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Evidence that DNA damage triggers interleukin 10 cytokine production in UV-irradiated murine keratinocytes

(photoimmunology/immune suppression/DNA repair/liposomes)

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ABSTRACT **UV irradiation interferes with the induction of T cell-mediated immune responses, in part by causing cells in the skin to produce immunoregulatory cytokines. Recent evidence implicates UV-induced DNA damage as a trigger for the cascade of events leading to systemic immune suppression *in vivo*. However, to date, there has been no direct evidence linking DNA damage and cytokine production in UV-irradiated cells. Here we provide such evidence by showing that treatment of UV-irradiated murine keratinocytes *in vitro* with liposomal T4 endonuclease V, which accelerates the repair of cyclobutylpyrimidine dimers in these cells, inhibits their production of immunosuppressive cytokines, including interleukin 10. Application of these liposomes to murine skin *in vivo* also reduced the induction of interleukin 10 by UV irradiation, whereas liposomes containing heat-inactivated T4 endonuclease V were ineffective. These results support our hypothesis that unrepaired DNA damage in the skin activates the production of cytokines that down-regulate immune responses initiated at distant sites.**

Excessive exposure of the skin to UV radiation has many biological consequences, including sunburn, solar keratosis, skin cancer, and immune suppression. Suppression of the immune response contributes to the formation of UV-induced skin cancers in mice by permitting the outgrowth of highly antigenic, UV-transformed cells (1). One mechanism proposed for UV-induced immune suppression is the elaboration of immunomodulatory cytokines by UV-irradiated keratinocytes (2). Studies by Streilein and Vermeer (3) implicated tumor necrosis factor α as an important mediator of the suppression of contact hypersensitivity responses in UV-irradiated mice. Ullrich and colleagues (4) demonstrated that interleukin 10 (IL-10) plays a crucial role in suppression of delayed type hypersensitivity (DTH) responses. They showed that IL-10 is produced and secreted by keratinocytes *in vitro* in response to UV irradiation and suggested that *in vivo* this cytokine diverts the DTH response toward a suppressor pathway (4, 5).

What has not yet been established is the identity of the initial photobiological reaction responsible for triggering the production of immunomodulatory cytokines. In previous studies, we provided evidence that DNA damage in the form of cyclobutyl pyrimidine dimers (CPD) is an initiating event in several models of UV-induced immune suppression (6, 7). These *in vivo* studies showed that reducing the number of CPD in UV-irradiated skin abrogated the systemic suppression of contact hypersensitivity and DTH responses. From these results we have inferred that unrepaired DNA damage stimulates keratinocytes to produce cytokines that modify critical steps in the immunological pathway, ultimately leading to reduced cell-mediated immune responses and suppressor cell generation. However, evidence of a direct effect of DNA damage on cytokine production has been lacking. In these studies, we address a pivotal aspect of this hypothesis by demonstrating that DNA damage triggers the production of immunomodulatory cytokines, both *in vitro* and *in vivo*.

The approach we employed involves the excision repair enzyme T4 endonuclease V (T4N5) encapsulated in liposomes. In previous studies we showed that application of T4N5 liposomes to UV-irradiated murine skin reduced the number of CPD, restored immune reactivity, and reduced the incidence of skin cancer (7, 8). Ultrastructural studies showed that the liposomes were taken up by keratinocytes and Langerhans cells in the epidermis and by cells of the transformed murine keratinocyte line PAM212 in culture and that the endonuclease reached the nuclei of these cells (9). Here we assessed the ability of T4N5 liposomes to reduce the immunosuppressive activity and IL-10 content of the culture supernatants from UV-irradiated PAM212 cells. In addition, we examined the effect of T4N5 liposomes on UV-induced IL-10 production in murine skin.

MATERIALS AND METHODS

Mice. Specific pathogen-free C3H/HeNCrMTV$^-$ and BALB/cAnNCr female mice were obtained from the Frederick Cancer Research Facility Animal Production Area (Frederick, MD). Age-matched mice between 10 and 12 wk of age were housed in filter-protected cages, and ambient lighting was controlled to provide 12-h light/12-h dark cycles. Autoclaved National Institutes of Health open formula mouse chow and water were provided ad libitum. The animal facility is accredited by the American Association for the Accreditation of Laboratory Animal Care; all procedures were approved by the Institutional Animal Care and Use Committee.

