# Ultraviolet-B–Induced Apoptosis of Keratinocytes: Evidence for Partial Involvement of Tumor Necrosis Factor-α in the Formation of Sunburn Cells

Agatha Schwarz, Ranjit Bhardwaj, Yoshinori Aragane, Karsten Mahnke, Helge Riemann, Dieter Metze, Thomas A. Luger, and Thomas Schwarz

Ludwig Boltzmann Institute for Cell Biology and Immunobiology of the Skin, Department of Dermatology, University Münster, Münster, Germany

**Irradiation with ultraviolet (UV) B radiation results in the formation of apoptotic keratinocytes called sunburn cells. Recently, it was demonstrated that keratinocytes can release tumor necrosis factor-α (TNF-α), which is known to cause apoptosis in particular cells. In addition, it has been shown that UVB light induces the release of TNF-α by keratinocytes and that keratinocytes express the 55-kD receptor for TNF-α. Therefore, we investigated whether TNF-α is involved in UV-induced apoptosis of keratinocytes. Normal human keratinocytes and HaCaT cells were exposed to UVB light, and apoptosis was examined by nick translation evaluated by fluorescence-activated cell sorter analysis. UVB induced apoptosis in a dose-dependent manner, which was confirmed by electron microscopy. Addition of a polyclonal antibody directed against human TNF-α immediately after UVB exposure was able to reduce DNA fragmentation. However, it was not possible to rescue all cells from apoptosis. To prove whether TNF-α is also involved *in vivo* in UVB-induced apoptosis of keratinocytes, Balb/c mice were exposed to UVB on their abdomens, skin biopsies were performed 24 h later, and sunburn cells were counted. A single dose of 2000 J/m$^2$ caused a significant induction of sunburn cells. Subcutaneous injection of a polyclonal antibody directed against murine TNF-α immediately after UVB treatment resulted in a significant but incomplete reduction of sunburn cells, whereas injection of a rabbit IgG as a control had no effect. In both the *in vitro* and *in vivo* systems, application of recombinant TNF-α alone either to untreated keratinocytes or into normal murine skin did not induce sunburn cells. Thus, these data demonstrate that TNF-α is involved in UVB-induced apoptosis, but by itself is not able to induce sunburn cells. This further supports the notion that UVB-induced apoptosis of keratinocytes is a multifactorial event.** ***J Invest Dermatol 104:922–927, 1995***

Two distinct forms of cell death have been recognized: necrosis and apoptosis. Necrosis, which once was thought to be the universal mode of cell death, is the consequence of major insults to the cellular environment. In contrast, apoptosis occurs under physiologic conditions when death is programmed [1,2]. Apoptosis is an active suicide program, given that protein synthesis and activation of kinases seem to be necessary [3]. Moreover, apoptosis is a response to less pathologic or sublethal stimuli, which often by themselves would not cause cell death. Ultraviolet (UV) light is one of the most important environmental influences. Besides its well-known advantages and indispensable effects on human life, UV light and in particular UVB (290–320 nm) can be a hazard to human health by inducing cancer, immunosuppression, premature skin aging, inflammation, and cell death. A consequence of acute UVB exposure is the occurrence of sunburn cells within the epidermis [4,5]. Merely by applying morphologic criteria, investigators have long appreciated these cells as keratinocytes undergoing apoptosis. Recently, by using more advanced techniques such as DNA ladder demonstration and nick translation, workers confirmed that UVB light induces apoptosis in keratinocytes and in HL 60 cells [6,7]. However, the particular mechanisms that activate the apoptosis program in keratinocytes upon UV irradiation remain poorly defined [6].

Tumor necrosis factor-α (TNF-α) was first described as a cytotoxic factor that preferentially kills growing tumor cells [8]. Recently, it was also recognized as an important mediator of immunity and inflammation [9]. Keratinocytes also exhibit the capacity to release TNF-α, and secretion of this cytokine can be induced by UVB light [10]. Moreover, it was found that keratinocytes express the p55 receptor for TNF-α [11]. Because TNF-α has been shown to initiate apoptotic cell death and DNA fragmentation in several mammalian cell lines [12–18], we were interested in studying whether TNF-α plays a role in UVB-induced apoptosis of keratinocytes. This study demonstrates both *in vitro* and *in vivo* that formation of sunburn cells after UVB irradiation can be partially inhibited by TNF-α antibodies, which suggests that TNF-α is involved in UVB-induced apoptosis of keratinocytes.

## MATERIALS AND METHODS

**Cell Culture and Irradiation** Human keratinocytes were obtained from neonatal foreskins as described previously [19] and cultured in Keratinocyte Growth Medium (KGM; Clonetics Corp., San Diego, CA). The spontane-

Manuscript received June 28, 1994; revised December 19, 1994; accepted for publication January 5, 1995.

Reprint requests to: Dr. Thomas Schwarz, Department of Dermatology, University Münster, Von-Esmarchstrasse 56, D-48149 Münster, Germany.

