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A role for interleukin-1 in epidermal differentiation: regulation by expression of functional versus decoy receptors

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SUMMARY

Although human epidermis contains levels of interleukin-1 (IL-1) up to 100 times higher than other tissues, the role of this cytokine in epidermal biology is unknown. Here, we show that interleukin-1 regulates the expression of mRNAs for two proteins associated with the differentiated phenotype of human keratinocytes, cellular retinoic acid-binding protein type II (CRABP II) and small, proline rich protein 1 (SPRR1). The ability of IL-1 to induce these transcripts correlates directly with keratinocyte expression of the IL-1 receptor type I (IL-1 RI) during differentiation and inversely with the expression of the type II IL-1 receptor (IL-1 RII), shown in other cell types to be a non-functional, decoy receptor. Furthermore, addition to keratinocyte cultures of an IL-1 RI-blocking, but not an IL-1 RII-blocking, antibody reduces the level of CRABP II and SPRR1 mRNAs in these cells. These data suggest that epidermal IL-1 functions to promote keratinocyte differentiation and that a change in the IL-1 receptor profile of these cells initiates this IL-1 response through a relative enhanced expression of functional IL-1 receptors.

Key words: interleukin-1, keratinocyte, epidermal differentiation, IL-1 receptor

INTRODUCTION

Interleukin-1 (IL-1) is a major epidermal cytokine synthesized constitutively by keratinocytes (Kupper et al., 1986; Sauder, 1985), the predominant epidermal cell type. IL-1 synthesis by keratinocytes can also be induced by other cytokines such as tumor necrosis factor α (TNFα) and granulocyte-macrophage colony stimulating factor (GM-CSF) (Partridge et al., 1991). Although keratinocytes synthesize both IL-1α and IL-1β, these cells are unable to convert the inactive IL-1β precursor form to the mature active IL-1β (Mizutani et al., 1989). Therefore, the vast majority of epidermal-associated IL-1 activity is accounted for by IL-1α. IL-1 synthesized by keratinocytes remains mostly cell-associated and is normally lost by desquamation of the stratum corneum (Blanton et al., 1989). Upon skin injury, however, a large pool of preformed IL-1 can be released and has been postulated to function in triggering a rapid immune response (Kupper et al., 1988). In addition to keratinocytes, IL-1 can be synthesized by other epidermal cells, such as Langerhans cells (Sauder et al., 1984), as well as by dermal fibroblasts (Kumar et al., 1992).

Two types of cell membrane-associated receptors for IL-1 have been identified and cloned. IL-1 receptor type I (IL-1 RI) is an 80 kDa protein and is the predominant IL-1 receptor on fibroblasts and T lymphocytes (Sims et al., 1989; Spriggs et al., 1990). IL-1 RII is a 68 kDa protein and is found on B lymphocytes, neutrophils and monocytes (McMahan et al., 1991; Spriggs et al., 1992; Benjamin et al., 1990; Horuk and McCubreg, 1989). Although the two receptors are ~28% homologous in their extracellular domains, IL-1 RII has a much shorter intracellular portion (Dower et al., 1990). IL-1α and IL-1β bind to both IL-1 RI and IL-1 RII but they do so with different affinities (McMahan et al., 1991). Recently it was demonstrated in polymorphonuclear cells and in monocytes that the IL-1 signal is transduced through IL-1 RI while IL-1 RII acts as a decoy receptor which binds to and effectively reduces the level of available IL-1 (Colotta et al., 1993).

Human keratinocytes not only synthesize Il-1 but also express IL-1 receptors (Blanton et al., 1989; Kupper et al., 1988; Dower and Urdal, 1987). Although not distinguished as IL-1 RI or IL-1 RII, the number of these receptors on keratinocytes in vitro was found to vary depending on culture conditions. Total receptor number decreased initially as keratinocyte cultures became confluent and more differentiated (Kupper et al., 1988), although induction of terminal differentiation by addition of phorbol 12-myristate 13-acetate (PMA) increased the level of IL-1 receptors up to 24-fold (Blanton et al., 1989).

