Evaluation of Apoptotic Cells Induced by Ultraviolet Light B Radiation in Epidermal Sheets Stained by the TUNEL Technique

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Two major components of epidermal cells, keratinocytes and Langerhans cells, are injured by ultraviolet light B radiation, resulting in sunburn cell (apoptotic cell) formation, impaired function, and a reduced number of Langerhans cells. Quantitative analysis of Langerhans cell damage is usually performed using epidermal sheets, whereas that of keratinocytes has been performed by counting the number of sunburn cells in vertical tissue sections. In this study we assessed the influences of ultraviolet light B radiation on epidermal cells by apoptotic cell formation, using murine epidermal sheets stained by terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling technique. Ten to 75 mJ per cm^2 of ultraviolet light B radiation induced apoptotic cells in abdominal skin of C3H mice. The cells were induced in 6 h after 50 mJ per cm^2 of ultraviolet light B irradiation with the peak in number in 24 h, 18.8 ± 5.0 per mm^2 and 97.7 ± 7.4 per mm^2, respectively. One week later, the apoptotic cells were not visualized. As C3H/He, BALB/C, and C57BL/6 mice showed almost the same frequency of apoptosis in epidermal sheets from 50 mJ per cm^2 ultraviolet light B-irradiated skin, the induction of the cells by ultraviolet light B radiation did not depend on the genetic trait of the mouse. Xeroderma pigmentosum type A gene-deficient mice, however, showed a greater induction of apoptotic cells (216.9 ± 25.2 per mm^2) by ultraviolet light B radiation than xeroderma pigmentosum type A wild-type mice (89.5 ± 13.6 per mm^2) and conventional mice. Pretreatment with a SPF 60 sunscreen agent was quite effective in reducing the induction of apoptotic cells. Using confocal laser scanning microscopy and double staining, 1.5 ± 2.7% of apoptotic cells were Ia-positive cells in 24 h after 50 mJ per cm^2 of ultraviolet light B radiation. Apoptotic Ia-positive cells were not observed 48 h after the radiation. On the other hand, no apoptotic dendritic epidermal T cells were observed in up to 75 mJ per cm^2 of ultraviolet light B radiated skin. Thus, nearly all apoptotic cells were keratinocytes, and Langerhans cells and dendritic epidermal T cells appeared resistant to ultraviolet light B-induced apoptosis. Compared with the assessment in vertical tissue sections, the terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling technique with epidermal sheets appeared to be a more physiologically relevant method for quantitative evaluation of apoptotic epidermal cells induced by ultraviolet light B radiation. ***Key words: apoptosis/epidermal sheet/Langerhans cell/sunburn cell/ultraviolet. J Invest Dermatol 113:802–807, 1999***

Ultraviolet light B (UVB) radiation can induce several types of epidermal injury, including sunburn cell (SBC) formation and functionally as well as morphologically impaired Langerhans cells. These injuries are considered to be associated with skin carcinogenesis and immunosuppression (Kripke, 1984; Ziegler *et al*, 1994). Therefore, the analysis of the mechanism and the intensity of epidermal injuries are significant for the consideration of the harmful effects of UVB radiation on the skin. The influence of UVB radiation on these epidermal components has previously been estimated by a variety of methods. Both quantitative and qualitative analysis have been performed on alterations in Langerhans cells induced by UVB radiation. Functionally, the antigen-presenting ability has been examined by using purified Langerhans cells or their cell line (Stingl *et al*, 1978; Schuhmachers *et al*, 1996). The density of Langerhans cells is quantitated in epidermal sheets stained with adenosine nucleotide phosphatase or specific monoclonal antibodies such as anti-CD1 antibody and anti-HLA-DR antibody (Mackenzie and Squier, 1975; Toews *et al*, 1980; Guo *et al*, 1991). On the other hand, the effect of UVB radiation on keratinocytes has been mainly estimated by the number of SBC. SBC are counted in vertical tissue sections of paraffin-embedded samples and expressed as the number per length of epidermis measured (Woodcock and Magnus, 1976; Danno *et al*, 1981).