Radiation Sources. A single FS40 sunlamp (National Biological, Twinsburg, OH) was used to irradiate keratinocytes, and a bank of six FS40 sunlamps was used to irradiate the mice. These lamps emit a continuous spectrum from 270 to 390 nm, with a peak emission at 313 nm; approximately 65% of the radiation is within the UV-B (280–320 nm) wavelength range. The irradiance of the single bulb averaged 1.43 J/m^2/sec at a tube-to-target distance of 23 cm, and that of the bank of six bulbs averaged 9 J/m^2/sec, as measured by an IL-700 radiometer with an SEE240 UV-B detector equipped with an A127 quartz diffuser (International Light, Newburyport, MA). The dose of 200 J/m^2 used for these experiments produced an

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Abbreviations: CPD, cyclobutyl pyrimidine dimer(s); DTH, delayed type hypersensitivity; HI, heat inactivated; IL-10, interleukin 10; T4N5, T4 endonuclease V.

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average of 146 CPD/10^6 bases 6 h after irradiation; to produce the same number of CPD with sunlight would require approximately 72 min of exposure to summer noonday sun in Dayton, OH (10). The dorsal fur of mice was removed with electric clippers, and the animals were then placed on a shelf 20 cm below the light source and irradiated. Control mice were treated in exactly the same way except they were not exposed to UV radiation.

Cell Line. A spontaneously transformed BALB/c keratinocyte cell line, PAM212, was obtained from Stuart Yuspa (National Cancer Institute, Bethesda, MD). Cells were maintained in Eagle's minimal essential medium (MEM), supplemented with 10% fetal bovine serum, nonessential amino acids, and L-glutamine (GIBCO/BRL/Life Technologies). Cells were maintained at 37°C in a 5% CO_2/95% air atmosphere.

T4N5 Liposomes. T4N5 liposomes were prepared by encapsulating purified, recombinant T4N5 in liposomes composed of phosphatidylcholine/phosphatidylethanolamine/oleic acid/cholesterol hemisuccinate (2:2:1:5 molar ratio) by the detergent dialysis method (11). The concentration of the entrapped enzyme was determined by ELISA (12) and was expressed as micrograms of T4N5 per milliliter of vehicle. The encapsulated activity was assayed by its ability to nick UV-irradiated supercoiled DNA with and without dissolution of the liposomes (12). Control preparations of liposomes contained heat-inactivated (65°C for 1 h) T4N5 heat inactivated (HI). The liposomes were diluted with MEM, supplemented with 1% fetal bovine serum, to a final concentration of 0.3 μg T4N5/ml of liposome-hydrogel mixture. After UV irradiation, cells were treated at 37°C for 1 h with 3 ml of liposomes (0.3 μg T4N5/ml) per 100-mm dish for collecting supernatants and 1.3 ml per 35-mm dish for immunohistochemistry.

UV Irradiation of Keratinocytes. Four × 10^6 keratinocytes were plated on 100-mm tissue culture dishes in 8 ml of medium. The next day the medium was removed and the cells were washed three times with PBS and overlaid with 0.5 ml of PBS. The cells were exposed to 200 J/m^2 of UVB and treated with liposomes for 1 h; then they were washed, and serum-free MEM was added. Twenty-four hours later, the supernatants were collected. Previous experiments determined that the activity of the supernatants increased up to a maximum of around 50% suppression of DTH to alloantigen with increasing UV doses up to 200 J/m^2; higher UV doses did not appreciably increase the suppressive activity and decreased cell viability (S.E.U., unpublished data). The protein concentration of the supernatants was determined using bicinchoninic acid (BCA protein assay reagent, Pierce); the supernatants were injected i.v. into mice.

DTH to Alloantigen. C3H mice received 20–25 μg of supernatant protein from keratinocytes treated with or without UV and with or without liposomes. Five days later, the mice were immunized by s.c. injection of 5 × 10^7 allogeneic BALB/c spleen cells into each flank. Six days later, the animals were challenged by injecting 10^7 BALB/c spleen cells into each hind footpad. Footpad swelling was measured at 24 h (4, 5).

Immunohistochemical Detection of IL-10. IL-10 was detected in PAM212 cells and mouse skin by immunoperoxidase staining using rat anti-mouse IL-10 monoclonal antibody (JES5-2A5 hybridoma, IgG1, PharMingen). PAM212 cells were grown on coverslips in 35-mm dishes. The next day, the cells were irradiated with 200 J/m^2 UVB, and some of them were treated with T4N5 or HI liposomes for 1 h immediately after UV irradiation. Nine and 24 h after UV irradiation cells were washed with PBS, fixed with 2% paraformaldehyde for 10 min at 25°C, and rinsed with PBS. The coverslips were placed in a humidified chamber and overlaid with PBS containing 1% normal goat serum and 10% normal horse serum for 20 min, followed by rat anti-mouse IL-10 antibody overnight at 4°C. The coverslips were washed with PBS and incubated for 1 h with peroxidase-conjugated anti-rat IgG1 (H+L) (Boehringer Mannheim), followed by diaminobenzidine (Research Genetics, Huntsville, AL) for 20 min at 25°C. For detection of IL-10 in mouse skin, skin samples were collected 1 and 3 days after UV irradiation and liposome treatment, embedded in OCT compound, and frozen in liquid N_2; 4 μm cryostat sections were fixed with 2% paraformaldehyde for 10 min. The slides were placed in a humidified chamber and endogenous peroxidase activity was blocked with 3% H_2O_2 in methanol for 12 min at 25°C. The sections were then rinsed with PBS and stained for IL-10 using the same procedure as that described above for PAM212 cells.

