Localization of DNA Damage and Its Role in Altered Antigen-presenting Cell Function in Ultraviolet-irradiated Mice

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Summary

Prior ultraviolet (UV) irradiation of the site of application of hapten on murine skin reduces contact sensitization, impairs the ability of dendritic cells in the draining lymph nodes (DLN) to present antigen, and leads to development of hapten-specific suppressor T lymphocytes. We tested the hypothesis that UV-induced DNA damage plays a role in the impaired antigen-presenting activity of DLN cells. First, we assessed the location and persistence of cells containing DNA damage. A monoclonal antibody specific for cyclobutyl pyrimidine dimers (CPD) was used to identify UV-damaged cells in the skin and DLN of C3H mice exposed to UV radiation. Cells containing CPD were present in the epidermis, dermis, and DLN and persisted, particularly in the dermis, for at least 4 d after UV irradiation. When fluorescein isothiocyanate (FITC) was applied to UV-exposed skin, the DLN contained cells that were Ia^+, $FITC^+$, and CPD^+; such cells from mice sensitized 3 d after UV irradiation exhibited reduced antigen-presenting function in vivo. We then assessed the role of DNA damage in UV-induced modulation of antigen-presenting cell (APC) function by using a novel method of increasing DNA repair in mouse skin in vivo. Liposomes containing T4 endonuclease V (T4N5) were applied to the site of UV exposure immediately after irradiation. This treatment prevented the impairment in APC function and reduced the number of CPD^+ cells in the DLN of UV-irradiated mice. Treatment of unirradiated skin with T4N5 in liposomes or treatment of UV-irradiated skin with liposomes containing heat-inactivated T4N5 did not restore immune function. These studies demonstrate that cutaneous immune cells sustain DNA damage in vivo that persists for several days, and that FITC sensitization causes the migration of these to the DLN, which exhibits impaired APC function. Further, they support the hypothesis that DNA damage is an essential initiator of one or more of the steps involved in impaired APC function after UV irradiation.

The ability of UV irradiation to interfere with the induction of contact hypersensitivity (CHS)[1] responses is well documented, both in mice (1, 2) and in humans (3, 4). Application of a contact-sensitizing hapten to the UV-irradiated skin of certain strains of mice (2) leads to a decreased CHS response and the induction of specific immunologic tolerance transferable by splenic T cells (1, 2). Numerous investigations over the past decade have addressed the cellular mechanisms of this phenomenon. Although not all steps have been fully delineated, certain events have been shown to contribute to the modification of immune responsiveness. Epidermal Langerhans cells (LC), the primary APC of the epidermis, are altered morphologically and functionally after exposure to sublethal doses of UV radiation (5), and in vitro UV irradiation of these cells impairs their ability to activate T helper 1 cells (6). Hapten-bearing LC and macrophages collected from the draining lymph nodes (DLN) of mice sensitized through UV-irradiated skin are deficient in their ability to induce CHS responses in vivo (7). Macrophages that infiltrate the skin several days

[1] *Abbreviations used in this paper:* CHS, contact hypersensitivity; CPD, cyclobutyl pyrimidine dimer(s); DLN, draining lymph node(s); FBS, fetal bovine serum; LC, epidermal Langerhans cell(s); PUVA, 8-methoxypsoralen plus UVA (320-400 nm) radiation; T4N5 liposomes, liposomes containing T4 endonuclease V; UCA, urocanic acid.

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after UV irradiation differ from normal LC in their antigen-presenting activity and may be responsible for T suppressor cell induction (8). TNF-α may play a central role in modifying this cellular response because administration of antibody against this cytokine permits the development of CHS in UV-irradiated mice (9, 10).

Along with the progress in our understanding of the cellular events leading to immune suppression, there has been increasing interest in delineating the precise molecular pathways that initiate these events. A theory that has received much attention involves the photo-isomerization of urocanic acid (UCA), which is a metabolite of the histidine synthesis pathway (11). This metabolite is abundant in the stratum corneum and is isomerized from the *trans* to the *cis* configuration upon exposure to UV radiation. *Cis*-UCA can be found in the circulation after the skin is exposed to UV radiation (12), and it has immunosuppressive activity, most notably, the ability to interfere with the induction of delayed-type hypersensitivity (DTH) to herpes simplex virus (13). *Cis*-UCA also causes morphological alterations in LC that resemble those induced by UV radiation (14). These and other observations support the hypothesis that UCA is a photoreceptor in the skin that transforms radiant energy into an immunosuppressive signal, ultimately impairing the induction of certain immune responses (15). The cellular target of *cis*-UCA and its relationship to cytokine mediators of immune suppression, such as TNF-α, remain undefined.

An equally compelling hypothesis is that DNA damage is the initiating event for immune suppression. Support for this idea comes from studies of the South American opossum *Monodelphis domestica*, whose cells contain a photoreactivating enzyme that absorbs visible light to repair UV-induced cyclobutyl pyrimidine dimers (CPD) in DNA. In these marsupials, exposure of the skin to visible light after UV irradiation prevents UV-induced local suppression of CHS (16) and altered morphology of LC (17), presumably by reversing CPD.

A third possibility was suggested by recent studies demonstrating that exposing cells in vitro to UV radiation can, by a DNA-independent mechanism, activate transcription factors such as AP-1 and nuclear factor κB (NFκB), which in turn increase production of TNF-α and other immunoregulatory and pro-inflammatory cytokines (18, 19). Thus, it is not clear at present which molecular events trigger the various forms of UV-induced immune suppression (e.g., local or systemic suppression of the induction or elicitation of CHS and DTH responses, inhibition of tumor rejection, etc; 1). Even within a single model of immune suppression, such as that described above, different steps in the pathway suppressor T cell leading to induction may be initiated by different molecular events.

