UVB Light Induces Nuclear Factor κB (NFκB) Activity Independently from Chromosomal DNA Damage in Cell-Free Cytosolic Extracts

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It has been shown previously that ultraviolet (UV) light (290–320 nm) activates keratinocytes to release proinflammatory cytokines including interleukin (IL)-6. Because the 5′ flanking region of the IL-6 gene contains a consensus NFκB binding sequence, the effect of UVB light on an NFκB-like binding activity was investigated in a human epidermoid carcinoma cell line (A431). Nuclear factor κB (NFκB) activation in the cytoplasm is known to be due to the dissociation of an inactive NFκB-inhibitor of nuclear factor κB (IκB) complex. Cytosolic extracts from cells harvested shortly after sublethal UVB irradiation showed a UVB dose-dependent increase of NFκB binding. The activation was reduced by radical scavenging chemicals, suggesting involvement of reactive oxygen intermediates. NFκB activation has been shown previously to be triggered by DNA lesions induced by UV light. To elucidate whether DNA damage is necessary and sufficient to mediate NFκB activation crude, cytosolic protein extracts obtained from unirradiated cells were exposed to UVB light. This *in vitro* UVB treatment led to activation of an NFκB-like binding activity, suggesting an additional signaling pathway independent of chromosomal DNA damage or byproducts of DNA damage. The activation process was dependent on the presence of membranes. The data suggest at least an additional signaling pathway for the early UVB response, including a component of the pathway residing at the cell membrane. *Key words: ultraviolet light/NFκB/interleukin 6/gene regulation. J Invest Dermatol 102:422–427, 1994*

Ultraviolet (UV) light, which can cause severe damage like induction and promotion of cancer, cutaneous inflammation, and immunosuppression [1], represents one of the most important environmental impacts for humans. These potentially harmful effects of solar light reaching the surface of the earth are mainly due to the UVB spectrum (290–320 nm) [2]. To better understand the biologic implications of solar irradiation it appears important to elucidate the molecular mechanism underlying the UV response. This issue, however, has been mostly studied by applying lethal doses of UVC light (e.g., 254 nm). Because UVC is strongly absorbed by ozone and consequently of minor biologic importance, the effects of the more biologically relevant UVB spectrum were addressed throughout this study.

Keratinocytes are natural target cells of UVB in humans and, upon UVB exposure, have been shown to release increasing amounts of a variety of immunologic and proinflammatory cytokines (for review see [3]) like interleukin (IL)-1 [4,5], IL-6 [6], and tumor necrosis factor α (TNFα) [7]. The molecular mechanisms particularly involved in the regulation, however, are not clear. As it recently turned out that a nuclear factor κB (NFκB) recognition sequence is an important regulatory element within the IL-6 promoter [8,9], we investigated the activation of NFκB by sublethal UVB irradiation. The transcriptional activator NFκB has been originally characterized as a nuclear factor binding to an enhancer sequence, κB, of the κ immunoglobulin light-chain gene [10]. NFκB is a multi-protein complex consisting of two subunits, p50 and p65 [11]. In addition, this complex can be kept in an inactive form by the binding of an inhibitory protein called IκB [12]. This inactive form is retained in the cytoplasm until the NFκB-inhibitor of NFκB (IκB) complex dissociates upon different types of stimuli, including IL-1 [13], TNF [14], phorbol esters [15], lipopolysaccharide [16], or lethal UVC irradiation [17]. Free NFκB is allowed to migrate into the nucleus, binds to NFκB-specific enhancer elements, and activates gene transcription (reviewed in [18]).

The main target of UV light was regarded to be chromosomal DNA damage and it was suggested that the UV light response is triggered by DNA damage events [17,19,20]. Because the consequences of this mechanism represent a severe vital impact, we used lower UVB doses in all our experiments, not sufficient to alter the survival rates of the cells. Survival rates were determined by trypan blue exclusion assay after 16 h of culture in Dulbecco's minimum essential medium (DMEM) with 10% fetal bovine serum (FBS) where unstained cells were defined as physiologically viable. Applying such low doses, we investigated whether the DNA damage events or additional alternative ones are involved in the signaling pathway of UVB induction of NFκB by UVB. We exposed cell-free cytosolic extracts obtained from untreated epidermal cell lines to UVB light, subsequently named *in vitro* irradiation. Here, we demonstrate 1) that NFκB-IκB dissociation can be induced in a dose-dependent manner by sublethal UVB irradiation in human epidermal cell lines and 2) that the dissociation can be triggered *in vitro* by UVB exposure of cytosolic protein extracts of untreated cells. As shown for other types of stimuli, the induction of NFκB binding activity is reduced to some extent in the presence of radical

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Abbreviations: EMSA, electrophoretic mobility shift assay; IκB, inhibitor of nuclear factor κB; NAC, N-acetylcysteine; NFκB, nuclear factor κB; PDTC, pyrrolidine-dithiocarbamate; ROI, reactive oxygen intermediates.

