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# Regulation of interleukin 1 and its receptor in human keratinocytes

**(cell culture/differentiation/autocrine growth)**

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**ABSTRACT** **Keratinocytes in culture synthesize and respond to interleukin 1 (IL-1). We have measured surface IL-1 receptor (IL-1R) on keratinocytes in culture using radiolabeled IL-1 binding assays. Surface IL-1R levels are <2000 receptors per cell in postconfluent cultures but increase 9- to 20-fold 24 hr after treatment with phorbol 12-myristate 13-acetate (PMA) at 10 ng/ml or after raising the extracellular $Ca^{2+}$ concentration to 2 mM. This induction of surface IL-1R can be blocked by the addition of retinoic acid and parallels induction of squamous differentiation markers. These results imply that IL-1R levels may be related to the degree of differentiation of these cells. In parallel studies IL-1 protein levels were determined by bioassay and by Western blotting (immunoblots). All detectable IL-1 protein and essentially all IL-1 activity was cell-associated. Although constitutive levels of IL-1 biological activity and protein are significant in these cultures, IL-1 levels increase when either PMA or retinoic acid alone are added to cultures. IL-1 does not increase when PMA and retinoic acid are added simultaneously to cultures; nor is it induced when extracellular $Ca^{2+}$ concentrations are raised to 2 mM. Thus, cell-associated IL-1 levels do not necessarily parallel surface IL-1R levels in these cultures. Taken together, these results demonstrate that IL-1 and surface IL-1R levels are differentially and complexly regulated in keratinocyte cultures. Possible implications of these results in terms of normal and abnormal regulation of proliferation and differentiation are discussed.**

Interleukin 1 (IL-1) was originally identified as a product of activated monocytes that could act as a co-mitogen for thymocytes (1). Since that time, it has become clear that IL-1 is a family of polypeptide hormones that has a wide range of biological effects (2–4). A summation and synthesis of these observations implies that IL-1 plays a major role in immune regulatory and inflammatory responses. Two distinct forms of IL-1, termed IL-1$\alpha$ and IL-1$\beta$, have been identified (5). The IL-1 polypeptides from these two genes share only 26% identity at the amino acid level, but both are capable of binding to the same receptor with similar affinities and mediating similar biological effects (6–8). An increasing number of reports have focused on IL-1 production and IL-1 response by epidermal keratinocytes. Human and murine primary cultured keratinocytes, as well as established keratinocyte and squamous carcinoma cell lines, have been shown to produce biologically active IL-1 (9, 10), and mRNAs for both IL-1$\alpha$ and IL-1$\beta$ can be demonstrated in cultured keratinocytes (11, 12). *In vivo*, significant IL-1 activity can be detected in stratum corneum and normal epidermis (13, 14).

Keratinocytes also possess receptors for IL-1, and recent reports have demonstrated that IL-1 can elicit a variety of responses in keratinocyte cultures, including proliferation (15), chemoattraction (16), and induction of various cytokines involved in immune responses, growth, and differentiation (17).

Keratinocytes in culture can express various functions associated with terminal differentiation in intact skin, and compounds capable of modulating the expression of these differentiation-specific markers receive intense study both *in vivo* and *in vitro* (for review, see ref. 18). Addition of $Ca^{2+}$ to an extracellular concentration of 1.2–2.0 mM or addition of phorbol 12-myristate 13-acetate (PMA) to keratinocyte cultures can induce or accelerate overlapping subsets of markers associated with squamous differentiation (19–24), and retinoic acid (RA) can block or reverse the appearance of these markers (25–28). Because keratinocyte cultures both produce IL-1 and respond proliferatively to its presence, we reasoned that IL-1 or IL-1 receptor (IL-1R) or both might be critically regulated as the cells cease to divide and commit to terminal differentiation in culture.

We present evidence that both PMA and $Ca^{2+}$ can induce surface IL-1 receptors in primary human neonatal keratinocyte cultures as measured by IL-1 binding assays and that RA can block this induction. We also extend results from several laboratories (10, 12, 17, 29) by showing that RA and PMA, but not $Ca^{2+}$, can induce IL-1 in these same cultures. Thus various combinations of PMA, $Ca^{2+}$, and RA can induce IL-1, IL-1R, or both. These results imply that IL-1 may regulate proliferation and differentiation in epidermis, in addition to its known immune functions, and that aberrant regulation of IL-1 or IL-1R may be involved in various disease states.