In studies reported here, we examined the role of DNA damage in a particular step of the pathway of UV-induced local suppression of CHS, namely, the alteration in the function of cutaneous APC that migrate to the DLN after epicutaneous sensitization. As a first step, we used a monoclonal antibody specific for CPD to assess the presence and persistence of cells with UV-damaged DNA in the skin and DLN of UV-irradiated C3H mice. We next determined whether the CPD^+ cells in the DLN were skin-derived immune cells that carry antigen to the DLN upon sensitization. Finally, we employed the DNA excision repair enzyme T4 endonucliase V encapsulated in liposomes (T4N5 liposomes) as a novel method of increasing DNA repair in murine skin in vivo, to investigate the role of DNA damage in UV-induced impairment of cutaneous APC function. In previous studies, we demonstrated that the liposomes are taken up by keratinocytes and LC in murine skin and that the endonuclease appears in both the nucleus and cytoplasm of these cells (20). Application of these T4N5 liposomes immediately after UV irradiation increases the rate of DNA repair in the skin (21, 22). Therefore, we used this approach to determine whether treatment of UV-irradiated skin with T4N5 in liposomes would decrease the number of CPD^+ dendritic cells in the DLN and restore APC function.

Materials and Methods

Mice. Specific pathogen-free female C3H/HeNCr(MTV^-) mice were supplied by the Animal Production Area of the Frederick Cancer Research Facility (Frederick, MD) or Charles River Breeding Laboratories, Inc. (Wilmington, MA). Specific pathogen-free, SCID mice were purchased from Harlan Sprague Dawley, Inc. (Indianapolis, IN). The mice were housed in a pathogen-free barrier facility accredited by the American Association for Accreditation of Laboratory Animal Care, in accordance with current U.S. Department of Agriculture, Department of Health and Human Services, and National Institutes of Health (NIH) regulations and standards. All animal procedures were approved by the Institutional Animal Care and Use Committee. The mice were given free access to NIH formula 31 mouse chow and sterilized water. Ambient lighting was controlled to provide regular 12-h light/12-h dark cycles. 10–12-wk-old, age-matched mice were used in the experiments.

T4N5 Liposomes. T4N5 liposomes were prepared by encapsulating purified T4N5 in liposomes as described previously (21, 23). Control preparations of liposomes contained boiled (enzymatically inactive) T4N5 (23). The liposomes were suspended in a 1.5% hydrogel lotion (Carbopol-941; BF Goodrich, Akron, OH) made with PBS and applied to mouse skin with a moist cotton swab. Immediately after UV irradiation, 250 μl of liposome suspension containing 0.5 μg T4N5 per ml of lotion was applied to the UV-irradiated skin of the mice.

UV Irradiation. UV irradiation was carried out using a bank of six FS40 sunlamps (National Biological Corp., Twinsburg, OH), as previously described (24). The irradiance of the source was ~9 W/m^2. The dorsal fur of groups of mice was removed with electric clippers, and, unless otherwise indicated, the animals were exposed to 5 kJ/m^2 UVB radiation, which is approximately twice the minimal erythemal dose for this strain (24). Except for exposure to UV radiation, control mice were treated exactly the same as UV-irradiated mice. For experiments involving psoralen plus UVA (PUVA) treatment, 400 μl of 2 mg/ml 8-methoxypsoralen in 70% ethanol was applied to shaved dorsal skin of mice. After 45 min in the dark, they were exposed to 20 kJ/m^2 UVA radiation from a lightbox (model 2000; Dermalight Systems, Sherman Oaks, CA), equipped with an H-1 filter to remove wavelengths below 320 nm.

APC Activity of DLN Cells. Mice were sensitized with 400 μl of 0.5% FITC (Isomer I; Aldrich Chemical Co., Milwaukee, WI) in acetone-dibutylphthalate (1:1, vol/vol) on shaved, UV-irradiated dorsal skin. 18 h later, a single-cell suspension was prepared from DLN (7), and 0.05 ml containing 10^6 cells was injected into each hind footpad of syngeneic recipients. In some experiments, the DLN cells were enriched for dendritic cells before injection. In this case, DLN cells were washed three times in RPMI 1640 with 5% fetal bovine serum (FBS), and layered on 3 ml of 18% metrizamide (Sigma Chemical Co., St. Louis, MO). The metrizamide gradient was centrifuged at 1,000 *g* for 10 min at 4°C. The dendritic cell–enriched interface cells were washed three times with 5% FBS/RPMI 1640 and suspended in RPMI 1640 at a concentration of 6×10^5 $FITC^+$ cells per ml, as determined by fluorescence microscopy. Mice were injected with 50 μl of cell suspension in each hind footpad; the remaining cells were suspended in FBS and collected on slides by cytospin centrifugation for immunohistochemistry. The recipients were challenged 5 d later by applying 5 μl of 0.5% FITC to the ventral and dorsal surfaces of both ears. The CHS response was determined by measuring the ear thickness with a micrometer before and 24 h after challenge, as described previously (24). The ability of the DLN cells to induce CHS in recipient mice is due to the presence of Ia^+, antigen-bearing dendritic cells (25).

Ia Staining. In some experiments, DLN cells were isolated from UV-irradiated mice that had been treated with FITC immediately after UV exposure. The purified DLN cells (isolated 18 h after UVB/FITC treatment) were incubated on ice for 30 min with 1 μg/ml PE-coupled antibody to Ia^k (PharMingen, San Diego, CA) and washed three times in RPMI 1640. The cells were resuspended in chilled 2% paraformaldehyde (EM Sciences, Fort Washington, PA) in RPMI 1640, and analyzed by flow cytometry.

