Ultraviolet Radiation-induced Interleukin 6 Release in HeLa Cells Is Mediated via Membrane Events in a DNA Damage-independent Way*

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Evidence exists that ultraviolet radiation (UV) affects molecular targets in the nucleus or at the cell membrane. UV-induced apoptosis was found to be mediated via DNA damage and activation of death receptors, suggesting that nuclear and membrane effects are not mutually exclusive. To determine whether participation of nuclear and membrane components is also essential for other UV responses, we studied the induction of interleukin-6 (IL-6) by UV. Exposing HeLa cells to UV at 4 °C, which inhibits activation of surface receptors, almost completely prevented IL-6 release. Enhanced repair of UV-mediated DNA damage by addition of the DNA repair enzyme photolyase did not affect UV-induced IL-6 production, suggesting that in this case membrane events predominate over nuclear effects. UV-induced IL-6 release is mediated via NFκB, since the NFκB inhibitor MG132 or transfection of cells with a super-repressor form of the NFκB inhibitor IκB reduced IL-6 release. Transfection with a dominant negative mutant of the signaling protein TRAF-2 reduced IL-6 release upon exposure to UV, indicating that UV-induced IL-6 release is mediated by activation of the tumor necrosis factor receptor-1. These data demonstrate that UV can exert biological effects mainly by affecting cell surface receptors and that this is independent of its ability to induce nuclear DNA damage.

Ultraviolet radiation (UV) and, in particular, UVB with a wavelength range between 290 and 320 nm represents one, if not the most, important environmental factor of inducible health hazards for mankind, which include the induction of skin cancer (1), suppression of the immune system (2), and chronic skin damage including premature skin aging (3). Similar to chemical agents, UV has the ability to alter mammalian gene expression (4–6). Elucidation of the mechanisms by which UV affects gene expression is crucial for understanding how UV exerts its biological effects and how it develops its pathogenetic properties. In this context, one of the most frequently but also controversially discussed issues is whether the cellular UV response is initiated at the cell membrane or in the nucleus (reviewed in Refs. 7 and 8).

The biological effects of UV are multiple and include the release of soluble mediators, the induction of apoptosis, and alterations of surface molecule expression, just to name a few. To exert these biological effects, UV must first be absorbed by a chromophore within the cell, which then transduces energy into a biochemical signal. A number of chromophores have been identified, e.g. porphyrins, aromatic amino acids, urocanic acid, and DNA. Among these, DNA has been regarded as the most important one, since the wavelength dependences of various UV effects match that of DNA absorption (9). In addition, removal of UV-induced DNA damage, e.g. by enhancing DNA repair, reduces or even inhibits some of the biological UV effects (10–13). Finally, lower UV doses induce some of the biological effects at the same magnitude in DNA repair-deficient cells as in cells with normal DNA repair (14). Considering these observations, it is understandable that for quite a long time DNA was regarded as the only molecular target for UV, and why the dogma existed that any biological effect must be a direct consequence of DNA damage.

On the other hand, a variety of groups have provided convincing evidence that UV may exert biological effects without the need of a nuclear signal. Utilizing enucleated cells, Devary et al. (15) demonstrated that activation of the transcription factor nuclear factor κB (NFκB)1 does not require a nuclear signal. This was confirmed by the observation that UV exposure of cytosolic extracts containing NFκB in its inactive form supplemented with cellular membranes causes activation of NFκB (16). In addition, growth factor receptors appear to be involved in the UV response since UV activates the epidermal growth factor receptor by directly initiating tyrosine phosphorylation (17). Rosette and Karin (18) reported for the first time that UV and osmotic shock, respectively, can activate cell surface receptors by inducing their oligomerization. Triggering of receptors in such a way by UV takes place without the binding of any ligand and independently of DNA damage (18, 19).