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scavenging chemicals [21,22], indicating an involvement of reactive oxygen intermediates (ROIs) as second messengers in the NFκB activation. Our data provide evidence for an at least additional chromosomal DNA damage-independent UVB signaling pathway. Moreover, a UVB target transmitting the signal to the NFκB-IκB complex appears to be localized in the membrane or associated with the membrane.

MATERIALS AND METHODS

Cell Lines and Culture Conditions The human epidermoid carcinoma cell line A431, obtained from ATCC, Rockville, MD (CRL 1555), was used throughout this study. A431 cells were propagated in DMEM supplemented with 10% FCS, 1% antibiotic/antimycotic solution, and 2 mM L-glutamine (all Gibco, Grand Island, NY). UVB irradiation was performed as described previously [7]. Briefly, cells were seeded in DMEM containing 10% FBS into dishes at a density of $7 \times 10^5/cm^2$ and grown for 16 h. Four hours prior to irradiation medium was changed for FBS-free DMEM. Immediately before irradiation, the medium was replaced by phosphate-buffered saline (PBS). For UVB irradiation, a bank of four FS20 bulbs (Westinghouse Electric Corp., Pittsburgh, PA), which emit most of their energy within the UVB range (290–320 nm) with an emission peak at 313 nm, was used. The UV output measured at 310 nm using an IL1700 research radiometer (International Light, Newburyport, MA) was 0.2 W/m^2 at a tube-to-target distance of 46 cm. Immediately after UVB treatment, PBS was replaced by serum-free DMEM. Cell viability was determined by trypan blue exclusion 16 h after irradiation cultivated in DMEM supplemented with 10% FBS. Survival rates were more than 90% in all experiments presented. Pyrrolidinedithiocarbamate (PDTC; Sigma, St. Louis, MO) was prepared freshly and added to the medium at a concentration of 20 μM 1 h prior to irradiation. After washing twice with PBS, UVB treatment was performed as described in PBS without PDTC. Immediately after irradiation, PBS was replaced by DMEM containing 20 μM PDTC. N-acteylcysteine (NAC; Sigma), adjusted to pH 7.5 with NaOH, was used at a final concentration of 20 mM, identically as described for PDTC.

Cytosolic and Nuclear Protein Extracts Cells ($1-5 \times 10^6$) were washed with PBS and detached from the dishes with a rubber policeman. Preparation of extracts was essentially performed as described [23]. Briefly, cells were allowed to swell in hypotonic buffer (10 mM HEPES, pH 7.8, 0.1 mM ethylenediamine tetraacetic acid [EDTA], 10 mM KCl, 1 mM dithiothreitol [DTT], phenylmethylsulfonyl fluoride [PMSF] for 15 min on ice. Cytosolic protein fraction was obtained after the addition of Nonidet P-40 (NP-40) (Sigma) to a final concentration of 0.5% and separation from the nuclei by centrifugation ($500 \times g$, 5 min). Nuclear proteins were extracted from the remaining pellet with hypertonic buffer (20 mM HEPES, pH 7.9, 350 mM NACl, 1 mM DTT, 1 mM PMSF). After clearing the extracts at $8000 \times g$ for 5 min, the protein content of all samples was adjusted to 2 μg/μl protein (determined by Biorad protein assay [Biorad, Munich, Germany]) with hypotonic buffer for cytosolic extracts and nuclear extracts, respectively. Extracts were used immediately or stored with 10% glycerol at −70°C. Membrane fraction was obtained by centrifugation of the cytosolic extract (40,000 × *g* at 4°C for 4 h), prepared by disruption of the cells with a dounce homogenizator in hypotonic buffer without detergent. The membrane pellet was resuspended in 100 μl hypertonic buffer (containing 1% NP-40) per 10^8 cells. Two microliters of membrane suspension were used for DNA binding reaction. UVB treatment of cytosolic extracts obtained from unirradiated cells was performed prior to electrophoretic mobility assay (EMSA) in 60 mM KCl, 1 mM DTT in reaction tubes; alternatively, drops of protein extract were irradiated on parafilm sheets. These samples were used for EMSA immediately after extraction. For estimation of the chromosomal DNA content in cytosolic extracts, cytosol from 10^7 cells was prepared as described and extracted once with buffer-saturated phenol/chloroform pH 8.0 and twice with chloroform. Nucleic acids were precipitated with ethanol and dissolved in 20 μl water. One-half was RNAse treated and used for agarose gel electrophoresis analysis; the other half was used as the template for polymerase chain reaction (PCR). The primer oligo nucleotides are derived from the glyceraldehyde-3-phosphate dehydrogenase genes 5′TCCCATCACCATCTTCCA3′ and 5′CATCACGCCACAGTTTCC3′, respectively.

