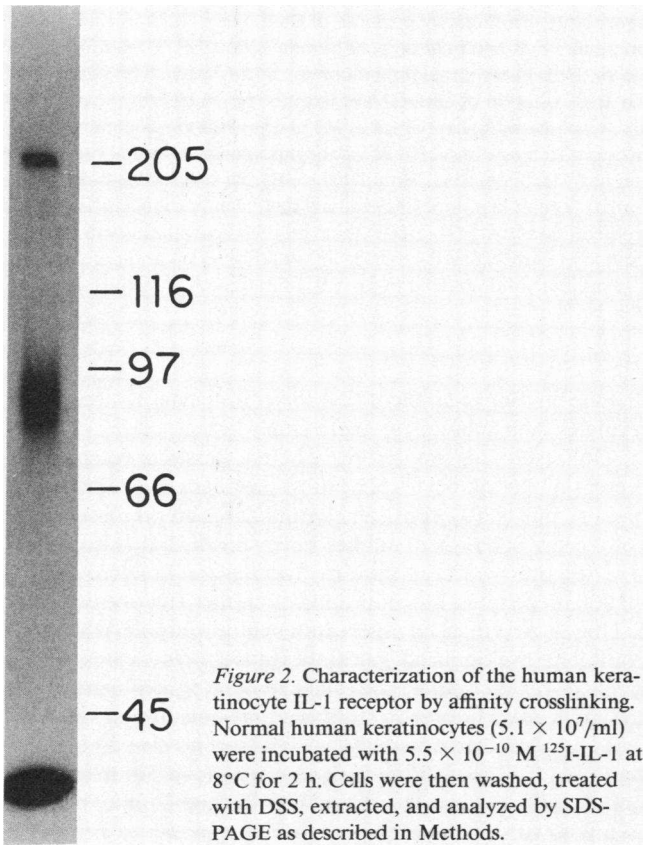

Figure 2. Characterization of the human keratinocyte IL-1 receptor by affinity crosslinking. Normal human keratinocytes (5.1×10^7/ml) were incubated with 5.5×10^{-10} M ^{125}I-IL-1 at 8°C for 2 h. Cells were then washed, treated with DSS, extracted, and analyzed by SDS-PAGE as described in Methods.

Purified rIL-1 alpha was added to near-confluent cultures of human keratinocytes, and total RNA was harvested at various times after stimulation. Fig. 3 shows an S1 nuclease analysis of RNA from normal human keratinocytes and the 5637 bladder carcinoma cell line. Lane *1* is the negative control, demonstrating that in the absence of RNA in the S1 mixture no radioactivity is observed. Lane *2* demonstrates that the bladder carcinoma cell line 5637 contains RNA strongly homologous to the protected fragment of human GM-CSF cDNA (see Methods). GM-CSF mRNA from 5637 cells protects a 590-bp fragment of human GM-CSF cDNA. Lane *3* shows RNA isolated from human keratinocytes grown in DME with 20% FCS under hydrocortisone-free conditions. A less intense but easily identifiable band of radioactivity that migrates indistinguishably from the GM-CSF RNA band in lane *2* (e.g., 590 bp) can be observed. Lane *4* represents RNA from keratinocytes grown under identical conditions to which 5 ng/ml of IL-1 alpha had been added 6 h previously. A band that migrates identically to the GM-CSF band can be observed; the intensity of this band is greatly increased in comparison with lane *3*. The lower molecular weight bands in lanes *2* and *4* represent highly reproducible minor S1 nuclease degradation products.

Fig. 4 shows the results of an S1 nuclease protection assay using RNA from keratinocytes grown in MCDB 153–defined medium. Lane *1* is the negative control containing no cellular RNA. Lane *2* shows mRNA from 5637 cells (positive control). Lane *3* shows RNA from human keratinocytes in the absence of exogenous stimulation. Under these conditions, no significant hybridization with the protected fragment of GM-CSF cDNA is observed. Lanes *4* and *5* show RNA from identical cultures to which rIL-1 alpha (final concentration 0.5 ng/ml and 5.0 ng/ml, respectively) had been added 6 h previously. There is a dose-dependent increase in the amount of radioactivity observed at the band consistent with GM-CSF mRNA. By comparison, lane *6* shows RNA from identical cultures to which phorbol myristate acetate (10 ng/ml) had been added 6 h previously.