0022-202X/95/$09.50 • SSDI0022-202X(95)00026-H 

ously transformed human keratinocyte cell line HaCaT was kindly provided by Dr. Fusenig (DKFZ, Heidelberg, Germany) [20]. UVB irradiation was performed as described [10]. Briefly, cells were seeded in KGM (normal human keratinocytes) or in Dulbecco's modified Eagle's medium supplemented with 5% fetal bovine serum (HaCaT) in tissue culture dishes at a density of $2 \times 10^5$/ml and grown until subconfluency. Immediately before UVB irradiation, cells were washed twice with prewarmed phosphate-buffered saline (PBS) and exposed to UVB in the presence of PBS. For UVB irradiation, we used a bank of 4 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. The output measured at 310 nm using an IL-1700 research radiometer (International Light, Newport, MA) was 8.0 W/m$^2$ at a tube-to-target distance of 28 cm. Immediately after UV treatment, PBS was replaced by the respective medium (10 ml per petri dish) and the cells were cultured for a further 16 h. Control cells were subjected to the identical procedure without UV exposure.

**Reagents** For the *in vitro* experiments, we used a polyclonal rabbit antibody (IgG fraction) directed against human TNF-α (Endogen, Boston, MA). A rabbit IgG fraction (Dianova, Hamburg, Germany) served as a control. For induction of cell necrosis, nigericine (Sigma) was used at a final concentration of 100 μM. The animal studies used a polyclonal rabbit antibody (IgG fraction) directed against murine TNF-α (Endogen). As a control, a rabbit IgG fraction (Endogen) was injected.

**Nick Labeling of Apoptotic DNA** Nick labeling of apoptotic DNA in cells was performed as described [21] with modifications. Briefly, $2 \times 10^6$ cells per sample were spun at 1200 rpm for 10 min, and pellets were gently resuspended in 250 μl PBS containing 0.1% sodium acide and 2% formaldehyde. After further incubation for 30 min on ice, pellets were spun down and resuspended in PBS and cold ethanol. Thereafter, the cells were washed twice with nick buffer (50 mM Tris/HCl, pH 7.8, 5 mM $MgCl_2$, 100 mM β-mercaptoethanol, 10 μg/ml bovine serum albumin). Ten microliters of cell suspension was incubated with 1 sample volume of Master Mix for 90 min at 15°C. Master Mix consisted of dATP, dCTP, and dGTP (0.2 mM, 1.3 μl each; Perkin Elmer, Norwalk, CT), 1.6 μl nick buffer, 0.3 μl 1-mM biotinylated 11-dUTP (Sigma), and 1 U Polymerase I (Promega, Madison, WI). Cells were washed with PBS supplemented with 0.1% Triton X-100 to block the polymerase. The pellet was resuspended in 40 μl 4 × sodium citrate/sodium chloride, 5% non-fat dry milk, 0.1% Triton X-100, and 2.5 μg/ml Avidin–fluorescein isothiocyanate (Vector, Burlingame, CA). After incubation for 30 min in the dark, cells were washed twice with PBS, suspended in PBS, and subjected to fluorescence-activated cell sorter (FACS) analysis (FACScan; Becton Dickinson or EPICS, Coulter).

**Electron Microscopy** Immediately after irradiation, the cells were washed twice with PBS, dehydrated in ethanol, fixed in glutaraldehyde and osmium tetroxide, and embedded in epon. Ultrathin sections were counterstained with lead citrate and uranyl acetate and examined in a Philips E410 electron microscope.

***In vivo* UVB Irradiation of Mice** Female Balb/c mice, 10–12 weeks old (obtained from the Versuchstierzuchtanstalt Hannover, Germany), were shaved on their abdomens using an electric clipper. UVB irradiation was performed with FS 20 lamps. The shaved skin areas were irradiated with 2000 J/m$^2$ given as a single exposure. Negative control mice were treated in an identical way, but only sham irradiated. Each group consisted of at least four animals. Twenty-four hours later, one biopsy sample each was taken from the irradiated and control areas, fixed in buffered formaldehyde, embedded in paraffin, sectioned (5 μm), and stained with hematoxylin and eosin. The numbers of sunburn cells, defined as apoptotic cells within the epidermis yielding a shrunken eosinophilic cytoplasm and a condensed nucleus, were counted throughout the epidermis of two sections per sample using a 1 × 1-cm grid inserted in a conventional microscope equipped with a 10× ocular lens and a 40× objective lens. Sunburn cells were counted per millimeter length of the epidermis. For each sample, at least five fields were evaluated and the mean value of the sunburn cells obtained. Immediately after irradiation into the UVB-exposed area, 100 μg of a polyclonal rabbit TNF-α antibody was injected subcutaneously. Injection of the same amounts of antibody into unirradiated skin did not induce any epidermal changes. As a control, 100 μg of a rabbit IgG (Endogen) was injected subcutaneously into UVB-exposed skin.

## RESULTS

**UVB Induces Apoptosis in Keratinocytes** HaCaT cell monolayers were irradiated with UVB to induce apoptotic death. Apoptosis was examined by nick translation and evaluated by FACS analysis. To test the sensitivity of this method, in the first approach we used low UVB doses and performed end labeling 16 h later. Application of 20 and 50 J/m$^2$, respectively, resulted in a slight dose-dependent increase in fluorescence activity, suggesting that particular cells underwent apoptosis **(Fig 1)**. As a negative control, we used both UVB-exposed and unirradiated cells in which either the polymerase I or the biotinylated 11-dUTP was omitted. The FACS profiles of these cells appeared identical to those of regularly labeled unirradiated cells (data not shown). To differentiate between apoptosis and necrosis, we used nigericine, a polyether ionophore that disrupts membrane potential. Addition of nigericine at a concentration of 100 μM to HaCaT cells results in remarkable necrosis, as checked by trypan blue exclusion (data not shown). When HaCaT cells were treated with 100 μM nigericine instead of UVB, no fluorescence shift was observed, indicating that this method detects apoptosis in a specific way. In addition, occurrence of apoptosis was confirmed by electron microscopy. Whereas sham-irradiated HaCaT cells were in good condition **(Fig 2*a*)**, cells exposed to UVB (250 J/m$^2$) showed marginal condensation of chromatin, fragmentation of