One of the most important biological effects of UV is the induction of apoptotic cell death (20). Convincing data exist that nuclear events especially UV-induced DNA damage determine whether a cell undergoes apoptosis or not (21). On the other hand, it was clearly demonstrated that activation of death receptors such as CD95 (Fas/APO-1) on the cell surface by UV induces the apoptotic machinery without being connected with DNA damage (22, 23). In addition, Sheikh et al. (19) proposed that UV-stimulated ligand independent activation of the tumor necrosis factor receptor plays a major role in mediating the apoptotic effects of UV. Considering these data,

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1 The abbreviations used are: NFκB, nuclear factor κB; IL, interleukin; TNF, tumor necrosis factor; TNF-R, TNF receptor; TRAF-2, TNF-R-associated factor-2; ELISA, enzyme-linked immunosorbent assay; FCS, fetal calf serum; PBS, phosphate-buffered saline; PCR, polymerase chain reaction.
which on first glance appear conflicting, we recently tried to
determine the relative contribution of nuclear and membrane
effects in UV-induced apoptosis (24). Removal of UV-induced
DNA damage by enhancing DNA repair using the repair en-
zyme photolyase significantly reduced the apoptosis rate
in HeLa cells. On the other hand, exposure of HeLa cells to UV at
4 °C, which prevents death receptor clustering (18, 22), also
reduced the apoptosis rate, although to a lesser extent. It is
important to mention that neither of these strategies alone was
able to completely prevent UV-mediated apoptosis. However,
when both strategies were combined, i.e. when cells were ex-
posed to UV at 4 °C and DNA damage removed by photolyase,
UV-induced apoptosis was completely inhibited (24). Hence,
these data indicated that, although nuclear effects are predom-
inant in comparison to membrane events, both are necessary to
obtain the complete apoptotic response.

Inspired by these observations, we were interested to deter-
mine whether the participation of both nuclear and membrane
components is specific for UV-induced apoptosis or is also
essential for other UV responses. Here, we studied the induction
of the release of the inflammatory cytokine interleukin 6 (IL-6)
in HeLa cells by UV. Using this system, we demonstrate that
removal of DNA damage by enhancing DNA repair does not
cause reduction of IL-6 release, implying that UV-induced DNA
damage is not an important intermediate in this type of UV
response. On the other hand, prevention of triggering cell sur-
face receptors by maintaining HeLa cells at 4 °C during UV
exposure resulted in complete inhibition of UV-mediated IL-6
secretion. Using dominant negative mutants, we provide evi-
dence that NFκB is involved in this signaling process and that
the tumor necrosis factor type 1 receptor (TNF-R1) seems to be
a major target at the cell membrane in this UV response.
Together, these data indicate that UV can also exert its biolog-
ic effects by exclusively acting on the cell membrane without
the necessity of a nuclear signal. In addition, our findings
suggest that the multiple biological effects of UV on mamma-
lian cells do not only differ in their final outcome but are also
dependent on how they are generated.

MATERIALS AND METHODS

Cells and Reagents—The human epithelial carcinoma cell line HeLa
(American Tissue Culture Collection) was cultured in RPMI 1640 with
10% FCS. Human recombinant tumor necrosis factor α (TNFα) was
obtained from Endogen (Woburn, CA). IL-6 release from cells was
measured by subjecting supernatants (10 μl each) to an IL-6 ELISA kit
(Diakine, Besancon, France). Measurements were performed according
to the manufacturer’s guidelines. The proteasome inhibitor MG132 was
purchased from Calbiochem (San Diego, CA). The plasmid allowing
overexpression of a mutated IκB variant was kindly provided by K.
Schulze-Osthoff (University of Münster, Münster, Germany) (25), the
plasmid overexpressing a dominant negative mutant of TRAF-2 was
kindly provided by David Goeddel (Tularik Inc., San Francisco, CA)
(26).

