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Despite the Presence of UVB-Induced DNA Damage, HLA-DR$^+$ Cells from *Ex Vivo* UVB-Exposed Human Skin Are Able to Migrate and Show No Impaired Allostimulatory Capacity

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In this study, we investigated the effect of ultraviolet B radiation on human Langerhans cell function. Normal human skin was irradiated *ex vivo* with single doses of ultraviolet B. For assessment of T-cell stimulatory function, cells that spontaneously migrated from epidermal sheets were used, whereas full-thickness skin biopsies were used to investigate alterations in migratory properties. The cells migrating from ultraviolet B-exposed epidermal sheets demonstrated a decrease in the percentage of HLA-DR positive Langerhans cells, as well as a reduced capacity to induce proliferation of allogeneic T cells, when compared with cells migrating from nonexposed sheets. When a correction was made for the decreased number of HLA-DR positive Langerhans cells migrating from ultraviolet B-exposed epidermis, however, it appeared that the capacity to induce T-cell proliferation was identical for Langerhans cells migrating from ultraviolet B-exposed and nonexposed epidermis. The presence of ultraviolet B-induced DNA damage could be demonstrated in the Langerhans cells from ultraviolet B-treated skin, indicating that the cells had received significant doses of ultraviolet B. As regards the effect of ultraviolet B on migratory properties of Langerhans cells, we found not only that reduced numbers of CD1a-positive Langerhans cells migrated from the ultraviolet B-exposed full-thickness skin, but also that there was a reduction in CD1a-positive Langerhans cells in the epidermis. This implies that ultraviolet B induces death of Langerhans cells as well as loss of cell surface molecules rather than altering Langerhans cells migration, whereas the Langerhans cells that were still able to migrate fully retained the capacity to activate allogeneic T cells. ***Key words: antigen presentation/skin organ culture/thymine dimers/ultraviolet. J Invest Dermatol 109:626–631, 1997***

Exposure of the skin to ultraviolet B (UVB) radiation causes suppression of cellular immune responses *in vivo*. UVB-irradiated mice fail to reject highly antigenic tumors (Fisher and Kripke, 1977; Kripke, 1990) and a decreased contact hypersensitivity response has been shown both in mice and in humans upon UVB exposure (Toews *et al*, 1980; Cooper *et al*, 1992). Furthermore, a long-lasting antigen-specific immunologic unresponsiveness can be acquired when antigen is applied on UVB-exposed human or murine skin, a phenomenon that in mice has been shown to be caused by the appearance of antigen-specific T suppressor cells (Elmets *et al*, 1983; Cooper *et al*, 1992).

One of the mechanisms by which UVB impairs skin immune responses appears to be the modulation of the function of the antigen-presenting cells (APC) of the epidermis, i.e., Langerhans cells that are highly sensitive to the effects of UVB. The reduced contact hypersensitivity response observed after treatment of murine or human skin with a single low dose of UVB *in vivo* is accompanied by a dose-dependent decrease in the number of ATP-ase and major histocompatibility complex class II positive cells (Toews *et al*, 1980; Hanau *et al*, 1985; Koulu *et al*, 1985). Depletion of Langerhans cells seems to be critically involved in the UVB-induced immunomodulation because recovery of the contact hypersensitivity response occurred at the same time that the number of Langerhans cells returned to normal (Toews *et al*, 1980). The function of Langerhans cells isolated from UV-exposed skin immediately after UVB treatment (before depletion occurs) is impaired: the capacity of epidermal cell suspensions from *in vivo* or *ex vivo* UV-exposed skin to stimulate allogeneic or antigen-specific T cells *in vitro* is reduced (Lynch *et al*, 1983; Cooper *et al*, 1985; Tang and Udey, 1991; El-Ghorr *et al*, 1994). Furthermore, when epidermal cells from UV-exposed mice are haptenated and subsequently injected into naïve syngeneic mice, a state of immunologic tolerance is acquired (Sauder *et al*, 1981). Epidermal cells irradiated *in vitro* with UVB also have an impaired capacity to stimulate allogeneic or antigen-specific T-cell proliferation (Stingl *et al*, 1981; Aberer *et al*, 1982; Austad and Braathen, 1985) and UVB-exposed murine Langerhans cells have been shown to induce anergy in Th1 clones (Simon *et al*, 1991).