Although the epidermis contains very high levels of IL-1, the function of this cytokine in the skin is largely speculative. Aside from a possible role in initiating an immune response following injury, other functions for epidermal IL-1 have been proposed. Some data suggest that IL-1 stimulates keratinocyte proliferation accompanying inflammation in vivo (Ristow, 1987) as well as in cultures of normal cells (Sauder et al., 1988; Sauder, 1985). However, this effect is very dependent on the cytokine concentrations used and varies to a great degree from one donor to another. Other investigators have found no effect of IL-1 on keratinocyte growth in vitro (Partridge et al., 1991).

Here we provide evidence that one of the functions of IL-1 in the epidermis is to promote keratinocyte differentiation by up-regulating the expression of certain differentiation-associated genes. Furthermore, we find that a change in the number and type of IL-1 receptors on these cells initiates this response through a relative enhanced expression of functional IL-1 receptors.

MATERIALS AND METHODS

Cell culture and northern analysis

CRABP II and SPR1 mRNA regualtion by IL-1α

Cultures of neonatal human keratinocytes were established from newborn foreskin (Gilchrest, 1979) and maintained in a hormone-supplemented, serum-free medium as described (Garmyn et al., 1991) except that no hydrocortisone was added. Serum-free medium was used in these experiments to eliminate the effects of IL-1 normally present in serum. Hydrocortisone was omitted because of the known effect of this corticosteroid in inhibiting the production and action of various cytokines (Kirnbauer et al., 1991; Lee et al., 1991). All keratinocyte cultures described here were derived from first passage cells. At about the time of confluence, IL-1α (Collaborative Biomedical Products, Bedford, MA) was added to a final concentration of 5 units/ml. Control cells received an equal volume of diluent (water). The cells were collected 3 days later and processed for RNA isolation using Tri-Reagent (Molecular Research Center Inc., Cincinnati, OH) in a protocol provided by the manufacturer. Total RNA (5 μg each sample) was analyzed by northern blot, transferred to Hybond-N membrane (Amersham, Arlington Heights, IL) and probed with CRABP II, SPRR1, and β-actin cDNAs as described (Eller et al., 1992). The CRABP II cDNA was generated by PCR as described previously (Eller et al., 1992). The SPRR1 cDNA was the kind gift of Dr C. Backendorf, Leiden, The Netherlands. The cDNA for β-actin, a constitutively expressed gene used to demonstrate even loading of the RNA samples, was purchased from the American Type Culture Collection (ATCC), Rockville, MD.

IL-1 RI and RII mRNAs in keratinocytes

Cultures were established and maintained in a DME-based medium containing 10% fetal calf serum as described (Gilchrest, 1979). At approximately 3 days pre-confluence and 3 to 5 days post-confluence, cells were harvested and total RNA isolated. Poly(A)$^+$ RNA was isolated from total RNA using an mRNA purification kit (Pharmacia, Milwaukee, WI). Approximately 5 μg poly(A)$^+$ RNA and 20 μg total RNA from the pre- and post-confluent cultures were analyzed by northern blot for IL-1 RI, IL-1 RII and CRABP II expression. Terminal differentiation was induced in keratinocytes by addition of 50 ng/ml tetradecanoylphorbol 13-acetate (TPA) (Sigma Chemical Co., St Louis, MO) to cultures approximately 3 days pre-confluence. The cDNA probe for IL-1 RI was generated by PCR using primers based on the published sequence (Sims et al., 1989): 5′ IL-1 RI: ATGACTACGTTGGGGAAGAC; 3′ IL-1 RI: AAGCCCTC-TACTCTGAAGTG. The IL-1 RII cDNA was generated using primers based on the published sequence (McMahan et al., 1991): 5′ IL-1 RII: GACCTGAGTGAATTCACCCG; 3′ IL-1 RII: TGAT-GAGGCCATAGCACAGT. Both cDNAs were generated from reverse-transcribed human keratinocyte RNA (Eller et al., 1992). Even loading of the RNAs samples is indicated by hybridization with a cDNA for glyceraldehyde-3-phosphate dehydrogenase (ATCC), a constitutively expressed gene, or ethidium bromide staining of the gel showing the 28 S and 18 S ribosomal RNAs.