Treatment of Cells—UV irradiation was performed as described pre-
viously with slight modifications (24). Briefly, subconfluent cells were
washed with PBS and exposed to UV light through colorless medium
with FCS. For UV irradiation, we used a bank of six TL12 fluores-
cent bulbs (Philips, Eindhoven, The Netherlands), which emit most of
their energy within the UVB range (290–320 nm) with an emission
peak at 313 nm. Throughout this study, a dose of 400 J/m² was used.
Control cells were subjected to the identical procedure without being
exposed to UV. UV irradiation at low temperature was carried out by
keeping cells at 4 °C for 10 min before UV exposure and during expo-
sure, which lasted 40 s. Cells were kept at 4 °C for another 20 min
before incubation at 37 °C for 16 or 24 h.

Osmotic shock was induced by incubating cells with 1 M sorbitol
(Sigma, Munich) in FCS-free medium for 30 min either at 37 °C or at
4 °C (18). Thereafter cells were washed with PBS, supplemented with
normal RPMI medium, and incubated for 16 h or 24 h at 37 °C.

Stimulation of cells with TNFα at low temperature was carried out by
adding TNFα (100 ng/ml) to cells that had been kept at 4 °C for 10
min. Cells were kept at 4 °C for another 20 min and then cultured at
37 °C for 16 or 24 h.

Induction of DNA Repair via Photoreactivation—Photolyase was en-
capsulated into liposomes (Photosomes®, AGI Dermatics, Freeport, NY)
at a concentration of 1.2 ng/ml (27). Liposomes consisted of the lysids
phosphatidylcholine, egg phosphatidyl ethanolamine, oleic acid,
and the membrane stabilizer cholesterol hemisuccinate. Empty liposomes
were used as negative controls, referred to as liposomes. For
photoreactivation, HeLa cells were irradiated as described above and
either Photosomes® or liposomes (40 μl/ml each) were added. Cells were
incubated at 37 °C for 1 h in the dark, followed by illumination with
photoreactivating light. A low light source for photoreactivating light,
UVA fluorescent bulbs (TL08, Philips) filtered through a 6-mm glass
plate with peak emission at 365 nm were used. Cells were exposed for
20 min, which corresponds to a photoreactivating light fluence of 12
kJ/m². After photoreactivation, cells were supplemented with normal
RPMI medium containing 10% FCS and incubated for 24 h at 37 °C.

Detection of Cell Death—16 h after stimulation cells were detached
from dishes, and apoptosis analyzed by a cell death detection ELISA
(Cell Death Detection ELISAPLISS, Roche Molecular Biochemicals).
The enrichment of mono- and oligonucleosomes released into the cytoplasm
of cell lysates is detected by biotinylated anti-histone- and peroxidase-
coupled anti-DNA antibodies and is calculated as follows: absorbance
of sample cells/absorbance of control cells. Unless otherwise stated, this
factor was used as a parameter of apoptosis and is given as the mean ±
S.D. of three independently performed experiments.

Semiquantitative Reverse Transcription Polymerase Chain Reaction
(RT-PCR)—At 4 h after stimulation, total RNA was extracted from cells
according to the protocol described by Chomczynski and Sacchi (28).
Total RNA (1 μg) was reverse transcribed with SuperScript RNase H
reverse transcriptase (Life Technologies, Inc.). The amount of template
needed was titrated by β-actin PCR in a 20-μl reaction utilizing the
RedTaq polymerase system from Sigma and evaluated densiometri-
cally. A hIL-6-amplimer set from CLONTECH (Palo Alto, CA) was used
as primers for hIL-6 PCR.

Western Blot Analysis—Western blot analysis was performed as re-
cently described (29). Briefly, cells were lysed in lysis buffer (50 mM
Hepes pH 7.5, 150 mM NaCl, 10% glycerol, 1% Triton X-100, 1.5 mM
MgCl₂, 1 mM EDTA, 10 mM Na₂GTA, 10 mM HEPES, 10 mM NaF,
10 mM Na₃VO₄, complete™ protease inhibitor mixture) for 15 min on ice.
After centrifugation, supernatants were collected, and the protein content
measured by Bio-Rad protein assay kit. The protein samples were
subjected to 12% SDS-polyacrylamide gel electrophoresis, blotted onto
nitrocellulose membranes, and incubated with antibodies directed
against hIL-6 (Upstate Biotechnology, Inc., Lake Placid, NY). Equal
loading was determined by reprobing membranes with an antibody directed
against α-tubulin (Calbiochem, San Diego, CA). Signals were detected
with an ECL™ kit (Amersham Pharmacia Biotech, Buckinghamshir,
United Kingdom).

