Analysis of the Mechanism of Ultraviolet (UV) B Radiation–Induced Prostaglandin E_2 Synthesis by Human Epidermoid Carcinoma Cells

Markus Grewe, Uwe Trefzer, Annette Ballhorn,* Karin Gyufko, Hanspeter Henninger,* and Jean Krutmann
Departments of Dermatology and *Biochemistry, University of Freiburg, Freiburg, Germany

Stimulation of cultured human keratinocytes with interleukin (IL)-1α is known to elicit prostaglandin (PG) E_2 release. Ultraviolet (UV) B radiation induces keratinocyte PGE_2 and cytokine production. The present study deals with the autocrine roles of UVB-induced, keratinocyte-derived cytokines IL-1 and tumor-necrosis-factor (TNF) α and their corresponding receptor molecules for UVB-induced PGE_2 release. *In vitro* exposure of transformed human keratinocytes (KB cells) induced PGE_2 production five- to eightfold. This increase was inhibited by 70%, if irradiated cells were cultured in presence of monoclonal antibody (MoAb) M4, which blocks IL-1 effects by binding to the type 1 IL-1 receptor (IL-1R). In contrast, MoAb M22, which blocks the type 2 IL-1R, had no significant effects. Addition of recombinant human TNFα to unirradiated KB cells resulted in five- to eightfold increased PGE_2 synthesis, and this increase could be mimicked by stimulation of KB cells with MoAb htr-9, which exerts TNFα-like bioactivity by binding to the 55-kD TNF receptor (TNFR). UVB-induced PGE_2 synthesis was blocked by 50% in the presence of neutralizing anti–TNFα-Ab, and was completely inhibited by addition of both anti–TNFα-Ab and MoAb M4. To elucidate a possible regulatory intracellular step in PGE_2 synthesis, specific cyclooxygenase activity in KB cells was determined. Following UVB treatment, cyclooxygenase activity increased twofold, but remained unaltered, if irradiated KB cells were cultured in the presence of anti–TNFα-Ab plus MoAb M4. These studies indicate that keratinocyte-derived TNFα and IL-1 together mediate UVB-induced PGE_2 release via specific cell surface receptors, and that one intracellular mechanism is an increased prostanoid-synthesizing capacity of irradiated cells.
Key words: cyclooxygenase/cytokines/receptors/keratinocytes. *J Invest Dermatol 101:528–531, 1993*

In vitro ultraviolet (UV) B (280–320 nm) radiation is a well-known inducer of human keratinocyte prostanoid synthesis and release [1,2]. There is increasing evidence that UVB exposure also induces the production of keratinocyte-derived cytokines including interleukin (IL) 1α [3]. Interleukin 1α previously was shown to be capable of eliciting PGE_2 release in unirradiated keratinocytes [3], and it has therefore been proposed that UVB-induced IL-1α may mediate PGE_2 release by irradiated keratinocytes. However, up to this point, no direct evidence proving an autocrine regulatory role of IL-1α in this system has been provided. Moreover, IL-1α may only partially account for UVB-induced keratinocyte PGE_2 production, because IL-1α was a significantly weaker stimulus, as compared to UVB irradiation [3]. In this regard it is of interest that tumor necrosis factor (TNF) α, another cytokine produced by UVB-irradiated keratinocytes [4], may induce PGE_2 synthesis in other cell systems [5,6].

In such an autocrine system, IL-1 receptor (IL-1R) molecules and/or TNF receptor (TNFR) molecules on the keratinocyte surface would play an important regulatory role. In studies employing ^{125}I–IL-1, high- as well as low-affinity IL-1R could be identified on the surface of unirradiated human keratinocytes [7,8]. This observation may reflect coexpression of the recently cloned type I and type II IL-1R molecules, as has been described for other cell types [9–11]. In addition, human keratinocytes were recently shown to express functionally active 55-kD TNF receptors, but to lack 75-kD TNFR expression [12,13]. The relevance of IL-1R and/or 55-kD TNFR for UVB-induced keratinocyte PGE_2 release is currently unknown.