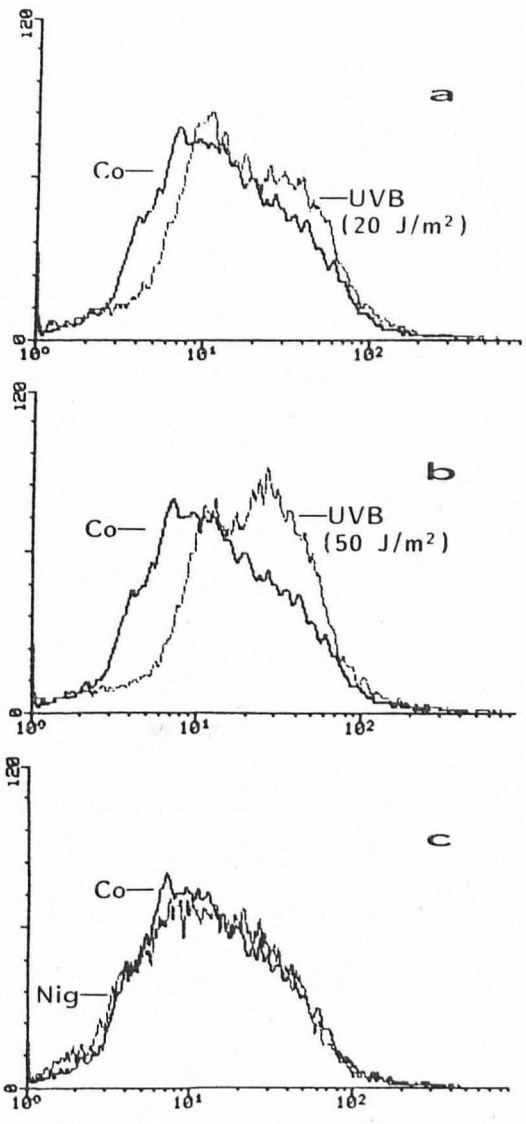


**Figure 1. UVB irradiation of HaCaT cells induces apoptosis.** Cells were nick end labeled, and nicked DNA was visualized by FACS analysis. Cells were left untreated (Co) or irradiated with 20 J/m$^2$ (*a*) or 50 J/m$^2$ (*b*), cultured for 16 h, end labeled, and subjected to FACS analysis. Unirradiated cells were incubated in the presence of nigericine (Nig; 100 μM) and end labeled 16 h later (*c*). Histograms show fluorescence intensity (x axis) versus cell number (y axis).