Transfection—HeLa cells (6 × 10⁶) were washed once with PBS and
resuspended in 600 μl of FCS-free RPMI medium, 2% Me₂SO. Cells
were electroporated with 20 μg of each plasmid DNA (pCMV-IκB-ΔN
or pRK-F-TRAF-2-DN) according to the method described by Melkonian et
al. (30). Transfection efficacy of cells cotransfected with a plasmid
encoding β-galactosidase (pcDNA6-VS-His-lacZ; Invitrogen, San Diego,
CA) was determined 36 h later by staining with 5-bromo-4-chloro-3-
indolyl-β-D-galactopyranoside (100 μg/ml) in 5 mM potassium ferricya-
ride, 5 mM potassium ferrocyanide, and 1 mM MgCl₂ in PBS. Transfec-
tion efficiencies ranged from 30% to 50%.

RESULTS

Keeping HeLa Cells at Low Temperature during Exposure to UV
Irradiation or Osmotic Shock Reduces Apoptosis to Different
Degrees—Both nuclear and membrane events have been shown previously to contribute independently to UV-induced
apoptosis (24). Since both components are essential to obtain
the complete apoptotic response, inhibition of aggregation of
death receptors expressed on the cell surface, such as CD95,
by incubating cells at 4 °C during UV exposure only partially inhibits
UV-induced apoptosis (Fig. 1). Osmotic shock was recently
demonstrated to induce receptor aggregation similar to UV
(18). However, in contrast to UV, osmotic shock does not induce
DNA damage and can thus be used as a stimulus that acts on
the cell membrane exclusively. Rosette and Karin (18) pre-
dicted that any receptor whose activation mechanism involves
Multimerization should be activable by UV or osmotic shock. Hence, osmotic shock should also activate the CD95 receptor and thus induce apoptosis. As predicted, exposure of HeLa cells to osmotic shock caused apoptotic cell death (Fig. 1). When HeLa cells were kept at 4 °C during osmotic shock, which prevents receptor clustering (18), apoptosis was completely inhibited. The complete prevention of apoptosis by inhibiting receptor aggregation thus confirms that osmotic shock, in contrast to UV, acts exclusively at the cell membrane when inducing apoptosis.

TNFα is known to induce apoptosis via activation of the TNF receptor 1 (TNF-R1) (31). To determine the effect of low temperature on TNFα-induced apoptosis, HeLa cells were exposed to TNFα, maintained at 4 °C during the first 30 min of exposure, and then kept at 37 °C for 16 h later apoptosis was measured. Under these conditions, low temperature had no effect on TNFα-induced apoptosis, since the death rate was the same irrespective of whether the cells were kept at 4 °C for the first 30 min or at 37 °C throughout the entire incubation period (Fig. 1).