## MATERIALS AND METHODS

**Preparation and Maintenance of Primary Human Foreskin Keratinocytes.** Foreskin keratinocyte cultures were prepared and maintained as described by Boyce and Ham (30). Foreskins were washed in phosphate-buffered saline (PBS), fatty tissue was removed, and foreskins were stored in 0.25% dispase (Boehringer Mannheim) overnight at 4°C. Epidermal sheets were peeled from the dermis, minced, and dispersed with 0.025% trypsin (GIBCO) by repeated pipetting. Cell suspensions were pelleted from the trypsin solution, sequentially resuspended, and washed with PBS/1% Chelex resin-treated fetal calf serum, and PBS alone, all by centrifugation at 1000 × *g* at 20°C for 5 min. Cell pellets were resuspended in modified MCDB 153 medium (KGM, Clonetics, San Diego, CA) and plated at $\approx 1 \times 10^6$ cells per ml. Cultures were fed every 3–4 days and subcultured by dispersal in 0.025% trypsin in PBS followed by washing as above and replated at a split ratio of 1:3 or less. Cultures were used between passages 2 and 5.

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Abbreviations: IL-1, interleukin 1; IL-1R, interleukin 1 receptor; RA, retinoic acid; PMA, phorbol 12-myristate 13-acetate; EGF, epidermal growth factor.

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**Preparation of Radioligand and Binding Measurements.** Recombinant human IL-1$\alpha$ was purified from *Escherichia coli* extracts (31) and radiolabeled with $^{125}$I to a specific activity of 1–3 $\times$ $10^{15}$ cpm/mmol as described (6).

For radioreceptor assays, keratinocyte cultures were grown in 24-well tissue culture trays (Costar), and assays were performed as described (32). In some experiments, endogenous bound ligand was stripped from cell surfaces by washing cultures with 0.1 M glycine buffer at pH 3.0 (32). Data were reduced and analyzed using RS/1, a data analysis package running on a Vax 11/750 under the VMS operating system (BBN Software Products, Cambridge, MA).

**Bioassays to Determine IL-1 Activity.** Culture supernatants from 60-mm tissue culture dishes (Costar) were decanted, centrifuged at 12,000 $\times$ *g* for 10 min, and stored at −35°C. Cell extracts were prepared by scraping PBS-washed monolayers into extraction buffer consisting of 10 mM Tris, pH 7.4/2 mM $MgCl_2$/150 mM NaCl/1% Triton X-100 in a volume equivalent to the original culture supernatant. Cell suspensions were Vortex mixed briefly and centrifuged at 12,000 $\times$ *g* for 10 min; then the supernatants were stored at −35°C. Bioassays were performed as described (33). A unit of IL-1 activity is based on purified recombinant human IL-1$\alpha$ as the standard.

**Western (Immunologic) Blotting to Visualize IL-1 Protein.** Monolayer cultures in 60-mm dishes were washed with PBS and extracted with 1 ml of extraction buffer as for the bioassays. Twenty five micrograms of protein were loaded per lane onto 15% polyacrylamide gels, separated by $NaDodSO_4$/PAGE (34), and transferred to nitrocellulose filters (35) by published procedures. Filters were soaked in 5% nonfat dry milk/0.9% NaCl/0.1% antifoam A (Sigma)/0.1% sodium azide/1 mM potassium iodide (milk buffer) for 1 hr at 4°C, rabbit antisera specific for IL-1$\alpha$ or IL-1$\beta$ (36) were added at a 1:500 dilution, and filters were incubated at 4°C overnight. All subsequent steps were carried out at room temperature. Blots were washed three times for 20 min with 0.5% sodium deoxycholate/0.1 M NaCl/0.5% Triton X-100/10 mM sodium phosphate, pH 7.5/0.1 mM phenylmethylsulfonyl fluoride (Western wash), washed once for 1 hr in milk buffer, incubated in milk buffer for 1 hr with $^{125}$I-labeled *staphylococcal aureus* protein A (Amersham) added at a final dilution of 1:1000, and finally washed three times for 20 min in Western wash. Filters were blotted dry and exposed to Kodak X-Omat film with intensifying screens at −70°C.