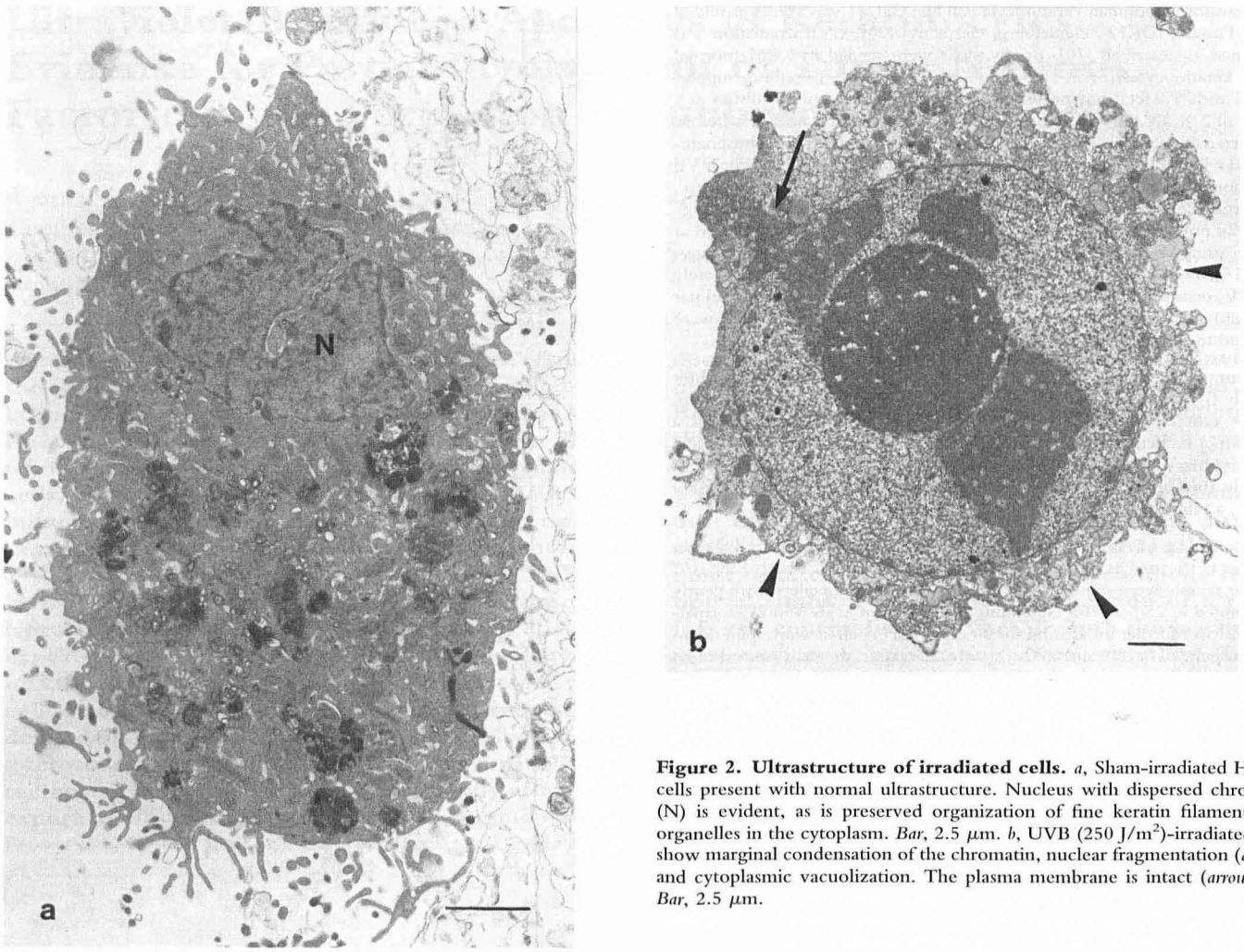


**Figure 2. Ultrastructure of irradiated cells.** *a*, Sham-irradiated HaCaT cells present with normal ultrastructure. Nucleus with dispersed chromatin (N) is evident, as is preserved organization of fine keratin filaments and organelles in the cytoplasm. *Bar*, 2.5 μm. *b*, UVB (250 J/m$^2$)-irradiated cells show marginal condensation of the chromatin, nuclear fragmentation (*arrow*), and cytoplasmic vacuolization. The plasma membrane is intact (*arrowhead*). *Bar*, 2.5 μm.

the nucleus, and intracellular vacuolization; cellular membranes and organelles remained intact **(Fig 2*b*)**.

**Reduction of UVB-Induced Apoptosis by Application of TNF-α Antibodies** To test whether TNF-α is involved in the UVB-induced apoptotic process, HaCaT cells were exposed to 250 J/m$^2$ UVB. This resulted in remarkable apoptosis, demonstrated by an increase in cell fluorescence **(Fig 3*a*)**. Application of a rabbit polyclonal antibody directed against human TNF-α immediately after UVB irradiation caused a decrease in fluorescence activity, indicating reduced apoptosis **(Fig 3*b*)**. In contrast, addition of a control antibody had no effect **(Fig 3*c*)**. These data suggest that neutralization of TNF-α can partially rescue cells from UVB-induced cell death. The less antibody we applied, the fewer cells were protected **(Fig 4)**. However, even by applying higher doses of TNF-α antibodies, beyond 100 μl/10 ml, we were not able to rescue all cells from apoptosis (data not shown).

HaCaT cells behave in many respects like normal human keratinocytes and thus are used frequently to study keratinocyte biology; however, the two are not absolutely comparable. Therefore, long-term cultured normal human keratinocytes were exposed to UVB light after the fifth passage and then immediately treated with TNF-α antibodies. As observed with HaCaT cells, normal human keratinocytes underwent apoptosis upon exposure to 250 J/m$^2$ UVB light, which could be partially prevented by addition of the TNF-α antibody immediately after UVB irradiation **(Fig 5)**. However, normal human keratinocytes appeared to be better protected by the addition of TNF-α antibody than were HaCaT cells. Therefore, both keratinocytes and HaCaT cells were exposed to UVB light (250 J/m$^2$), and TNF-α levels were measured in supernatants 16 h later. Normal human keratinocytes appeared to be stronger in their capacity to release TNF-α (80.3 ± 2.7 pg/ml) upon UVB exposure as compared with HaCaT cells (21.6 ± 5.9 pg/ml).

**Reduction of *In Vivo* Formation of Sunburn Cells by Injection of TNF-α Antibodies** To answer whether TNF-α is also involved *in vivo* in UVB-induced apoptosis, Balb/c mice were irradiated with 2000 J/m$^2$ UVB given as a single dose on the shaved abdomen. Immediately after UVB treatment, a polyclonal rabbit antibody directed against murine TNF-α was injected subcutaneously into the center of the UVB-exposed area. Biopsies were performed 24 h later and the number of sunburn cells was counted. A single exposure of 2000 J/m$^2$ induced numerous sunburn cells, which were defined as shrunken cells within the epidermis exhibiting an eosinophilic cytoplasm and a condensed nucleus. Injection of a rabbit-derived polyclonal antibody directed against murine recombinant TNF-α (100 μg) resulted in a significant reduction of sunburn cells, whereas administration of control IgG had no effect on the formation of sunburn cells **(Fig 6)**. When the antibody was preincubated before injection with equivalent amounts of recombinant murine TNF-α, formation of sunburn cells was not changed. However, as in the *in vitro* data, we were not able to reduce sunburn cell formation completely even by injecting higher doses of the TNF-α antibodies (data not shown). Moreover, by injecting recombinant TNF-α, we could not obtain morphologic changes of keratinocytes similar to those with UVB light (data not shown). Thus, these data suggest that TNF-α is involved in the formation of