The depletion of Langerhans cells from the epidermis after *in vivo* UVB exposure may be caused by several, not mutually exclusive, mechanisms, such as enhanced migration of Langerhans cells or cell death. Electron microscopy studies of UVB-irradiated skin revealed the presence of residual cells with Langerhans cells characteristics although with morphologic alterations, such as loss of dendrites and a

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Abbreviations: APC, antigen-presenting cells; MLR, mixed leukocyte reaction; PBS, phosphate-buffered saline; UVB, ultraviolet B.

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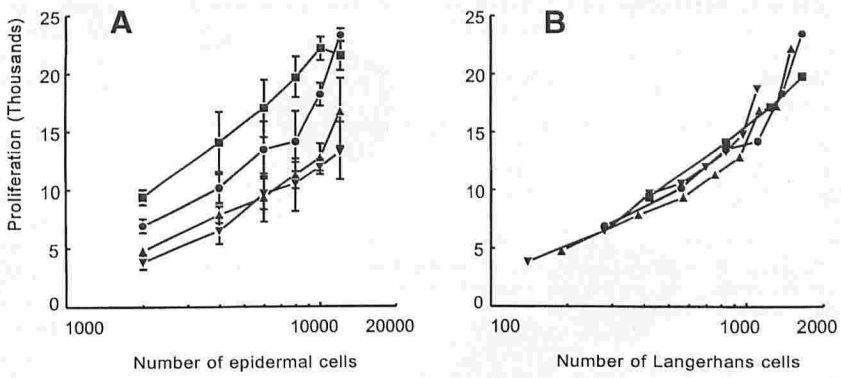

Figure 1. UVB irradiation of epidermal sheets does not impair Langerhans cells function. Epidermal sheets were irradiated with graded single doses of UVB and cultured overnight. Cells migrating from the sheets were harvested from the culture medium and used as stimulators in an MLR. Proliferation of responding allogeneic T cells was assessed by incorporation of ^{3}H-TdR during the last 18 h of a 6-d culture and expressed as counts per minute ± SD (n = 2). (*A*) Proliferation of T cells plotted against total number of epidermal cells. (*B*) Proliferation of T cells plotted against the number of DR$^+$ Langerhans cells within the stimulator epidermal cell suspensions. ■, 0 J per m^2; ●, 200 J per m^2; ▲, 400 J per m^2; ▼ 800 J per m^2.

rounded shape (Aberer *et al*, 1981). This indicates that Langerhans cells are partly retained as viable cells within the epidermis, but are not detectable by immunostaining due to downregulation of their characteristic cell surface molecules (Aberer *et al*, 1981). Interestingly, after *in vitro* irradiation of epidermal cells, no reduction is seen in the expression of HLA-DR or CD1a on Langerhans cells. This shows that the functional impairment of Langerhans cell function by *in vitro* UVB exposure of epidermal cell suspensions is not caused by downregulation of HLA-DR expression (Czernielewski *et al*, 1984; Rattis *et al*, 1995). Thus, *in vitro* irradiation of single cell suspensions is probably not an accurate system by which to study *in vivo* effects of UVB on Langerhans cells function. Only limited information exists on the functional capacity of Langerhans cells irradiated within intact skin. To be able to investigate the modulation by UVB of Langerhans cell function in their natural environment of the skin, we have used *ex vivo* model systems: epidermal sheets and skin biopsies derived from normal human skin. The present study shows that Langerhans cells spontaneously migrating from UVB-exposed epidermal sheets do not have an impaired function as stimulators of the mixed leukocyte reaction (MLR), even though a dose-dependent increase in UVB-induced DNA damage could be demonstrated. Langerhans cells with DNA damage were also able to migrate out of full-thickness skin biopsies, indicating that DNA damage does not prevent migration.

