Ultraviolet (UV) B radiation may trigger cutaneous inflammatory responses by directly inducing epidermal keratinocytes to elaborate specific cytokines such as interleukin (IL)-1 and IL-6. Because IL-1 is a potent inducer of IL-6, one may speculate that the release of IL-6 by keratinocytes after UV exposure is mediated via the release of IL-1 in an autocrine or paracrine manner. We demonstrated that UVB irradiation upregulated IL-1α mRNA at a lower dose (15 mJ/cm²) and then downregulated IL-1α mRNA expression at high doses (30–40 mJ/cm²). The kinetic profile of IL-1α mRNA expression showed a biphasic response, with the early increase by 1 h after UV exposure and the secondary increase at 6 h after UV. On the other hand, the expression of IL-6 mRNA was increased with increasing doses of UVB (0–45 mJ/cm²) and showed a single peak at 6 h post-UV. These results may indicate that UVB radiation could regulate the expression of IL-1α and IL-6 mRNA in keratinocytes by different mechanisms. Our data show that anti-human IL-1α antibody inhibits UV-induced IL-6 production and mRNA expression in cultured keratinocytes. The addition of recombinant IL-1α to the medium increased IL-6 synthesis and augmented IL-6 production and mRNA expression in cultured human keratinocytes by UVB irradiation. These results support the hypothesis that UVB irradiation-enhanced IL-6 production and mRNA expression may be mediated by IL-1α.

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**MATERIALS AND METHODS**

**Keratinocyte Culture** Human keratinocytes derived from normal foreskin were isolated as described previously (Boyce and Ham, 1983). Keratinocytes were cultured in keratinocyte growth medium (Clonetics, San Diego, CA) composed of MCDB 153 medium supplemented with epidermal growth factor (10 ng/ml), bovine pituitary extract (70 μg/ml), hydrocortisone (0.5 μg/ml), penicillin (100 μg/ml), streptomycin (100 μg/ml), and fungizone (0.25 μg/ml). Third-passage keratinocytes were used. Briefly, the epidermal sheet was separated from the dermis after 90 min exposure to collagenase, and cells were liberated from the epidermal sheet after 10 min exposure to trypsin–ethylenediamine tetraacetic acid (0.025–0.01%). The cells were cultured until 80% confluent and stored in liquid nitrogen until use. At the time of the experiment, the cells were thawed and cultured until 80% confluent, inoculated onto 10-cm culture dishes, and cultured until 80% confluent at 37°C in 5% CO₂ before exposure to UV radiation.

**IL-1α and Anti-IL-1α Antibody** Recombinant mouse IL-1α and rabbit anti-human IL-1α antibody were obtained from Genzyme (Cambridge, MA).

**UVB Irradiation** The UVB light source was Waldmann UV-800 (Waldmann Co., VS-Schwenningen, Germany), which used a fluorescent lamp (Philips type TL 20W/12) that emitted 2.5 mW/cm² of UV light between 285 and 350 nm (peak 310–315 nm) at a distance of 25 cm. The energy was measured with a Waldmann UV meter (model no. 585100; Waldmann).

The keratinocytes used in each experiment originated from different batches of cells from a single parent plate. The cells were grown in 10-cm or 3.5-cm culture dishes (Falcon, Lincoln Park, NJ) until subconfluent. Subsequently, the medium was replaced by 4 ml or 1 ml of phosphate-buffered saline, and the cells were exposed to UVB (0–45 mJ/cm²) light. After irradiation, the cells were washed with phosphate-buffered saline and
cultured in the keratinocyte growth medium for either 6 h before RNA preparation or 0–36 h before IL-1α and IL-6 enzyme-linked immunosorbent assay (ELISA).

Cell Viability Assay  Cell viability was determined by the MTT assay (Mosmann, 1983), which is based on reduction of soluble yellow MTT tetrazolium salt [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide] to a blue insoluble MTT formazan product by mitochondrial succinic dehydrogenase. After UV irradiation, the cells were cultured for 24 h; 20 μl of MTT (5 mg/ml) was added to each well, and the cells were incubated for 4 h at 37°C. The supernatant was removed, and 200 μl of dimethylsulfoxide was added to each well to dissolve formazan products. The absorbance was determined spectrophotometrically at 570 nm with an ELISA reader. The number of viable cells remaining in each well was calculated from a standard curve of optical density obtained by serial dilutions of control keratinocytes. The results were expressed as a percentage of control in triplicate cultures.