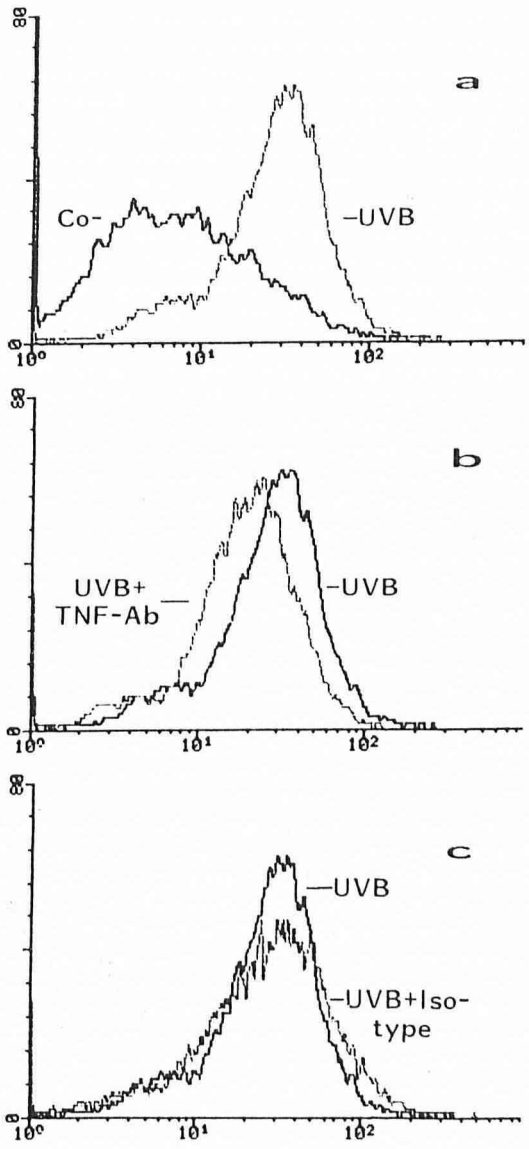


**Figure 3. Reduction of UVB-induced apoptosis by TNF-α antibody.** HaCaT cells were left untreated (Co) or irradiated with 250 J/m$^2$ UVB (*a*). Immediately after UVB exposure, TNF-α antibody (TNF-Ab) (*b*) or an isotype control (*c*) was added. At 16 h after treatment, cells were end labeled and subjected to FACS analysis. Histograms show fluorescence intensity (x axis) versus cell number (y axis).

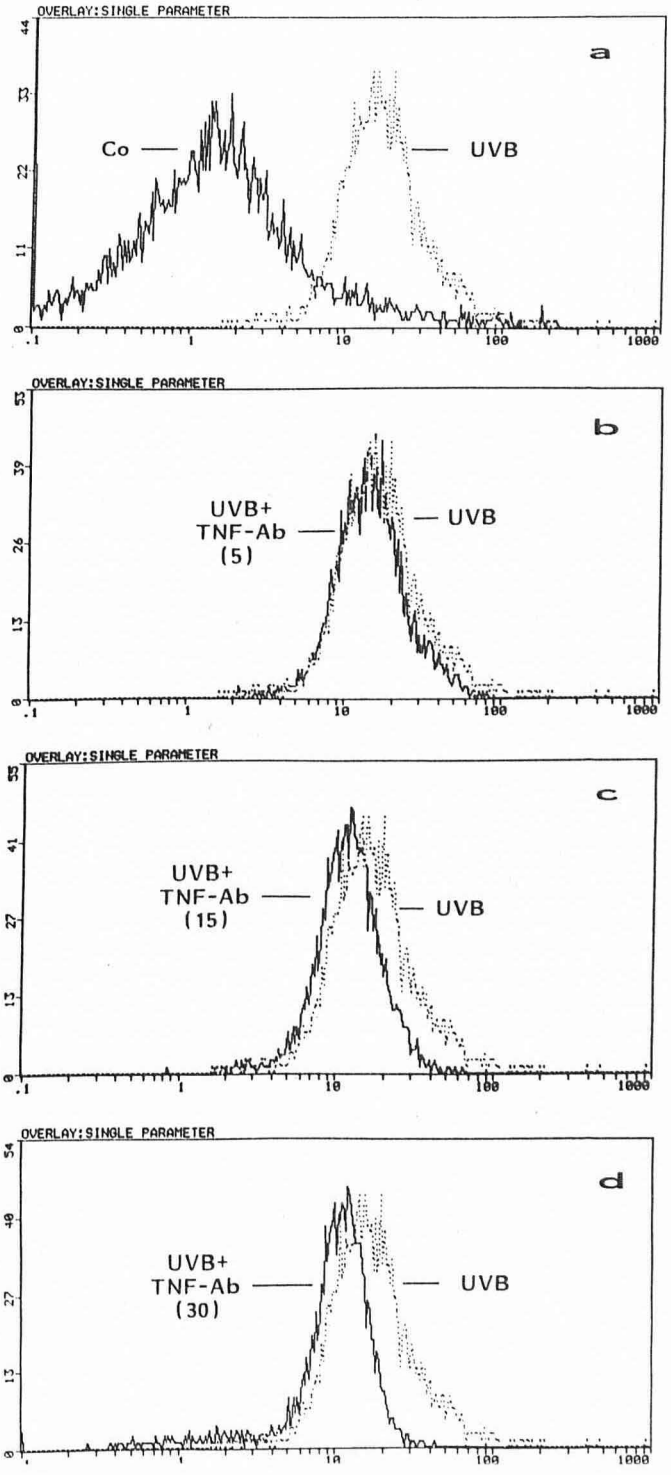


**Figure 4. Reduction of UVB-induced apoptosis by TNF-α antibody.** HaCaT cells were left untreated (Co) or irradiated with 250 J/m$^2$ UVB (*a*). Immediately after UVB exposure, 5 μl (*b*), 15 μl (*c*), or 30 μl (*d*) TNF-α antibody (TNF-Ab) was added. At 16 h after treatment, cells were end labeled and subjected to FACS analysis. Histograms show fluorescence intensity (x axis) versus cell number (y axis). Mean fluorescence intensity: Co, 1.6; UVB, 17.4; 5 μl, 13.4; 15 μl, 12.2; 30 μl, 9.6.

sunburn cells, but by itself is not sufficient to induce apoptosis in keratinocytes.