## RESULTS

**Quantitation of IL-1 Receptors on Human Keratinocytes After PMA Treatment.** We have measured the number of surface receptors for IL-1 present on postconfluent human foreskin epidermal cells cultured in serum-free medium in the absence of contaminating fibroblasts or feeder layers (30). Fig. 1 shows the results of one such experiment. Cells were grown to confluence in KGM medium, the medium was changed to either normal epidermal growth factor (EGF)-containing medium, medium without EGF, or medium without EGF but containing PMA at 10 ng/ml. Cultures were assayed for IL-1 binding 48 hr later. Two classes of binding sites can be detected in all of the cultures. The more abundant class of receptors has a $K_a$ of between 2 $\times$ $10^9$ and 4 $\times$ $10^9$, which is similar to that reported for other cell types, whereas the less abundant class has a $K_a$ of $>10^{12}$. Detection of the high-affinity class of receptor was variable, and even when it could be detected with confidence, low levels precluded accurate measurements of the site number and affinity. We have therefore limited our observations to the abundant class of receptors, where reliable quantitative data could be obtained. The significance of the high-affinity receptors is unclear. Although high-affinity receptors are found on a variety of cell types (7, 37, 38), the more abundant class of receptors can transmit biologic signals in T cells.

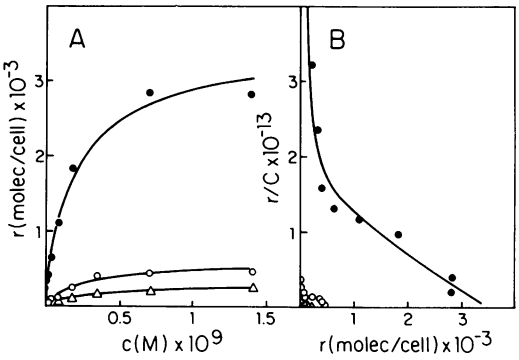


FIG. 1. Binding of $^{125}$I-labeled IL-1 to postconfluent primary human foreskin keratinocyte cultures. Cultures were grown to confluence in 24-well trays (Costar), refed with the indicated medium, and incubated for 48 hr. $^{125}$I-labeled IL-1 binding assays were performed as described. (*A*) Binding of $^{125}$I-labeled IL-1$\alpha$ to cells refed with medium only (△), medium containing EGF (○), or medium containing PMA (●). (*B*) Data from *A* replotted in the Scatchard coordinate system. r, molecules of IL-1 bound per cell; c(M), concentration of unbound IL-1; r/c, ratio of bound to unbound IL-1.

For the predominant class of surface IL-1R, we detected 600–300 receptors per cell in medium with or without EGF, respectively, and 3400 receptors per cell in 48-hr PMA-treated cultures (Fig. 1). EGF has minimal effects on the number of surface IL-1R. In early experiments, we compared cultures with and without EGF and found no reproducible effect of EGF on either constitutive or induced surface IL-1R levels. The 5- to 10-fold increase in surface IL-1R seen in cultures 48 hr after PMA treatment prompted us to look at the kinetics of this induction (Fig. 2). Confluent cultures were refed, and a single dose of PMA or solvent alone was added at various intervals. All cultures were assayed for IL-

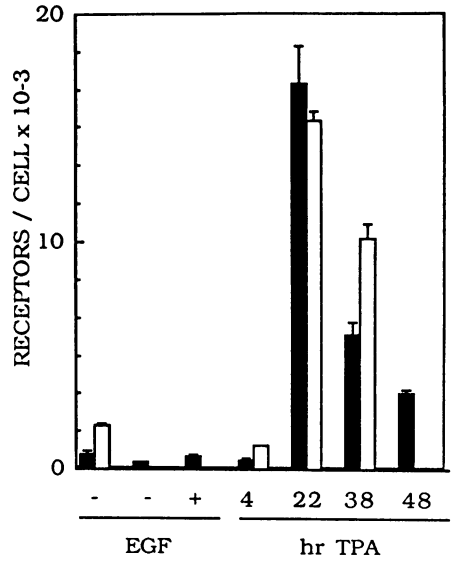


FIG. 2. Measurement of the number of surface IL-1 receptors per cell in postconfluent primary human keratinocyte cultures. Cultures were grown to confluence, refed, and incubated for 48 hr. PMA (TPA) was added to individual cultures to a final concentration of 10 ng/ml at various times as indicated. Two sets of cultures were incubated for the final 48 hr in medium without EGF. IL-1 binding assays were performed as described in Fig. 1. Closed bars, IL-1R measurements for unbound surface receptors; open bars, total surface receptor measurements determined after stripping endogenous bound ligand from the surface of cells.