MATERIALS AND METHODS

UVB irradiation of skin specimens Normal human skin was obtained from patients undergoing reconstructive surgery of the breast or abdomen. Skin sheets of 0.4 mm (for epidermal sheets) or 0.6 mm (for skin organ cultures) were obtained using a keratome. For preparation of epidermal sheets, the skin was incubated in 0.30% dispase (Sigma, St Louis, MO) for 30 min at 37°C to separate epidermis from dermis. Epidermal sheets were then put in petri dishes in phosphate-buffered saline (PBS) (dermal side down) and irradiated with Philips TL12 lamps as described previously for cell suspensions (Kremer *et al*, 1995). These lamps emit UV primarily in the UVB range (280–320 nm) and have an output of 1 J per m^2 per s at a distance of 40 cm. After irradiation, the epidermal sheets were transferred to petri dishes containing culture medium: IMDM (Gibco, Paisley, U.K.) supplemented with 5% heat-inactivated human serum and gentamycin (50 μg per ml; Sigma). The skin organ cultures were slightly modified from the method described previously by Le Poole *et al* (1994). Briefly, 6-mm skin biopsies were cut from the 0.6-mm keratomed sheets and cultured on stainless steel grids with the support of Millipore filter paper (pore size 1.2 μm), epidermal side up. The whole system was placed in petri dishes containing PBS during UVB irradiation and these were then placed in culture medium. To determine the number of cells migrating out of full-thickness (epidermis plus dermis, 0.6 mm) skin sheets were floated on culture medium in petri dishes, epidermal side up.

Preparation of Langerhans cells-enriched cell suspensions Epidermal sheets were cultured overnight and the cells that migrated out of the sheets were harvested from the culture medium and filtered through gauze to obtain a single cell suspension. Epidermal cells were enriched for Langerhans cells by centrifugation on a Nycodenz (1.138 g per ml; Nyegaard, Oslo, Norway) gradient; 62.5% Nycodenz in IMDM with 2% fetal bovine serum. Viability of cells harvested from the interface was > 90%. The percentage of HLA-DR$^+$ cells (Langerhans cells) within the epidermal cell suspensions was determined by flow cytometry, as described previously (Kremer *et al*, 1995). The epidermal cells were also analyzed by immunohistochemistry of cytospin preparations, using CD1a (Becton Dickinson; Mountain View, CA), HLA-DR (Becton Dickinson), NKI-beteb (Sanbio, The Netherlands), and CK1 (DAKO, Glostrup, Denmark) as primary antibodies and peroxidase conjugated rabbit anti-mouse (DAKO) as secondary antibody. In order to estimate the percentage of positive cells, at least 1000 cells per cytospin were counted.

MLR T cells were isolated from heparinized blood of healthy human volunteers as described previously (Kremer *et al*, 1995) by centrifugation on Lymphoprep (1.077 g per cm^3; Nycomed, Oslo, Norway) and Percoll (Pharmacia, Uppsala, Sweden), followed by rozetting to amino-ethyl-isothiouronium bromide (Sigma) treated sheep erythrocytes and incubation on plastic petri dishes. Proliferation assays were performed by incubating 1×10^5 T cells with 2–15 $\times 10^3$ γ-irradiated epidermal cells in round-bottom 96-well plates for 6 d, the last 18 h in the presence of 0.3 μCi ^{3}H-TdR per well (Amersham, Aylesbury, U.K.). Incorporation of the isotope was determined by liquid scintillation counting.

Detection of UVB-induced thymine dimers in Langerhans cells Cytospins of Langerhans cells migrating from epidermal sheets were double-stained with anti-HLA-DR and H3 (Roza *et al*, 1991), an antibody specific for thymine dimers in DNA (a kind gift of Dr. L. Roza; T.N.O., Rijswijk, The Netherlands) as described previously (Sontag *et al*, 1995). First, acetone-fixed slides were incubated with anti-HLA-DR for 60 min. After rinsing in PBS, slides were incubated with rabbit-anti-mouse alkaline phosphatase (DAKO) for 30 min. alkaline phosphatase activity was visualized by incubation in a freshly made solution of 5-bromo-4-chloro-indolyl-phosphate-4-nitro blue tetrazolium chloride in 10 ml 0.1 M Tris-HCl (pH 9.5) and levamisole (Sigma). Slides were fixed in acetic acid 25% (vol/vol) with 0.02% H_2O_2 for 10 min, followed by rinsing in PBS and incubation in 0.07 N NaOH in 70% EtOH for 2 min. Slides were washed and incubated with 10% normal mouse serum for 20 min, immediately followed by incubation with the biotin-conjugated murine anti-thymine dimer mAb H3 for 60 min. Staining was visualized by incubation with an avidin-biotin complex conjugated with HRP (DAKO) for 60 min, followed by incubation in freshly made 3-amino-9-ethylcarbazole (Sigma) for 15 min.