Assay for IL-10 in Supernatants. IL-10 production by PAM212 cells was measured by ELISA of supernatants collected 24 h after UV irradiation. Purified rat anti-mouse IL-10 produced by hybridoma clone JES5-2A5 was used as a capture antibody in combination with the biotinylated detection monoclonal antibody SXC-1 (Rat IgM, PharMingen). ELISA was carried out according to the manufacturer's procedures, and the IL-10 concentration was determined from the linear portion of a standard curve obtained using recombinant IL-10 (PharMingen).

Assays for DNA Damage. CPD were detected by immunoperoxidase labeling using a thymine dimer-specific monoclonal antibody (13). PAM212 cells were plated on coverslips in 35-mm dishes. The next day, cells were mock-irradiated or UV-irradiated with doses of UVB ranging from 100 to 200 J/m^2. Some coverslips were also incubated for 1 h with T4N5 or HI liposomes. Six hours after UV irradiation the cells were washed with PBS three times and fixed with 70% ethanol for 10 min at 25°C. Coverslips were rinsed with PBS and incubated with the anti-CPD monoclonal antibody for 2 h at 25°C, followed by horseradish peroxidase-conjugated goat anti-mouse secondary antibody for 1 h at 25°C. Peroxidase activity was visualized by incubating the samples with diaminobenzidine. The alkaline agarose gel technique was used to measure DNA breaks produced at CPD sites in unlabeled DNA by digesting with purified, recombinant, CPD-specific T4N5. PAM212 cells were exposed to 200 J/m^2 of UV radiation and treated with liposomes containing T4N5 or heat-inactivated T4N5 for 1 h. Six hours later DNA was isolated and 1 μg of DNA was incubated with 32 μg/ml of T4N5 for 60 min at 37°C. DNA samples were electrophoresed in a 0.5% alkaline agarose gel, and the amount of DNA fragmentation was determined by densitometry scanning of the gels (7).

RESULTS

T4N5 Liposomes Reduce the Immunosuppressive Activity of Supernatants from UV-Irradiated PAM212 Cells. Supernatants from UV-irradiated PAM212 keratinocytes suppress the DTH response to alloantigen when injected into mice (4). To investigate the role of DNA damage in generating suppressive activity, supernatants were collected from cells 24 h after exposure to 200 J/m^2 UVB radiation and treatment with T4N5 liposomes and injected i.v. into mice. We determined previously that this dose of UV has no effect on cell viability but results in maximal immunosuppressive activity in the supernatants. As shown in Fig. 1, supernatants from UV-irradiated cells reduced the DTH response by 50% compared with that in untreated mice ($P < 0.001$, Student's t test), whereas supernatants from unirradiated cells had no effect on the DTH response. Treatment of UV-irradiated PAM212 cells with T4N5 liposomes abrogated the suppressive activity of the supernatants (Fig. 1). This reversal was specific for the DNA repair activity because heat-inactivated T4N5 in liposomes had no effect. To test whether T4N5 liposomes affected the ability of keratinocytes to secrete immunosuppressive mediators in the absence of DNA damage, control groups were included in which supernatants from unirradiated PAM212 cells treated with T4N5 or HI liposomes were injected into mice. These

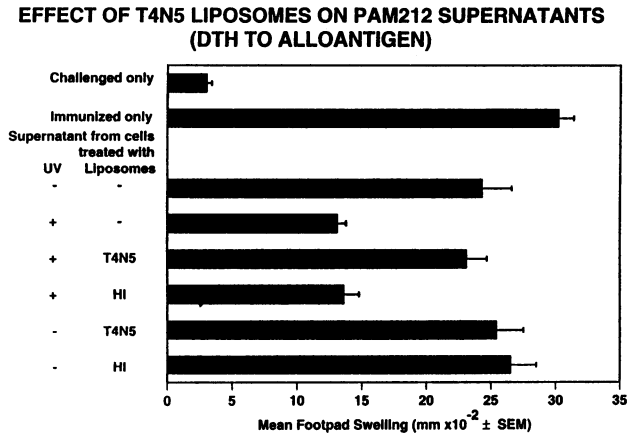

FIG. 1. Suppression of the DTH response to alloantigen by supernatants from UV-irradiated PAM212 cells. PAM212 cells were exposed to 200 J/m^2 UVB (+) or not exposed (−) and incubated for 1 h with active (T4N5) or inactive (HI) T4N5 in liposomes. Serum-free supernatants were collected at 24 h and injected i.v. into C3H mice, which were immunized 5 days later with BALB/c spleen cells s.c. DTH was assessed by footpad challenge 6 days later, and footpad swelling was measured at 24 h. $P < 0.001$, Student's t test.

supernatants had no effect on the DTH response (Fig. 1), indicating that the liposomes were active only in the presence of the UV-irradiated DNA substrate. This result argues against the possibility that active T4N5 or the liposomes themselves act by a mechanism other than DNA repair.