Stimulation of prostanoid formation requires the involvement of several enzymatic steps. Liberation of arachidonic acid is indispensable for prostanoid formation. Within the arachidonic acid cascade PGH_2 synthase (cyclooxygenase) is the rate-limiting enzyme for prostaglandin production and the specific activity of this enzyme may increase after stimulation of cells [14–17]. In the present *in vitro* study, by employing KB cells as a model for transformed human keratinocytes, the potential autocrine role of keratinocyte derived IL-1 and/or TNFα and their respective receptor molecules in UVB-induced PGE_2 release and cyclooxygenase activation was assessed.

MATERIALS AND METHODS

Chemicals Tritiated prostanoid standards for calibration of high-performance liquid chromatography (HPLC) and (1-^{14}C) arachidonic acid were from Amersham Buchler, Braunschweig, Germany. Trifluoroacetic acid and HPLC grade S acetonitrile was from Sigma, Munich, Germany. All other chemicals used were of analytical grade.

Cytokines and Antibodies Recombinant human (rh) TNFα was kindly provided by Boehringer Mannheim, Mannheim, Germany. Monoclonal antibody (MoAb) htr-9 (mIgG1) against the human 55-kD TNFR was a gift from M. Brockhaus, Hoffmann-LaRoche Ltd, Basel, Switzerland. The generation and characterization of this antibody, which was purified from hybridoma supernatants by using rabbit–anti-mouse Ig linked to Sepharose 4b (Pharmacia, Freiburg, Germany), previously has been described [18]. Monoclonal antibody M4 (rat IgG2b), which blocks IL-1 effects by binding to the

Manuscript received January 11, 1993; accepted for publication June 1, 1993.

Reprint requests to: Dr. Jean Krutmann, Department of Dermatology, Hauptstrasse 7, D-7800 Freiburg i.Br., Germany.

0022-202X/93/$06.00

human type I IL-1R [19] and MoAb M22 (rat IgG2b), which functionally blocks the human type II IL-1R (J.E. Sims, S.K. Dower, unpublished observation) were kindly provided by J.E. Sims, Immunex Corporation, Seattle, WA. Both antibodies were used at a concentration of 10 μg/ml. The polyclonal rabbit antibody IP 300 (IgG and IgM), which was purchased from Genzyme Corporation, Boston, MA, was used at a concentration of 10 μg/ml, which corresponds to a neutralizing activity of 10,000 units TNFα bioactivity, as assessed by the L929 cell cytotoxicity assay. Mouse and rat isotype control antibodies were from Dianova, Hamburg, Germany. As a control for the polyclonal rabbit antibodies, a rabbit pre-immune serum was used (kindly provided by M. Brockhaus, Hoffmann-LaRoche Ltd, Basel, Switzerland).

Cell Culture The human carcinoma cell line KB (American Type Culture Collection, Rockville, MD) was maintained in monolayer cultures in Dulbecco's modified Eagle's medium (Gibco, Berlin, Germany) containing 10% fetal calf serum (Gibco) in a humidified atmosphere containing 5% CO_2 as previously described [20]. Supplementation of medium with 10% fetal bovine serum (FBS) did not increase basal PGE_2 release by cultured KB cells and did not interfere with the PGE_2 RIA. This cell line was derived from an epidermoid carcinoma in the mouth of an adult man and has previously been used as a model for transformed human keratinocytes [4,12,20,21].

Ultraviolet Radiation For UV radiation, medium was replaced by phosphate-buffered saline (PBS), lids were removed, and cells were exposed as previously described to UV radiation (100 J/m^2) using a bank of four FS20 sunlamp bulbs (Westinghouse Electric Corp., Pittsburgh, PA), which are known to primarily emit in the UVB range (280–320 nm) [13,20]. The UVB output was monitored by means of an IL-1700 research radiometer and SEE 240 UVB photodetector (International Light, Newburyport, MA) and was approximately 24×10^{-5} W/cm^2 at a tube to target distance of 22 cm. This UVB dose previously was found to enhance several cellular functions including the expression of cytokine and adhesion molecule mRNA [4,13,20] and does not affect cell viability as assessed by trypan blue exclusion experiments. After irradiation, cells were washed and cultured in medium in the presence or absence of the indicated antibodies. As controls, KB cells were washed and allowed to sit in PBS for the irradiation period.