Flow Cytometry. DLN cells from UV-irradiated mice treated with FITC immediately after irradiation were metrizamide purified and suspended in 5% FBS/RPMI 1640 at a concentration of 10^6 cells/ml. They were sorted into $FITC^+$ and $FITC^-$ populations in a cell sorter (Coulter Corp., Hialeah, FL). In experiments in which DLN cells were treated with PE-coupled anti-Ia^k antibody, the $FITC^+$ cells were sorted into Ia^+ and Ia^- populations. The cells were then suspended in FBS and collected on slides by cytospin centrifugation for immunohistochemistry.

Immunohistochemical Staining for CPD. CPD were detected using a mouse monoclonal antibody specific for CPD, according to the method of Roza et al. (26, 27), with a minor modification: instead of a fluorescent second antibody, a horseradish peroxidase (HRP)–conjugated goat anti–mouse antibody (Boehringer Mannheim Corp., Indianapolis, IN) was used to visualize the CPD-specific antibody. HRP activity was detected by incubation in diaminobenzidine (Research Genetics, Huntsville, AL) for 20 min at 22°C. The controls for the assay included frozen skin sections from UV-irradiated and nonirradiated mice and samples stained with only the second antibody. The stained samples were mounted in Universal Mount (Research Genetics), and the percentage of CPD^+ cells was counted under a light microscope. Five fields from each sample, each containing at least 200 cells, were counted. For experiments on whole tissues, skin biopsies and DLN were taken from mice at various times after UV irradiation. The tissues were embedded in Tissue-Tek OCT medium (Miles Laboratories Inc., Elkhart, IN) and snap frozen in liquid nitrogen, and 6-μm cryostat sections were prepared and used for immunohistochemistry.

Statistics. The significance of differences between experimental groups was determined using Student's two-tailed *t* test. A difference was considered to be statistically significant when the probability of no difference was $p \leq 0.05$. For CHS experiments, each experimental group contained at least five mice.

Results

Localization and Persistence of CPD. Shaved dorsal skin of C3H mice was exposed to doses of UVB ranging from 0.5 to 5 kJ/m^2; immediately thereafter, the mice were killed and skin was removed. Immunoperoxidase staining of cryostat sections revealed CPD^+ cells in skin exposed to 1 kJ/m^2 but not in skin exposed to lower doses. For all subsequent experiments, mice received 5 kJ/m^2 UVB (about twice the minimum erythemal dose), which produced heavy, fairly uniform labeling of nuclei in the epidermis (Fig. 1 *B*). This dose of UVB also produced CPD in cells in the dermis within the upper 0.2 mm of the skin; the intensity of staining and number of positive cells decreased with increasing distance from the epidermal surface. In normal skin, which served as negative control, no dimers were detected (Fig. 1 *A*). Remarkably, CPD^+ cells were still present in the skin 4 d after UV irradiation, particularly in the dermis, although they were less numerous and stained less intensely (Fig. 1 *C*).

A recent report by Sontag et al. (28) indicated that cells containing CPD could be detected in the DLN of hairless mice exposed to UVB 24 h earlier. To determine whether such cells could be detected in our C3H mouse model, and whether these cells persisted in the DLN, we examined the DLN of UV-irradiated C3H mice from days 0 to 4 after UV irradiation. Cytospin preparations of DLN cells showed that DNA-damaged cells were present in the DLN of UV-irradiated mice 24 h after UV irradiation, but in very small numbers. Separation of the cells on a metrizamide gradient, which resulted in populations of cells at the interface containing up to 93% dendritic cells, greatly increased the proportion of CPD^+ cells; no CPD^+ cells could be detected in the pellets. In the experiment shown in Fig. 2, ~10% of the metrizamide-purified cells that were collected 1 d after UV irradiation contained detectable CPD; this percentage declined to 1–2% over the next 3 d (not shown). This observation demonstrates that cells with unrepaired DNA damage persist for a surprisingly long period of time in the LN of UV-irradiated C3H mice. The presence of nuclear rather than cytoplasmic labeling argues against the unlikely possibility that the UV-damaged cells were macrophages that had ingested CPD-containing DNA from damaged epidermal cells. No CPD^+ cells were detected in DLN removed immediately after UV irradiation, which suggests that the CPD^+ cells were not derived from blood-borne cells exposed in the circulation to UVB, but rather from cells that migrated to the DLN from the skin after UV irradiation. No UV-damaged cells were detected in any skin or DLN preparation taken from nonirradiated mice.

Identity of the CPD^+ DLN Cells. To help identify the CPD^+ cells, a similar experiment was performed with C3H SCID mice, which lack T and B cells. Cryostat sections of