Detection of IL-10 in PAM212 Cells and Supernatants. Because IL-10 is an essential mediator of immune suppression in this system (4, 5), we examined the effect of T4N5 liposomes on production and release of IL-10 by UV-irradiated PAM212 cells. Fig. 2 shows the induction of IL-10 in PAM212 cells detected by immunoperoxidase staining 24 h after exposure to 200 J/m^2 UVB radiation (compare *A* with *B*). Treatment of the cells with T4N5 liposomes after UV irradiation markedly reduced the amount of IL-10 (*C*), whereas treatment with HI liposomes had no effect (*D*). IL-10 was also secreted by

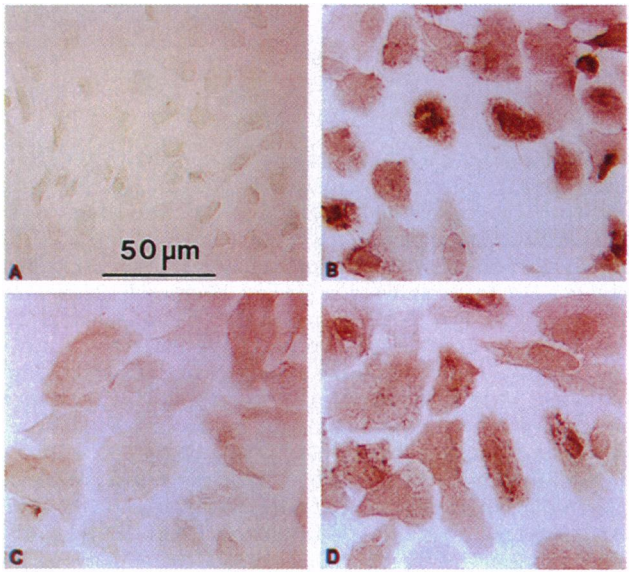

FIG. 2. Effect of T4N5 liposomes on UV induction of IL-10 in PAM212 cells. PAM212 cells were plated on coverslips and treated 18 h later with UV and liposomes as described in Fig. 1. The cells were fixed with paraformaldehyde 24 h after irradiation and stained with rat anti-mouse IL-10, followed by peroxidase-labeled anti-rat IgG1 and incubation with diaminobenzidine. (*A*) No UV. (*B*) UV alone. (*C*) UV + T4N5 liposomes. (*D*) UV + heat-inactivated T4N5 in liposomes.

PAM212 cells as determined by ELISA of culture supernatants, as shown in the representative experiment presented in Table 1. IL-10 was not detected in supernatants from unirradiated PAM212 cells, although small amounts were occasionally found in supernatants of unirradiated cells treated with T4N5 liposomes. By contrast, supernatants from UV-irradiated keratinocytes contained large amounts of IL-10. Treatment of UV-irradiated cells with T4N5 liposomes, but not HI liposomes, reduced the amount of IL-10. The reduction varied from 26 to 90% in five independent experiments, but the amount of IL-10 was always significantly less in supernatants from keratinocytes treated with active liposomes than in those from cells treated with inactive liposomes.

T4N5 Liposomes Reduce UV-Induced IL-10 Production in Murine Skin *in Vivo*. The induction of immunosuppressive factors, particularly IL-10, by UV irradiation of cultured murine keratinocytes has been used as a model of the induction of immunosuppression by UV irradiation *in vivo* (4, 5). We would expect therefore, that exposing mouse skin to UV *in situ* would induce the production of IL-10 and that treatment of UV-irradiated skin with T4N5 liposomes would reduce this effect. Since IL-10 is also present in the serum of mice after UV irradiation (2), we would expect, in addition, that T4N5 liposomes would reduce serum IL-10 as well, thereby minimizing systemic immune suppression. We looked for IL-10 in sections of mouse skin by immunoperoxidase staining and in serum by ELISA 3 days after exposure to 10 kJ/m^2 UVB. This dose of UV produces approximately 110 CPD/10^6 bases in epidermal DNA 6 h after UV irradiation of this mouse strain (7), which is similar to the number of CPD produced in PAM212 cells exposed to 200 J/m^2 of UV radiation *in vitro*. More importantly, this UV dose abrogates the DTH response to *Candida albicans*, and we showed previously that *in vivo* treatment with T4N5 liposomes completely reverses the immune suppression observed at this UVB dose (7).