SBC are UV-damaged keratinocytes with pyknotic nuclei and eosinophilic cytoplasm (Olson *et al*, 1974). Although the cells are

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Abbreviations: SBC, sunburn cell; TUNEL, terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling.

known to be apoptotic keratinocytes (Weedon *et al*, 1979; Danno and Horio, 1982; Ziegler *et al*, 1994; Kane and Maytin, 1995; Schwarz *et al*, 1995), the mechanism of the formation remains unknown. Reactive oxygen intermediates, tumor necrosis factor-α, Fas/Fas ligand system, cell cycle, DNA repair activity, DNA photoproducts, and p53 have been reported to be involved (Brenner and Gschnait, 1979; Hanada *et al*, 1991; Ziegler *et al*, 1994; Schwarz *et al*, 1995; Wolf *et al*, 1995; Leverkus *et al*, 1997). In respect to death receptors in UV-induced apoptosis, Rehemtulla *et al* (1997) reported a germicidal UV light activates the Fas pathway through receptor aggregation and subsequent recruitment of the Fas-associated protein with death domain (FADD). Aragane *et al* (1998) recently reported that UVB directly stimulates Fas receptor of human keratinocyte cell line and thereby activates the Fas-dependent apoptosis pathway.

Apoptosis is a cell death process with specific distinct morphologic, biochemical, and molecular alterations, including chromatin condensation, DNA fragmentation, and cellular shrinkage and collapse (Raskin, 1997). Recently, many strategies have been developed for the detection of apoptosis and adapted for the examination of keratinocyte death. Alterations in cellular and nuclear morphology can be seen by electronmicroscopy as chromatin condensation, DNA organization, and cytoplasmic integrity. Cleavage of nuclear DNA is detected by the DNA ladder on agarose gels during electrophoresis and enzymatic *in situ* labeling of DNA polymerase and terminal deoxynucleotidyl transferase(TdT) described as the TUNEL technique (Gavrieli *et al*, 1992).

We investigated apoptosis of epidermal cells induced by UVB exposure to murine abdominal or ear skin, using epidermal sheets stained with the TUNEL technique. This technique allowed the direct detection of DNA fragmentation of UVB-irradiated epidermal cells in vertical tissue sections by fluorescence microscopy (Ziegler *et al*, 1994; Bernerd and Asselineau, 1997). Our modified method using epidermal sheets was more physiologically relevant for the quantitative assessment of epidermal cell injuries induced by UVB exposure than the semiquantitative assay of apoptotic cells or SBC in vertical tissue sections, and also identified apoptosis of Langerhans cells using double staining with monoclonal anti-Ia antibody.

MATERIALS AND METHODS

Animals C3H/He, BALB/C, and C57/BL female mice, 8 wk of age were purchased from the Shimizu Laboratory Animal Center, Japan. Xeroderma pigmentosum group A (XPA) gene-deficient homozygous (−/−) and XPA wild-type (+/+) male mice, 8–10 wk of age were also used (Nakane *et al*, 1995).

UV radiation UVB was administered by a bank of seven tubes of fluorescent sunlamp (FL 20SE, Toshiba Medical Supply, Tokyo, Japan) with an emission spectrum of 275–375 nm peaking at 305 nm, emitting mainly within the UVB range. The irradiance was 0.35 mW per cm^2 at a distance of 35 cm measured by a radiometer (UVR-305/365 D(II), Toshiba Medical Supply). To expose razor-shaved abdominal skin, mice were anesthetized with an intraperitoneal injection of 0.27 ml of 5 mg per ml pentobarbital sodium solution (Dainabot, Osaka, Japan) and were placed in the supine position. To expose the ear skin, mice were placed in wire cages, permitted to freely move and irradiated uniformly. The mice received a total energy dose of 10–300 mJ per cm^2 during one exposure. To determine the effect of a sunscreen agent on UV-induced epidermal cell injuries, 50 μl of a SPF 60 sunscreen agent (Shiseido, Tokyo, Japan) was applied 30 min before irradiation.

Contact sensitization To determine the effect of UVB radiation on the immune reaction, contact hypersensitivity was evaluated as previously reported (Okamoto and Kripke, 1987). A 0.5% fluorescein isothiocyanate (Dojin, Kumamoto, Japan) solution was prepared in a solvent composed of equal volumes of acetone and dibutyl phthalate. For sensitization, 0.4 ml of the solution was applied to shaved abdominal skin. Five days later, the contact hypersensitivity reaction was elicited by applying 10 μl of the 0.5% fluorescein isothiocyanate solution to each ear. The ear thickness was measured with a spring-loaded caliper (Mitutoyo, Japan) before and 24 h after applying the challenge dose. Each group contained five mice, and each experiment was performed three times.