RESULTS

Dose-dependent reduction of the MLR induced by UV-exposed epidermal cells To study the effect of UVB on human Langerhans cell function in the natural skin environment, human epidermal sheets were exposed to graded single doses of UVB radiation *in vitro*. Langerhans cells spontaneously migrate from the sheets during overnight culture and can be harvested from the culture medium, avoiding trypsin treatment of the cells. When cells migrating from UVB-exposed epidermal sheets were used as stimulators in the MLR, a UVB dose-dependent reduction was seen in the proliferation of the responding allogeneic T cells (**Fig 1*a***). This result is in agreement with previously published studies using UVB-irradiated (*in vivo*, *ex vivo*, or *in vitro*) Langerhans cells (Lynch *et al*, 1983; Cooper *et al*, 1985; Tang and Udey, 1991; El-Ghorr *et al*, 1994).

Stimulatory function of HLA-DR$^+$ Langerhans cells from UV-exposed epidermal sheets not impaired Next, we wanted to investigate whether exposure of the epidermal sheets to UVB had an effect on the capacity of Langerhans cells to migrate out of the

Table I. Percentage of HLA-DR positive cells is decreased after UVB[a]

UVB dose (J per m^2)	DR^+ cells (%)	± SD
0	20.7	0.2
200	13.5	1.5
400	8.8	0.9
800	6.7	0.5

[a]Epidermal cells were obtained as described for **Fig 1** and analyzed for HLA-DR expression by flow cytometry. The percentage of $HLA\text{-}DR^+$ cells ± SD is shown in the total epidermal cell suspension (n = 2).

Table II. UVB induces dose-dependent increase in thymine dimers in Langerhans cells[a]

UVB dose (J per m^2)	Thymine-dimer staining Langerhans cells (% of DR^+ cells)			
	–	+	++	+++
0	100 ± 0.0	0 ± 0.0	0 ± 0.0	0 ± 0.0
200	88	9	2	1
400	66 ± 18.3	13 ± 2.8	8 ± 3.7	13 ± 11.6
800	55 ± 2.1	16 ± 2.0	7 ± 2.5	22 ± 6.7

[a]Cytospins were made of cells harvested from the culture medium of UVB exposed or nonexposed control epidermal sheets and doublestained with mAb for HLA-DR and thymine dimers (H3). Thymine-dimer positive cells were divided into three groups on the basis of intensity of staining: +, slightly positive; + +, medium positive; + + +, strongly positive. Results for thymine-dimer staining are presented as percentage ± SD of $HLA\text{-}DR^+$ cells (n = 2).