EMSA Synthetic double-stranded oligonucleotides with 5′ protruding *Xho*1-compatible ends were provided by H. Zeller, Department of Experimental Dermatology, University of Muenster. κB-IL6 is derived from the IL-6 promoter (−121 to −138): 5′TCGAGTGTGGGATTTTCCCATGAC3′. The κB-immunoglobulin (IG) oligonucleotide is derived from the κ immunoglobulin light chain described previously [24], 5′TCGAGAGAGGGGACTTTTCCGAGAC3′. For nonspecific competition the following double-stranded oligonucleotide was used: 5′TCGATGGAGTCTCCA3′. Binding reactions were performed with 6 μg of protein, ^{32}P-labeled double-stranded oligonucleotide, 50 mM KCl, and μg poly dIdC (Boehringer Mannheim, Mannheim, Germany), for 45 min at room temperature. *In vitro* UVB irradiation of protein extracts was performed immediately before the binding reaction.

RESULTS

Sublethal UVB Doses Induce NFκB-like Binding Activity In humans, epidermal cells, by virtue of their anatomic location, represent the main target cells of UVB light. Therefore, the human epidermoid carcinoma cell line A431 was selected for this study. As with other epidermal cells [6], A431 cells respond to UVB light with increased cytokine release including IL-6 (unpublished data). First, we investigated whether preexisting inactive NFκB could be activated upon UVB. Therefore we analyzed DNA binding activity in cytosolic extracts of UVB-treated cells. As shown in Fig 1, NFκB-like binding activity to the ^{32}P-labeled κB-IL6 DNA fragment derived from the IL-6 promoter is induced in cytosolic extracts after UVB irradiation of the A431 cells *(lane 3)*. The extracts were prepared 20 min after irradiation with 200 J/m^2 UVB, a dose after which more than 90% of UV-irradiated cells were physiologically viable. The specificity of the binding is demonstrated by competition of the binding protein with either unlabeled κB-IL6 *(lane 4)* or with the unlabeled NFκB binding element residing in the promoter of the κ immunoglobulin light-chain gene *(lane 6)*. A nonspecific unlabeled oligonucleotide is unable to compete for the binding protein *(lane 7)*. Thus, as described for other stimuli, low-dose UVB is able to induce dissociation of a preexisting cytosolic NFκB-IκB complex, leading to a release of active NFκB in a fast reaction. Next, we determined whether NFκB after activation in the cytosol is properly imported into the nucleus and whether the NFκB activation is UVB dose dependent. Consequently, nuclear protein extracts were prepared from cells 1 h after UVB treatment and tested for NFκB binding activity. As shown in Fig 2 *(lanes 1–3)*, NFκB-like binding activity is increased upon irradiation. A similar result is obtained with cytosolic extracts prepared from cells irradiated with different UVB doses (data not shown). Taken together, it can be concluded that preexisting inactive NFκB complexes are dissociated upon UVB irradiation *in vivo* in a dose-dependent manner and that binding active NFκB is subsequently imported into the nucleus. Survival rates of irradiated cells determined by trypan blue exclusion were comparable to untreated controls and not essentially diminished even after 24 h. Because we employed a trypan blue exclusion assay to determine the cell viability we cannot exclude that the UVB treatment caused an increased percentage of reproductively inactive cells not able to form colonies. However, UVB doses exceeding 300 J/m^2 resulted in a dramatic increase of cells susceptible to trypan blue staining. UVB induction of NFκB binding activity is not restricted to A431 cells. Similar observations have been made using a human transformed keratinocyte cell line (HaCaT, kindly provided by N. Fusenig, DKFZ, Heidelberg, Germany) and less pronounced using a human histiocytic lymphoma cell line (U937) (data not shown).