Preparation of epidermal sheets Shaved abdominal skin was removed from the mice and the dermal and adipose tissues were scraped off with fine curved forceps. Ear skin was split into two pieces. The skin was washed once with phosphate-buffered saline and incubated in a phosphate-buffered saline/20 mM ethylenediamine tetraacetic acid solution (pH 7.4) at 37°C for 1 h. The epidermal sheets were peeled from the skin and washed twice with phosphate-buffered saline.

***In situ* nick end labeling** To detect DNA breaks *in situ*, TUNEL was performed by using a detection kit (Boehringer Mannheim, Mannheim, Germany). Epidermal sheets were cut into small pieces, approximately 4 mm^2 and fixed with 4% paraformaldehyde solution for 30 min at room temperature. After two rinses with phosphate-buffered saline, the sheets were incubated with the TUNEL reaction mixture for 1 h at 37°C in dark conditions. The positive cells with fluorescence were observed by fluorescence microscopy (Nikon, Tokyo, Japan). Five randomly selected fields per piece from four pieces were examined under 10 × ocular and 40 × (22 mm) object lenses (one field was 0.2375 mm^2) and the apoptotic cell induction was expressed as cells per mm^2 (mean ± SD). To identify the cell type of apoptotic cells, double staining was performed with anti-Iak or Thy-1 antibody for Langerhans cells or dendritic epidermal T cells, respectively, together with the TUNEL technique. Paraformaldehyde-fixed epidermal sheets were incubated with phycoerythrin-conjugated anti-Iak antibody or phycoerythrin-conjugated Thy-1 antibody (Caltag, Burlingame, CA) for 1 h at 37°C. After two rinses with phosphate-buffered saline, the sheets were subjected to TUNEL staining. Immunostained sheets were then examined by confocal laser scanning, Fluoview (Olympus, Tokyo, Japan).

Sunburn cell counting Abdominal or ear skin was removed after UVB radiation and the specimens were fixed in 10% formalin and embedded in paraffin. Five micrometer thick sections were stained with hematoxylin–eosin. To compare apoptotic cell induction with SBC formation in vertical sections, dewaxed paraffin sections were incubated with 20 μg per ml proteinase K (Wako Life Sci, Osaka, Japan) for 20 min at room temperature. After two rinses with phosphate-buffered saline, the sections were incubated with the TUNEL reaction mixture. The total number of SBC or apoptotic cells in the epidermis was counted and the length of the epidermis was measured with a scale bar under 10 × ocular and 40 × object lenses. SBC or apoptotic cell counts per millimeter of epidermis were calculated after observing five different sections per specimen.

RESULTS

Comparison of SBC and *in situ* nick end labeling of skin sections Tissue sections from abdominal or ear skin of C3H mice 24 h after irradiation were examined with hematoxylin and eosin staining and TUNEL staining. Fifty or 75 mJ per cm^2 of UVB exposure to abdominal skin produced 17 ± 7 cells per cm SBC and 26 ± 7 cells per cm apoptotic cells or 20 ± 3 cells per cm SBC and 32 ± 6 cells per cm apoptotic cells, respectively. The number of TUNEL staining-positive apoptotic cells was higher than that of SBC (**Fig 1**). To induce similar number of SBC and apoptotic cells in ear skin, 200 mJ per cm^2 of UVB radiation was necessary: 16 ± 8 cells per cm SBC and 23 ± 9 cells per cm apoptotic cells. It may be due to the presence of hairs on the ear. Serial sections showed almost the same number of SBC, but samples from selected different areas showed a high mean deviation of the number, particularly in ear skin.

Quantitative analysis of apoptotic cells in epidermal sheets The TUNEL technique identified apoptotic cells in epidermal sheets from UVB-irradiated mice. To determine the effect of UVB dose on the frequency of apoptotic cells, 10–300 mJ of UVB radiation per cm^2 was applied to the abdominal skin of C3H mice. No macroscopic changes were observed in the skin 24–48 h after 10–75 mJ of UVB radiation per cm^2. More than 100 mJ per cm^2 of UVB radiation produced erythematous changes. The density of apoptotic cells augmented with increasing dose of UVB radiation up to 75 mJ per cm^2 (**Fig 2**). When abdominal skin of mice was irradiated with more than 100 mJ UVB per cm^2, however, the skin was damaged and it was often difficult to prepare intact