In situ hybridization

In situ hybridization of newborn foreskin tissue sections was carried out essentially as previously described (Eller et al., 1994). Briefly, the IL-1 RII, CRABP II and SPRR1 cDNAs were cloned into the pGEM 3Z transcription vector (Promega, Madison, WI) containing SP6 and T7 RNA polymerase promoter sites. Sense and anti-sense transcripts were identified by northern blot hybridization to keratinocyte RNA. Tissue samples were fixed for 24 hours in 4% paraformaldehyde/PBS, dehydrated though a series of alcohol washes and embedded in paraffin blocks. Three-micron sections were cut, placed on slides, deparaffinized with xylene, and the cells permeabilized by proteinase K (Gibco/BRL, Gaithersburg, MD) digestion. Hybridization with the anti-sense probe detected the IL-1 RII mRNA and hybridization with the sense probe was used as a negative control. After hybridization and washing, the slides were dipped in NTB-2 liquid emulsion (Kodak, Rochester, NY) and exposed for 14 days. After developing in Kodak D-19 developer, the sections were stained with hematoxylin and eosin. Light field illumination is presented for better histologic representation. Dark field illumination is presented to show silver granules (bright dots).

Immunoperoxidase staining

Immunoperoxidase staining was carried out on newborn foreskin tissue, fixed overnight in Histochoice fixative (Amresco, Solon, OH) and embedded in paraffin. Three-micron thick sections were deparaffinized and blocked with normal rabbit serum provided in the Vectastain kit (Vector Laboratories, Burlingame, CA). The anti-IL-1-RII monoclonal antibody M22 was kindly provided by the Immunex Corp. (Seattle, WA) and was used as a 1/50 dilution of the 2 mg/ml stock. The general protocol for the immunoperoxidase staining was provided by the manufacturer of the Vectastain kit. Primary antibody-treated and non-treated (control) sections were reacted with the AEC peroxidase substrate for 20 minutes. Finally, the sections were counter-stained with hematoxylin for histologic representation.

RESULTS

Because IL-1 is known to have profound effects on the growth and differentiation of many cell types (Dinarello, 1991; Schwarz and Luger, 1992), we examined the effect of IL-1 on keratinocyte differentiation by studying the expression of two differentiation-associated mRNAs, those coding for cellular retinoic acid-binding protein type II (CRABP II) and small, proline rich protein type I (SPRR1). CRABP II, the major isoform of CRABP in the epidermis, is up-regulated at the mRNA and protein level when keratinocytes are cultured under conditions which promote differentiation (Eller et al., 1992; Aström et al., 1991; Siegenthaler et al., 1992). Also, CRABP II transcript levels are essentially undetectable by in situ hybridization in the proliferative, basal layer of keratinocytes in the epidermis, but are abundant in the more differentiated, suprabasal cells (Eller et al., 1994). Although CRABP II is thought to mediate the effects of retinoids on keratinocyte growth and differentiation, the actual function of this protein is not known. SPRR1, also called pancornulin, is a precursor of the cornified envelope, the product of terminally differentiated keratinocytes in the stratum corneum (Marvin et al., 1992; Fujimoto et al., 1993). Like CRABP II, SPRR1 mRNA is induced during keratinocyte differentiation in vitro (Kartasova et al., 1987, 1988; Gibbs et al., 1993; Yaar et al., 1995). We found that exogenously added IL-1α increased the steady-state level of both CRABP II and SPRR1 mRNAs in keratinocytes cultured in a serum-free, defined medium (Fig. 1). The level of β-actin mRNA, a constitutively expressed control mRNA, was