PGE_2 Measurements and Determination of Cyclooxygenase Activity PGE_2 in the culture medium was determined by radioimmunoassay (RIA) as previously described [22]. After stimulations, culture medium of KB cells was removed and used for PGE_2 RIA, and the corresponding cells were washed twice with PBS and then frozen in liquid nitrogen. Cells were disrupted, incubated with [1-^{14}C] arachidonic acid and resulting metabolites were extracted and analyzed by HPLC as described previously [23]. In brief, cells were disrupted using a tight Dounce homogenizer and were then incubated with 20 μM [1-^{14}C] arachidonic acid (Amersham Buchler, Braunschweig, Germany) in a 100 mM Tris/HC1 buffer (pH 8.0) containing 1 mM ethylenediaminetetraacetic acid, 1 mM reduced glutathione, and 0.6 μM bovine hemoglobin (all ingredients from Sigma, Munich, Germany). After 10 min at 25°C, reaction was stopped by adding tenfold −20°C methanol, denatured protein was centrifuged, and the supernatant was evaporated to dryness. Residues were taken up in 20% methanol in water and subjected to reverse-phase HPLC using an acetonitrile/0.1% trifluoroacetic-acid in water gradient. Radioactivity was measured by an on-line continuous fluid scintillation counter.

RESULTS

Exposure of KB cells to 100 J/m^2 UVB radiation increased PGE_2 release into the culture medium five- to eightfold, as compared to basal secretion (Fig 1), thus confirming previous studies employing long-term cultured normal human keratinocytes [3]. The time course of UVB-induced PGE_2 production was characterized by a lag phase of 4 to 6 h and reached maximum levels 18 to 24 h post-exposure (Fig 1).

To assess the role of the keratinocyte-derived cytokine IL-1 in UVB-induced PGE_2 release, in the following experiments, irradiated KB cells were cultured in the presence of MoAbs that were known to functionally block either the type I (MoAb M4) or type II (MoAb M22) IL-1R. Addition of MoAb M4 to cultures with UVB-irradiated KB cells inhibited UVB-induced PGE_2 release by 70% (Fig 1). This inhibition was relatively specific, because MoAb M22, even if used over a wide dose range (0.1–100 μg/ml), did not exert any significant effects in this system (Fig 1; shown for 10 μg/ml).

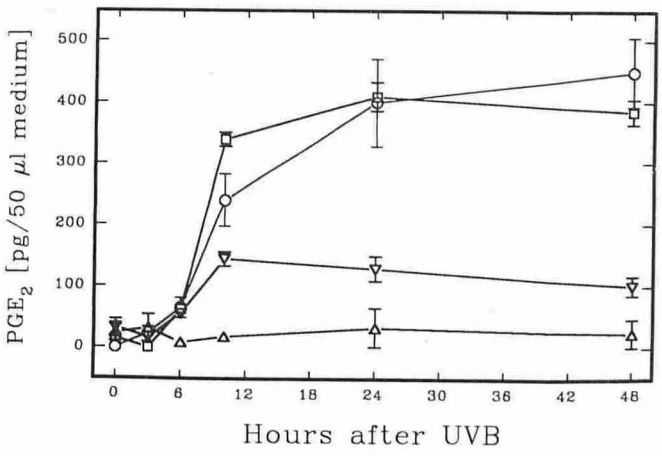

Figure 1. Influence of anti-IL-1R antibodies on UVB-induced PGE_2 synthesis of KB cells. Transformed human keratinocytes (KB cells) were UVB irradiated as described in *Materials and Methods* and culture medium was assessed for PGE_2 content 3, 6, 10, 24, and 48 h after irradiation. *Curves* represent unirradiated controls (△), UVB-irradiated cells (○), UVB-irradiated cells cultured in the presence of MoAb M22 (□), and UVB-irradiated cells cultured in the presence of MoAb M4 (▽). Data are given as means ± SD of three independent experiments.