Biological assays for GM-CSF are nonspecific and often difficult to analyze quantitatively. Recently, an assay using blast cells from peripheral blood of CML patients was reported (22). We used this assay in conjunction with an antibody to rGM-CSF that neutralizes its activity in this assay to assess GM-CSF activity in conditioned medium from normal and

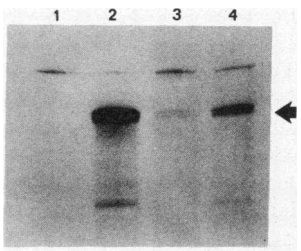

Figure 3. S1 nuclease protection assay for human GM-CSF RNA in keratinocytes grown in serum containing hydrocortisone-free medium. Lane *1*, no RNA; lane *2*, 5637 poly A+ RNA (5 μg); lane *3*, normal human keratinocyte total RNA (200 μg); lane *4*, total RNA (200 μg) from human keratinocytes stimulated 6 h previously with 5.0 ng/ml purified rIL-1 alpha. Arrow, 590 bp.

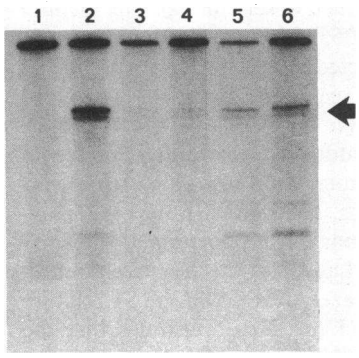

Figure 4. S1 nuclease protection assay from human GM-CSF RNA in human keratinocytes grown in serum-free MCDB 153 medium. Lane *1*, no RNA; lane *2*, 5637 poly A+ RNA (5 μg); lane *3*, total RNA (200 μg) from unstimulated human keratinocytes; lane *4*, total RNA (200 μg) from human keratinocytes stimulated 6 h previously with purified rIL-1 alpha (0.5 ng/ml); lane *5*, total RNA from human keratinocytes stimulated 6 h previously with purified rIL-1 alpha (5.0 ng/ml); lane *6*, total RNA from human keratinocytes stimulated 6 h previously with phorbol 12-myristate 13-acetate (10 ng/ml).

IL-1–induced keratinocytes. Fig. 5 shows that recombinant GM-CSF induces proliferation of CML blasts and that this proliferation can be blocked with polyclonal goat antibody to GM-CSF. In contrast, this antibody does not block proliferation of CML cells induced by IL-3 (15). Fig. 5 further shows that conditioned medium from unstimulated human keratinocytes contains little CML blast–stimulating activity. In contrast, conditioned medium from keratinocytes stimulated 18 h previously with human IL-1 alpha contains significant CML blast–stimulating activity; compared with the human rGM-CSF standard, this sample contains 1,250 half maximal units. The majority (84%) of this activity could be neutralized with a goat polyclonal antibody to GM-CSF. Therefore, the conditions shown to induce GM-CSF mRNA also induce GM-CSF biological activity. The addition of antibodies to IL-3 did not lead to further inhibition of this proliferative response. This is consistent with the observation that anti–IL-3 antibodies alone had no effect on keratinocyte-conditioned medium–induced CML blast proliferation.