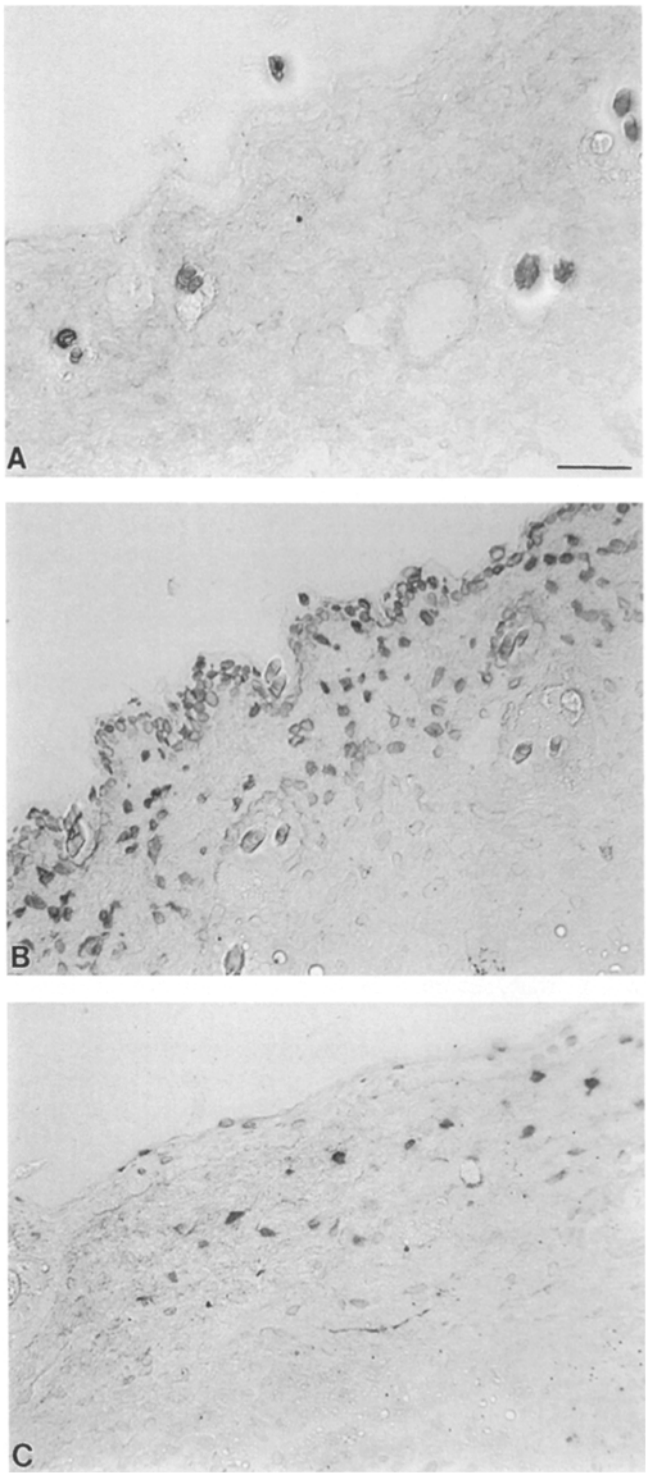


Figure 1. Detection of CPD in C3H mouse skin by immunoperoxidase staining. Shaved back skins of C3H mice were exposed to 5 kJ/m^2 UVB, and cryostat sections from skin biopsies taken immediately (*B*) or 4 d (*C*) after UV irradiation were fixed and stained with anti-CPD antibody, using an immunoperoxidase detection method (see Materials and Methods). Skin from nonirradiated mice served as control (*A*). The dark-stained nuclei are indicative of the reddish-brown, CPD-specific peroxidase stain as visualized by light microscopy. Note that hair shafts (as shown in *A*) are also dark, and should not be mistaken for cells with DNA damage. Bar, 32 μm.

DLN collected 24 h after UV irradiation of C3H SCID mice contained many CPD$^+$ cells, as detected by indirect immunoperoxidase staining (Fig. 3), as did cytospin preparations from DLN cell suspensions (not shown). This result suggests that these cells are macrophages or dendritic cells rather than recirculating lymphocytes or epidermal T cells.

To determine whether the CPD$^+$ cells were skin-derived immune cells, we used FITC as a contact sensitizer, which enabled us to follow the migration of APC from the skin to the DLN (7, 29, 30). Antigen was applied to the skin immediately after UV irradiation, and DLN cells were collected 24 h later and enriched for dendritic cells by centrifugation in metrizamide. FITC$^+$ cells were then isolated by cell sorting and stained for the presence of dimers. All CPD$^+$ cells were present in the FITC$^+$ population; none was present in the FITC$^-$ population. The number of CPD$^+$ cells in the FITC$^+$ population was enriched relative to the starting preparation, but only a portion of the antigen-bearing cells contained detectable levels of DNA damage (not shown).

Our previous studies demonstrated that in this model, all of the FITC$^+$ cells in the LN are Ia$^+$ APC (30), and some contain Birbeck granules, indicating that they originated as epidermal LC (29). Therefore, we predicted that the CPD$^+$ cells would also be Ia$^+$. To test this prediction, metrizamide-purified DLN cells from UV-irradiated, FITC-sensitized mice were incubated with PE-labeled anti-Ia^k and sorted sequentially by cell sorter for PE and FITC. Analysis of the sorted populations revealed that virtually all of the FITC$^+$ cells were contained within the Ia$^+$ population, as expected (29). Immunoperoxidase labeling of cytospin preparations of these cells demonstrated that all DNA-damaged cells were present in the FITC$^+$ Ia$^+$ population; none was detected in the FITC$^-$ Ia$^+$ population. These results provide strong evidence that the CPD$^+$ DLN cells were skin-derived Ia$^+$ cells.

Effect of Contact Sensitization on the Number of CPD$^+$ DLN Cells. Application of antigen on murine skin increases the number of Ia$^+$, dendritic APC in the DLN (31, 32). To determine whether FITC application increased the number of CPD$^+$ cells in the DLN of UV-irradiated mice, mice were sensitized with FITC immediately or 3 d after UV irradiation, and their DLN were collected 24 h later. Application of FITC immediately after UV irradiation slightly increased the number of CPD$^+$ cells in the DLN 24 h later, but this increase was not statistically significant. However, application of FITC on day 3 after UVB followed by collection of DLN cells on day 4 approximately doubled the number of CPD$^+$ cells in the DLN compared to that in unsensitized mice (Fig. 4 *A*). The observed additional migration of CPD$^+$ cells implies that many of the residual DNA-damaged cells present in the skin 3 d after UV irradiation are APC that migrate and carry antigen to the DLN after contact sensitization.