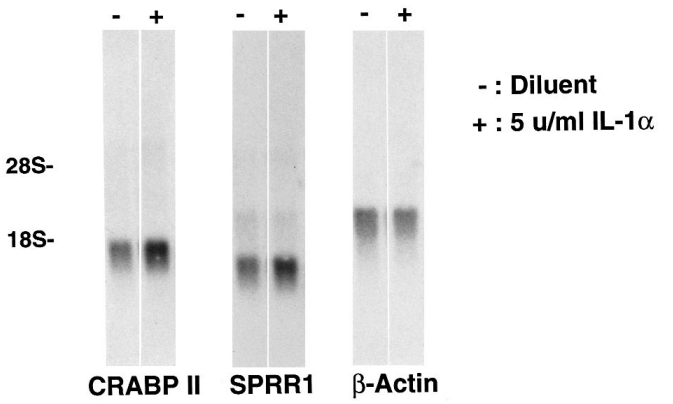

Fig. 1. The upregulation of CRABP II and SPRR1 mRNAs by IL-1α. Total RNA (5 μg) from diluent (–) and IL-1α (+) treated keratinocytes was examined for CRABP II, SPRR1 and β-actin mRNA expression by northern blot as described in Materials and Methods. Hybridization with the β-actin cDNA demonstrates even loading of the RNA samples.

unaffected by IL-1α. These data suggest that epidermal IL-1 may play a role in expression of the differentiated keratinocyte phenotype.

Next we examined the levels of the two IL-1 receptors, IL-1 RI and RII, expressed by keratinocytes at various stages of differentiation. Here, we induced differentiation in cultures by growth to confluence. Post-confluent keratinocyte cultures are stratified and express higher levels of markers of differentiation such as involucrin, CRABP II, SPRR1 and transglutaminase than do pre-confluent cultures (Eller et al., 1994; Yaar et al., 1995; Pillali et al., 1990). Using northern blot hybridization with PCR-generated IL-1 RI and RII cDNAs, we found that IL-1 RI mRNA is up-regulated greater than 3-fold (determined by densitometric analysis) in post-confluent, more differentiated cells (Fig. 2a). This increase is in parallel with a greater than 7-fold increase in CRABP II mRNA. On the other hand, IL-1 RII mRNA levels are regulated inversely to those for CRABP II, being abundant in pre-confluent, less-differentiated cells, but barely detectable in the post-confluent cells (Fig. 2b). The level of SPRR1 mRNA is regulated similarly to CRABP II in these cells (data not shown). Thus, the relative level of the mRNAs for IL-1 RI and RII changes in vitro depending on the state of differentiation of the cells.

We also studied the distribution of the SPRR1, CRABP II and IL-1 RII mRNAs and IL-1 RII protein in human epidermis. In situ hybridization of neonatal foreskin sections with an antisense IL-1 RII riboprobe detects this transcript in the proliferative, basal layer cells and in the most differentiated cells of the stratum granulosum (Fig. 3a). As would be expected from the northern analysis of cultured keratinocytes, the cells in the epidermis which express the highest levels of IL-1 RII transcript also express the least CRABP II and SPRR1 mRNAs. In situ hybridization with CRABP II and SPRR1 antisense riboprobes detects little, if any, of these messages in the basal layer of keratinocytes and in the stratum granulosum, but readily detects transcripts in the mid-epidermis (Fig. 3a). Immunoperoxidase staining of similar skin sections using the IL-1 RII-specific monoclonal antibody M22 (Immunex Corp.) also detects this protein in the basal layer of keratinocytes and in the terminally differentiating cells of the stratum granulosum (Fig. 3b). IL-1 RI mRNA and protein were below the level of detection by in situ hybridization and immunoperoxidase staining in these skin sections.