1-binding ability 48 hr after the medium change. We detected 1800–300 surface receptors per cell in cultures fed with medium plus or minus EGF or in cultures treated for 4 hr with PMA. In cultures treated with PMA for 22 hr, the number of surface IL-1R increased to ≈16,000 per cell, and with longer PMA treatment surface IL-1R levels gradually declined.

PMA also induces IL-1 biological activity (29) and mRNA (11) in these cultures, so it was possible that changes in detectable surface receptor levels could be due to changes in receptor occupancy. We have ruled this out by performing parallel binding assays after stripping endogenous bound ligand from cell surfaces with a pH 2 wash (Fig. 2). These experiments demonstrate that total surface receptor levels increase in parallel with unbound receptor levels.

It is possible that ligand binding and receptor internalization (2, 7, 32) are responsible for the decline in surface IL-1R seen at 38 hr and 48 hr after PMA treatment, because receptor occupancy appears to increase somewhat at 38 hr. On the other hand, prolonged treatment with PMA results in cell aggregation and sloughing of cells into the medium. In the experiment shown in Fig. 2, cell counts for PMA-treated cultures at the time of the IL-1 binding assay had dropped to 93% and 84% of solvent-treated controls at 38 hr and 48 hr, respectively, and it is possible that subpopulations of cells with the highest numbers of IL-1R are selectively lost.

**Effects of $Ca^{2+}$ and RA on Surface IL-1R Levels.** PMA has diverse and profound effects on cells, and only a subset of these effects may be related to squamous differentiation in keratinocytes (39). We were interested in analyzing the effects of other agents known to have an impact on squamous differentiation in these cultures. We reasoned that if IL-1R induction was related to squamous differentiation, we might see a similar induction with 2 mM $Ca^{2+}$ and that this induction might be blocked with RA. Fig. 3 shows results from a series of receptor binding experiments in which $CaCl_2$ or RA was added to cultures to final concentrations of 2 mM and $10^{-6}$ M, respectively. IL-1 binding assays were done 24–36 hr later as indicated. At either time, we detect an induction of surface IL-1R in $Ca^{2+}$-treated cultures similar in magnitude to that seen in PMA-treated cultures, whereas RA-treated cultures maintain surface receptor numbers similar to solvent-treated controls. Cultures treated with either $Ca^{2+}$ or PMA and also treated with RA have surface receptor levels only marginally higher than those seen in untreated controls. Interestingly, addition of RA as late as 8 hr after addition of $Ca^{2+}$ appears to substantially block induction of IL-1R (Fig. 3).

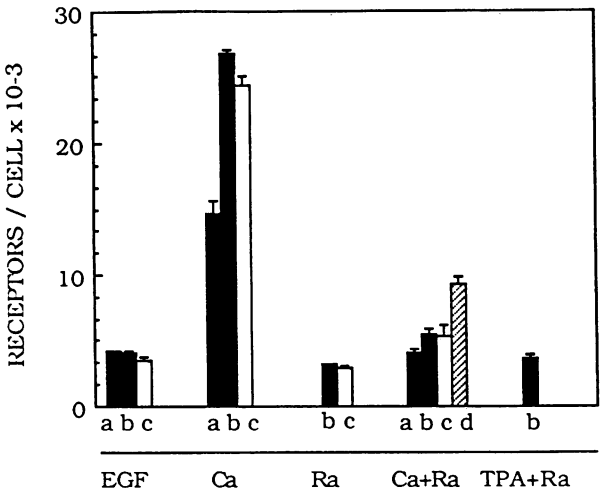


FIG. 3. Measurement of the number of surface IL-1 receptors per cell in postconfluent primary human keratinocyte cultures. Cultures were grown to confluence, refed, and various combinations of $Ca^{2+}$, RA, and PMA (TPA) were added to cultures to final concentrations of 2 mM, $10^{-6}$ M, and 10 ng/ml, respectively. Binding assays were performed 24–36 hr later as for Fig. 2. Unbound surface IL-1 receptor measurements were determined at 24 hr after treatment (*a*), unbound (*b*), and total surface IL-1 receptor measurements were determined at 36 hr after treatment in an independent experiment (*c*), unbound surface IL-1 receptor measurements from the same experiment as *b* and *c*, but in this culture RA was added 8 hr after the addition of $Ca^{2+}$ (*d*). Ra, retinoic acid.