In Vivo APC Activity of DLN. Having demonstrated that skin-derived immune cells containing DNA damage and antigen are present in the DLN after UV irradiation and sensitization of the skin, we investigated the in vivo

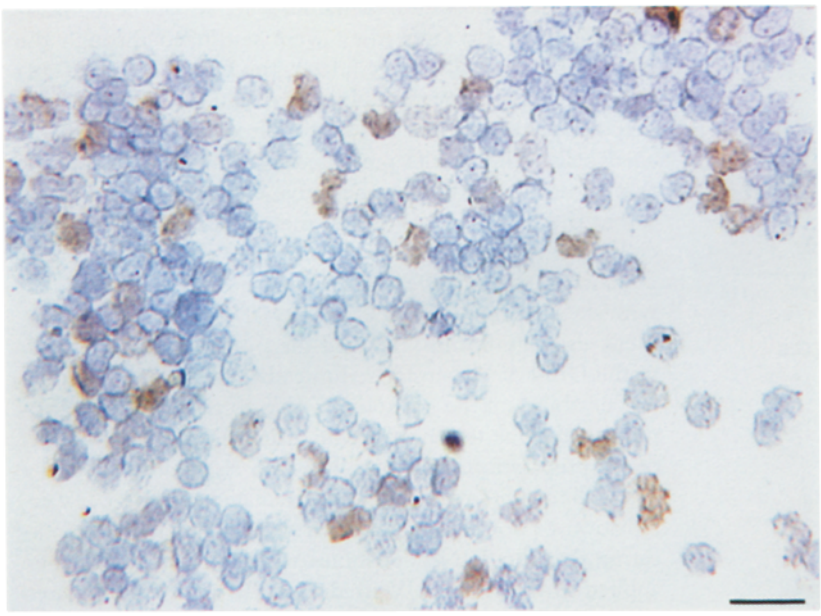
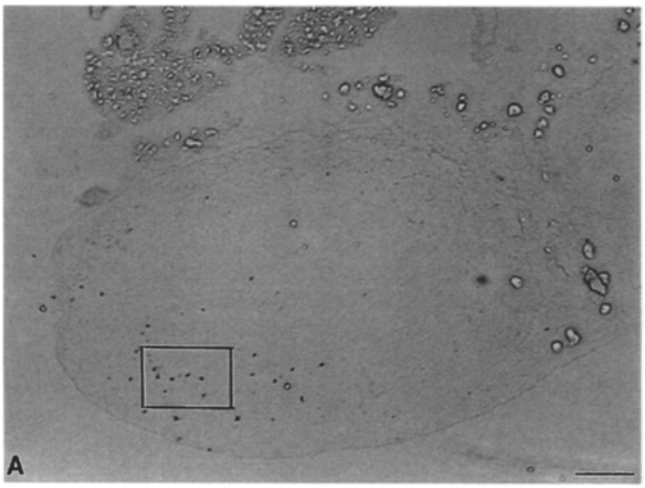
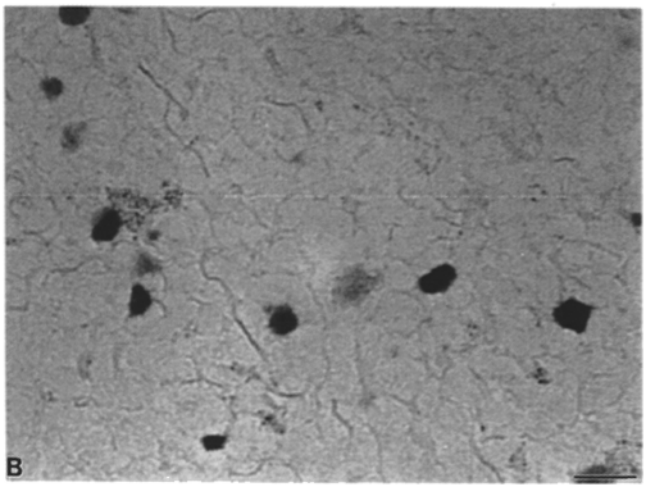


Figure 2. CPD$^+$ DLN cells. 1 d after UVB irradiation (5 kJ/m^2) of C3H mouse skin, DLN cells were isolated, and metrizamide-interface cells were collected on microscopic slides by cytospin centrifugation. The presence of CPD in the nuclei of the DLN cells was revealed by immunoperoxidase staining (reddish-brown precipitate). Nuclei are lightly counterstained with haematoxylin (*blue*). Bar, 14 μm.





APC activity of the DLN cells. DLN cells from mice sensitized immediately or 3 d after UV irradiation and collected 24 h later were injected into the hind footpads of normal syngeneic mice. The recipients were challenged on the ears 5 d later to assess their CHS response. As shown in Fig. 4 *B*, DLN cells from nonirradiated, FITC-sensitized mice or mice sensitized immediately after UV irradiation induced CHS in the recipient animals. However, mice immunized with DLN cells from donors sensitized 3 d after UV irradiation exhibited a significantly reduced CHS response. As indicated in Fig. 4 *A*, the percentage of CPD$^+$ cells in the metrizamide-purified DLN cell population collected on day 4 is lower than that collected on day 1. However, because the percentage of FITC-bearing DLN cells was higher on day 1 than on day 4 after UV irradiation, more cells of the latter group were used for adoptive transfer in order to inject equal numbers of FITC-bearing cells (6×10^4 per mouse). As a result, there was only a slight (not statistically significant) difference in the absolute number of CPD$^+$ cells injected into the recipient mice (not shown). Therefore, these data indicate that although CPD are present immediately after UV irradiation, the impairment of APC activity develops over time.

Effect of T4N5 Liposomes on the Number and Activity of CPD$^+$ DLN Cells. We demonstrated previously that DLN cells from mice sensitized with FITC on UV-irradiated skin 3 d after UV irradiation are deficient in their ability to induce CHS when the DLN cells are injected into the footpads of normal, syngeneic mice; instead, the cells induce antigen-specific suppression transferable by splenic T

Figure 3. CPD$^+$ cells in DLN of SCID mice. 1 d after UVB irradiation, DLN were isolated from SCID mice, and cryostat sections were prepared and used for immunoperoxidase detection of CPD. Bars: (*A*) 83 μm; (*B*), 15 μm.