## DISCUSSION

The most characteristic histologic change of acutely UVB-damaged skin is the appearance of sunburn cells [4,5,22]. The mechanisms involved in the generation of sunburn cells remain ill defined. It has been suggested that increased rupture of lysosomes [23], decreased DNA repair [24], and reactive oxygen intermediates may be important [25–27]. Although just by applying morphologic criteria, investigators have long known that sunburn cells are apoptotic cells, functional evidence for UV-induced apoptosis has been obtained only recently based on the characteristic ladder pattern of fragmented DNA [6,7]. Occurrence of apoptosis in keratinocytes was also observed by nick end labeling and visualization of nicked DNA *in situ* [7]. None of the studies revealing a new pathway involved in the formation of sunburn cells were able to reduce sunburn cell formation completely, which suggests that this is a multifactorial event. TNF-α is a cytokine that was first described according to its ability to kill preferentially growing tumor cells [28]. There is good evidence that TNF-α is able to induce apoptosis

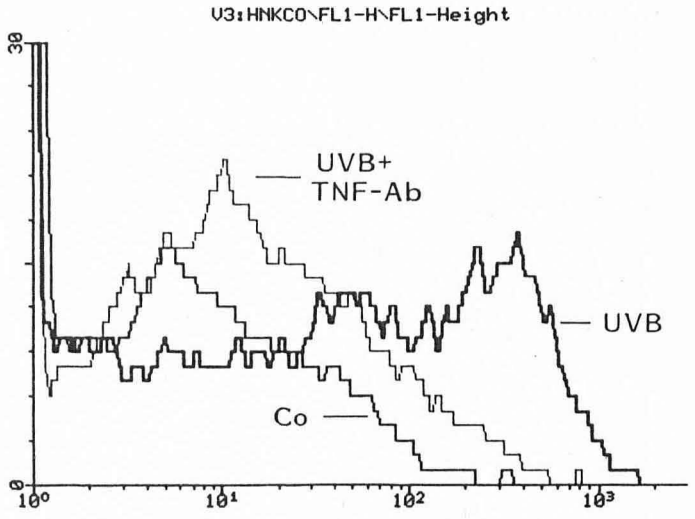


**Figure 5. UVB-induced apoptosis in normal human keratinocytes.** Normal human keratinocytes were left untreated (Co) or irradiated with 250 J/m$^2$ UVB. Immediately after UVB exposure, TNF-α antibody (TNF-Ab) was added (20 μl). At 16 h after treatment, cells were end labeled and subjected to FACS analysis. Histograms show fluorescence intensity (x axis) versus cell number (y axis). Mean fluorescence intensity: Co, 19.2; UVB, 128.5; UVB plus TNF-α antibody, 34.5.

of certain cells [12–18,28,29]. Bearing in mind the observations that TNF-α can induce apoptosis, that the release of TNF-α by keratinocytes is induced by UV light, and that keratinocytes express receptors for TNF-α, we postulated that TNF-α might be involved in the generation of sunburn cells.

The present study demonstrates that *in vitro* irradiation of normal human keratinocytes and HaCaT cells induces apoptosis and that DNA fragmentation can be reduced by the addition of TNF-α antibodies to the culture immediately after UV treatment. Apoptosis was determined by nick translation evaluated by FACS analysis, with slight modifications as reported [30]. In our hands, this seems to be the most sensitive method to detect apoptosis. Because of this sensitivity, we applied rather low doses of UVB (20–250 J/m$^2$) and were able to find DNA fragmentation even after a single dose of 20 J/m$^2$. Doses above 300 J/m$^2$ were not used in this *in vitro* study because we know from previous work that such doses can start to induce necrosis in some cells (unpublished observation). This bimodal response is not specific for UVB; apoptosis can be induced by low concentrations of several toxins ($H_2O_2$, ethanol, chemotherapeutic agents) [31], which at higher concentrations cause necrosis. Mildly injurious, sublethal stimuli may therefore cause the cell to trigger its endogenously programmed cell-suicide mechanism before it loses its control. We initially determined apoptosis by checking for the DNA ladder pattern in gel electrophoresis and could detect DNA fragmentation in UVB-exposed keratinocytes (data not shown). However, using this system we were unable to estimate whether there is a reduction in DNA fragmentation after application of TNF-α antibodies. Because we anticipated that only a minority of cells would be protected by TNF-α neutralization, we used the more sensitive nick labeling method. With this technique, we could show that TNF-α antibodies can reduce DNA fragmentation. However, even by adding higher doses of antibody, we could not increase the number of protected cells beyond a certain level. The failure to prevent apoptosis entirely with TNF-α antibodies suggests that TNF-α contributes to the formation of sunburn cells, but it does not seem to be the only mechanism involved. Consequently, it was not surprising to observe that the mere addition of recombinant human TNF-α to the culture failed to induce apoptosis in normal keratinocytes (data not shown).