Anti-oxidants Suppress the UVB-induced NFκB-like Activity NFκB is activated by a diverse set of substances (for review see [18]), acting in obviously different signaling pathways. The observation has been made that thiol-containing reducing agents like NAC or PDTC are able to suppress NFκB activation triggered by phorbol esters, TNFα, lipopolysaccharide or (LPS) [22,25], suggesting an involvement of radicals in the activation mechanism. To test whether this suppression could also be observed after UVB irradiation, cells were preincubated in serum-free DMEM with 20 μM PDTC for 1 h and washed twice with PBS prior to UVB treatment. After irradiation, PBS was replaced by DMEM containing 20 μM PDTC. Nuclear extracts were prepared 1 h after irradiation while the cells were incubated with DMEM containing 20 μM PDTC. Controls were treated identically, but kept without PDTC. Figure 2 demonstrates the effect of PDTC on UVB-induced NFκB activation. 150 J/m^2 and 300 J/m^2 of UVB induce a marked NFκB

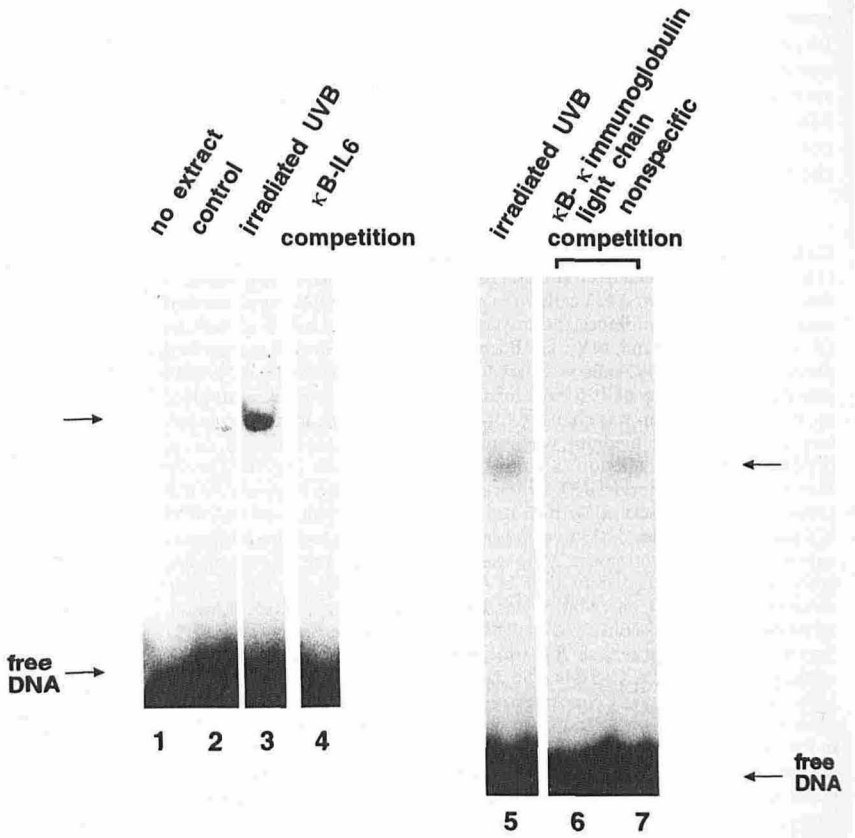

Figure 1. NFκB-IκB dissociation in cytosolic protein extracts of A431 keratinocytes. EMSA was performed with κB-IL6 double-stranded ^{32}P-labeled oligonucleotide without protein extract *(lane 1)*, with cytosolic extract from untreated *(lane 2)* or UVB-treated (200 J/m²) cells *(lane 3)*. Extracts were prepared 20 min after irradiation of the cells. Specificity of the DNA binding: competition with fiftyfold molar excess of κB-IL6 *(lane 4)*. Cytosolic extracts from irradiated A431 cells were used for EMSA with κB-IL6 *(lane 5)*. Competition was performed with a fiftyfold molar excess of unlabeled κB-κIG *(lane 6)* and a fiftyfold excess of unspecific double-stranded oligonucleotide.

binding activity *(lanes 2 and 3)*, whereas in the presence of PDTC the binding activity is suppressed *(lanes 5 and 6)*. Because PDTC turned out to be toxic for A431 as well as for other keratinocyte cell lines at concentrations used in other studies [22], in particular after UVB treatment, PDTC was used at a concentration of 20 μM. This ensured that the viability of irradiated cells determined by trypan blue exclusion after 16 h was more than 90%. Similar suppression of UVB-mediated NFκB activation as found with PDTC was observed by the addition of 20 mM NAC. However, NAC seems to have lower efficiency (data not shown).