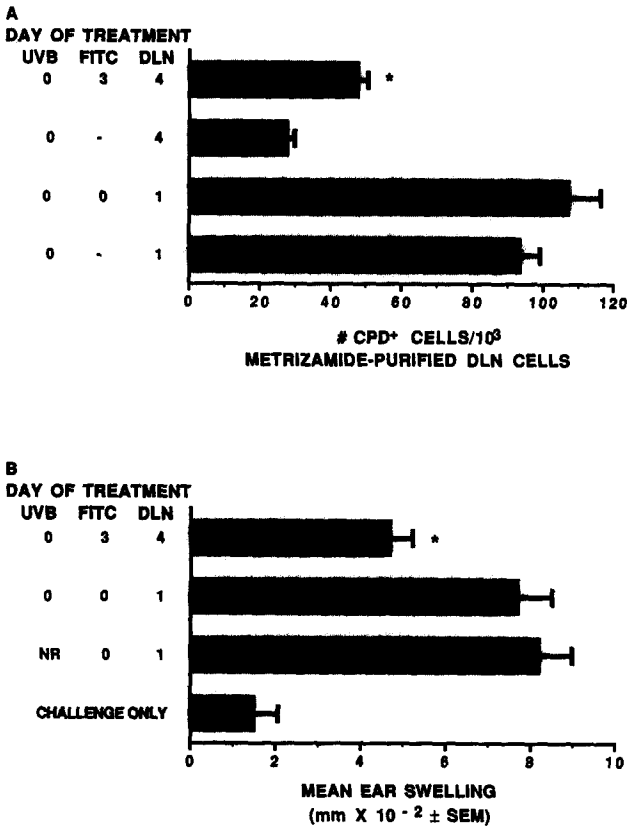


Figure 4. (*A*) Effect of FITC application on the number of CPD^+ DLN cells. Mice were UVB irradiated, and their exposed skins were treated with FITC either immediately or 3 d after UV exposure. UV-irradiated, nonsensitized animals served as controls. On days 1 and 4 (24 h after FITC application), DLN cells were isolated, purified on a metrizamide gradient, and collected on slides for staining with the CPD-specific antibody. Five fields from each sample, each containing at least 200 cells, were counted, and the number of CPD^+ cells per 10^3 metrizamide-purified DLN cells was calculated. At 1 d after UV irradiation, the number of DNA-damaged cells was significantly higher than that on day 4 ($P < 0.05$). (*) $P < 0.05$ versus day 4 unsensitized group. (*B*) Impaired APC activity of DLN cells with persistent DNA damage. Donor mice were exposed to UVB and sensitized with FITC immediately or 3 d after irradiation. 3×10^4 metrizamide-purified DLN cells from FITC-sensitized, UV-irradiated or nonirradiated mice were injected into each hind footpad of normal recipients in a volume of 50 μl to investigate their ability to induce CHS. DLN cells were also collected on slides for immunostaining to determine the percentage of CPD^+ cells and the absolute number of CPD^+ cells used for adoptive transfer. 5 d after adoptive transfer, recipient mice were challenged on the ears with FITC and the mean ear swelling was determined 24 h later. (*) $P < 0.05$ versus nonirradiated and UV-irradiated groups.

cells (7). Furthermore, cell sorter-purified, $FITC^+$ immune cells from UV-irradiated donors are markedly impaired in their ability to induce CHS in recipient mice (30). We therefore wished to determine whether applying T4N5 liposomes to the skin immediately after UV irradiation, which increases the rate of DNA repair (21, 22), would prevent the UV-induced impairment in antigen-presenting activity of such DLN cells and also reduce the number of CPD-containing cells.

Mice were exposed to 5 kJ/m^2 of UVB and immediately treated with liposomes containing active or heat-inactivated T4N5. 3 d later, they were sensitized through the treated skin with FITC. 24 h later, the DLN cells were collected and tested for their ability to induce CHS in syngeneic recipients, as well as for the presence of DNA-damaged cells. The results presented in Table 1 demonstrate that liposomes containing the active enzyme completely restored the APC activity of the metrizamide-purified DLN cells obtained from UV-irradiated mice, whereas liposomes containing the inactive enzyme had no effect. Coincident with the restoration of APC activity, T4N5 liposome treatment also significantly reduced the number of CPD^+ cells in the DLN of UV-irradiated mice by ~50%.

The ability of the liposomal T4N5 to restore APC activity was not due to a nonspecific, immunostimulatory effect of the preparation. As shown in Fig. 5 *A*, application of the T4N5 liposomes to ventral, nonirradiated skin after UV irradiation of dorsal skin did not restore APC activity, indicating that the T4N5 liposomes were only effective when applied to the site of UV irradiation. In addition, impaired APC function resulting from PUVA treatment, in contrast to that resulting from UVB treatment, could not be restored by T4N5 liposome treatment (Fig. 5 *B*). Because PUVA treatment induces monofunctional adducts and cross-links in DNA rather than CPD, this experiment demonstrates the specificity of the T4N5 liposomes for UVB-induced DNA damage and provides additional evidence against the notion that T4N5 liposome treatment works by means of a nonspecific, immunostimulatory effect.