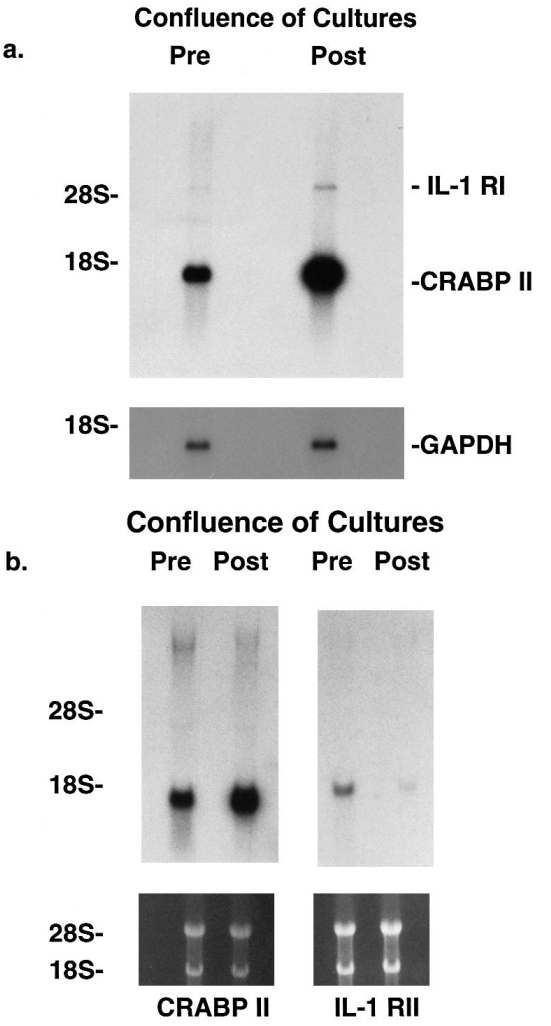

Fig. 2. Expression of IL-1 RI and RII mRNAs in cultured keratinocytes. Cultures of human neonatal keratinocytes were maintained and processed as described in Materials and Methods. (a) Approximately 5 μg poly(A)$^+$ RNA from pre- and post-confluent keratinocyte cultures was analyzed by northern blot for IL-1 RI and CRABP II mRNA. Hybridization with a glyceraldehyde-3-phosphate dehydrogenase (GAPDH) cDNA, a constitutively expressed gene, demostrates even loading of the RNA samples. (b) Two identicle blots containing 20 μg total RNA from pre- and post-confluent keratinocyte cultures were hybridized with CRABP II and IL-1 RII cDNAs. Ethidium bromide stained 28 S and 18 S RNAs are shown to indicate even loading of the RNA samples.

In order to examine the expression of IL-1 RII in terminally differentiating keratinocytes, cultures were treated with tetradecanoylphorbol 13-acetate (TPA), known to induce terminal differentiation in these cells (Yaar et al., 1993; Younus and Gilchrest, 1992). We found that the IL-1 RII mRNA is up-regulated as early as 8 hours after addition of TPA and continues to increase for at least 24 hours (Fig. 4). Thus, keratinocytes terminally differentiating in response to TPA mimic those cells in the stratum granulosum in vivo and re-express the IL-1 RII.