Monoclonal antibody M4 only partially inhibited UVB-induced PGE_2 release, indicating the involvement of one or more additional mediators. Accordingly, stimulation of unirradiated KB cells with recombinant human (rh) TNFα significantly enhanced PGE_2 release (Fig 2). This increase could be mimicked by stimulating cells with MoAb htr-9 (100 μg/ml), which exerts TNFα-like bioactivity by binding to the 55-kd TNFR on human KB cells (Fig 2). In contrast, equivalent concentrations of an isotype control antibody did not significantly affect KB cell PGE_2 production. Incubation of KB cells with neutralizing anti-TNFα antibody inhibited UVB-induced PGE_2 release by 50% (Fig 3). This inhibitory effect could be abolished by adding an excess amount of exogenous rhTNFα (data not shown). Because both anti-TNFα antibody and MoAb M4, if

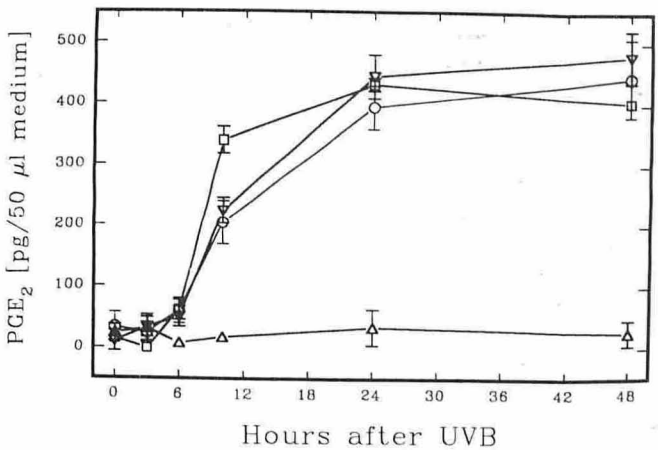

Figure 2. Effect of a TNFα and UVB irradiation on PGE_2 synthesis of KB cells. PGE_2 in culture media was determined by RIA at the indicated time points. Transformed human keratinocytes (KB cells) were UVB irradiated as described in *Materials and Methods* (○), or left unirradiated and treated with IgG1 control antibodies (△), or left unirradiated and treated with MoAb htr9 (100 μg/ml) (□), or left unirradiated and treated with rhTNFα (100 U/ml) (▽). Data are given as means ± SD of three independent experiments.

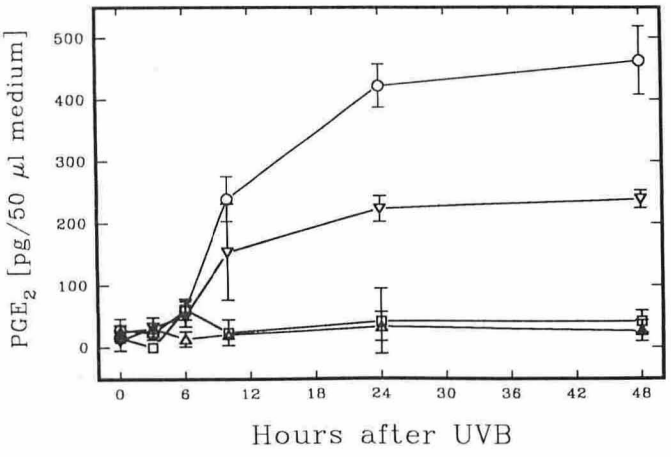

Figure 3. Effect of neutralizing anti-TNFα-antibody and anti-IL-1R type I antibody M4 on UVB-induced PGE_2 synthesis of KB cells. Transformed human keratinocytes (KB cells) were UVB irradiated as described in *Materials and Methods* and culture medium was assessed for PGE_2 content at the indicated time points after irradiation. Cells were left unirradiated and cultured in the presence of IgG1 isotype control antibodies (△), or UVB irradiated and cultured in the presence of a rabbit control serum (○), or UVB irradiated and cultured in the presence of neutralizing anti-TNFα antibodies (▽), or UVB irradiated and cultured in the presence of both anti-TNFα antibodies plus MoAb M4 (□). Culture of UVB-irradiated cells in the presence of both a rabbit control serum plus IgG1 isotype control antibodies did not significantly decrease PGE_2 synthesis (data not shown). Data are given as means ± SD of three independent experiments.

given alone, only partially decreased UVB-induced PGE_2 production, in the following experiments UVB-irradiated KB cells were incubated in the presence of both antibodies. As is shown in Fig 3, combined treatment of UVB-irradiated KB cells with anti-TNFα antibody plus MoAb M4 lead to complete inhibition of UVB-induced PGE_2 release.