Discussion

In these studies, we have attempted to delineate the molecular event that triggers one step in the cascade of cutaneous responses initiated by UV irradiation that culminates in impaired induction of CHS. Specifically, we focused on the impaired antigen-presenting activity of hapten-bearing LN dendritic cells, which originate from contact-sensitized skin (29). As an initial step in assessing the role of UV-induced DNA damage in this effect of UV irradiation, we asked the following questions. Are there CPD-containing APC in UV-irradiated skin? Are they still present in the skin at the time of deficient APC function? Do these cells reach the DLN after contact sensitization? Does the application of T4N5 liposomes, which increases the rate of DNA repair after UV irradiation, restore APC function and decrease the number of DNA-damaged cells in the DLN?

To address these questions, we used a model in which mice were sensitized with FITC through UV-irradiated or nonirradiated skin, which enabled us to follow the migration of FITC-bearing APC to the DLN. Using a monoclonal antibody specific for CPD, we found that DNA-damaged cells were present not only in the epidermis of UV-irradiated mice but also in the dermis up to a depth of ~0.2 mm. This finding suggests that dermal macrophages, as well as epidermal LC, are potential immunological targets for the DNA-damaging effects of UV radiation, and complements the study of Kurimoto et al. (33) indicating

Table 1. *Effect of T4N5 Liposomes on Number of CPD⁺ DLN Cells and In Vivo APC Activity*

Treatment of DLN donors	Experiment 1		Experiment 2	
	No. CPD⁺ cells ± SEM per 10^3 metrizamide-purified DLN cells	Mean ear swelling (mm × 10^{-2}) ± SEM in recipients of DLN cells	No. CPD⁺ cells ± SEM per 10^3 metrizamide-purified DLN cells	Mean ear swelling (mm × 10^{-2}) ± SEM in recipients of DLN cells
	% UV control	*% suppression*	*% UV control*	*% suppression*
No cells	--	1.8 ± 1.7	--	2.3 ± 1.4
UVB*	27 ± 2 (100)	3.0 ± 0.7 (72)	62 ± 3 (100)	5.3 ± 1.6 (64)
UVB/HI*‡	32 ± 5 (119)	3.9 ± 0.7 (51)	54 ± 2 (87)	5.5 ± 2.6 (62)
UVB/T4N5*§	12 ± 1 (44)‖	6.6 ± 0.4 (0)‖	39 ± 6 (63)‖	9.9 ± 2.4 (10)‖
NR*	0 (0)	6.1 ± 0.3 (0)	0 (0)	10.7 ± 3.2 (0)
NR/HI*‡	ND	ND	0 (0)	12.1 ± 3.7 (0)
NR/T4N5*§	ND	ND	0 (0)	8.1 ± 2.0 (4)

*DLN cells from UV-exposed (5 kJ/m² UVB) and nonirradiated (NR) C3H mice isolated at 18 h after FITC sensitization (at 3 d after UV) were stained with a CPD-specific antibody and injected into recipient mice (6 × 10^4 FITC⁺ cells per mouse) to test APC activity.
‡Liposomes containing heat-inactivated T4N5 (HI) served as controls and were applied to UV-exposed skin immediately after UV and also to normal skin (Exp. 2).
§Liposomes containing active T4N5 were applied to UV-exposed skin immediately after UV and also to normal skin (Exp. 2).
‖$P < 0.05$ versus UV control.

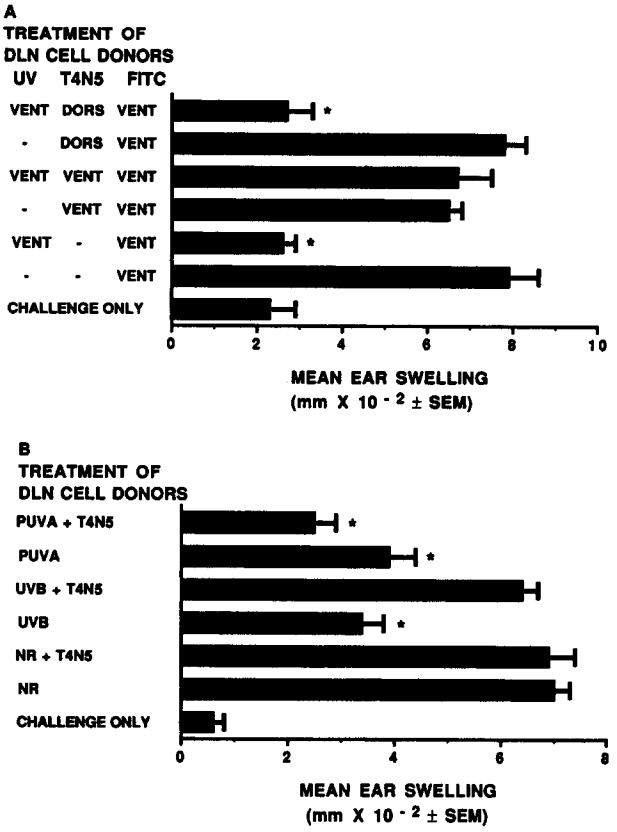


Figure 5. (*A*) Effect of T4N5 liposome treatment of UV-irradiated versus unirradiated skin on APC activity of DLN cells. Mice were exposed to 2 kJ/m² UVB on ventral skin and immediately treated with T4N5 liposomes on ventral or dorsal skin. 3 d later, they were sensitized with FITC on ventral skin, and DLN cells were collected 24 h later. 3 × 10^6 unpurified DLN cells were injected into each hind footpad of normal mice, which were challenged on the ears with FITC 5 d later. Ear swelling was measured 24 h later. (*B*) Effect of T4N5 liposome treatment on UVB- versus PUVA-induced impairment of APC activity. Mice were exposed to 2 kJ/m² UVB or 2 mg/ml 8-methoxypsoralen plus 20 kJ/m² UVA and immediately treated with T4N5 liposomes on the irradiated site. The APC activity of DLN cells was assayed as described in *A*. (*) $P < 0.001$ versus matching control group.

the importance of these cells in UV-induced local suppression of CHS. Although the number of CPD⁺ cells decreased over time, cells containing DNA damage persisted in the skin for a surprisingly long period of time, particularly in the dermis. Thus, at the time when contact sensitization was impaired (3 d after UV irradiation), DNA-damaged cells were still present in the UV-irradiated skin. Loss of CPD⁺ cells from the skin during this period could have been due to DNA repair, cell death, terminal differentiation, emigration of cells from the skin to the LN, or dilution of DNA damage during cell division. Most likely, all of these processes contributed to the decreased number of CPD⁺ cells over time.