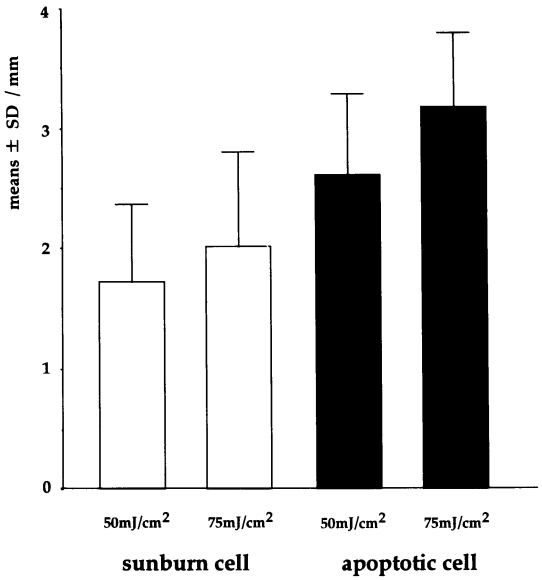

Figure 1. The number of SBC and apoptotic cells on vertical section.

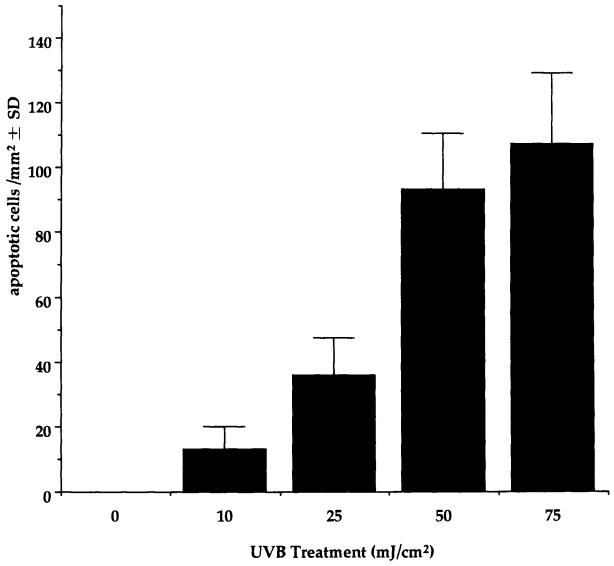

Figure 2. Correlation of the density of apoptotic cells with dose of UVB radiation. The density of apoptotic cells in function of UVB dose.

epidermal sheets. To examine whether the induction of apoptotic cells depends on time after UVB radiation, epidermal sheets were prepared from abdominal skin 6–72 h after 25 or 50 mJ per cm^2 of UVB radiation. Apoptotic cells were observed in 6 h (7.8 ± 1.2 per mm^2 by 25 mJ of UVB radiation per cm^2 and 18.8 ± 5.0 by 50 mJ of UVB radiation per cm^2) with a maximum density in 24 h after UVB radiation (45.6 ± 8.2 per mm^2 by 25 mJ of UVB radiation per cm^2 and 97.7 ± 17.4 per mm^2 by 50 mJ of UVB radiation per cm^2) (**Fig 3**). The apoptotic cells did not appear in any obvious geometrical pattern.

Double staining of epidermal sheets from 50 mJ per cm^2 UVB-irradiated mice Murine epidermal cells damaged by UVB radiation include keratinocytes, Langerhans cells, and dendritic epidermal T cells. To identify in which cell apoptosis is induced by UVB radiation, double staining was performed by the TUNEL technique and specific monoclonal antibody staining for Langerhans cells or dendritic epidermal T cells. Twenty-five to 75 mJ per cm^2 of UVB radiation decreased the number of Ia-positive cells and Thy-1-positive cells (**Table I**). Although