The role of keratinocyte-derived IL-1 and TNFα in UVB-induced PGE_2 production was corroborated in experiments, in which specific cyclooxygenase activity in UVB-irradiated KB cells was assessed after cell homogenization. Prostaglandin E_2 and 6-keto-$PGF_{1\alpha}$ were produced by cell homogenates with PGE_2 being the main product with approximately 60% of cyclooxygenase products, whereas 6-keto-$PGF_{1\alpha}$ accounted for the other 40%. None of the stimulations lead to production of other prostanoids nor did the ratio of the mentioned prostaglandins change upon stimulations.

In contrast to unirradiated cells, homogenates of UVB-irradiated cells revealed a more than twofold increase of cyclooxygenase activity (Fig 4). This increase could be detected after a lag phase of 8 h, yielding a maximum at 24 h post exposure. A partial but significant inhibition of induction of cyclooxygenase activity could be observed after MoAb M4 treatment of UVB-irradiated cells (Fig 4), whereas neutralizing anti-TNFα-Ab did not significantly alter the upregulation of this enzyme activity (data not shown). A combination of anti-TNFα-Ab and MoAb M4 was able to completely inhibit the rise of cyclooxygenase activity as measured *in vitro* in the corresponding cell homogenates (Fig 4).

DISCUSSION

The presented data clearly demonstrate the involvement of IL-1 and TNFα in UVB-elicited PGE_2 synthesis by human keratinocytes. In previous studies, stimulation of long-term cultured, normal human keratinocytes with rhIL-1α was found to induce PGE_2 release in unirradiated cells, but only indirect evidence based on the time kinetics of UVB-induced PGE_2 and IL-1α production was given to support a potential role of endogenously produced IL-1 in UVB-induced prostanoid production [3]. In the present study, functional blocking of the type I IL-1R on the surface of UVB-irradiated KB cells was found to suppress UVB-induced PGE_2 release by 70%, definitely proving the autocrine role of IL-1 in this system. In addition, these data for the first time demonstrate the presence of functionally active type I IL-1R molecules on the surface of transformed human keratinocytes. Recently, two types of human IL-1R have been identified: the type I IL-1R, an 80-kD molecule and the type II IL-1R, a 68-kD molecule, which both may be present on the same cells, but probably mediate distinct functions [10]. Binding studies employing ^{125}I-IL-1α revealed the presence of two types of IL-1 receptors on the surface of cultured human keratinocytes [8], which may reflect co-expression of both types of IL-1R molecules. It is therefore of interest that in the present study only the anti-type I IL-1R antibody, but not the anti-type II IL-1R antibody, inhibited UVB-induced PGE_2 release, indicating that only the type I IL-1R has intrinsic activity with respect to keratinocyte PGE_2 production. Further studies to examine the expression and function of the type II IL-1R on the surface of human keratinocytes are currently underway.

IL-1R blockage only partially inhibited UVB-induced PGE_2 synthesis, thus supporting the assumption [3] that IL-1α is responsible only in part for UVB-evoked PGE_2 release. In the search for a second mediator involved in this system, TNFα appeared to be a likely candidate. Accordingly, it has been reported for a variety of other cell types, including macrophages, that TNFα is a potent inducer of PGE_2 synthesis [5,6]. Moreover, recent studies indicate that cultured human keratinocytes are both a source and a target for TNFα [4,12,13] and that *in vitro* UVB irradiation may cause binding of UVB-induced, endogenous TNFα to TNFR on the keratinocyte surface [13]. In the present study, rhTNFα stimulation of unirradiated keratinocytes induced PGE_2 release, and the time course and quantity of TNFα- and of UVB-induced PGE_2 release was consistent with a cause/effect relationship. The observation that TNFα stimulated PGE_2 release in transformed human keratinocytes was corroborated in experiments, in which stimulation of