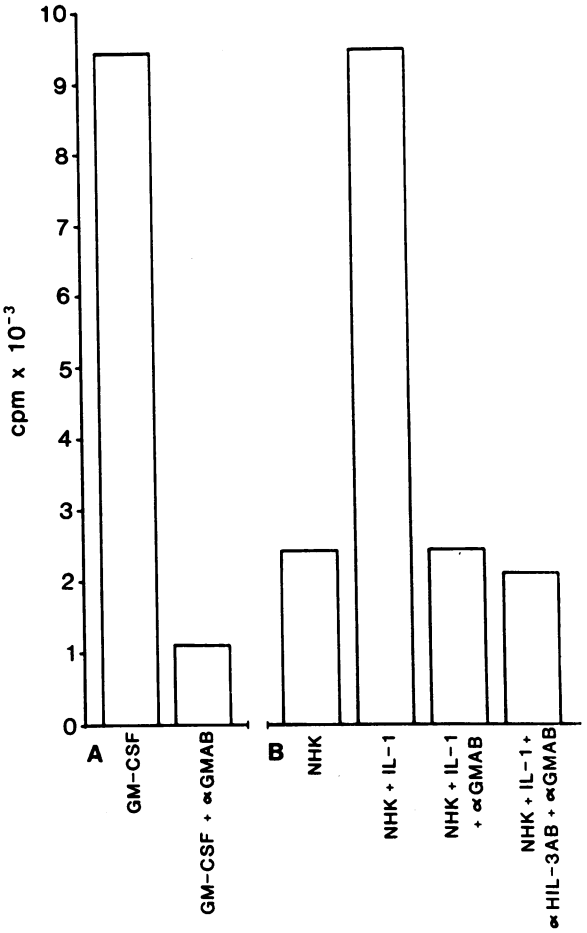

Figure 5. (*A*) Proliferative response of CML blasts at 72 h in response to 5 half maximal units of human rGM-CSF. The proliferation of CML blasts in medium alone is 1,115.67 cpm. The presence of a 1:500 dilution of anti–GM-CSF antibody neutralizes the proliferative response significantly. (*B*) A 1:250 final dilution of conditioned medium from unstimulated keratinocytes induces minimal proliferation of CML blasts. IL-1–stimulated (5 ng/ml purified rIL-1 alpha) normal human keratinocytes produce significantly more CML blast proliferative activity ($P < 0.01$) that could be neutralized with the antibody to GM-CSF ($P < 0.01$). The addition of an anti–human IL-3 antibody did not lead to further inhibition of proliferation (P = NS).

Discussion

In this report, we have demonstrated that cultured human keratinocytes express specific high- and low-affinity cell surface receptors that bind IL-1 alpha and beta with comparable but not identical affinities. We have further shown that the addition of rIL-1 to cultured keratinocytes induces the accumulation of GM-CSF mRNA. This accumulation of mRNA is associated with the secretion of GM-CSF into the culture medium, as measured by the CML assay and confirmed with neutralizing antibody.

The observation of IL-1 receptors on keratinocytes was not unexpected, since we and others have demonstrated that IL-1 is mitogenic for keratinocytes under certain conditions (9, 10). However, the capacity for expression of IL-1 receptors on a cell that normally produces IL-1 in vitro suggests an autocrine role for IL-1 in the activation of keratinocytes. The presence of IL-1 activity in keratinocyte-conditioned medium did not interfere with our detection of IL-1 receptors. The preincubation of human keratinocytes in a pH 3 glycine buffer (which has been demonstrated to lead to dissociation of IL-1 from its receptor in vitro) before the initiation of the binding assays did not alter the number of receptors detected under a wide variety of conditions (not shown). We cannot rule out the possibility that the binding of ambient IL-1 to keratinocyte IL-1 receptors regulates their subsequent expression.

Our results suggest that two discrete classes of receptor are present on keratinocytes. The most prevalent class of receptor has a K_a of 4×10^9, consistent with that found on most eukaryotic cells. Subsequent studies have shown that this class of IL-1 receptors varies in number from several hundred to over 40,000, depending on conditions of culture (Kupper, T., and S. Dower, unpublished observations). For example, while subconfluent proliferating keratinocyte cultures express thousands of IL-1 receptors, the establishment of confluence leads to significant decreases in IL-1 receptors on cultured keratinocytes (unpublished observations). The second class of receptor has a K_a of 1.2×10^{12} and is seen on human fibroblasts (23) and T cell lines (24). The function of the high-affinity receptor is unknown, but its presence does not appear to be strictly

required for transduction of an activating signal to the cell, since T cell lines that lack this receptor can respond to IL-1 (unpublished observations). Regulation of receptor number on keratinocytes may be a means by which autocrine effects of IL-1 can be regulated.

The majority of IL-1 activity produced by the keratinocyte in vitro is cell-associated, residing either in the cytoplasm or in a membrane-associated fraction (2, 6). We have shown that keratinocytes grown under conditions that allow for in vitro differentiation (e.g., DME with 20% FCS) release more IL-1 activity into the culture medium than do keratinocytes grown in MCDB 153 (unpublished observation), a low-calcium medium that prevents differentiation and stratification (4). We have demonstrated that keratinocytes grown in DME and 20% FCS in the absence of hydrocortisone produce significant basal levels of GM-CSF mRNA (Fig. 2). This may be a result of autocrine stimulation via ambient levels of IL-1 present in this conditioned medium. In contrast, keratinocytes grown in MCDB 153 release less IL-1 into the culture medium (unpublished observations). Cells grown under these conditions contain little measurable GM-CSF mRNA, and minimal GM-CSF activity can be detected in either the cell extract or conditioned medium from these cultures. While it is likely that the release of IL-1 from keratinocytes and its interaction with keratinocyte IL-1 receptors accounts for this difference in basal GM-CSF production by keratinocytes under these two conditions, we cannot rule out the contribution of components of FCS (e.g., PDGF) to this effect.