To test whether TNF-α also might be involved in the formation of sunburn cells *in vivo*, we exposed Balb/c mice to UVB light (2000 J/m$^2$) at their shaved abdomens. In our hands, this dose induces a significant number of sunburn cells but does not cause necrosis. Subcutaneous injection of a polyclonal TNF-α antibody resulted in a significant reduction of sunburn cells, whereas rabbit IgG had no effect on the number of sunburn cells. Compared with the *in vitro* data, again we observed only a reduction in the number of sunburn cells, and it was infeasible to overcome sunburn cell formation completely even by increasing the antibody doses. Accordingly, just the injection of recombinant TNF-α failed to induce sunburn cell formation *in vivo* (data not shown). Therefore, TNF-α is not the only factor involved in the formation of sunburn cells. We cannot exclude with absolute certainty that under different experimental conditions, e.g., increased dose, multiple injections, or different times of application, injection of TNF-α could induce sunburn cells. However, it appears much more likely that additional factors could be involved. It has been clearly demonstrated that upon exposure to UVB light, keratinocytes release a variety of cytokines [32], which also may contribute to the formation of sunburn cells. Moreover, we are currently addressing the effects of UVB light on other pathways that play an important role in the activation of apoptosis. Of particular interest in this respect is the effect of UVB light on the expression of the Fas-antigen, which induces apoptosis [33], and on BCL-2, which prevents activation of the cell-suicide program [34].

The present study demonstrates that TNF-α appears to be involved in UVB-mediated apoptosis, but is not sufficient by itself to induce the formation of sunburn cells. Therefore, this study supports the concept that UVB-induced apoptosis and formation of sunburn cells is a multifactorial event.

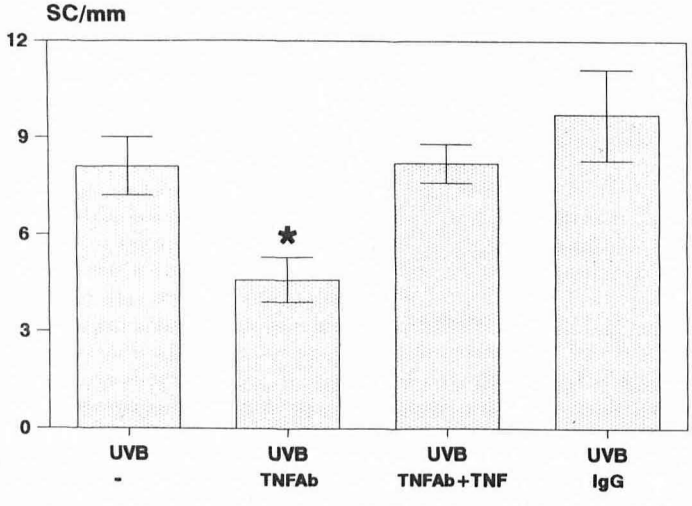


**Figure 6. *In vivo* reduction of sunburn cells by TNF-α antibody.** Balb/c mice were irradiated with UVB (2000 J/m$^2$) on the shaved abdomen. Immediately thereafter, TNF-α antibody (TNF-Ab) was injected into the UVB-exposed skin area. As controls, rabbit IgG and TNF-α antibody preincubated with recombinant TNF-α (TNF), respectively, were injected into UVB-exposed skin. Biopsies were taken 24 h later and sunburn cells (SC) were counted per mm length of epidermis (mean ± SD). In sham-irradiated skin, no sunburn cells were found. Data show one representative of three experiments. *$p < 0.05$ (Student t test).

*We thank B. Brückler, Department of Dermatology, Hospital Vienna Lainz, Vienna, Austria for excellent technical assistance in preparing histologic sections, and D. Wagner for secretarial assistance. This study was supported by a grant from the Bundesministerium für Forschung und Technik (07UVB53-7) and by the European Community (EV5V-CT94-0564).*