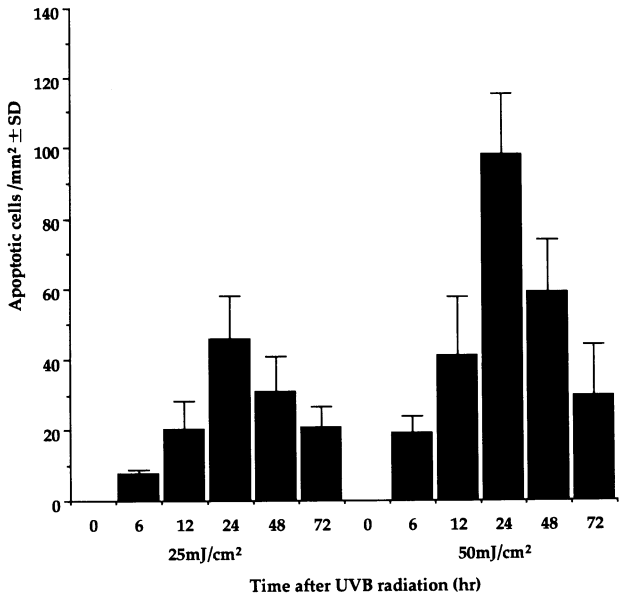

Figure 3. Time course of apoptotic cells in 25 or 50 mJ per cm^2 UVB radiation. Epidermal sheets were prepared from abdominal skin 6–72 h after 25 or 50 mJ per cm^2 UVB radiation.

Table I. Ia positive or Thy-1 positive epidermal cells 24 h after UVB radiation[a]

			Apoptotic cells[b]	
UVB (mJ per cm^2)	Ia^+ cells (per mm^2)	Thy-1^+ cells (per mm^2)	Ia^+/total (%)	Thy 1^+/total (%)
0	894.4 ± 61.9	635.6 ± 53.4	0	0
25	748.1 ± 109.5	585.0 ± 60.5	0.6 ± 2.0	0
50	540.0 ± 80.4[c]	472.5 ± 103.4[d]	1.5 ± 2.7	0
75	433.1 ± 102.9[c]	399.4 ± 93.5[c]	1.9 ± 3.2	0

[a]C3H mice were irradiated with 0–75 mJ per cm^2 of UVB. Twenty-four hours later, epidermal sheets were prepared from the irradiated abdominal skin and stained with monoclonal anti-Ia or Thy 1 antibody.
[b]Epidermal sheets were also double stained with each monoclonal antibody and TUNEL technique, and observed with a confocal laser scanning microscopy.
[c]$p<0.001$ *versus* no-irradiated skin.
[d]$p<0.05$ *versus* no-irradiated skin.

immunofluorescence microscopy identified a cell positive for Ia and TUNEL, there is a possibility that an Ia-positive, TUNEL-negative cell, and an Ia-negative, TUNEL-positive cell are overlapped. Therefore, immunostained sheets were examined with a confocal laser scanning microscopy and analyzed by serial optical sectioning along the z-axis. Ia-positive cells were infrequently identified in apoptosis (**Fig 4**). The frequency of Ia-positive apoptotic cells induced by UVB radiation was expressed as Ia-positive apoptotic cells/total apoptotic cells (**Table I**). Approximately 1–2% of apoptotic cells were Ia-positive 24 h after 50 or 75 mJ per cm^2 of UVB radiation. Such apoptotic cells were not observed 48 h after the radiation. Thy-1-positive apoptotic cells were not observed 24–48 h after up to 75 mJ of UVB radiation per cm^2. Immunologically, the contact hypersensitivity reaction to fluorescein isothiocyanate painted on 50 mJ per cm^2 of UVB irradiated skin 4 d before was significantly suppressed, compared with that in sensitization on unirradiated skin (data not shown).

Apoptotic cells in various strains of mice Effects of UVB radiation on cutaneous or immunologic responses, for example, contact hypersensitivity and carcinogenesis, are sometimes dependent on the genetic background of mice. To determine the influence of the genetic background on the induction of apoptosis by UVB radiation, various strains of mice were examined. There was no significant difference in the frequency of apoptotic cells induced