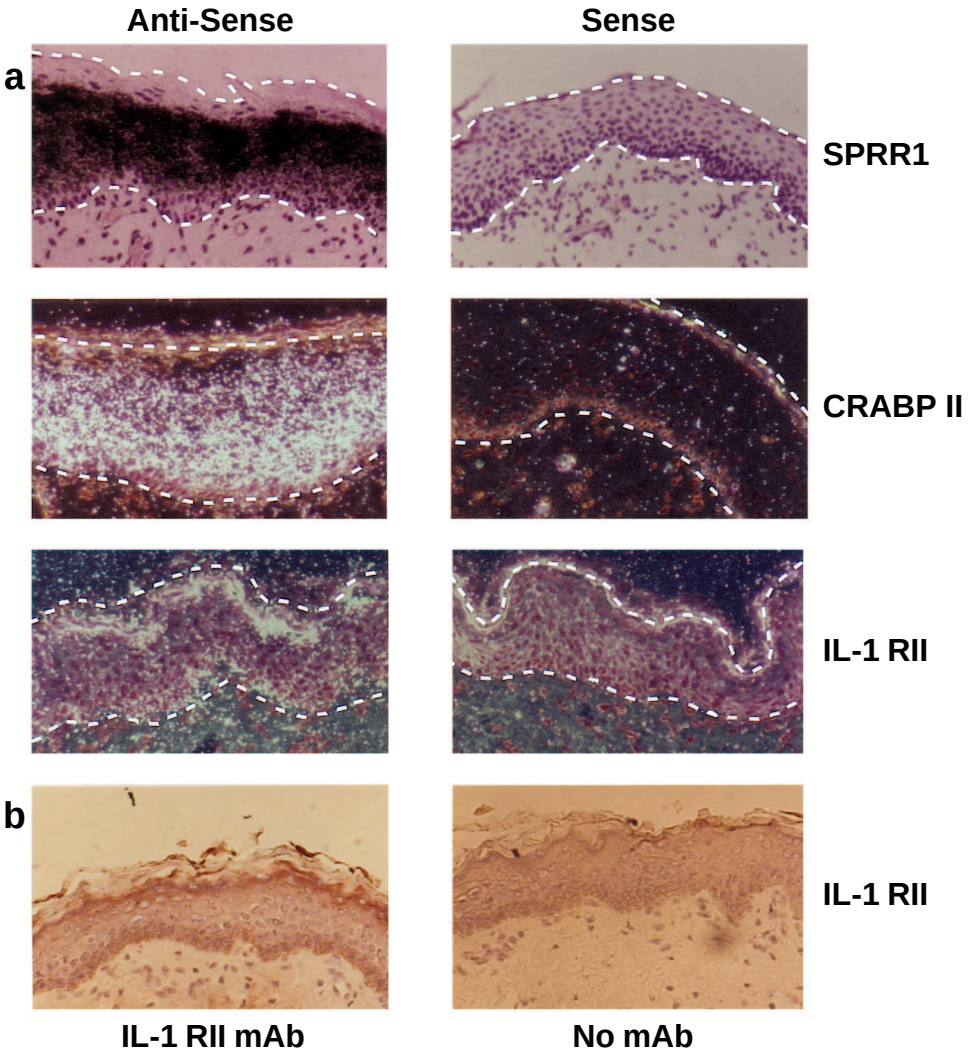

Fig. 3. Expression of SPRR1, CRABP II and IL-1 RII in neonatal skin. (a) In situ hybridization of newborn foreskin tissue sections with SPRR1, CRABP II and IL-1 RII riboprobes was carried out as described in Materials and Methods. Sense transcripts were used as a negative control. For CRABP II and IL-1 RII, dark field illumination was used for detection of silver granules (white dots). Because of the abundance of SPRR1 mRNA transcripts in the epidermis, light field presentation offers clearer representation of the silver granules. The upper broken white line designates the stratum corneum and the lower line, the dermal-epidermal junction. The somewhat broken or 'patchy' appearance of the hybridization of the IL-1 RII riboprobe to the basal layer reflects the actual low level expression of this message in these cells. (b) Immunoperoxidase staining was carried out on newborn foreskin sections with the IL-1 RII-specific monoclonal antibody M22 (Immunex Corp., Seattle, WA). Staining without reaction with the primary antibody was used as a negative control.