UVB Exposure of Cytosolic Extracts Leads to Increased NFκB-like Binding Activity It has been reported previously that DNA is one of the targets of UVC light in bacteria (SOS-response) [26] and fungi [27], as well as in higher eukaryotes [28]. Repair mechanisms are thought to be turned on as a response to DNA damage or byproducts of the damaging process. A similar inducing mechanism has been proposed for the mammalian UV response also with respect to NFκB [29]. To investigate whether the UVB response with respect to activation of NFκB is mediated independently from chromosomal DNA damage we performed UVB exposure experiments in a cell-free system, subsequently referred to as *in vitro* irradiation. Crude cytosolic protein extracts from unirradiated cells were exposed to UVB light and analyzed for NFκB binding activity. Figure 3 shows a representative UVB exposure experiment (200 J/m²) performed with an identical protein extract at 0°C *(lane 2)* and at room temperature *(lane 4)*. Controls were kept at 0°C *(lane 1)* or at room temperature *(lane 3)*, respectively. The binding reaction was performed at room temperature immediately after irradiation. Both conditions enable UVB-mediated induction of NFκB binding activity. The temperature during UVB exposure appeared to be of minor relevance. Samples treated at low temperatures exhibited lower binding activity in the control as well as in the induced extract. These findings were confirmed by determining the radioactivity contained in the bands by scintillation counting, showing an essentially similar induction factor under both conditions. The enhanced levels of binding activity observed in the unirradiated controls are likely to be due to the prolonged handling time during the *in vitro* irradiation. Seemingly, *in vitro* UVB irradiation of crude cytosolic extracts obtained from untreated cells also induces the release of binding active NFκB from the NFκB-IκB complex, suggesting a UVB target localized in the cytoplasm, in the membrane, or associated with the membrane. To estimate the DNA content in th cytosolic extracts two methods were used. The cytosolic extracts were extracted by phenol/chloroform to purify nucleic acids and analyzed first by agarose gel electrophoresis for chromosomal DNA. After RNAse digestion no ethidium bromide stain was observed. DNA contaminations derived from mitochondria were considered unlikely because the content of mitochondrial DNA is some four to five orders of magnitude smaller than that of the diploid human genome. In addition, the organelles are likely to be removed from the soluble cytosolic fraction during the 8000 × *g* centrifugation step described in *Materials and Methods*. As a second approach, nucleic acids extracted from cytosolic extracts were used as templates for the PCR amplification of a fragment of the glycerinaldehyde-3-phosphate dehydrogenase. No PCR product was observed, indicating that only low amounts of intact chromosomal DNA were contained in the cytosolic extracts. Compared to the control chromosomal DNA sample, where a PCR product could be amplified with 5 ng, but not with 500 pg of chromosomal DNA as PCR template, the total amount of chromosomal DNA contained in cytosolic extract from 10^7 cells was less than 5 ng (data not shown).

Induction of NFκB-like Binding Activity is Enhanced by the Presence of the Membranes Recently, one target of the mammalian UVC response was shown to be mediated by membrane-associated protein kinases [30]. Therefore, we next investigated whether the activation of NFκB-like binding activity is dependent on the presence of membranes or membrane-associated proteins. The crude cytosolic protein extracts used in the previous experiments contained membrane components. To separate the mem-