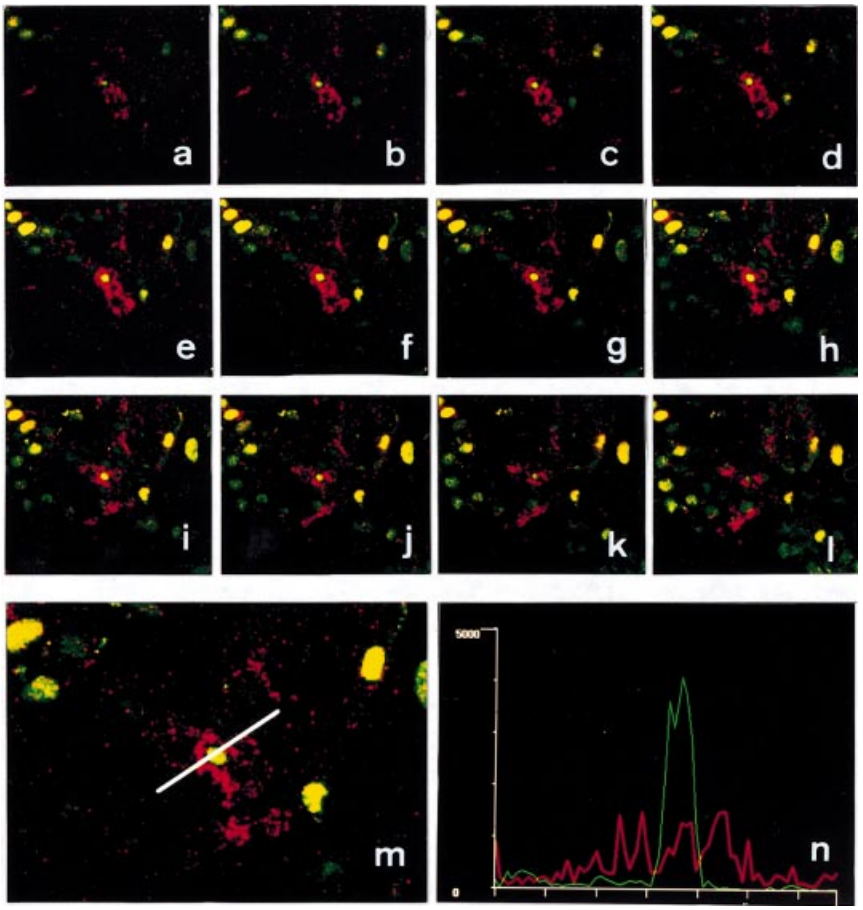

Figure 4. Double staining of apoptotic cells with specific antibody for Langerhans cells. Epidermal sheets were stained with phycoerythrin-conjugated specific monoclonal antibody for Langerhans cells and then subjected to the TUNEL technique. Sequential display of optical sections in the z-axis (*a–l*) and a histogram of analyzed images using a line probe (*m*, *n*).

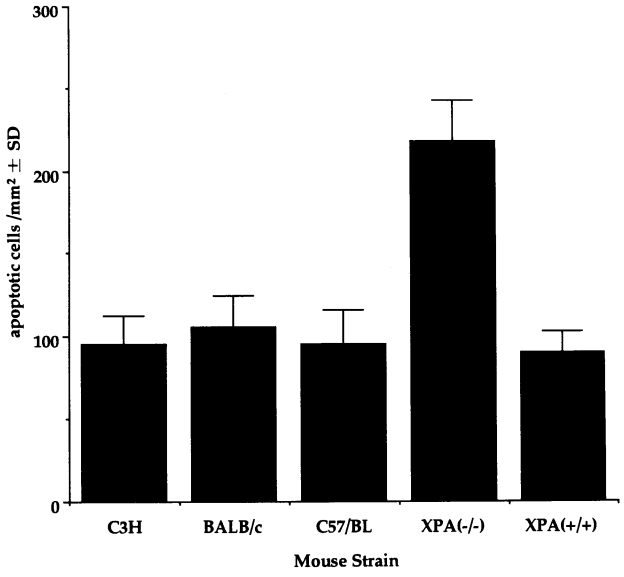

Figure 5. Apoptotic cells in various strains of mice. The density of apoptotic cells in epidermal sheets of various mouse strains after irradiation with 50 mJ per cm^2 UVB.

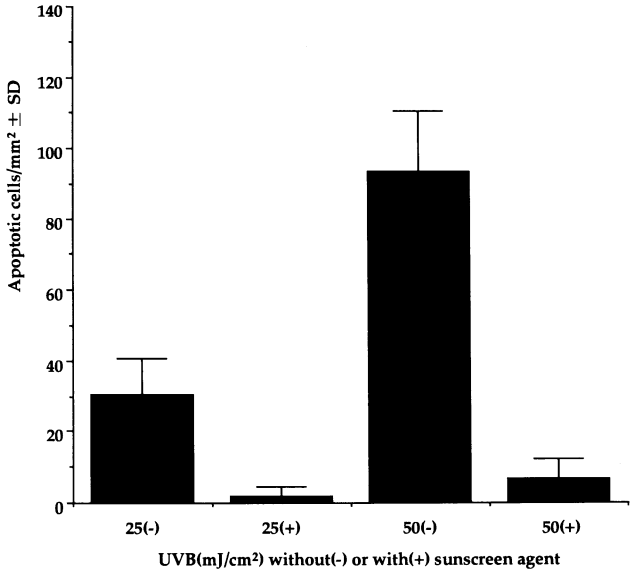

Figure 6. Effect of sunscreen agent on the induction of apoptotic cells. The density of apoptotic cells in epidermal sheets of mice pretreated (+) or not (–) with an SPF 60 sunscreen agent before irradiation with 50 mJ per cm^2 UVB.