epidermal sheets. Therefore, we performed HLA-DR staining to determine whether the percentage of Langerhans cells in the cell suspensions migrating from UVB-exposed epidermal sheets was reduced as compared with control sheets. Because epidermal sheets were used and no dermal component was present we could be sure that all HLA-DR positive cells in the suspensions were Langerhans cells of epidermal origin. After exposure of the epidermal sheets to UVB, the migrating cells contain a decreased percentage of HLA-DR positive cells (**Table I**). The mean fluorescence intensity of HLA-DR on the positive cells, however, was not affected by UVB (data not shown). This finding prompted us to re-evaluate the previously described MLR data, because the capacity of these cells to stimulate an MLR is related to the number of majorhistocompatability complex class II positive cells. We calculated the exact number of HLA-DR positive Langerhans cells present in the UV-exposed stimulator cell suspensions (**Fig 1*b***). This correction clearly shows that on a per cell basis the capacity to induce T-cell proliferation is identical for Langerhans cells migrating from the UV-exposed and nonexposed control sheets. Characterization of the HLA-DR negative population by immunohistochemistry revealed no significant differences in the composition of this HLA-DR negative population between the irradiated and the unirradiated groups. This population contained 4.2 ± 1.1% T cells ($CD3^+$) and 4.8 ± 1.8% melanocytes ($NKI\text{-}beteb^+$), with the remainder of the cells being keratinocytes ($CK1^+$). Furthermore, none of the HLA-DR negative cells exhibit the morphology of dendritic cells. Thus, it does not seem to be likely that the UVB-induced reduction in percentage of HLA-DR is due to downregulation of HLA-DR or the appearance of HLA-DR negative Langerhans cells.

Thymine-dimer formation in UV-exposed Langerhans cells is not correlated with APC function It is possible that the APC function of the Langerhans cells migrating from the UV-exposed epidermal sheets was retained because they were protected during the irradiation procedure by the surrounding keratinocytes. To determine whether these Langerhans cells had actually received UVB radiation we determined the amount of DNA damage in the migrating epidermal cells. Cytospins of epidermal cells were double-stained with an mAb recognizing UV-induced thymine-dimer formation and HLA-DR to identify Langerhans cells. As expected, migrating cells from nonexposed skin did not contain any thymine dimers, but upon exposure to UVB, thymine dimers could clearly be observed in a considerable number of $HLA\text{-}DR^+$ Langerhans cells (**Table II** and **Fig 2*a–d***). Cells staining positively for the thymine dimers were divided into four groups on the basis of the intensity of the staining (–, not stained; +, slightly positive; + +, medium positive; + + +, strongly positive). The thymine-dimer staining dose-dependently increased (**Table II**); not only was the percentage of HLA-DR positive cells containing dimers larger when a higher UV dose was administered, but also the intensity of the staining increased, indicating an increase in the number of dimers per cell. These results show that the Langerhans cells derived from the UV-exposed epidermal sheets had received enough UVB to induce significant DNA damage. Combining this observation with the data from **Fig 1*b*** we may conclude that the presence of DNA damage in the Langerhans cells does not lead to functional impairment concerning their capacity to stimulate allogeneic T cells.

UVB-induced DNA damage does not prevent migration of Langerhans cells UVB exposure of the skin has been reported to influence the migratory properties of epidermal Langerhans cells; however, conflicting studies describe either an enhancement or an inhibition of Langerhans cells migration after UVB (Mommaas *et al*, 1993; Moodycliffe *et al*, 1994). The epidermal sheets used in the previous experiments were not useful for experiments concerning migration of Langerhans cells, because most Langerhans cells migrate out of the sheets spontaneously during overnight culture. Therefore, we used a full-thickness skin organ culture model containing an epidermal as well as a dermal component, in which it was previously demonstrated that a normal location and number of Langerhans cells was retained up to 3 d of culture (Rambukkana *et al*, 1995). Irradiation of skin biopsies with UVB did not have an effect on the number of Langerhans cells, as determined by CD1a staining, in the epidermis at 6 h after the exposure. At 24 h after UVB treatment of the biopsies, a concentration-dependent decrease of $CD1a^+$ cells was observed, which became more apparent at 48 h after UVB exposure (**Fig 3**). This reduction in $CD1a^+$ cells may be explained by an accelerated migration of Langerhans cells out of the biopsies; however, a decreased number of APC, as identified by CD1a or HLA-DR, was harvested from the culture medium of UVB-exposed full-thickness skin sheets that were cultured for 2 d. This latter finding obviously does not support the possibility of accelerated migration. The presence of thymine dimers could be identified in the epidermis-derived $CD1a^+$ cells migrating from the full-thickness sheets (21 ± 7% of $CD1a^+$ cells were H3 positive; **Table III**). Furthermore, 24 h after irradiation of skin biopsies with UVB, the CD1a-positive Langerhans cells were found exclusively in a basal location, in contrast to the predominant suprabasal location in nonirradiated skin (data not shown). Thus, a subset of UVB-exposed and thymine-dimer positive Langerhans cells exists that still has intact migratory properties.