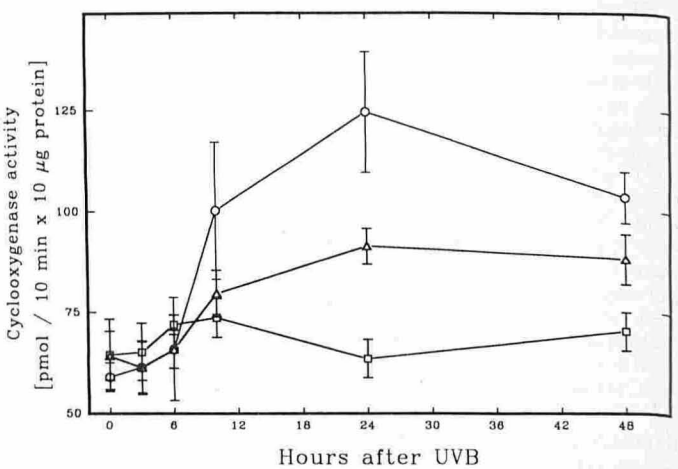

Figure 4. Cyclooxygenase activity of KB cells after UVB treatment. Transformed human keratinocytes (KB cells) were UVB irradiated as described in *Materials and Methods*. At the indicated time points after irradiation KB cells were homogenized and incubated with (1-^{14}C) arachidonic acid, and cyclooxygenase products were determined by HPLC. Cyclooxygenase activity was defined as the sum of all prostanoids formed by 10 µg protein in 10 min. The *curves* represent cyclooxygenase activity of UVB-irradiated KB cells (○), cyclooxygenase activity of UVB-irradiated KB cells cultured in the presence of MoAb M4 (△), and cyclooxygenase activity of UVB-irradiated KB cells cultured in the presence of MoAb M4 plus anti-TNFα antibody (□). Cyclooxygenase activity of unirradiated KB cells did not differ from that of time point 0 h by more than ±7%. Incubation of UVB-irradiated KB cells in the presence of the respective isotype control antibodies did not alter the UVB effects on cyclooxygenase activity of KB cells (data not shown). Data are given as means ±SD of three independent experiments.

KB cells with MoAb htr-9, which previously was shown to exert TNFα-like bioactivity by binding to the 55-kD TNFR on the surface of KB cells [12], mimicked TNFα-induced PGE_2 release. Direct evidence for the autocrine role of TNFα in UVB-evoked PGE_2 synthesis came from experiments in which neutralizing anti-TNFα-Ab reduced UVB-induced PGE_2 production by 50%. Interestingly, TNFα stimulation of unirradiated cells increased PGE_2 release to an extent essentially identical to that observed after UVB stimulation, whereas anti-TNFα treatment of UVB-irradiated cells only partially inhibited UVB-elicited PGE_2 synthesis. This discrepancy may at least partially be explained by the possibility that UVB irradiation additionally leads to the production of molecules that are capable of inhibiting TNFα effects. In this regard it is of interest that human keratinocytes including KB cells recently were shown to produce significant amounts of soluble 55-kD TNFR molecules [13]. Taken together, the present study indicates that IL-1 and TNFα alone only partially contribute to UVB-elicited PGE_2 release. Addition of both anti-type I IL-1R and anti-TNFα antibodies to UVB-irradiated keratinocytes, however, decreased PGE_2 release to background levels, strongly indicating that UVB-elicited PGE_2 synthesis in human keratinocytes is under the control of both cytokines acting together.

This conclusion is supported by experiments in which specific cyclooxygenase activity rather than PGE_2 release was assessed in UVB-irradiated KB cells. Although untreated keratinocytes revealed a significant basal cyclooxygenase activity, UVB-irradiated keratinocytes showed a time-dependent twofold increase of this enzyme activity. This upregulation could be inhibited to some extent by anti-type I IL-1R antibodies, whereas anti-TNFα antibodies alone did not exert significant inhibitory effects. However, if irradiated cells were cultured in the presence of both anti-IL-1R and anti-TNFα antibodies, complete inhibition of UVB-induced cyclooxygenase activity in irradiated KB cells was observed. Thus, upregulation of cyclooxygenase activity appears to critically depend on endogenously produced IL-1 and, to a minor part, TNFα. The capacity of IL-1 to induce cyclooxygenase activity is not specific for keratinocytes, because a similar effect could be observed in human fibroblasts [14].