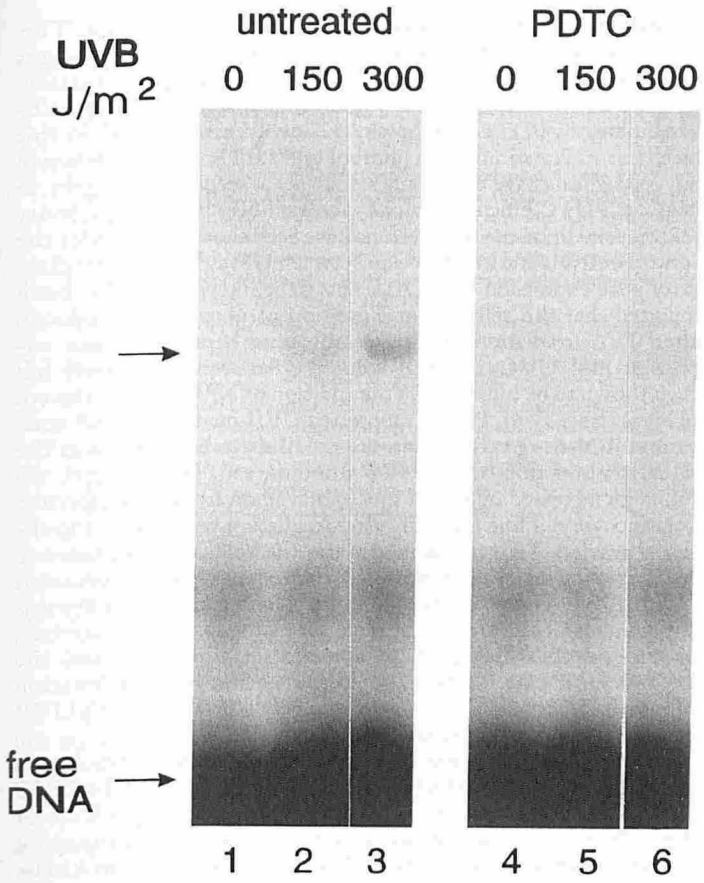

Figure 2. UVB dose dependency of NFκB activation and effect of the antioxidant PDTC. EMSA was performed with radiolabeled oligonucleotide κB-IL6 and nuclear protein extracts obtained from A431 cells harvested 1 h after UVB treatment. Cells were irradiated as described with 150 J/m² (*lanes 2* and *5*), 300 J/m² (*lanes 3* and *6*), or left untreated (*lanes 1* and 4). Protein extracts used in *lanes 4, 5,* and *6* were obtained from cells incubated with PDTC, whereas the others (*lanes 1, 2,* and *3*) were left untreated. For PDTC treatment cells were preincubated with 20 μM PDTC in DMEM for 1 h, washed twice with PBS, and irradiated. After UVB exposure, PBS was replaced by DMEM containing 20 μM PDTC.

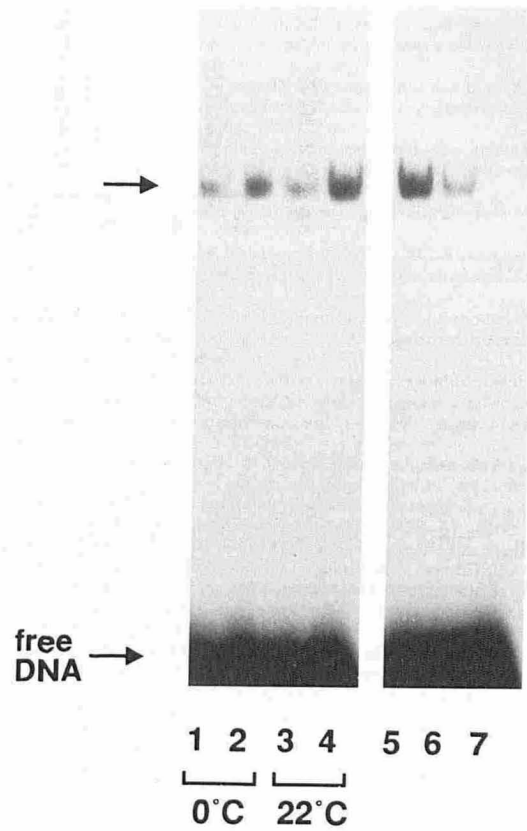

Figure 3. Effect of UVB on NFκB binding activity *in vitro.* An EMSA was performed with radiolabeled oligonucleotide κB-IL6. Cytosolic extracts obtained from unirradiated A431 cells were exposed to UVB (200 J/m², *lanes 2* and *4*) or left untreated (*lanes 1* and *3*). Samples were kept on ice (*lanes 1* and *2*) or at room temperature (*lanes 3* and 4) throughout the UVB treatment. Extracts were used immediately after irradiation for the DNA binding reaction. Competition of the DNA binding activity induced by *in vitro* UVB treatment *(lane 5)* was performed with κB-IL6 (tenfold molar excess, *lane 6;* one-hundredfold, *lane* 7). Radioactivity contained in the bands were counted. *Lane 1,* 2020 cpm; *lane 2,* 6212 cpm; *lane 3,* 2758 cpm; and *lane 4,* 8551 cpm.

brane from the soluble fraction by centrifugation a detergent-free disruption method was chosen. The protein extract for all lanes presented in Fig 4 was obtained from untreated cells and irradiated with UVB *in vitro.* The crude, irradiated cytosolic extract was able to mediate UVB-dependent NFκB activation (*lane 2,* 300 J/m²), whereas the soluble fraction obtained from the same extract by centrifugation (*lane 3,* 300 J/m² and *lane 4,* 150 J/m²) exhibits less NFκB-like binding activity. The addition of the membrane fraction to the soluble fraction enables UVB induction of binding activity (*lane 5,* 300 J/m² and *lane 6,* 150 J/m²), suggesting an involvement of the components of the membrane, e.g., lipide peroxide, to be necessary for the signaling pathway.