DISCUSSION

In this study we demonstrate that UVB-irradiation of human epidermal sheets do not impair the capacity of the migrating Langerhans cells to activate allogeneic T cells, but rather decrease the number and percentage of HLA-DR positive migrating Langerhans cells. A dose-dependent decrease in the allostimulatory function was seen if no correction was made for the reduced numbers of $HLA\text{-}DR^+$ cells in the epidermal cell suspensions. These data seem to contradict two previous studies in which epidermal cells from *ex vivo* irradiated human (Cooper *et al*, 1985) or murine (Tang and Udey, 1991) skin showed a reduced capacity to activate allogeneic T cells; however, in these studies single cell suspensions were obtained by trypsin treatment of the epidermal sheets immediately after UVB exposure, whereas we used the epidermal cells that spontaneously migrate from UVB-irradiated epidermal sheets during overnight culture. It may be that in our experimental set-up, we excluded the severely damaged Langerhans cells with reduced APC function that were not capable of spontaneous migration and those that died during the overnight culture. Thus we

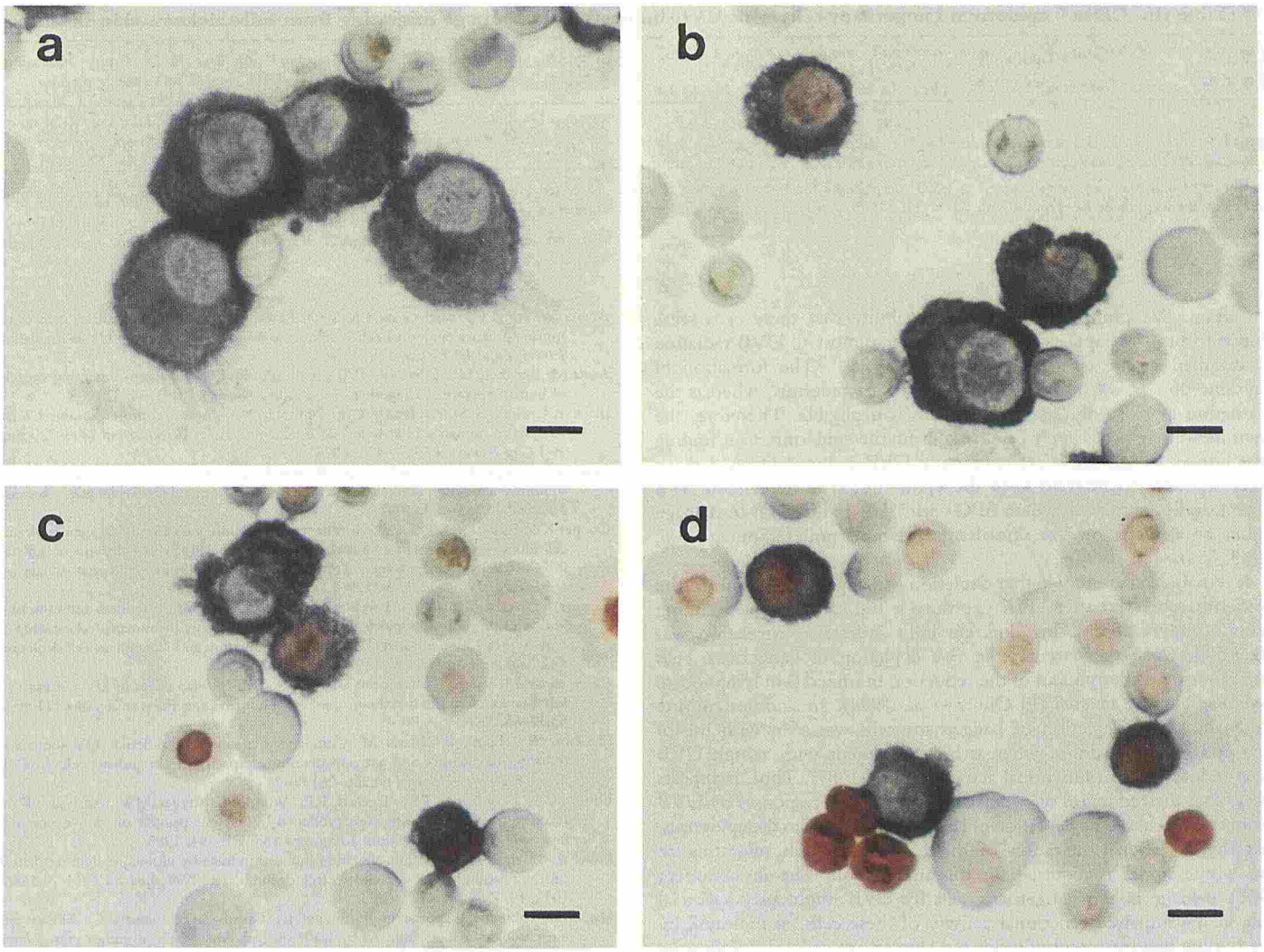

Figure 2. UVB induces dose-dependent increase in thymine dimers in Langerhans cells. Representative photographs of nonexposed (*a*) or UVB-exposed (*b*, 200 J per m^2; *c*, 400 J per m^2; *d*, 800 J per m^2) epidermal cells stained with mAb specific for HLA-DR (blue staining) and thymine dimers (red staining), as described for **Table II** (n = 2). A UVB dose-dependent increase in the intensity of the red staining can be observed for the blue stained, HLA-DR^+ cells. *Scale bars*, 12.5 μm.

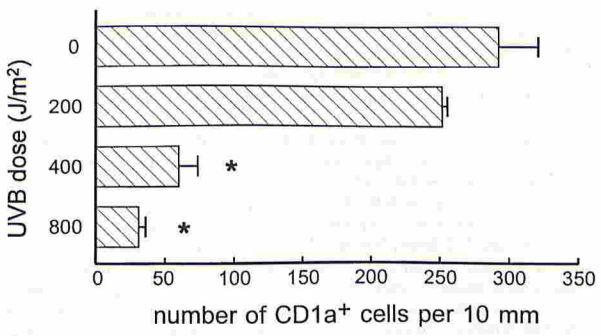

Figure 3. UVB exposure of skin biopsies reduces number of $CD1a^+$ epidermal Langerhans cells. Skin biopsies (0.6 mm) were exposed to UVB and cultured for 48 h on stainless steel grids after which biopsies were snap frozen in liquid nitrogen. Cryostat sections (0.6 μm) were stained with CD1a and the number of positive cells per 10 mm was counted (n = 2; error bars, mean ± SD; * $p < 0.01$).