These data suggest that the IL-1 RII is suppressing the action of IL-1 in inducing and/or maintaining the expression of the differentiation-associated genes CRABP II and SPRR1. We next used the monoclonal IL-1-blocking antibodies M4 (anti-IL-1 RI) and M22 (anti-IL-1 RII) (Spriggs et al., 1990; McMahan et al., 1991) to assess the function of each of these receptors in mediating the IL-1 response in cultured keratinocytes. Pre-confluent cultures were treated for 24 hours with M4, M22 or M8 (non-blocking antibody to IL-1 RI) or diluent alone. Since non-saturating levels of IL-1, which are synthesized constitutively by keratinocytes (Kupper et al., 1986; Sauder et al., 1988), were necessary to study the effect of displacement of ligand from one receptor (due to antibody binding) to another, no exogenous IL-1 was added to the cultures. Addition of the IL-1 RI non-blocking antibody has no effect on CRABP II or SPRR1 mRNA levels compared to cells receiving no antibody (Fig. 5). Addition of the IL-1 RI-blocking antibody lowers the steady-state level of both of these transcripts, consistent with a role of IL-1 in their induction. The relatively modest reduction in the CRABP II mRNA level (~20%) most likely reflects the extreme stability of this message in keratinocytes (Eller et al., 1995). Addition of the IL-1 RII-blocking antibody results in increased levels of CRABP II and SPRR1 mRNAs, consistent with displacement

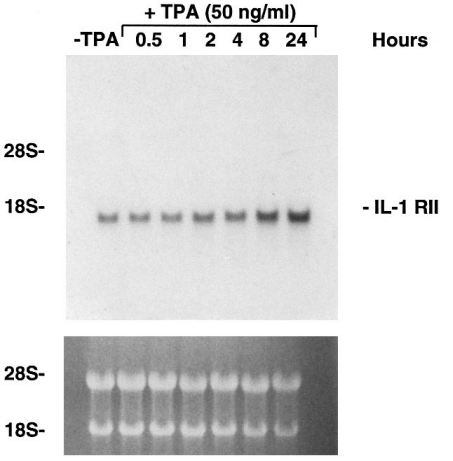

Fig. 4. Effect of terminal differentiation on IL-1 RII mRNA expression in cultured keratinocytes. Pre-confluent cultures of human neonatal keratinocytes were induced to terminally differentiate by the addition of 50 ng/ml TPA. Untreated cells and cells treated with TPA for the designated times were collected and analyzed for IL-1 RII mRNA by northern blot. The ethidium bromide stained 28 S and 18 S ribosomal RNAs are shown to demonstrate even loading of the RNA samples.

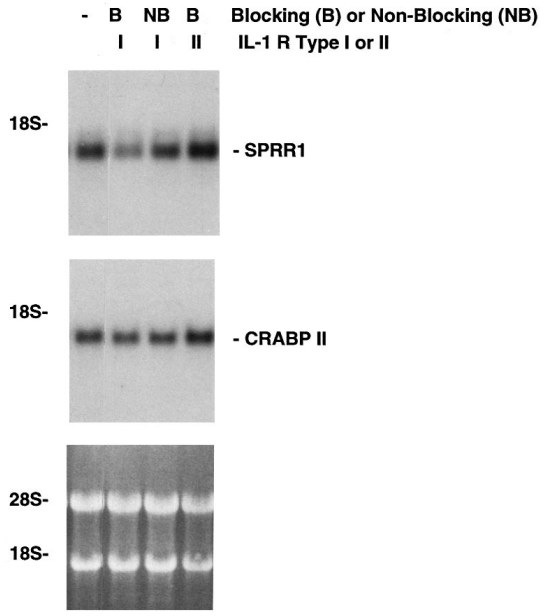

Fig. 5. Effect of IL-1 RI and RII antibodies on CRABP II and SPRR1 mRNA expression in keratinocytes. Newborn keratinocyte cultures were maintained in a serum-containing medium without added hydrocortisone until approximately 80% confluent. The cells were re-fed and monoclonal antibodies (Immunex Corp.) were added at a final concentration of 10 μg/ml. The antibodies used were as follows: blocking to IL-1 RI, mAb M4; blocking to IL-1 RII, mAb M22; non-blocking to IL-1 RI, mAb M8. Control cells not exposed to monoclonal antibodies are designated as (–). One day after addition of the antibodies, the cells were harvested and analyzed by northern blot for CRABP II and SPRR1 mRNA levels. The ethidium bromide stained gel showing the 28 S and 18 S ribosomal RNAs demonstrates even loading of the RNA samples. Relative densitometry readings for control:IL-1 RI-blocking:IL-1 RI non-blocking:IL-1 RII-blocking are 1:0.6:1:1.7 for SPRR1 and 1:0.8:1:1.4 for CRABP II.