IL-1α and IL-6 ELISA  Commercially available ELISAs were used for measuring human IL-1α (R&D System, Minneapolis, MN) and IL-6 (Genzyme) protein levels in cell-free supernatants. The detection limits of these kits are 0.3 pg/ml IL-1α and 18 pg/ml IL-6. These kits do not cross-react with any other known cytokine, including human IL-1β, TNF-α, or TNF-β.

Northern Blot Analysis  Northern blot analysis of IL-1α and IL-6 mRNA expression was performed as described previously (Thomas, 1980). Briefly, total RNA was isolated by the acid guanidinium thiocyanate-phenol-chloroform extraction method (Chomczynski and Sacchi, 1987). After denaturation with 50% formamide and 0.6% formaldehyde, 20 μg of total RNA was size fractionated in 1% agarose gel containing formaldehyde and was transferred to nylon membranes by using blotting papers. The transferred RNA was hybridized with 32P-labeled human IL-1α or IL-6 probes (obtained from American Type Culture Collection, no. 65259 and no. 68636, respectively) under stringent conditions. The specific activities of the probes were between 2 and 8 × 106 cpm/μg DNA. After washing (twice in 2 × sodium citrate/sodium chloride, 0.1% sodium dodecyl sulfate for 10 min at room temperature and twice in 0.1% sodium citrate/sodium chloride, 0.1% sodium dodecyl sulfate for 10 min at 60°C), the membrane was exposed to x-ray film with an intensifying screen at −70°C.

Densitometry  A densitometer was used for normalization. The autoradiogram was scanned, and the peak areas were measured for the relative mRNA levels (IL-1α mRNA/glyceraldehyde-3-phosphate dehydrogenase [GAPDH] mRNA, IL-6 mRNA/GAPDH mRNA) in the tested sample. The relative measurements of the mRNA from the irradiated keratinocytes to the mRNA from the control keratinocytes were calculated from the densitometer readings.

RESULTS

Differing Regulation of IL-1α and IL-6 mRNA Expression by UVB  To determine whether the UVB-mediated increases of IL-1α mRNA and IL-6 mRNA are dose dependent, we irradiated keratinocytes with various amounts of UVB (5–45 mJ/cm²). UVB-induced expression of IL-1α and IL-6 mRNA was evaluated 6 h after UV irradiation by Northern blot analysis. Figure 1 shows densitometric scanning of Northern blot analysis of IL-1α mRNA expression after various doses of UVB irradiation. Each lane was normalized to the level of GAPDH mRNA. Unirradiated normal keratinocytes produced a large amount of IL-1α mRNA constitutively. UVB radiation, however, altered IL-1α mRNA expression in a dose-dependent fashion. As shown in Fig 1, at a low dose (5–10 mJ/cm²) of UVB, IL-1α mRNA expression remained at a steady-state level. At the dose of 15 mJ/cm², UVB irradiation significantly upregulated IL-1α mRNA; IL-1α mRNA expression was downregulated at high doses (30–40 mJ/cm²). As shown in Fig 2, the expression of IL-6 mRNA by normal keratinocytes could not be demonstrated by Northern blot analysis in our experiments. After 6 h post-UV, IL-6 mRNA expression could be detected from the dose of 15 mJ/cm² of UVB, and the maximum expression of IL-6 mRNA was found at 30 and 45 mJ/cm².

At a dose of 45 mJ/cm² or less of UVB, the viability of cultured keratinocytes measured by the MTT assay at 6 h or 36 h after UV irradiation did not show a significant decrease (data not shown).

Because we observed that 15 mJ/cm² was optimal for inducing IL-1α mRNA expression in our experimental setting, the keratinoocytes were exposed to 15 mJ/cm² to determine the kinetics of IL-1α and IL-6 mRNA expression. As shown in Fig 3, at 15 mJ/cm² of UVB, the response of IL-1α mRNA was biphasic. IL-1α mRNA increased as early as 1 h after UV irradiation, and then
kinetic profiles of gene expression for the two cytokines after UV irradiation differed from each other.