DISCUSSION

Normal human keratinocytes, as well as keratinocyte cell lines such as A431 or HaCaT, have been shown to release a variety of immunologic and proinflammatory cytokines like IL-1, IL-6, and TNFα in response to UVB [3]. It was the aim of our studies to get more insight into the molecular basis of the UVB response of IL-6 expression using biologically relevant energy doses. One transacting factor involved in the regulation of IL-6 expression is NFκB [8]. This transcriptional regulator was previously shown to be the target of other lymphokines [9] and is thought to be involved in the immediate early response to DNA damage (reviewed in [31]). As previously described for lethal UVC [17], our results (Figs 1 and 2, *lanes 1–3*) demonstrate that NFκB-like binding is induced after low-dose UVB treatment in a dose-dependent manner.

In the last few years, several molecular mechanisms for NFκB activation have been presented, suggesting that a variety of different signaling pathways affect NFκB activity. The cytosolic inhibitory subunit IκB was demonstrated to be a target for modification by protein kinases. High protein kinase C (PKC) activities *in vitro* were shown to mediate the dissociation process by IκB phosphorylation [32]. Cyclic adenosine monophosphate has been found to be involved in stimulating gene transcription by IL-1 *via* an NFκB recognition sequence residing in the κ immunoglobulin light-chain gene promoter [13,33]. In addition, DNA damage events were shown to upregulate AP-1 and NFκB binding activity by demonstrating a correlation between the DNA lesion density and the gene transcription rate of immediate early genes [17]. Seemingly, there are additional alternative targets for UVB light as well as for ionization by γ irradiation. PKC, for example, was demonstrated to be activated by γ irradiation in a very fast reaction, suggesting a more direct, DNA damage-intermediate independent mechanism [34]. Here, we show by *in vitro* activation in cell-free cytosolic extracts that UVB mediates the activation of NFκB also if only minimal amounts of chromosomal DNA serving as chromophore are present. Because the concept of using DNA damage products as second messengers for immediate early gene regulation is found also

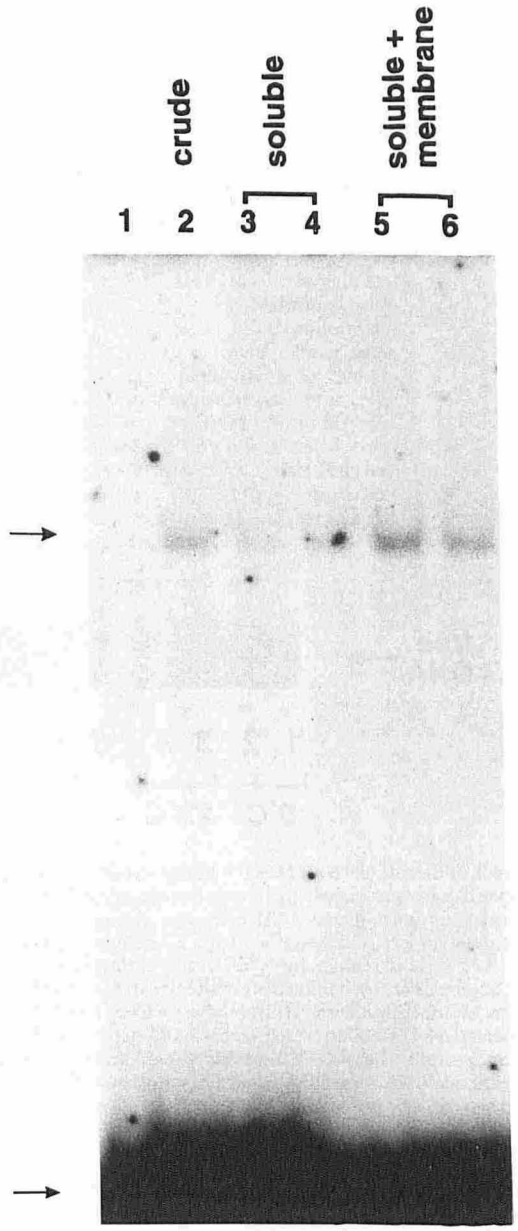

Figure 4. Influence of the membrane fraction for induction of NFκB binding activity *in vitro*. EMSA was performed with radiolabeled oligonucleotide κB-IL6 (without protein extract, *lane 1*). Crude protein extract obtained from untreated A431 cells was divided before irradiation. One part was stored, the other was centrifuged to separate the soluble from the membrane fraction as described in *Materials and Methods*. All extracts were UVB irradiated and kept at room temperature throughout the experiment. EMSA was performed with irradiated extracts. *Lane 1*, no extract; *lane 2*, crude extract, 300 J/m²; *lane 3*, soluble fraction, 300 J/m²; and *lane 4*, soluble fraction, 150 J/m²; *lane 5*, soluble fraction with membranes added before irradiation, 300 J/m²; *lane 6*, soluble fraction with membranes, 150 J/m². All samples were added to the binding reaction mix and incubated at room temperature for 45 min.

in bacteria, from the standpoint of evolution this mechanism is a successful and therefore well conserved one. But it cannot satisfy the necessity for a step preceeding the DNA damage, which could upregulate protection programs and thereby stabilize the genome information. These arguments and data suggest that DNA damage events are clearly a step in a certain signaling pathway, but are not necessary to establish NFκB-like binding activity after UVB treatment.