UV irradiation resulted in increased keratinization and proliferation of the epidermis, strong induction of IL-10 in the epidermis, and the appearance of IL-10-producing dendritic cells in the dermis (Fig. 3*A*). Treatment of the skin with T4N5 liposomes immediately after UV irradiation appeared to reduce the amount of UV-induced IL-10 in the epidermis and the number of IL-10$^+$ cells in the dermis (Fig. 3*B*); liposomes containing inactive T4N5 had no effect (not shown). The effect was specific for skin containing DNA damage because application of T4N5 liposomes to unirradiated skin (Fig. 3*C*) had no effect on epidermal proliferation or IL-10 production compared with untreated skin (not shown).

Circulating IL-10 was measured in the serum of these groups of mice by ELISA. No IL-10 was detected in the serum of any group of non-UV-irradiated mice; however, 3 days after UV,

Table 1. Effect of liposomal T4N5 on UV-induced IL-10 secretion by PAM212 cells

Treatment		
UV*	Liposomes†	IL-10, pg/ml‡
−	None	0
−	T4N5	0
−	HI	0
+	None	662 ± 158
+	T4N5	73 ± 42
+	HI	973 ± 98

*PAM212 cells were exposed to 200 J/m^2 UVB (+) or were not exposed (−).

†Liposomes containing active enzyme (T4N5) or heat-inactivated enzyme (HI) were added to cultures for 1 h immediately after UV. After excess liposomes were washed off, serum-free medium was added.

‡Supernatants were harvested 24 h after UV irradiation, and IL-10 was measured by ELISA. Values are mean ± SD of triplicate samples.

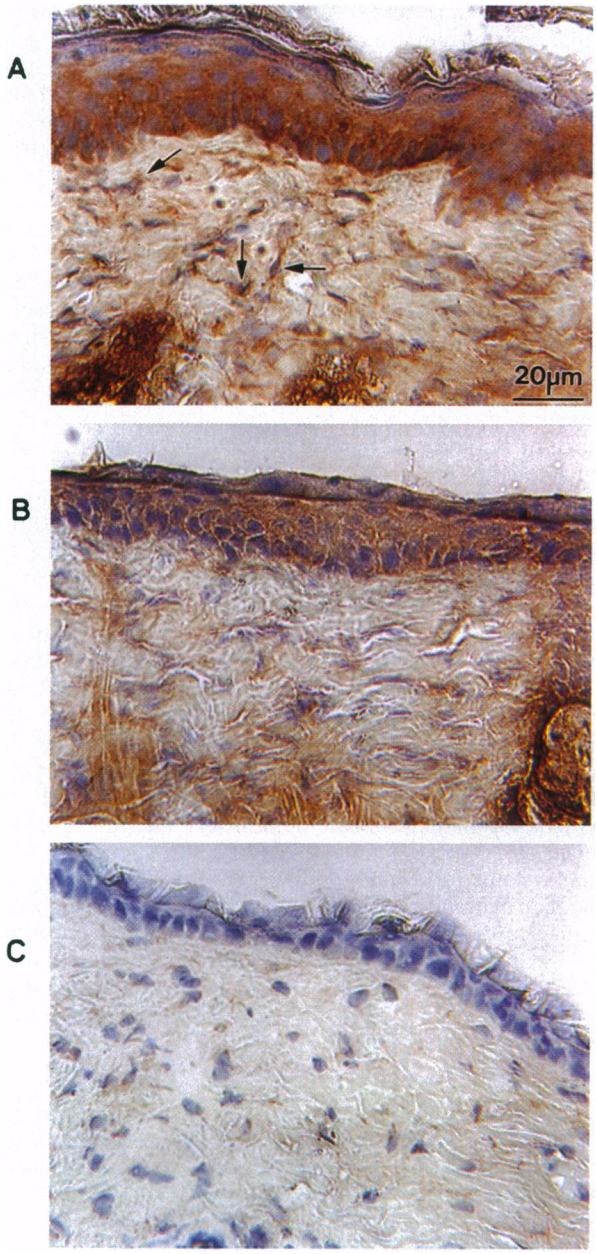

FIG. 3. Effect of T4N5 liposomes on UV induction of IL-10 in murine skin *in vivo*. Mice were exposed to 10 kJ/m^2 UVB on shaved dorsal skin, which was immediately treated with active or inactive T4N5 in liposomes. Skin samples were taken 3 days after UV, and cryostat sections were fixed and stained for IL-10 by immunoperoxidase labeling as described for Fig. 2. (*A*) UV alone (arrows indicate IL-10 positive dendritic cells in dermis). (*B*) UV + T4N5 liposomes. (*C*) T4N5 liposomes alone.

the serum of UV-irradiated mice contained 6.4 ± 0.7 ng/ml of IL-10, whereas at the same time the serum of mice treated with active T4N5 liposomes after UV irradiation contained 1.2 ± 0.7 ng/ml of IL-10 ($P < 0.0001$). This reduction was associated with the DNA repair activity of the T4N5 liposomes, since treatment of mice with inactive T4N5 in liposomes after UV irradiation did not significantly reduce the amount of IL-10 in the serum (6.0 ± 0.9 ng/ml).