We have used different lots of cells for IL-1 binding experiments and have never failed to detect a large induction of IL-1R with $Ca^{2+}$ or PMA, or to block this induction with RA. However, basal levels of surface IL-1R varied from experiment to experiment (compare Figs. 2 and 3). We feel these fluctuations may be donor specific, because basal levels of surface IL-1R in untreated control cultures varied with the lot of cells, but not from experiment to experiment with cells derived from the same individual.

**Induction of Biologically Active IL-1 in Keratinocyte Cultures.** IL-1 has been identified as a constitutive product in primary keratinocyte cultures (12). IL-1 in epidermal keratinocyte cultures is reported to be inducible by PMA (11, 29) and in gingival keratinocyte cultures by RA (40). Given the contrasts between the induction of surface IL-1R reported here and induction of IL-1 in dissimilar culture systems used by others, we have assayed IL-1 in our cultures by bioassay and Western blotting. Table 1 shows the results of bioassays on culture supernatants and cell extracts from cells treated as described in Fig. 1. Untreated cultures had $2.9 \times 10^3$ units of IL-1 in cell extracts, but little detectable IL-1 activity in the culture medium. At 24, 36, and 48 hr after PMA treatment, there was a slight increase in detectable IL-1 released to the medium, but the cell-associated IL-1 activity increased dramatically to 345-, 4500-, and 1200-fold over untreated cells, respectively.

Table 2 shows bioassay experiments where various combinations of $Ca^{2+}$, RA, and PMA were added to cultures for 24 or 36 hr. Cell-associated IL-1 in RA-treated cultures increased 16- to 17-fold at 24 hr in one experiment and 300-fold at 36 hr in a separate experiment, irrespective of whether $Ca^{2+}$ was also present. Low levels of supernatant IL-1 activity are also detected after 36 hr of RA. Cultures treated with $Ca^{2+}$ alone were more similar to untreated controls. When PMA and RA are added simultaneously to cultures, there is no induction of IL-1 activity, even though each compound alone is capable of inducing IL-1 activity.

**Western Blotting to Detect IL-1 Proteins.** mRNA for both IL-1$\alpha$ and IL-1$\beta$ has been detected in keratinocyte cultures (11, 12). In monocytes, both $\alpha$ and $\beta$ primary translation products have an apparent molecular mass of ≈31 kDa and are proteolytically processed into mature forms of ≈17 kDa (4, 36). Fig. 4 shows a Western blot of cell extracts reacted with mouse polyclonal antiserum specific for IL-1$\alpha$. The only IL-1 band detected in these blots is a single band with an apparent molecular mass of 31 kDa. Except for occasional, faint low-molecular mass bands assumed to be degradation products, no processed or mature forms of IL-1$\alpha$ were detected. Blots treated with polyclonal antiserum specific for IL-1$\beta$ were negative, as were blots made from culture medium proteins and treated with either IL-1$\alpha$ or IL-1$\beta$ antiserum. Results with Western blots reflect and extend the

Table 1. Biologically active IL-1 in culture medium and cell extracts

| | Untreated | 24-hr PMA | 36-hr PMA | 48-hr PMA |
|---|---|---|---|---|
| Culture medium* | 6 | 24 | 10 | 350 |
| Cell pellets* | $2.9 \times 10^3$ | $1 \times 10^6$ | $1.3 \times 10^7$ | $3.5 \times 10^6$ |
| Increase† | | 345-fold | 4500-fold | 1200-fold |

*, Units of IL-1.
†, Fold-increase of cell-associated IL-1 activity relative to that in untreated cells.