Evidence for the emigration of cells from the skin was provided by our demonstration that UV-damaged cells could be detected in DLN from 1 to 4 d after exposure of the skin to UV radiation. In accordance with a previous study with hairless mice (28), no CPD⁺ cells were detected in the DLN when mice were killed within minutes after UV irradiation, which implies that CPD⁺ cells in the DLN did not originate from UV-irradiated circulating leukocytes, but rather from cells that emigrated from the skin. Our demonstration that the DNA damage–containing cells in the DLN were dendritic, antigen-bearing, Ia⁺ cells, copurified with dendritic APC, and were present in SCID

mice, identifies these cells as belonging to a lineage of nonlymphoid APC. It is unlikely that B cells contributed significantly to this population in the C3H mice because very few B cells would have been present in the skin at the time of UV irradiation.

There were striking differences in the activity of DLN cells recovered on different days after UV irradiation. When sensitization with FITC occurred immediately after UV, DLN cells collected 24 h later contained DNA damage, but showed no impairment in APC activity. In contrast, sensitization 3 d after UV resulted in a population of DLN cells with impaired activity, although the number of CPD^+ cells used for adoptive transfer was approximately the same. This result demonstrates that the CPD^+ cells that leave the skin immediately after UV irradiation do not contribute to the impaired APC function in UV-irradiated mice. These cells could represent severely damaged cells being cleared from the skin or cells that had insufficient time to modulate their APC activity. On the other hand, APC that were present in the skin 3 d after UV and migrated to the DLN upon FITC sensitization exhibited impaired function. This implies that the antigen-bearing cells in this population containing DNA damage represent a different, UV-resistant or less UV-damaged subpopulation of APC, or that several days are required to modify the activity of cutaneous APC after UV irradiation.

If DNA damage was responsible for the impaired function of $FITC^+$ DLN cells, then increasing the rate of DNA repair by applying T4N5 liposomes to the irradiated skin should both restore immune function and reduce the number of CPD^+ cells in the DLN. This was indeed the case. T4N5 liposome treatment completely restored the APC activity of the DLN cells and concomitantly reduced the number of CPD^+ cells in the DLN cell population used for immunization. Liposomes containing heat-inactivated T4N5 and liposomes containing active enzyme, but applied to a nonirradiated site were ineffective in restoring APC activity. Furthermore, APC activity impaired by PUVA treatment, which does not produce CPD, was not restored by T4N5 in liposomes. These results rule out the possibility that the T4N5 liposomes restore immune function by means of a nonspecific immunostimulatory effect and demonstrate the specificity of the T4N5 liposomes for repairing UV-induced CPD.

That not all CPD were removed by the liposome treatment could have been due to some cells containing so many dimers that they died, and therefore could neither be rescued by T4N5 liposome treatment nor contribute to APC function. Second, there were probably many cells containing CPD that were below the threshold of detection with the antibody technique employed. Thus, there could have been considerably more DNA damage and repair in the DLN cells than was detectable by our method. Third, because we only determined the presence or absence of DNA damage at 4 d after UV irradiation, the rate of repair was not measured. It may be that the number of CPD present at some critical time point after UV determines APC function. Also, there could have been differences in CPD removal in different APC subsets. For example, some CPD^+ cells may have emigrated to the DLN immediately after UV irradiation and thus were not affected by the T4N5 liposomes applied to the skin. Finally, it has been demonstrated that CPD in actively transcribed genes are repaired preferentially (34). Thus, restoration of APC function may not require repair of DNA damage throughout the entire genome of a cell, but only in a limited number of critical sites.

Of course, we would like to have determined directly whether the CPD^+ cells were deficient in APC activity. However, at present, it is not possible to isolate viable, CPD^+ cells and test their function because CPD can only be detected after fixation of the cells by a method that permeabilizes the nucleus. Thus, our results establish a correlation between altered APC function and the presence of CPD in cutaneous APC, but they do not rule out the possibility that CPD in other cells contribute to impaired APC function. For example, in response to DNA damage, keratinocytes may produce soluble mediators that alter the migration and activity of both resident and infiltrating APC (9), and therefore, increased DNA repair in keratinocytes may be responsible for restoration of APC function.

However, we can conclude from our studies that: (*a*) APC in both the dermis and the epidermis are directly damaged in the skin upon exposure to UVB; (*b*) the damaged cells persist, mainly in the dermis, for at least 4 d after irradiation; (*c*) some of the damaged cells migrate to the DLN within 24 h, but the DLN cell population exhibits normal APC activity at this time; (*d*) other cells remain in the skin and migrate to the DLN only after epicutaneous sensitization 3 d after UV exposure, and this DLN cell population has impaired APC function; and (*e*) reducing the number of CPD^+ cells in the DLN using T4N5 liposomes correlates with abrogation of UV-induced impairment of APC activity. Further studies in which CPD are removed selectively from APC, but not from keratinocytes, are needed to determine whether the latter correlation represents a causal relationship between DNA damage to APC and their reduced antigen-presenting activity. Nonetheless, these studies provide strong evidence in support of the hypothesis that DNA damage is an essential triggering event in the impaired activity of APC that results from in vivo UV irradiation.

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