Reduction of CPD in UV-Irradiated PAM212 Cells by T4N5 Liposomes. Treatment of murine fibroblasts in culture and mouse skin *in vivo* with T4N5 liposomes after exposure to UVC radiation increases the rate of CPD removal from DNA (8). To confirm that T4N5 liposomes reduced the number of CPD in PAM212 cells, we first used a monoclonal antibody to visualize CPD in cells by immunoperoxidase labeling (13). PAM212 cells were exposed to doses of UVB ranging from 100 to 400 J/m^2 and stained 6 h later to detect CPD. Fig. 4*A* shows that increasing the dose of UV led to an increased intensity of nuclear staining by the anti-CPD monoclonal antibody. UV-irradiated cells treated with an isotype-matched control antibody and the developing reagents showed no staining above the background levels observed in the unirradiated cells (not shown).

To assess the effect of T4N5 liposomes on CPD, PAM212 cells were exposed to 200 J/m^2 UVB and treated for 1 h with liposomes containing active or heat-inactivated T4N5. After an additional 5 h of incubation, the cells were fixed, stained, and examined for CPD. As shown in Fig. 4*B*, treatment with T4N5 liposomes (*Center*) reduced the dimer content of cells, but liposomes containing heat-inactivated endonuclease had little effect (*Right*) compared with UV-irradiated controls (*Left*). Quantitation of the effect of T4N5 liposomes on CPD removal was performed by measuring endonuclease-sensitive sites using the alkaline agarose gel technique. At 6 h after irradiation, the number of CPD/10^6 bases was 146 in cells treated with 200 J/m^2 UVB and 132 in cells treated with HI liposomes, but was 116 in UV-irradiated cells treated with T4N5 liposomes. Relative to the UV-irradiated cells, this represented a 20% reduction in the number of CPD. An additional kinetic study revealed that the majority of CPD remain unrepaired at 24 h and that nearly all repair occurred during the first 6 h after UV irradiation. The percent CPD remaining at 6, 18, and 24 h in the UV-irradiated PAM212 cells was 71, 77, and 64%, respectively. In UV-irradiated cells treated with T4N5 liposomes, there was again a 19.6% percent reduction in the number of CPD at 6 h. The percent of CPD remaining was 57, 56, and 50% at 6, 18, and 24 h, respectively, and the number of CPD was significantly less than controls at all time points ($P < 0.01$, paired t test).

DISCUSSION

In these studies, treatment of UV-irradiated murine keratinocytes with liposomal T4N5 *in vitro* reduced the immunosuppressive activity and IL-10 content of culture supernatants. Immunocytochemical staining of the cells demonstrated that T4N5 liposome treatment reduced both the IL-10 content of the keratinocytes and the number of CPD, although the effects on IL-10 and suppressive activity were more dramatic than the effect on CPD removal, as we discuss below. The ability of the liposomes to abrogate these effects of UV seemed to be associated with the repair of UV-irradiated DNA because control liposomes containing heat-inactivated enzyme or treatment of unirradiated cells with active T4N5 liposomes did

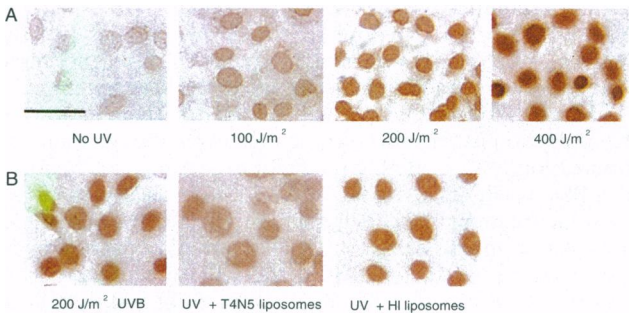

FIG. 4. Visualization of CPD in UV-irradiated PAM212 cells. (*A*) PAM212 cells were treated with increasing doses of UVB and fixed 6 h later with 70% ethanol. CPD were detected using a dimer-specific monoclonal antibody in an immunoperoxidase method. (Bar = 20 μm.) (*B*) PAM212 cells were treated with 200 J/m^2 UVB and incubated for 1 h with active (T4N5) or inactive (HI) T4N5 in liposomes. Five h later, they were washed, fixed, and stained by immunoperoxidase using anti-CPD antibody.