**IL-1α Is Increased in Culture Supernatant Immediately After UVB Irradiation** To detect the amounts of IL-1α and IL-6 in UV-irradiated keratinocyte-conditioned medium, ELISA was performed. To evaluate the time course of IL-1α and IL-6 production, we tested supernatants from each time interval (0, 3, 6, and 24 h) for IL-1α and IL-6 protein. As seen in Table I, there was an immediate, significant increase of IL-1α at 30 min after irradiation. The production of IL-6 protein increased after 6 h, and large amounts of IL-6 protein were produced after 24 h.

**Anti-IL-1α Antibody Inhibits IL-6 Production and mRNA Expression** Because it is well known that IL-1 can induce the production of IL-6 in keratinocytes (Ansel et al, 1990), we designed experiments to investigate whether UVB irradiation-enhanced IL-6 is mediated by IL-1α.

To determine the effect of anti-human IL-1α antibody on IL-6 production, we used diluted anti-human IL-1α antibody and measured human IL-6 protein levels in cell-free supernatants with a commercially available ELISA kit (Genzyme). The antibody was added to the medium immediately after UV irradiation, and the cells were incubated for 36 h. Unirradiated keratinocytes synthesized IL-6 protein below the detectable level (18 pg/ml) of the ELISA kit (Fig 5). In vitro exposure of keratinocytes to UVB radiation increased IL-6 protein synthesis in a dose-dependent manner. The amount of IL-6 secreted by keratinocytes was 80.1 ± 0.6 pg/ml (mean ± SD) at 15 mJ/cm² of UVB and 360 ± 44.3 pg/ml at 30 mJ/cm² of UVB. At 15 mJ/cm² of UVB, the addition of anti-human IL-1α antibody (20 μg/ml, 40 μg/ml) decreased the IL-6 secretion to below the detectable level (18 pg/ml) of the

**Table I. UVB Causes IL-1α Release in Culture Supernatants Immediately After UVB Irradiation**

<table>
<thead>
<tr>
<th>Time Post-UV (h)</th>
<th>IL-1α (pg/ml)</th>
<th>IL-6 (pg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>86.8</td>
<td></td>
</tr>
<tr>
<td>1/2</td>
<td>48.6</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>79.3</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>102.4</td>
<td>47.5</td>
</tr>
<tr>
<td>6</td>
<td>132.9</td>
<td>265.0</td>
</tr>
</tbody>
</table>

* The supernatants of keratinocyte cultures from various time intervals after UVB exposure (45 mJ/cm²) were assayed for IL-1α and IL-6 protein using ELISA kits. Blank space indicates value below the detectable level of the ELISA kit.
IL-6 mRNA expression. The antibody was added to the culture medium immediately after UVB irradiation (15 mJ/cm²). The keratinocytes were incubated 6 h before RNA preparation. Twenty micrograms of total RNA was electrophoresed, transferred to a nylon membrane, and hybridized with [3P]-labeled IL-6 cDNA and GAPDH cDNA probes.

ELISA kit. At 30 mJ/cm², antibody-dilution-dependent inhibition of IL-6 production was observed. In a control experiment, pre-immune rabbit serum did not show any effects on IL-6 production (data not shown).

To determine the effect of anti-human IL-1α antibody on IL-6 mRNA expression, we added the antibody (20 µg/ml) to the medium immediately after UV irradiation (15 mJ/cm²). The keratinocytes were incubated for 6 h and harvested for RNA preparation. We analyzed the ability of the antibody to inhibit UV-induced IL-6 mRNA expression, and indeed observed an inhibition of IL-6 mRNA expression by anti-human IL-1α antibody (Fig 6).

IL-1α Increases IL-6 Production and mRNA Expression

To investigate the effect of recombinant IL-1α on the production of IL-6, we added various concentrations (2–32 ng/ml) of IL-1α to the culture media of unirradiated keratinocytes. Immediately after UVB irradiation (15 mJ/cm²), the cells were incubated for 36 h. As shown in Fig 7, the addition of recombinant IL-1α at less than 4 ng/ml was not able to induce IL-6 production above the detectable level (18 pg/ml) of the ELISA kit, but concentrations greater than 8 ng/ml increased IL-6 production in a dose-dependent fashion.