The addition of IL-1 to cultured keratinocytes induced the accumulation of GM-CSF mRNA in a dose-dependent fashion. This increase in mRNA was associated with an increase of GM-CSF biological activity in keratinocyte-conditioned medium. This biological activity could be completely neutralized by a polyclonal antibody to human GM-CSF. The CML assay for GM-CSF also detects IL-3 activity; in contrast to previous reports (25), we did not observe IL-3 activity in conditioned medium of either resting or IL-1–stimulated keratinocytes. Since it is now clear that human IL-3 does not bind to the murine IL-3 receptor (26), reports of putative human IL-3 stimulation of murine IL-3–dependent cell lines require reinterpretation and probably represent the activity of human keratinocyte IL-6 on murine IL-3–dependent myelomonocytic cell lines (unpublished observations). We have not found conditions under which RNA homologous to human IL-3 cDNA can be identified by Northern blot analysis in human keratinocytes (unpublished observations).

IL-1 production and release by keratinocytes is enhanced by injurious stimuli. It now appears that IL-1 can mediate several important autocrine functions in the keratinocyte, including growth, production of GM-CSF, and production of IL-6/BSF-2/beta 2 IFN (unpublished observation). It is not difficult to imagine that after damage to the skin, local mitogenesis of keratinocytes may enhance the rate of wound reepithelialization, and that the production of GM-CSF, which can activate mature granulocytes and macrophages as well as enhance Langerhans cell function, might serve to transiently enhance local antimicrobial defenses at the site of injury. Therefore, preformed IL-1 in normal epidermis, when released by injury, may stimulate both keratinocytes and dermal cells to participate in wound healing.

Regulation of IL-1 receptor expression in normal epidermis may explain why normal epidermis can contain IL-1 without resulting in persistent activation of resident keratinocytes. Specifically, it is likely that high numbers of IL-1 receptors are not normally expressed by keratinocytes in vivo, and are induced only after injury or in disease. These speculations are consistent with the unpublished observations that in vitro, confluent contact-inhibited cells express low levels of detectable IL-1 receptors, in contrast to the subconfluent cultures analyzed in this study. Experiments are underway to determine the nature of IL-1 receptors in normal and diseased epidermis.

GM-CSF was originally described according to its activities on bone marrow–derived hematopoietic precursors (27). It is now clear that IL-1 has significant effects on hematopoietic cells, and that GM-CSF and IL-1 can act synergistically (28, 29). Since it has been demonstrated that bone marrow–derived hematopoietic precursors circulate in peripheral blood, it is conceivable that human epidermis that produces (at least) IL-1, GM-CSF, and IL-6/BSF-2 may serve to induce local differentiation of these cells. Some evidence suggests that certain T cell lymphomas, leukemias, and cell lines, as well as a subpopulation of double negative thymocytes, can respond to GM-CSF (22, 30, 31). That epithelial tissues, including skin and thymus, produce GM-CSF, IL-1, and thymic hormones (32, 33) may relate to the differentiation of T cells in these tissues from bone marrow–derived precursors.

The effect of GM-CSF on B cell and T cell immune responses (34, 35) was recently shown to represent a specific effect of GM-CSF on antigen-presenting cells (36). Furthermore, GM-CSF has been shown to induce the proliferation of cells of the macrophage lineage (37) and to induce proliferation and enhance antigen presentation in murine Langerhans cells (38). It would not be surprising if one effect of IL-1 released in human epidermis is to enhance antigen presentation by Langerhans cells. Alternatively (or in addition), GM-CSF could expand the Langerhans cell population in epidermis by either inducing mature cells to proliferate or inducing maturation of such cells in situ (38).

The observation of IL-1 in normal epidermis, coupled with the demonstration that keratinocyte IL-1 gene expression can be enhanced by various stimuli, suggests that cutaneous IL-1 might mediate systemic effects after injury. The present study indicates that the potential exists for autocrine effects of IL-1 in the epidermis, with keratinocyte growth and cytokine production being among the consequences.

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