Table 2. Biologically active IL-1 in culture medium and cell extracts

| | Untreated | RA | $RA^+Ca^{2+}$ | $Ca^{2+}$ | PMA+RA |
|---|---|---|---|---|---|
| 24 hr* | | | | | |
| Culture medium† | 120 | 70 | 40 | 15 | ND‡ |
| Cell pellets† | $2.4 \times 10^4$ | $4.0 \times 10^5$ | $3.4 \times 10^5$ | $6.6 \times 10^4$ | ND |
| Increase§ | | 17-fold | 16-fold | <3-fold | ND |
| 36 hr* | | | | | |
| Culture medium† | 85 | 850 | 918 | 21 | 22 |
| Cell pellets† | $6.3 \times 10^3$ | $1.9 \times 10^6$ | ND | $3.4 \times 10^4$ | $1.3 \times 10^4$ |
| Increase | | 300-fold | ND | 5-fold | 2-fold |

*, Twenty-four-hr and 36-hr measurements were determined in independent experiments.
†, Units of IL-1.
‡, Not determined.
§, Fold-increase of cell-associated IL-1 activity relative to that in untreated controls.

observations made with bioassays. We detected an increase in cell-associated IL-1 protein with the addition of $10^{-6}$ M RA to cultures in the presence or absence of 2 mM $Ca^{2+}$ and a much larger increase in IL-1 protein with PMA treatment. As was seen in the bioassays, PMA and RA added together reproducibly did not increase IL-1 protein in multiple blotting experiments. The blotting data indicate that virtually all IL-1 in keratinocyte cultures is unprocessed IL-1$\alpha$.

## DISCUSSION

In this report we demonstrate that IL-1 and surface IL-1R are significantly and differentially modulated in primary human foreskin keratinocyte cultures by $Ca^{2+}$, PMA, and RA. $Ca^{2+}$ and PMA can induce surface IL-1R in these cultures, and this induction can be blocked by RA. No induction of this magnitude for surface IL-1R has been reported for any cell type. These compounds also modulate the level of IL-1 protein and biologically active IL-1 in these cultures, but the modulation of IL-1 does not necessarily parallel the modulation of IL-1R. PMA and, to a lesser extent, RA increase the level of IL-1 in these cultures, whereas PMA and RA added simultaneously to cultures do not induce IL-1. This last result is difficult to interpret but suggests that IL-1 induction by these two compounds occurs by separate mechanisms. IL-1 induction is often associated with subsequent rapid negative feedback regulation (41), and separate RA- and PMA-induced negative regulatory circuits may cross-cancel the different induction processes. In contrast to PMA or RA, 2 mM $Ca^{2+}$ has only minor effects on IL-1 levels.

$Ca^{2+}$ and/or PMA induce a variety of changes associated with squamous differentiation, including inhibition of cell division, alterations in keratin synthesis and keratinization,

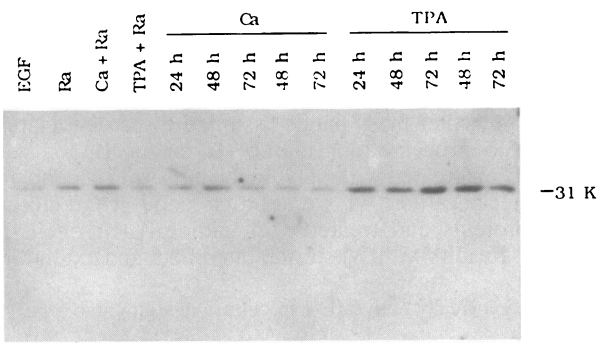


FIG. 4. Western blot of proteins extracted from keratinocyte cultures. Cellular proteins were extracted and electrophoresed on a 15% $NaDodSO_4$/polyacrylamide gel, transferred to nitrocellulose, and visualized with polyclonal anti-IL-1$\alpha$ antiserum, $^{125}I$-labeled *Staphylococcal aureus* protein A, and autoradiography as described. The first 4 lanes are from cells harvested 24 hr after treatment. Duplicate lanes at 48 and 72 hr represent duplicate cultures. TPA, PMA; K, kDa; h, hr.

and ability and competence to form cornified envelopes, whereas RA blocks the appearance of these markers (for review, see ref. 18). The correlation of surface IL-1R induction with induction of squamous differentiation markers is striking, but whether, in fact, surface IL-1R induction is related to squamous differentiation or to other PMA- or $Ca^{2+}$-induced changes remains to be established. One established keratinocyte cell line isolated in our laboratory (42) is blocked in differentiation and fails to induce surface IL-1R after PMA or $Ca^{2+}$ treatment, but does modulate IL-1 gene expression in response to $Ca^{2+}$, PMA, and RA in a manner similar to primary keratinocyte cultures; other keratinocyte cell lines should be analyzed for IL-1R under these treatments.