In vivo exposure of mice to UVB radiation previously was shown to effectively inhibit selected cell-mediated immune responses, e.g., induction of contact hypersensitivity reactions [24]. Recent studies indicate that this immunosuppressive may be prevented, if UVB-irradiated animals are treated with anti-TNFα antibodies, and it has therefore been suggested that UVB-induced, keratinocyte-derived TNFα is an important mediator in this system [25]. Based on the present observation, that keratinocyte-derived TNFα acts in an autocrine manner to induce keratinocyte prostanoid production, it is proposed that the immunomodulatory capacity of UVB-induced TNFα may at least partially be attributed to TNFα-induced keratinocyte PGE_2, which may inhibit the function of immunocompetent epidermal cells such as Langerhans cells. This hypothesis is supported by previous reports demonstrating that UVB-induced inhibition of contact hypersensitivity reactions may be effectively prevented by treating UVB-irradiated mice with indomethacin, a potent inhibitor of PGE_2 production [26,27].

In summary, the present study provides clear evidence for the involvement of endogenously produced IL-1 and TNFα in UVB-elicited PGE_2 release by transformed, human keratinocytes. Interleukin-1 was found to act via the type I IL-1R and to account for the major part of PGE_2 released, whereas TNFα acted via the 55-kD TNFR, contributing a minor effect. Increasing release of PGE_2 is partly due to upregulation of specific cyclooxygenase activity of keratinocytes, which was again mediated in an autocrine manner by the combined action of IL-1 plus TNFα.

This work was supported by a grant from the Deutsche Forschungsgemeinschaft Kr 871/3-2 and a grant from the Volkswagenstiftung I/67 421.