As an alternative mechanism it has been reported that intracellular thiols and ROIs are involved in NFκB regulation [21,15]. This concept suggests ROIs to be natural fast-acting second messengers probably for a variety of regulation processes. In the present study, we show the decrease in the UVB-dependent activation of NFκB-like binding by PDTC and thereby the involvement of ROIs in this mechanism. For stimuli like phorbol esters, LPS, or TNF, reactive oxygen radicals have been suggested to act as second messengers, as NAC or PDTC efficiently block increased NFκB binding activity [22]. Several light-mediated effects have been shown to be under the control of ROIs and a connection between UV light and immediate early gene expression *via* ROIs seems to be likely [35]. It has been reported that the cellular concentration of glutathion is reduced after UVA irradiation, leading to enhanced heme oxygenase expression [36]. UVC (254 nm)-induced c-*Jun* promoter activity has been shown to be inhibited by the addition of NAC [30]. As shown in Fig 2 *(lanes 4–6)*, PDTC suppressed UVB-mediated NFκB activation. ROI-triggered mechanisms are likely to be involved in the signal transduction of the UVB stimulus, too. Furthermore, we found an increased release of superoxide anion from a transformed keratinocyte cell line (HaCaT) after irradiation with UVB (unpublished results). Figure 4 demonstrates the role of the membrane fraction of cytosolic extracts for induction of UVB-mediated NFκB-like binding activity and suggests lipid peroxides to serve as components of the signal transduction pathway. Taken together, because NFκB is the target of different signaling pathways, the UVB-induced NFκB activity could be mediated by a combination of signals. The one under investigation here is dependent on a UVB target localized in the membrane.

Recently, the c-*Src* tyrosine kinase family was identified as a component of the signaling pathway for transduction of the UVC stimulus leading to the phosphorylation of the transcription factor AP-1 [30,37]. c-Src was shown to be the first detectable participant in the signaling pathway. In addition, the *Raf*-1 protein kinase residing downstream of c-*Src* in the signaling pathway is proposed to be a central checkpoint for the signal transduction for phorbol esters as well as UV-induced DNA damage events to the AP-1 induction [37,38]. The same components of the signaling pathway were shown to be involved in UVC-mediated activation of cytosolic NFκB [39]. Because the *Src* protein kinases, seemingly the first targets of the UVC signal, are thought to be localized at the inner side of the plasma membrane [40], it was proposed that the UVC signal is received at the plasma membrane rather than at the nucleus and that ROIs are involved in the mechanism. This model is further supported by our finding that in nucleus-free cytosolic extracts, NFκB activity is turned on upon exposure to UVB light, even if the stimulus does not severely affect the physiologic cell viability. We also observed the downregulation of NFκB binding by radical scavengers and that *in vitro* activation of NFκB by UVB is decreased by the lack of membrane components.

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BRITISH SOCIETY FOR INVESTIGATIVE DERMATOLOGY

The Annual Meeting of the British Society for Investigative Dermatology will be held on Thursday and Friday, 15–16 September 1994 at the University of Bath.

Abstracts are invited for oral and poster presentations at this meeting, and awards will be made for outstanding presentations.

The meeting format includes an overseas guest speaker, Dr. Madeleine Duvic, talking about "Recent Advances in Psoriasis," and a local expert on "Connective Tissue Disease," and abstracts are particularly invited on these subjects.

Young Investigators may apply for bursaries to assist attendance at the meeting provided that they are co-presenters at the meeting, and there are two BSID Young Investigator Awards (one for the UK and one for Eastern Europe and the Developing World) from competitive applications.

Details of how to apply for Bursaries and Fellowships, and Abstract Forms are obtainable from Valerie Brame, BSID Conference Office, PO Box 32, Woodford Green, Essex IG8 OUR. Tel, 071 377 7724; Fax, 071 247 6509.

Deadline for receipt of Applications and Abstracts is Friday, 22 April 1994.