selected for a population of viable stimulator Langerhans cells that were fairly resistant to UVB.

Different mechanisms may be responsible for the reduced number of HLA-DR^+ cells migrating from UVB-exposed epidermal sheets: (i) cell death due to photo-toxic effects of UVB on Langerhans cells, (ii) downregulation of Langerhans cells specific surface marker expression, or (iii) reduced migration of Langerhans cells out of epidermal sheets. Using two different *ex vivo* model systems (epidermal sheets and full-thickness skin), we observed decreased numbers of Langerhans cells in the culture medium of irradiated skin explants, suggesting that the migratory properties were impaired. This result is in agreement with a recent study by Richters *et al* (1996) in which spontaneous migration of Langerhans cells out of cultured skin could be inhibited by UVB exposure. Using cryostat sections of UVB-exposed biopsies, however, we found that the numbers of $CD1a^+$ Langerhans cells in the epidermis of the cultured UVB-exposed biopsies were reduced as well. This suggests that only for a subset of Langerhans cells were the migratory properties not affected and that in the rest of the Langerhans cells population downregulation of Langerhans cells surface molecules or cell death had occurred. Downregulation of CD1a expression on Langerhans cells by UVB has previously been shown using electron microscopy of human skin irradiated with UVB *in vivo* (Aberer *et al*, 1981); a residual population with Langerhans cells characteristics could be observed that lacked staining with ATP-ase or HLA-DR. Our analysis of the cells that migrated from the UVB-exposed full-thickness explants revealed that the HLA-DR^+/thymine-$dimer^+$ cells outnumbered the $CD1a^+$/thymine-$dimer^+$ cells, making the epidermal origin of the former double positive cell population