When recombinant IL-1α was added to the culture medium immediately after UVB irradiation (15 mJ/cm²), IL-1α augmented IL-6 production in a dose-dependent manner at 2 ng/ml or more. As shown in Fig 8, the addition of recombinant IL-1α at 2 ng/ml augmented IL-6 mRNA expression in keratinocytes by 30 mJ/cm² of UVB (lane 4), as in the case of IL-6 production by IL-1α, but recombinant IL-1α alone at 2 ng/ml was not able to induce IL-6 mRNA expression (lane 3). Concentrations of recombinant IL-1α greater than 8 ng/ml increased IL-6 mRNA expression (data not shown).

**DISCUSSION**

Human epidermal cells are capable of secreting various cytokines with immunologic, inflammatory, and proliferative properties (Shimada and Katz, 1988; Kupper, 1988, 1989; Ansel et al., 1990) and are the principal source of cytokines in the epidermis. Because human skin cells, by virtue of their anatomic location, are uniquely exposed to UV light, the most relevant physical injury to human skin is UVB radiation. UV radiation may trigger cutaneous inflammatory responses by directly inducing keratinocytes to elaborate specific cytokines such as IL-1, IL-6, and TNF (Kupper, 1989). These cytokines are thought to mediate systemic inflammatory reactions, such as fever, as well as local inflammatory responses in the skin after UVB irradiation.

IL-1 is characterized by its broad range of inflammatory and proliferative activities in many different cell types such as Langerhans cells, fibroblasts, and endothelial cells. In keratinocytes, IL-1 can upregulate its own production and so promote increased keratinocyte proliferation (Ansel et al., 1990). In addition, IL-1 increases keratinocyte production of other cytokines, such as TNF-α, IL-6, IL-8, and granulocyte-macrophage colony-stimulating factor (GM-CSF) (Kupper, 1990). IL-6 is a multifunctional...
cytokine exhibiting a variety of biologic effects on keratinocytes in vitro (Kirnbauer et al., 1991; de Vos et al., 1994), and increased serum levels of IL-6 have been found in patients exposed to UVB (Urbanski et al., 1990). IL-6 may participate in inflammatory reaction following solar exposure. IL-6 has been demonstrated to induce keratinocyte proliferation, and serum IL-6 levels correlate with the severity of sunburn reaction (Urbanski et al., 1990). It is not clear, however, whether IL-6 is induced directly by UVB radiation or indirectly via other mediators. Because IL-1 is a potent inducer of IL-6 (Ansel et al., 1990), one may speculate that the release of IL-6 by keratinocytes after UV exposure is mediated by the release of IL-1 from keratinocytes in an autocrine or paracrine manner. We performed this study to examine the hypothesis that UVB could trigger IL-1α release by keratinocytes, which could then act in an autocrine or paracrine manner to stimulate IL-6.

In the present study, we have shown that UVB radiation may regulate the expression of IL-1α and IL-6 mRNA in keratinocytes by different mechanisms, as we found differences in the dose responses and kinetics of IL-1α and IL-6 mRNA expression in cultured keratinocytes after UV irradiation. Ansel et al. (1988) reported that high-dose UV radiation inhibited IL-1α mRNA expression at 24 and 48 h after irradiation, whereas a lower dose of UV induced IL-1α mRNA expression in murine PAM212 keratinocytes. We have demonstrated similar dose-dependent effects of UVB on IL-1α mRNA expression in normal human keratinocytes. The kinetic profile of IL-1α mRNA expression in human keratinocytes showed a biphasic response: an early increase by 1 h after irradiation and a secondary increase at 6 h. This biphasic response of IL-1α mRNA expression by UVB has also been reported in murine PAM212 keratinocytes: an initial increase at 3–6 h after UV and a secondary increase at 24–48 h (Nozaki and Sander, 1994). The mechanism for this biphasic response is unclear.

On the other hand, we demonstrated that the expression of IL-6 mRNA was increased with increasing doses of UVB and showed a single peak at 6 h post-UV. Elder et al. (1992) failed to demonstrate enhanced IL-6 mRNA expression after UVB exposure in normal human keratinocytes, but other investigators found a dose-dependent increase of IL-6 mRNA expression and a single peak 6 h after exposure (Kirnbauer et al., 1991; de Vos et al., 1994), as shown in our results.