There remain a variety of other explanations for the induction of surface IL-1R. One possibility is that surface IL-1R induction is related to proliferation, because others have shown that IL-1 can stimulate proliferation in these cultures (15). Primary keratinocyte cultures contain a subpopulation of epithelial cells that may be stimulated to proliferate by PMA (20, 22), and it has been proposed that $Ca^{2+}$ stimulates some aspects of proliferation that are superseded by differentiation processes in keratinocyte cultures (43). On the other hand, EGF is essential for proliferation in these cultures but appears to have little effect on the numbers of surface IL-1R in postconfluent cultures, and RA may induce proliferation in these cultures (27) but blocks the induction of surface IL-1R.

Another possibility is that surface IL-1R induction may be related to epithelial migratory or proliferative responses related to wound-healing. Keratinocytes in culture have many properties that are associated with both wound-healing responses and certain hyperproliferative skin diseases, but not with normal epidermis (44, 45). Recently Martinet *et al.* (16) demonstrated that IL-1 is a chemoattractant for primary keratinocytes and postulated that IL-1 released from damaged epithelial cells at a wound site may induce epithelial cell migration into the wound.

We have proposed that IL-1 may be involved in hyperproliferative skin diseases such as psoriasis (46, 47). Psoriatic keratinocytes hyperproliferate in culture relative to normal cells, but both cell types produce similar amounts of IL-1-like activity (48). It will be interesting to compare the number of IL-1 surface receptors present on normal and psoriatic keratinocytes, especially since retinoids are often therapeutic for psoriasis. Preliminary results suggest that synthesis of a keratin pair associated with hyperproliferative disease states and wound-healing (44) may increase under some culture conditions after addition of exogenous IL-1.

Aberrant regulation of IL-1 or IL-1R may be relevant to a variety of other abnormal conditions, including malignant progression. In the mouse skin model of tumor promotion (49) RA can block tumor promotion by PMA (50). Our results present the possibility that simultaneous induction of surface

IL-1R and IL-1 by PMA is related to promotion and that RA reverses this process by blocking one or both of these induction processes.

mRNAs for both $\alpha$ and $\beta$ IL-1 can be isolated from keratinocytes (11, 12), although the mRNA for IL-1$\alpha$ predominates and IL-1 activity from similar cultures is neutralized with IL-1$\alpha$-specific sera (11). All IL-1 protein that we detect on Western blots is uncleaved cell-associated IL-1$\alpha$. This contrasts with the situation in monocytes, where the majority of IL-1 is proteolytically processed IL-1$\beta$ (36). IL-1$\alpha$, unlike IL-1$\beta$, is bioactive in the uncleaved form (37) and could be involved in intracellular autocrine signaling in keratinocytes. The putative advantage of IL-1$\alpha$ in human epidermis has been discussed in detail (17, 46, 47).

We detect relatively little IL-1 activity released to the culture medium under normal culture conditions or after the addition of PMA or $Ca^{2+}$. However, inhibitors of IL-1 and hydrocortisone present in the culture medium (51) can interfere with IL-1 activity in the bioassays we have used, and it is probable that IL-1 is present and inhibited in culture supernatants but at levels undetected by Western blotting. We do detect somewhat larger amounts of IL-1 activity in the medium after induction with RA by both bioassay and Western blotting. We have observed that culture systems containing serum or feeder layers have more supernatant IL-1 activity than cultures using serum-free medium (17, 46, 47), and Brown *et al.* (26) demonstrated that RA induces a variety of ultrastructural changes associated with enhanced secretory activity in keratinocytes. We suggest that the supernatant IL-1 detected in serum-containing cultures may be partly due to induction of this IL-1 activity by retinoids present in serum (25).

The complexities of IL-1 and surface IL-1R regulation described here imply that IL-1, in addition to its known immune and inflammatory functions, is involved in proliferation and differentiation of keratinocytes. However, many basic questions concerning the interplay of IL-1, IL-1R, and IL-1 inhibitors remain unanswered. More importantly, we do not yet know whether IL-1 and IL-1R are regulated in similar ways in intact epidermis or in laboratory situations that more closely parallel intact epidermis.

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