**Low Temperature Inhibits UV-induced IL-6 Release**—To further determine whether the involvement of both nuclear and membrane events is unique for UV-mediated apoptosis or also relevant for other biological effects caused by UV, we studied UV-mediated release of IL-6 by HeLa cells. HeLa cells do not constitutively secrete IL-6. However, UV irradiation resulted in a significant secretion of IL-6 (Fig. 2). Exposure of HeLa cells to TNFα, a well known inducer of IL-6, also caused enhanced IL-6 levels in the supernatants. Exposing HeLa cells to osmotic shock also induced IL-6 production, which may best be explained by activation of TNF-R1 (18). Accordingly, osmotic shock-induced IL-6 release was drastically reduced, when cells were kept at 4 °C during exposure to osmotic shock (Fig. 2). Likewise, when cells were stimulated with TNFα at 4 °C for 30 min, TNFα removed after that period by medium change and cells cultured for another 24 h at 37 °C, no induction of IL-6 was observed (data not shown). In contrast, keeping TNFα-stimulated cells at 4 °C for 30 min did not have any inhibitory impact on TNFα-induced IL-6 release provided that TNFα was not washed off but left in the medium for the rest of the incubation period of 24 h at 37 °C (Fig. 2). This confirms that keeping cells at low temperature for such a limited period by itself does not cause reduced IL-6 production by inhibition of molecular processes within the cell, e.g. transcription, but just interferes with membrane receptor activation (18, 22). Surprisingly, when HeLa cells were kept at 4 °C during UV exposure, UV-induced IL-6 release was strongly reduced close to base-line levels. Since inhibition of UV-induced IL-6 release was almost as pronounced as the inhibition of IL-6 release induced by osmotic shock, a purely membrane-located event, this implies that UV-induced IL-6 release appears to be primarily mediated via membrane and not nuclear events.

**Enhancement of DNA Repair Does Not Affect UV-mediated IL-6 Release**—To confirm the above presented observations, we tested the effect of accelerated removal of UV-mediated DNA damage by enhancing DNA repair on UV-mediated IL-6 release. This approach was used previously to demonstrate the importance of DNA damage in mediating UV effects (10–13, 24). To induce repair of UV-mediated DNA damage, we utilized the photoreactivating enzyme photolyase. Photolyase binds to UV-induced cyclobutane pyrimidine dimers in DNA and catalyzes its splitting by electron transfer from absorbing wave-
In addition, cells were stimulated with TNF-α or alternatively exposed to osmotic shock at either 37 °C or 4 °C. HeLa cells were UV-irradiated at 37 °C or at 4 °C, as described before, or incubated with empty liposomes followed by exposure to photosensitizing light. Experiments were performed either at 37 °C (black bars) or at 4 °C (white bars). 24 h later amounts of IL-6 were measured using an IL-6 ELISA. IL-6 concentrations (pg/ml) are shown on the y axis (mean ± S.D. of triplicate samples). Data presented show one representative experiment of three independently performed experiments.

Mechanism of UV-induced IL-6 Release

Keeping Cells at Low Temperature during UV Exposure Inhibits IL-6 mRNA Expression—Next, we addressed whether blocking UV-mediated receptor oligomerization by keeping cells at low temperature inhibits induction of IL-6 mRNA transcription. Therefore, semiquantitative RT-PCR utilizing primers amplifying parts of the IL-6 gene was performed. HeLa cells were UV-irradiated at 37 °C or at 4 °C, as described before, or alternatively exposed to osmotic shock at either 37 °C or 4 °C. In addition, cells were incubated with empty liposomes at two temperatures above 320 nm (photoreactivating light) (32). To enable uptake of the enzyme into the cells, photolyase was encapsulated into liposomes (Photosomes®) (27).

HeLa cells were irradiated with 400 J/m² UV. Immediately thereafter, Photosomes® or empty liposomes were added and cells kept in the dark for 1 h, followed by exposure to photoreactivating light. As demonstrated previously (24), the combination of Photosomes® and photoreactivating light significantly reduces UV-induced DNA damage in HeLa cells. However, enhancement of DNA repair by Photosomes® had no effect on UV-induced IL-6 release, implying that in this case DNA damage is not an important mediator (Fig. 3). Likewise, addition of empty liposomes did not affect UV-stimulated IL-6 secretion. As already demonstrated in Fig. 2, the most effective way to inhibit UV-induced IL-6 release was keeping cells at 4 °C during UV exposure. The combination of inhibiting aggregation of cell surface receptors by keeping cells at low temperature and enhancement of DNA repair by adding Photosomes® did not result in further inhibition of UV-mediated IL-6 release (Fig. 3), although UV-induced pyrimidine dimers were reduced by 50% to 70%, as demonstrated by Southwestern dot blot analysis using an antibody directed against pyrimidine dimers (24). Taken together, these data indicate that DNA damage might not be of importance for mediating IL-6 release following UV exposure, further suggesting that membrane events may predominate over nuclear events concerning UV-induced IL-6 release in HeLa cells.