We demonstrated that anti-human IL-1α antibody inhibited the UV-induced IL-6 production of keratinocytes completely at 15 mg/ml and decreased IL-6 production at 30 mg/ml of UVB; the reduction was dependent on the antibody concentration. Addition of anti-human IL-1α antibody to the medium also decreased UV-induced IL-6 mRNA expression in cultured keratinocytes after UV irradiation. The addition of recombiant IL-1α to the culture medium increased IL-6 production by keratinocytes and augmented UVB-enhanced IL-6 production and mRNA expression in keratinocytes. These results support the idea that UVB irradiation-enhanced IL-6 production and mRNA expression may be largely mediated by IL-1α released by keratinocytes after UVB irradiation.

In this study, we determined the kinetics of two cytokines produced in the culture supernatant; this was necessary to confirm our hypothesis that UVB irradiation induction of IL-6 may be mediated by the early release of IL-1α from human keratinocytes. We demonstrated that the increase of IL-1α occurred immediately after UVB irradiation and preceded IL-6 mRNA expression. Nozaki et al. (1991) also observed an early increase in IL-1 bioactivity in the supernatant of keratinocyte cultures after UVB irradiation. It is well known that IL-1α mRNA is constitutively expressed by keratinocytes cultured in keratinocyte growth medium (Lee et al., 1991; Chung and Youn, 1995), and keratinocytes normally contain prodigious amounts of IL-1α (Kupper, 1989). Because IL-1 lacks a hydrophobic leader sequence necessary for transmembrane secretion, it has been proposed that it can be released only after some type of cell injury or membrane perturbation (Oppenheim et al., 1986). thermal, radiant, and kinetic energy can injure cells, and injured cells can leak intracellular proteins to the extracellular spaces (Kupper, 1990). These same injurious stimuli, including UV radiation, can passively release cellular contents, including preformed IL-1α. The IL-1α released from the keratinocyte may in turn induce IL-6 mRNA expression and IL-6 protein synthesis successively. We have demonstrated that these processes can be blocked by adding anti-IL-1α antibody to the culture medium of keratinocytes.

Normal human epidermis also contains considerable amounts of IL-1β (Didierjean et al., 1989). In contrast to IL-1α, IL-1β occurs only in its biologically inactive 31-kDa precursor form, as keratinocytes lack proteases to convert it into its active 17-kDa form (Mizutani et al., 1991). Thus, IL-1β could not be involved in the regulation of IL-6 production by UVB-irradiated keratinocytes.

In this study, the addition of anti-IL-1α antibody did not inhibit the production of IL-6 protein completely at 30 μg/ml of UVB and did not decrease the expression of IL-6 mRNA to the unirradiated level. This may be because the amount of antibody we used may not be adequate to block IL-1α completely. Partridge et al. (1991) demonstrated that secretion of IL-6 by keratinocytes was increased after the addition of TNF-α, GM-CSF, IL-1α, or transforming growth factor-β to the culture medium. It is known that the production of TNF-α (Oxholm et al., 1988) and GM-CSF (Nozaki et al., 1991) by human keratinocytes is enhanced upon UV exposure. Thus, in addition to IL-1α, cytokines such as TNF and GM-CSF might have played some role in inducing IL-6 by UVB irradiation.

The mechanism by which UVB radiation increases IL-6 mRNA expression has been unclear. In general, the increased levels of IL-6 mRNA may be caused by two different mechanisms: an increased rate of transcription of the respective gene or an increased stabilization of its preexisting mRNA (Hentze, 1991). A proposal has been made recently that UVB radiation may regulate the expression of IL-6 transcripts in cultured keratinocytes at a post-transcriptional level by increasing IL-6 mRNA stability, instead of directly increasing the transcription rate of the IL-6 gene in these cells (de Vos et al., 1994). Similarly, stimulation of human thymic epithelial cells with the growth factors epidermal growth factor or transforming growth factor-α increased IL-6 mRNA steady-state levels without affecting the transcription rate of the IL-6 gene (Le et al., 1991). It also was reported that IL-1 clearly was able to augment PAM212 IL-1α transcription rates and to increase IL-1 mRNA half-life. It is tempting, therefore, to speculate that UVB radiation induces IL-1 release from keratinocytes, and in turn, the increased IL-1α may increase the IL-6 mRNA half-life, thus contributing to the IL-6 mRNA accumulation after UV irradiation.

The present study indicates that an autocrine or paracrine mechanism involving IL-1 and IL-6 exists in epidermal keratinocytes and supports the theory that UVB irradiation-enhanced IL-6 production and mRNA expression in human keratinocytes may be mediated by IL-1α.

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