Table III. CD1a$^+$ epidermal Langerhans cells with UVB-induced DNA damage migrating from full-thickness skin sheets[a]

UVB (J per m^2)	Total cells (per cm^2)	CD1a$^+$ cells (per cm^2)	DR$^+$ cells (per cm^2)	H3$^+$ (% of CD1a$^+$)	H3$^+$ (% of DR$^+$)
0	48,000	2,304 ± 528	39,984 ± 2707	0 ± 0	0 ± 0
800	18,667	429 ± 168	9,707 ± 27	21 ± 7	8 ± 1

[a]Sheets of skin (0.6 mm) were exposed to UVB and cultured for 48 h. Migrating cells were harvested from the culture supernatant. Total number of migrating cells was determined and cytospins were made for staining with CD1a, HLA-DR, and H3. Results are presented as number of cells per cm^2 skin ± SD (n = 2).

uncertain. We cannot rule out the possibility that these cells were derived from the papillary dermis; however, almost all UVB radiation is absorbed in the epidermis (Bruls *et al*, 1984). The formation of thymine dimers will usually take place in the epidermis, whereas the formation of these dimers in the dermis is negligible. Therefore, the thymine-dimer$^+$ cells likely originated from the epidermis. Our finding that human Langerhans cells containing UVB-induced DNA damage were still able to migrate from the epidermis, is in agreement with recent studies in mice in which APC with UVB-induced DNA damage could be detected in the skin-draining lymph nodes (Sontag *et al*, 1995; Vink *et al*, 1996).

It remains uncertain whether depletion of Langerhans cells from the epidermis observed after UVB exposure is the cause of the resulting immunosuppression. Using different light sources a correlation was shown between the extent of *in vivo* depletion of Langerhans cells from murine epidermis and of the reduction in mixed skin lymphocyte response activity *in vitro* (El-Ghorr *et al*, 1994). In another murine study, however, depletion of Langerhans cells was seen with use of low and high UV doses, whereas only treatment with a high UVB dose induced loss of functional activity (Orita, 1987). Thus, it appears likely that the reduction of contact hypersensitivity responses by UVB is not merely caused by depletion of Langerhans cells from the epidermis, but that functional alteration of the Langerhans cells migrating to the lymph nodes is required for this suppression. The induction of DNA damage in the Langerhans cells by UVB might play a crucial role in the impaired functional activity of these cells, as indicated by the finding that treatment of irradiated Langerhans cells with liposomes containing T4 endonuclease immediately after exposure could restore the antigen-presenting function (Vink *et al*, 1996). This finding that Langerhans cells with UVB-induced DNA damage show an impaired stimulatory function seems to contradict our results. A possible explanation for this discrepancy might be that the requirements for allostimulation are less stringent than those for antigen processing and presentation to antigen-specific T cells. The modulation of Langerhans cells function may also be caused by indirect effects of UVB on Langerhans cells function, e.g., by an increased production of suppressive mediators such as IL-10 or PGE_2 (Black *et al*, 1978; Kang *et al*, 1994). These direct or indirect effects of UVB can affect the expression of co-stimulatory molecules that are involved in T-cell activation, such as B7–1 and B7–2 or ICAM-1 (Tang and Udey, 1991; Weiss *et al*, 1995), or decrease the production of stimulatory cytokines, such as IL-12, by the Langerhans cells.[1]

From our study it appears that a subset of Langerhans cells is resistant to the effects of UVB with regard to migration and to activation of allogeneic T cells, even though UVB-induced formation of thymine dimers can clearly be demonstrated in these cells. Although these UVB-exposed Langerhans cells normally stimulate an MLR *in vitro*, the differentiation of responding T cells *in vivo* may be altered with respect to, for example, cytokine production.

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