Keeping Cells at Low Temperature during UV Exposure Inhibits IL-6 mRNA Expression—Next, we addressed whether blocking UV-mediated receptor oligomerization by keeping cells at low temperature inhibits induction of IL-6 mRNA transcription. Therefore, semiquantitative RT-PCR utilizing primers amplifying parts of the IL-6 gene was performed. HeLa cells were UV-irradiated at 37 °C or at 4 °C, as described before, or alternatively exposed to osmotic shock at either 37 °C or 4 °C. In addition, cells were incubated with empty liposomes at 37 °C or 4 °C. After incubating cells at 37 °C for another 4 h, RNA was extracted and RT-PCR performed using primers for IL-6 and β-actin, respectively. Co, control.

Data presented show one representative experiment of three independently performed experiments. These data exclude the unlikely possibility that low temperature inhibits UV-induced IL-6 release by interfering with protein translation or secretion.

UV-induced IL-6 Release Is Mediated via Activation of NFκB—TNFα stimulates HeLa cells to produce enhanced amounts of IL-6 via activation of TNF-R1. TNF-R1 belongs to the group of receptors that are only biologically active when trimerized. Since UV directly induces receptor oligomerization independently of the respective ligands (18, 19, 22, 23), UV-induced IL-6 release may be due to direct activation of TNF-R1 by UV light. One consequence of triggering TNF-R1 is activation of the transcription factor NFκB. In addition, the IL-6 promoter contains several NFκB binding sites (34). Hence, we postulated that, if activation of TNF-R1 is the initial signaling step in UV- or osmosis-induced IL-6 release, NFκB should be involved in the signaling cascade. Activation of NFκB is associated with degradation of the inhibitory protein IκB by the proteasome pathway. Upon activation, IκB is phosphorylated at two serine residues (Ser-32 and Ser-36); this acts as a signal for ubiquitination, followed by its degradation (35, 36). Since
IκB is degraded by the 26 S proteasome, NFκB activation can be blocked by proteasome inhibitors (36). Thus, we tested whether the proteasome inhibitor MG132 inhibits IL-6 release induced by UV and osmotic shock, respectively. Therefore, HeLa cells were stimulated with UV, osmotic shock, or TNFα at 37 °C either with or without pretreatment with 5 μM MG132 for 1 h. 24 h later supernatants were harvested and analyzed for their IL-6 levels. As demonstrated in Fig. 5, MG132 inhibited IL-6 release, irrespective of whether cells were stimulated with UV, osmotic shock, or TNFα. This indicates that NFκB is involved in the signaling of all three stimuli and may also imply that they use the same signaling pathway.

On the other hand, the approach using proteasome inhibitors provides only indirect evidence that induction of IL-6 by UV, osmotic shock, or TNFα is due to activation of NFκB since one cannot exclude that inhibitors like MG132 may affect other pathways as well (37). Thus, we determined whether activation of NFκB is involved in IL-6 induction by overexpressing a super-repressor form of IκB. In this mutant form, two point mutations (Ser-32 → Ala, Ser-36 → Ala) prevent phosphorylation and subsequent proteasomal degradation of IκB (25). As a consequence, NFκB release, nuclear translocation, and functional DNA binding are prevented. Although HeLa cells transfected with the empty CMV vector were not impaired in their IL-6 release upon stimulation with UV or osmotic shock, cells transiently transfected with the IκB dominant negative mutant exhibited a significant reduction in the release of IL-6 upon these two stimuli (Fig. 6). Together, these data indicate that activation of NFκB is involved in signaling IL-6 release induced either by UV or by osmotic shock.