## REFERENCES


1. Kerr JFR, Harmon BV: Definition and incidence of apoptosis: an historical perspective. In: Tomei LD, Cope FO (eds.). *Apoptosis: The Molecular Basis of Cell Death. Current Communications in Cell and Molecular Biology*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, 1991
2. Schwartz LM, Osborne BA: Programmed cell death, apoptosis and killer genes. *Immunol Today* 14:582–590, 1993
3. Cohen JJ: Apoptosis. *Immunol Today* 14:126–130, 1993
4. Danno K, Horio T: Sunburn cell: factors involved in its formation. *Photochem Photobiol* 45:683–690, 1987
5. Young AR: The sunburn cell. *Photodermatology* 4:127–134, 1987
6. Martin SJ, Cotter TG: Ultraviolet B irradiation of human leukaemia HL-60 cells in vitro induces apoptosis. *Int J Radiat Biol* 59:1001–1016, 1991
7. Casciola-Rosen LA, Anhalt G, Rosen A: Autoantigens targeted in systemic lupus erythematosus are clustered in two populations of surface structures on apoptotic keratinocytes. *J Exp Med* 179:1317–1330, 1994
8. Carswell EA, Old LJ, Kassel RL, Green S, Fiore N, Williamson B: An endotoxin-induced serum factor that causes necrosis of tumors. *Proc Natl Acad Sci USA* 72:3666–3670, 1975
9. Beutler B, Cerami A: Cachectin (tumor necrosis factor): an endogenous mediator of shock and inflammatory response. In: Oppenheim JJ, Shevach EM (eds.). *Immunophysiology. The Role of Cells and Cytokines in Immunity and Inflammation*. Oxford University Press, New York, 1990, pp 226–237
10. Köck A, Schwarz T, Kirnbauer R, Urbanski A, Perry P, Ansel JC, Luger TA: Human keratinocytes are a source for tumor necrosis factor alpha. Evidence for synthesis and release upon stimulation with endotoxin or ultraviolet light. *J Exp Med* 172:1609–1614, 1990
11. Trefzer U, Brockhaus M, Lötscher H, Parlow F, Budnik A, Grewe M, Christoph H, Kapp A, Schöpf E, Luger TA, Krutmann J: 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* 92:462–470, 1993
12. Elias E, Moore PB, Rose SM: Tumor necrosis factor induced DNA fragmentation in HL-60 cells. *Biochem Biophys Res Commun* 157:963–969, 1988
13. Greenblatt MS, Elias L: The type B receptor for tumor necrosis factor-alpha mediates DNA fragmentation in HL-60 and U937 cells and differentiation in HL-60 cells. *Blood* 80:1339-1346, 1992
14. Wright SC, Kumar P, Tam AW, Shen N, Varma M, Larrick JW: Apoptosis and DNA fragmentation precede TNF-induced cytolysis in U937 cells. *J Cell Biochem* 48:344–355, 1992
15. Kyprianou N, Alexander RB, Isaacs JT: Activation of programmed cell death by recombinant human tumor necrosis factor plus topoisomerase II-targeted drugs in L929 tumor cells. *J Natl Cancer Inst* 83:346–350, 1991
16. Fehsel K, Kolb-Bachofen V, Kolb H: Analysis of TNF alpha induced DNA strand breaks at the single cell level. *Am J Pathol* 139:251–254, 1991
17. Gromkowski SH, Mama K, Yagi J, Sen R, Rath S: Double stranded RNA and bacterial lipopolysaccharide enhance sensitivity to TNF-alpha-mediated cell death. *Int Immunol* 2:903–908, 1990
18. Fulton AM, Chong YC: The role of macrophage-derived TNF alpha in the induction of sublethal tumor cell DNA damage. *Carcinogenesis* 13:77–81, 1992
19. Eisinger M, Jisootee J, Hefton M, Darzynkiewicz Z, Chiao JW, de Harven E: Human epidermal cell cultures: growth and differentiation in the absence of dermal components of medium supplements. *Proc Natl Acad Sci USA* 76:5340–5344, 1979
20. Boukam P, Petrussevska RT, Breitkreutz D, Hornung J, Markhan A, Fusenig NE: Normal keratinization in a spontaneously immortalized aneuploid human keratinocyte cell line. *J Cell Biol* 106:761–771, 1988
21. Gavrieli Y, Sherman Y, Ben-Sasson SA: Identification of programmed cell death in situ via specific labeling of nuclear DNA fragmentation. *J Cell Biol* 119:493–501, 1992
22. Gilchrest BA, Soter NA, Stoff JS, Mihm MC: The human sunburn reaction: histologic and biochemical studies. *J Am Acad Dermatol* 5:111–122, 1981
23. Johnson BE, Daniels F Jr. Lysosomes and the reactions of skin to ultraviolet radiation. *J Invest Dermatol* 53:85–94, 1969
24. Brenner W, Gschnait F: Decreased DNA repair activity in sunburn cells. *Arch Dermatol Res* 266:11–16, 1979
25. Miyachi Y, Horio T, Imamura S: Sunburn cell formation is prevented by scavenging oxygen intermediates. *Clin Exp Dermatol* 8:305–310, 1983
26. Danno K, Horio T, Takigawa M, Imamura S: Role of oxygen intermediates in UV-induced epidermal cell injury. *J Invest Dermatol* 83:166–168, 1984
27. Youn JI, Gange RW, Maytum D, Parrish JA: Effect of hypoxia on sunburn cell formation and inflammation induced by ultraviolet radiation. *Photodermatology* 5:252–256, 1988
28. Larrick JW, Wright SC: Cytotoxic mechanism of tumor necrosis factor-α. *FASEB J* 4:3215–3223, 1990
29. Jarvis WD, Kolesnick RN, Fornari FA, Traylor RS: Induction of apoptotic DNA damage and cell death by activation of the sphingomyelin pathway. *Proc Natl Acad Sci USA* 91:73–77, 1994
30. Haimovitz-Friedman A, Kan CC, Ehleiter D, Persaud RS, McLoughlin M, Fuks Z, Kolesnick RN: Ionizing radiation acts on cellular membranes to generate ceramide and initiate apoptosis. *J Exp Med* 180:525–535, 1994
31. Lemon SV, Martin S, Cotter TG: Induction of apoptosis (programmed cell death) in tumour cell lines by widely diverging stimuli. *Biochem Soc Trans* 18:343–345, 1990
32. Schwarz T, Urbanski A, Luger TA: Ultraviolet light and epidermal cell-derived cytokines. In: Luger TA, Schwarz T (eds.). *Epidermal Growth Factors and Cytokines*. Marcel Dekker, New York, 1994, pp 303–324
33. Itoh N, Yonehara S, Ishii A, Yonehara M, Mizushima S, Sameshima M, Hase A, Seto Y, Nagata S: The polypeptide encoded by the cDNA for human cell surface antigen Fas can mediate apoptosis. *Cell* 66:233–243, 1991
34. Hockenbery DM, Zutter M, Hickey W, Nahm M, Korsmeyer SJ: BCL-2 protein is topographically restricted in tissues characterized by apoptotic cell death. *Proc Natl Acad Sci USA* 88:6961–6965, 1991