of IL-1 to active IL-1 RI receptors. These data suggest that, as in polymorphonuclear leukocytes and monocytes (Colotta et al., 1993), the IL-1 signal in keratinocytes is transmitted through the IL-1 RI while IL-1 RII is an inactive receptor which effectively lowers the local availability of IL-1.

DISCUSSION

The results presented here suggest that one function of epidermal IL-1 is to regulate the expression of at least a subset of differentiation-associated genes in keratinocytes. However, these data do not rule out additional regulatory effects on these genes by other factors. In normal skin, the increased response to IL-1 upon initiation of the differentiation process appears to be accomplished by a change in the number and type of IL-1 receptors: the inhibitory IL-1 RII is down-regulated, allowing more IL-1 to interact with the functional IL-1 RI. Whether the IL-1 RI is upregulated in the suprabasal cells of the epidermis, as in more differentiated cultured keratinocytes, remains to be determined. Of note, because the IL-1 RI message level is extremely low and detectable only in poly(A)$^+$ RNA, even when it is up-regulated with differentiation, the total IL-1 receptor (type I and type II) message level decreases in post-confluent cells. This decrease is in agreement with the observation (Kupper et al., 1988) that the total number of IL-1-binding sites on cultured keratinocytes decreases dramatically at confluence. Interestingly, the in situ analysis shows that IL-1 RII is up-regulated in the stratum granulosum, at or just before terminal differentiation and assembly of the cornified envelope. This up-regulation was not noticed in normal keratinocytes in vitro possibly because cultures were not examined at extended times post-confluence, or possibly because cells differentiating under submerged culture conditions do not express the full spectrum of events reflecting the differentiation process in vivo (Sanquer et al., 1993). Treatment of these cells with TPA, known to induce terminal differentiation, did increase IL-1 RII transcript levels in vitro, in agreement with the pattern of expression in vivo. Although phorbol esters such as TPA have also been shown to increase IL-1 synthesis by cultured human keratinocytes (Blanton et al., 1989), the vast majority of this IL-1 remains cell-associated and therefore its availability to modulate keratinocyte function is unknown. These data do suggest, however, that the relationship between IL-1 and IL-1 receptors expression in differentiating keratinocytes in the epidermis is likely to be complex.

The source of the IL-1 affecting these changes during differentiation in vivo is unclear. Although keratinocytes constitutively synthesize high levels of IL-1, most is thought to remain intracellular or membrane associated and may not be available to exert autocrine or paracrine effects (Blanton et al., 1989; Kupper, 1987). Alternatively, other cells in the skin such as Langerhans cells or dermal fibroblasts may provide the IL-1 to mediate these effects. If so, then the previously reported increase in epidermal CRABP II in response to inflammation (Eller et al., 1994; Hirschel-Scholz et al., 1989) may result from IL-1 released from the inflammatory cell infiltrate.

Our data also indicate that at or just before terminal differentiation, the IL-1 RII is re-expressed, presumably to remove the IL-1 influence from these cells. Interestingly, it has been proposed that IL-1 promotes survival of polymorphonuclear leukocytes by inhibiting the programmed death of these cells (Colotta et al., 1992). Perhaps IL-1 has a similar effect on keratinocytes and therefore the reappearance of IL-1 RII in the stratum granulosum may act to allow cell death and terminal differentiation. Regardless, the data presented here suggest that IL-1 plays a significant role in the regulation of gene expression during keratinocyte differentiation. Furthermore, the influence of IL-1 appears to be regulated by the presence of functional versus non-functional IL-1 receptors on these cells.

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