Keeping Cells at Low Temperature Inhibits Activation of NFκB Induced by UV, Osmotic Shock, or TNFα—It has previously been reported that UV activates NFκB independent of a nuclear signal (15, 16). Hence, we postulated that if activation of NFκB by UV or osmotic shock is due to direct activation of surface receptors, e.g. TNF-R1, activation of NFκB should be prevented when receptor aggregation is inhibited by keeping cells at low temperature. Therefore, HeLa cells were exposed to UV, osmotic shock, or TNFα either at 37 °C or at 4 °C. Protein extracts were prepared 30 min later and subjected to Western blot analysis using an antibody directed against IκB (Fig. 7). At 37 °C all three stimuli caused degradation of IκB, UV being the weakest stimulus. In contrast, when cells were exposed to osmotic shock or UV at 4 °C, degradation of IκB was significantly reduced, indicating that activation of NFκB under these conditions is inhibited. Taken together, these data confirm previous findings localizing activation of NFκB by UV close to the membrane (15, 16).

Inhibition of Signaling of TNF-R1 by a Dominant Negative Mutant of TRAF-2 Reduces UV-induced IL-6 release—The data
so far suggest that UV induces IL-6 release in HeLa cells by primarily acting on the cell membrane rather than affecting the nucleus. Reduction of IL-6 release upon UV exposure at 4 °C implies that UV radiation activates a cell surface receptor, which ultimately induces IL-6 release. TNF-R1 appears to be the best candidate for several reasons; (i) TNF induces IL-6 release in HeLa cells, (ii) triggering of TNF-R1 activates NFκB (26, 38, 39), and (iii) UV or osmotic shock cause clustering of TNF-R1 (18). To finally prove whether TNF-R1 is involved in UV-mediated induction of IL-6 release, a dominant negative mutant for the TNF-R1 signaling protein TNF-R-associated factor-2 (TRAF-2) was used (26). Upon activation, TNF-R1 trimerization is followed by the binding of the adapter protein TNF-R-associated death domain protein, subsequent recruitment of the signaling protein TRAF-2 and activation of the NFκB pathway (38). The TRAF-2 dominant negative mutant plasmid is a truncated version of TRAF-2 lacking its N-terminal RING finger domain (26). HeLa cells were transiently transfected with this mutant encoding plasmid and exposed to UV or osmotic shock. Transient transfection with TRAF-2 dominant negative mutant resulted in significant reduction of IL-6 release caused by UV or osmotic shock (Fig. 8). Taken together, these data indicate that induction of IL-6 release by UV is initiated at the cell membrane by direct activation of TNF-R1. As a consequence of TNF-R1 triggering, NFκB is activated, which ultimately results in induction of IL-6 gene transcription and its secretion.

**DISCUSSION**

The observations that removal of UV-mediated DNA damage by enhancing DNA repair results in the inhibition of certain biological UV effects strongly supported the idea that UV-induced DNA damage is crucially involved in mediating these effects (10–13). On the other hand, numerous studies provided clear evidence that UV can also affect targets at the cell membrane, suggesting UV responses being independent of nuclear events (reviewed in Refs. 7 and 8). Bender et al. (40) recently reported that UV activates NFκB sequentially in a DNA damage-independent and -dependent way. The discovery of a dual mechanism finally solved the apparent discrepancy between previous studies favoring either nuclear or non-nuclear pathways only. Along this line, using UV-induced apoptosis as the biological read-out-system, we currently observed that nuclear and membrane events are not mutually exclusive but that both components are essential to obtain the complete apoptotic response to UV (24). To determine whether the participation of both nuclear and membrane components is specific for UV-induced apoptosis or is also essential for other UV responses, we used UV-induced release of IL-6 in HeLa cells as an additional biological read-out-system.

Surprisingly, the data obtained provide evidence that induction of IL-6 release in HeLa cells by UV is predominantly due to membrane events, whereas UV-induced DNA damage, if at all, might be of minor importance. These conclusions are based on several experimental approaches. First, keeping HeLa cells at 4 °C during UV exposure drastically reduced IL-6 release. Keeping cells at low temperature prevents multimerization of cell surface receptors and thus inhibits their activation (18, 22). The mechanisms by which low temperature inhibits receptor aggregation is not clear, but changes in membrane fluidity have been suggested (18).