not have such effects. Treatment of UV-irradiated mouse skin *in vivo* with T4N5 liposomes also reduced the appearance of IL-10 in both skin and serum. Whether the serum IL-10 is derived from keratinocytes, dermal macrophages, or some other source is not clear. It is possible that keratinocyte-derived IL-10 enters the circulation directly via skin capillaries; alternatively, other immunoregulatory cytokines produced by UV-activated keratinocytes may enter the circulation and stimulate blood monocytes to secrete IL-10. For example, treating blood monocytes with prostaglandin E_2, an immunomodulatory agent produced by UV-irradiated keratinocytes, induces IL-10 secretion (14). Similarly, tumor necrosis factor α, which is produced *in vivo* following UV irradiation (3), activates blood monocytes to secrete IL-10 (15–17). Regardless of the cellular source of the IL-10, however, it is clear that DNA damage can initiate the cascade of events that results in increased IL-10 in serum and ultimately the suppression of DTH.

Our findings from the *in vitro* studies of murine keratinocytes provide the first direct evidence that UV radiation-induced DNA damage initiates cytokine production and support our hypothesis that DNA damage in the form of CPD triggers the production of immunomodulatory epidermal cytokines. This conclusion differs somewhat from that of Simon *et al.* (18), who showed that UV irradiation of human keratinocytes activated the transcription factor NFκB by a DNA-independent mechanism. These and other similar studies (19, 20) imply that UV irradiation can activate transcription factors NFκB and AP-1 by a membrane-mediated mechanism that triggers signal transduction pathways. Since the promoter region of some cytokine genes have NFκB- or AP-1-binding sites, it has been inferred that production of at least some cytokines would be induced by a DNA damage-independent mechanism in UV-irradiated cells, although as yet no UV-sensitive chromophore has been identified in the cell membrane. However, this does not seem to be the case for IL-10 production in murine keratinocytes.

In murine skin *in vivo* and PAM212 cells *in vitro*, T4N5 liposome treatment reduced, but did not completely abrogate, IL-10 production. Nonetheless, the immunosuppressive effects of the supernatants and immune suppression *in vivo* (7) were blocked. This implies either that IL-10 is only one of several critical mediators of immune suppression induced by DNA damage or that a threshold amount of IL-10 is required to effect immune suppression *in vivo*. It was interesting to note that although systemic suppression of the DTH response was completely abrogated by T4N5 liposome treatment (7), other responses of the skin to UV, such as epidermal hyperplasia (Fig. 3) and skin swelling (21), were not appreciably altered. This implies that direct damage to the DNA of target cells in the skin is only one of several mechanisms by which UV radiation modifies the physiology of the skin.

As we have noted before in our *in vivo* studies (7), the number of CPD in UV-irradiated epidermal cells, measured 6 h after UV irradiation, is significantly lower when UV irradiation is followed by liposomal T4N5 treatment; however, the reduction is far from complete, even though immune suppression is abrogated. One might expect that the majority of CPD would have to be removed from DNA by T4N5 liposome treatment before immune suppression was inhibited. However, in mouse cells, not all CPD are of equal biological importance: only 10 to 20% of the CPD are excised from UV-irradiated mouse cells in culture in 24 h, and these cells survive as well as human cells, which excise around 80% of their CPD in that time period (22). In this study, repair of CPD in UV-irradiated PAM212 cells treated with T4N5 liposomes was increased by around 20% over the first 24 h. This increased repair was sufficient to eliminate the immunosuppressive activity and reduce the IL-10 content of the culture supernatants. Others have also found that small, but selective increases in repair have dramatic effects on responses to UV, such as increased cell survival (23), and the most important repair may be that which occurs selectively in actively transcribed genes (24). Thus, DNA damage in actively transcribed genes might signal a series of damage responses, including cytokine induction, and active genes should be among the first to undergo repair. Complete repair of all CPD would not be necessary to remove the signal for UV-induced activation of damage response genes. In summary, the evidence presented here demonstrates that DNA damage is a vital signal for cytokine induction in mouse cells, and it is the same type of DNA damage that is recognized and repaired by T4 endonuclease V. These data further suggest that removal of a subset of UV-induced CPD can abrogate the IL-10 induction signal. This challenges the assumption that biological responses are always proportional to the overall global level of genomic damage and opens new questions about the impact of gene-specific and cell-type-specific repair in expression of inducible cytokines.