REFERENCES

1. DeLeo VA, Horlick H, Hanson D, Eisenger M, Harber L: Ultraviolet radiation induces changes in membrane metabolism of human keratinocytes in culture. *J Invest Dermatol* 83:323–326, 1984
2. Pentland A, George J, Moran C, Needleman P: Cellular confluence determines injury-induced prostaglandin E_2 synthesis by human keratinocyte cultures. *Biochem Biophys Acta* 919:71–78, 1987
3. Pentland A, Mahoney M: Keratinocyte prostaglandin synthesis is enhanced by IL-1. *J Invest Dermatol* 94:43–46, 1990
4. Köck A, Schwarz T, Kirnbauer R, *et al*: Human keratinocytes are a source for tumor necrosis factor α: evidence for synthesis and release upon stimulation with endotoxin of ultraviolet light. *J Exp Med* 172:1609–1614, 1990
5. Dayer JM, Beutler B, Cerami A: Cachectin/tumor necrosis factor stimulates collagenase and prostaglandin E_2 production by human synovial cells and dermal fibroblasts. *J Exp Med* 162:2163–2168, 1985
6. Schultz RM, Pavlidis NA, Stylos WA, Chirigos MA: Regulation of macrophage tumoricidal function: a role for prostaglandins of the E series. *Science* 202:320–323, 1978
7. Kupper TS, Lee F, Birchall N, Clark S: Interleukin 1 binds to specific receptors on human keratinocytes and induces granulocyte macrophage colony-stimulating factor mRNA and protein. *J Clin Invest* 82:1787–1792, 1988
8. Blanton RA, Kupper TS, McDougall JK, Dower S: Regulation of interleukin 1 and its receptor in human keratinocytes. *Proc Natl Acad Sci USA* 86:1273–1277, 1989
9. McMahan CJ, Slack JL, Mosley B, *et al*: A novel IL-1 receptor, cloned from B-cells by mammalian expression, is expressed in many cell types. *EMBO J* 10:2821–2832, 1991
10. Munoz E, Zubiaga AM, Sims JE, Huber BT: IL-1 signal transduction pathways. I. Two functional IL-1 receptors are expressed in T-cells. *J Immunol* 146(1):136–143, 1991
11. Giri JG, Robb R, Wong WL, Horuk R: HepG2 cells predominantly express the type II interleukin 1 receptor (biochemical and molecular characterization of the IL-1 receptor). *Cytokine* 4(1):18–23, 1992
12. Trefzer U, Brockhaus M, Loetscher H, *et al*: 55-kd tumor necrosis factor receptor is expressed by human keratinocytes and plays a pivotal role in regulation of human keratinocyte ICAM-1 expression. *J Invest Dermatol* 97:911–916, 1991
13. Trefzer U, Brockhaus M, Loetscher H, *et al*: The 55-kd tumor necrosis factor receptor on human keratinocytes is regulated by tumor necrosis factor alpha and by ultraviolet B radiation. *J Clin Invest* (in press)
14. Raz A, Wyche A, Siegel N, Needleman P: Regulation of fibroblast cyclooxygenase synthesis by interleukin-1. *J Biol Chem* 263:3021–3028, 1988
15. Barr RM, Symonds PH, Akpan AS, Greaves MW: Culture of human dermal fibroblasts in collagen gels: modulation of interleukin 1-induced prostaglandin E_2 synthesis by an extracellular matrix. *Exp Cell Res* 198(2):321–327, 1992
16. Aizu E, Yamamoto S, Nishikawa K, Kato R: Synergistic stimulation of prostaglandin E_2 release by epidermal growth factor and a tumor promotor anthralin from primary cultures of mouse epidermal cells. *Jpn J Pharmacol* 57(3):361–366, 1991
17. Coyne DW, Nickols M, Bertrand W, Morrison AR: Regulation of mesangial cell cyclooxygenase synthesis by cytokines and glucocorticoids. *Am J Physiol* 263 (1 Pt 2):F97–F102, 1992
18. Brockhaus M, Schoenfeld HJ, Schlaeger EJ, *et al*: Identification of two types of tumor necrosis factor receptors on human cell lines by monoclonal antibodies. *Proc Natl Acad Sci USA* 87:3127–3131, 1990
19. Sims JE, Acres RB, Grubin CE, *et al*: Cloning the interleukin 1 receptor from human T cells. *Proc Natl Acad Sci USA* 86:8946–8950, 1989
20. Krutmann J, Köck A, Schauer E, *et al*: Tumor necrosis factor beta and ultraviolet radiation are potent regulators of human keratinocyte ICAM-1 expression. *J Invest Dermatol* 95:127–131, 1990
21. Kirnbauer R, Köck A, Schwarz T, Urbanski A, Krutmann J, Borth W, Damm D, Shipley G, Ansel JC, Luger TA: IFN-β2, B cell differentiation factor 2, or hybridoma growth factor (IL-6) is expressed and released by human epidermal cells and epidermoid carcinoma cell lines. *J Immunol* 142:1922–1928, 1989
22. Eyhorn S, Schlayer HJ, Henninger H, *et al*: Rat hepatic sinusoidal endothelial cells in monolayer culture. Biochemical and histological characteristics. *J Hepatol* 6:23–35, 1988
23. Grewe M, Duyster J, Dieter P, Henninger H, Schulze-Specking A, Decker K: Prostaglandin D_2 and E_2 syntheses in rat Kupffer cells are antagonistically regulated by lipopolysaccharide and phorbol ester. *Biol Chem Hoppe-Seyler* 373:655–664, 1992
24. Krutmann J, Elmets CA: Recent studies on mechanisms in photoimmunology. *Photochem Photobiol* 48:787–798, 1989
25. Yoshikawa T, Kurimoto I, Streilein JW: Tumor necrosis factor-alpha mediates ultraviolet light B-enhanced expression of contact hypersensitivity. *Immunology* 76:264–271, 1992
26. Chung H, Burnham DK, Roberston B, Roberts LK, Daynes RD: Involvement of prostaglandins in the immune alteration caused by the exposure of mice to ultraviolet radiations. *J Immunol* 137:2478–2484, 1986
27. Jun BD, Roberts LK, Cho BH, Roberston B, Daynes RD: Parallel recovery of epidermal antigen-presenting cell activity and contact hypersensitivity responses in mice exposed to ultraviolet irradiation: the role of a prostaglandin-dependent mechanism. *J Invest Dermatol* 90:311–316, 1988