by 50 mJ of UVB radiation per cm^2 in all conventional mice examined, C3H (94.8 ± 17.6 per mm^2), BALB/C (105.2 ± 19.7 per mm^2), and C57/BL (95.4 ± 20.1 per mm^2) mice.

XPA(–/–) mice, however, showed an apparently greater induction of the cells (216.9 ± 25.2 per mm^2) compared with the conventional or XPA(+/+) mice (89.5 ± 13.6 per mm^2) (**Fig 5**). Macroscopically, XPA(–/–) mice showed erythematous changes in 50 mJ per cm^2 of UVB irradiated skin.

Effect of sunscreen agents on the induction of apoptotic cells For further application of this TUNEL method with epidermal sheets as an evaluation of skin damage, we examined the effect of sunscreen agents on the induction of apoptotic cells by UVB radiation. **Figure 6** showed that a SPF 60 sunscreen agent was quite effective in reducing the induction of apoptotic cells.

DISCUSSION

Our study confirmed that apoptotic cells were induced in the epidermis by UVB radiation, using murine epidermal sheets stained by the TUNEL technique; 93.1 ± 17.3 per mm^2 apoptotic cells in epidermal sheets appear to be equivalent to 17 ± 7 cells per cm SBC or 26 ± 7 cells per cm apoptotic cells in vertical sections induced by 50 mJ of UVB radiation per cm^2. Compared with counting the cell number in vertical tissue sections which estimates only the areas of 4–6 μm × a section length, the examinations with epidermal sheets can cover all the areas of irradiated skin. There is also a possibility that SBC and apoptotic cells may be incorrectly counted in the vertical examinations as skin is not uniformly damaged by UVB radiation. Furthermore the comparison study of apoptotic cells and SBC in vertical sections showed that the number of apoptotic cells was higher than that of SBC, suggesting that the TUNEL technique is more sensitive for the detection of early changes of UVB-induced epidermal damage than the morphologic observation. An early appearance of apoptotic cells has been shown in thymocytes induced by toxin and hormones (Gavrieli *et al*, 1992; Waring *et al*, 1997). Thus examinations of apoptotic cells with epidermal sheets is more physiologically relevant for UVB-induced epidermal damage than previous methods such as examination of SBC in tissue section stained with hematoxylin and eosin.

The frequency of apoptotic cells was dependent on the dose of and time after UVB radiation. Apoptotic cells in epidermal sheets increased in number between 10 and 75 mJ per cm^2 UVB. When more than 100 mJ per cm^2 of UVB irradiated skin was examined, it was sometimes difficult to prepare the epidermal sheets, due to tissue damage. In an assay for SBC in tissue sections reported by Woodcock and Magnus (1976), the epidermis from 24 to 48 h after 70 mJ per cm^2 of 254 nm of UVB radiation was too damaged for satisfactory counting. Therefore, the assessment of the *in vivo* effects of UVB radiation on keratinocytes by SBC and apoptotic cells should be designed for a low dose of UVB radiation. Our technique appears to be suitable for estimating skin damage induced by less than 100 mJ of UVB radiation per cm^2. Apoptotic cells appeared within 6 h with maximum density in 24 h after UVB radiation. The previous report of semiquantitative analysis showed that SBC in mice appeared 5 h after 80 mJ per cm^2 of 300 nm UVB radiation and reached a maximum about 24 h after irradiation (Woodcock and Magnus, 1976). This time course of SBC formation has been also observed in rabbit skin (Danno and Horio, 1980).