The finding that UV-induced apoptosis is completely prevented only by inhibition of death receptor aggregation and removal of DNA damage by enhanced DNA repair (24) clearly indicates that in UV-induced apoptosis both membrane and nuclear events are critically involved. In contrast, induction of apoptosis by osmotic shock, which acts exclusively at the cell membrane and does not induce DNA damage, was completely prevented when cells were kept at low temperature during exposure to 1 M sorbitol (Fig. 1). Osmotic shock also induced IL-6 release presumably via triggering cell surface receptors, since IL-6 induction was profoundly inhibited by keeping cells at 4 °C. Surprisingly, low temperature also drastically reduced UV-mediated IL-6 production. Inhibition of IL-6 release by low temperature is not due to induction of stress proteins induced by cold shock (22). In addition, keeping cells at low temperature does not interfere with transcription in general, since TNF-induced IL-6 expression was not affected when HeLa cells were kept at 4 °C for 30 min and then cultured at 37 °C in the presence of TNFα for another 24 h (Fig. 2; Ref. 24). It is also unlikely that low temperature interferes with UV-induced DNA damage, since generation of cyclobutane pyrimidine dimers is not prevented in the cold (41). Together, these findings suggest that receptor aggregation may be the predominant event in UV-mediated IL-6 release, whereas UV-induced DNA damage may be of minor importance in this type of response.

To further examine the role of DNA damage in this system, DNA damage was reduced by enhancing DNA repair. For this purpose, we used the repair enzyme photolyase, which has already been successfully utilized for such purposes (24). As demonstrated previously, delivery of photolyase to UV-exposed HeLa cells via the liposome route followed by exposure of cells to photoreactivating light successfully enhances DNA repair, resulting in significantly reduced amounts of thymine-thymine cyclobutane pyrimidine dimers (24). In contrast to UV-induced apoptosis, UV-mediated IL-6 release by HeLa cells was not affected at all by accelerated removal of pyrimidine dimers. These observations are in contrast to a recent publication by Petit-Frere et al. (9), in which they reported that photoreactivation led to a reduction of IL-6 release in KB cells and normal human keratinocytes. However, in that case inhibition of UV-induced IL-6 release was not complete, implying that other pathways may be involved as well. In addition, photoreactivating light itself in the absence of photolyase reduced UV-induced IL-6 secretion in KB cells, although no reduction in pyrimidine dimers was observed under these conditions, a finding that needs further investigation. Furthermore, the relevant experiments in the study by Petit-Frere et al. were done with UVC, whereas we have used the biologically and physiologically more
relevant UVB spectrum.

The failure of enhanced DNA repair to reduce IL-6 release on the one hand, and the marked inhibition of IL-6 production by keeping cells at 4 °C on the other hand, suggested that UV might mediate IL-6 release via activation of cell surface receptors rather than via induction of DNA damage. In this scenario, activation of TNF-R1 appears to be of critical importance. TNF-R1 was among the receptors initially found to be aggregated by UV (18). Examining the role of TNF-R1 as a mediator of the activation of Rel/NFκB proteins in keratinocytes, Tobin et al. (33) demonstrated ligand-independent activation of TNF-R1 by UV leading to recruitment of the transducer TRAF-2. Based on these findings and our own observation that TNFα induces the release of IL-6 in HeLa cells, we postulated that UV-induced IL-6 release may be due to direct activation of TNF-R1.

Upon activation, TNF-R1 becomes trimerized, which leads to the induced association of the intracellular TNF-R-associated death domain protein and subsequent recruitment of further proteins, including TRAF-2 and Fas-associated death domain protein and subsequent recruitment of further proteins. This pathway may be involved in the final processes they induce within the cell but also in the ways they are generated.

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