From these results, we would predict that cytokine production is a highly sensitive and tightly regulated systemic indicator of localized cellular DNA damage and repair. The elevation of serum IL-10 in addition to induction of IL-10 in the skin shows that localized DNA damage can signal changes at distant, unirradiated sites. Because cytokines produced in response to UV radiation include ones involved in hematopoiesis, tissue repair, and inflammation (25), in addition to immune suppression, the close linkage of their production to DNA damage and repair suggests that they may function in the recovery of tissues injured by UV radiation. In fact, the immune suppression observed after UV irradiation may be an undesirable but minor consequence of a mechanism designed to restore homeostasis in organ systems following genotoxic insults. The finding that Fanconi's anemia, a condition characterized by chromosome fragility following ionizing radiation, is associated with a cytokine dysregulation involving tumor necrosis factor α and IL-6 (26), supports the concept that certain cytokines may be involved in restoring both cellular and tissue processes that are perturbed following DNA damage.

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1. Fisher, M. S. & Kripke, M. L. (1982) *Science* **216,** 1133–1134.
2. Rivas, J. M. & Ullrich, S. E. (1994) *J. Leukocyte Biol.* **56,** 769–775.
3. Vermeer, M. & Streilein, J. W. (1990) *Photodermatol. Photoimmunol. Photomed.* **7,** 258–265.
4. Rivas, J. M. & Ullrich, S. E. (1992) *J. Immunol.* **149,** 3865–3871.
5. Ullrich, S. E. (1995) *J. Invest. Dermatol.* **105,** 30S–36S.
6. Applegate, L. A., Ley, R. D., Alcalay, J. & Kripke, M. L. (1989) *J. Exp. Med.* **170,** 1117–1131.
7. Kripke, M. L., Cox, P. A., Alas, L. G. & Yarosh, D. B. (1992) *Proc. Natl. Acad. Sci. USA* **89,** 7516–7520.
8. Yarosh, D., Alas, L., Yee, B., Oberyszyn, A., Kibitel, J., Mitchell, D., Rosenstein, R., Spinositz, A. & Citron, M. (1992) *Cancer Res.* **52,** 4227–4231.
9. Yarosh, D. B., Bucana, C., Cox, P. A., Alas, L. G., Kibitel, J. & Kripke, M. L. (1994) *J. Invest. Dermatol.* **103,** 461–468.
10. Kantor, G. & Ritter, C. (1983) *Photochem. Photobiol.* **37,** 533–538.
11. Yarosh, D. B., Tsimis, J. & Yee, V. (1990) *J. Soc. Cosmet. Chem.* **41,** 85–92.
12. Ceccoli, J., Rosales, N., Tsimis, J. & Yarosh, D. B. (1989) *J. Invest. Dermatol.* **93,** 190–194.
13. Roza, L., van der Wulp, K. J. M., MacFarlane, S. J., Lohman, P. H. M. & Baan, R. A. (1988) *Photochem. Photobiol.* **48,** 627–634.
14. Strassmann, G., Patil-Koota, V., Finkelman, F., Fong, M. & Kambayashi, T. (1994) *J. Exp. Med.* **180,** 2365–2370.

15. Platzer, D., Meisel, C., Vogt, K., Platzer, M. & Volk, H.-D. (1995) *Int. Immunol.* **7,** 517–523.
16. van der Poll, T., Jansen, J., Levi, M., ten Cate, H., ten Cate, J. W. & van Deventer, S. J. H. (1994) *J. Exp. Med.* **180,** 1985–1988.
17. Wanidworanun, C. & Strober, W. (1993) *J. Immunol.* **151,** 6853–6861.
18. Simon, M. M., Aragane, Y., Schwarz, A., Luger, T. A. & Schwarz, T. (1994) *J. Invest. Dermatol.* **102,** 422–427.
19. Devary, Y., Rosette, C., DiDonato, J. A. & Karin, M. (1993) *Science* **261,** 1442–1445.
20. Coffer, P. J., Burgering, B. M., Peppelenbosch, M. P., Bos, J. L. & Kruijer, W. (1995) *Oncogene* **11,** 561–569.
21. Wolf, P., Yarosh, D. & Kripke, M. L. (1993) *J. Invest. Dermatol.* **101,** 523–527.
22. Bohr, V. A. (1987) *Dan. Med. Bull.* **34,** 309–320.
23. Cleaver, J., Charles, W. C., McDowell, M. L., Sadinsky, W. J. & Mitchell, D. L. (1995) *Cancer Res.* **55,** 6152–6160.
24. Ruven, H. J. T., Berg, R. J. W., Seelen, J. J., Dekkers, A. J. M., Lohman, P. H. M., Mullenders, L. H. J. & van Zeeland, A. A. (1993) *Cancer Res.* **53,** 1642–1645.
25. Luger, T. A. & Schwarz, T. (1995) in *Photoimmunology*, eds. Krutmann, J. & Elmets, C. A. (Blackwell, Oxford), pp. 55–76.
26. Rosselli, F., Sanceau, J., Wietzerbin, J. & Moustacchi, E. (1992) *Hum. Genet.* **89,** 42–48.