The effect of UVB radiation on skin immunity depends on the genetic background of mice. Impaired induction of contact dermatitis following UV exposure in mice has been found to be under genetic control (Noonan and Hoffman, 1994). There was no genetic influence on epidermal apoptotic cell production by UVB radiation when various conventional mouse strains were studied. A significantly higher number of apoptotic cells, however, were induced by low doses of UVB radiation in XPA(–/–) mice than in XPA(+/+) mice. These results are in agreement with the previous findings that SBC are higher in XPA(–/–) mice than XPA(+/+) mice (Miyauchi-Hashimoto *et al*, 1996). Although the precise mechanism by which SBC are formed is unknown, keratinocytes in the process of DNA replication may be highly influenced by UVB radiation, resulting in transition into SBC (Brenner and Gschnait, 1979; Danno and Horio, 1982). XPA has a defect in nucleotide excision repair ability and an increased susceptibility to the cell damage by UVB radiation. Application of an agent increasing DNA repair has been documented to reduce the appearance of SBC (Wolf *et al*, 1995). Thus in XPA mice SBC induced by a low dose of UVB radiation are higher than in corresponding wild-type mice. Our findings suggest that DNA damage plays an important part in the generation of UVB-induced apoptosis.

Double staining with epidermal sheets allows for the identification of epidermal cells that are undergoing apoptosis. The cell type in apoptosis can be determined by flow cytometry by staining for membrane determinants and for apoptotic cells by the TUNEL technique (Sgonc *et al*, 1994). We examined whether TUNEL-positive cells included Langerhans cells or dendritic epidermal T cells using their specific antibodies. No Thy-1-positive cells (dendritic epidermal T cells) were seen in apoptosis induced by 25–75 mJ of UVB radiation per cm^2. On the other hand, Ia-positive cells (Langerhans cells) in apoptotic epidermal cells were infrequently identified in the same radiation condition. Langerhans cells are known to be sensitive to UVB radiation, resulting in the decreased number of the cells. There are several hypotheses for explaining the mechanism of decreased number of Langerhans cells by UVB radiation, including cell death, loss of membrane specificity to specific antibodies (Aberer *et al*, 1981; Humm and Cole, 1986), and migration from epidermis (Moodycliffe *et al*, 1994; Kremer *et al*, 1997). Kitajima *et al* (1996) reported that apoptosis can be induced in cultured Langerhans cells exposed to UVB radiation together with stimulation by both an antigen and T lymphocytes activated by the antigen, but not in nonactivated Langerhans cells. Most recently, Rattis *et al* (1998) demonstrated that *in vitro* UVB radiation induced apoptosis of purified human Langerhans cells. We observed apoptotic Langerhans cells were induced by *in vivo* UVB exposure to mice but its frequency was very low. Fifty millijoules of UVB radiation per cm^2 induced about 100 per mm^2 apoptotic cells and 1.5% of such cells were Ia-positive cells, meaning that only about 1.5 cells per mm^2 were apoptotic Langerhans cells in the irradiated skin. Therefore, it might be very difficult to detect apoptotic Langerhans cells in vertical sections. Although there is a possibility that there are Ia-negative apoptotic Langerhans cells, because UVB radiation can deplete Ia expression from Langerhans cells, the main mechanism by which the number of Langerhans cells is reduced by UVB radiation does not appear to be an apoptotic process, but probably necrosis or migration out of the epidermis. On the other hand, Thy-1-positive apoptotic cells were not recognized in up to 75 mJ per cm^2 UVB-irradiated skin. Thus, Langerhans cells and dendritic epidermal T cells appear to be more resistant to UVB-induced apoptosis than keratinocytes. As the contact hypersensitivity reaction to fluorescein isothiocyanate painted on 50 mJ per cm^2 of UVB irradiated skin was suppressed, apoptosis of Langerhans cells in the epidermis is not essential for UVB-induced immunosuppression.

There are two factors of biologic importance of UVB exposure to the skin: suppression of skin immunity and carcinogenesis. Although apoptotic cells are considered to be UV-damaged keratinocytes, the apoptotic process of keratinocytes is also important for removal of cells with mutations potentially involved in carcinogenic transformation (Ziegler *et al*, 1994; Bernerd and Asselineau, 1997). On the other hand, impaired Langerhans cells contribute to the immunosuppressive reaction to antigen applied to UVB-irradiated skin. Therefore, quantitative assessment as well as analysis at the cellular level of epidermal cell injuries is of significance in understanding the UVB effects on the skin. The TUNEL technique with epidermal sheets is beneficial for quantitative assessment of apoptotic keratinocytes and further investigation of Langerhans cell apoptosis.

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