Mechanism Involved in the Systemic Suppression of Antigen-Presenting Cell Function by UV Irradiation

Keratinocyte-Derived IL-10 Modulates Antigen-Presenting Cell Function of Splenic Adherent Cells[1]

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Exposure to UV radiation suppresses tumor rejection and delayed-in-time hypersensitivity reactions and depresses splenic APC function. Because almost all of the UV radiation is absorbed in the upper layers of the skin, it appears unlikely the direct irradiation of APC can account for the impaired ability of splenic adherent cells to present Ag after total-body UV exposure. Because UV-irradiated keratinocytes release IL-10, and in light of the well-documented effects of IL-10 on Ag presentation, we tested the hypothesis that keratinocyte-derived IL-10 is responsible for the systemic impairment of APC function following UV exposure. Injecting supernatants from UV-irradiated keratinocytes suppressed the ability of splenic adherent cells to present Ag. Treating the supernatants with anti-IL-10 mAb neutralized the suppressive effect. Similarly, when splenic adherent cells were isolated from mice exposed to UV radiation, APC function was suppressed. Injecting the UV-irradiated animals with anti-IL-10 restored APC function. In addition, spleen cells from UV-irradiated mice did not efficiently present Ag to Th1 clones, and injecting anti-IL-10 after UV exposure restored APC function. The reverse was observed when spleen cells from UV-irradiated mice were used to present Ag to Th2 clones; in which case, UV exposure enhances APC function, and anti-IL-10 reverses this effect. These findings suggest that UV-induced, keratinocyte-derived IL-10 can modulate splenic APC function. *Journal of Immunology,* 1994, 152: 3410.

The UV radiation found in sunlight is carcinogenic and the major cause of nonmelanoma skin cancers (1). Moreover, UV radiation is immunosuppressive, and studies with experimental animals (2–4) and, recently, with biopsy-proven skin cancer patients (5) have indicated that there is a link between the immunosuppressive effects of UV radiation and its carcinogenic potential. Because skin cancer is the most prevalent form of human cancer and considering that exposure to UV radiation occurs daily and may be increasing because of depletion of atmospheric ozone by environmental pollutants, it is important to determine how UV exposure suppresses the immune response. Three well-described immunologic defects are observed in mice following whole-body UV exposure: suppression of the ability to reject highly antigenic, UV-induced skin tumors; suppression of delayed-in-time hypersensitivity reactions; and a defect in the ability of splenic adherent cells (SAC)[3] to present Ag (6). Although a variety of studies have demonstrated that direct irradiation of APC by UV can alter APC function (7), the limited ability of UV radiation to penetrate beyond the dermis (8) suggests that direct irradiation of splenic APC probably does not occur following in vivo UV exposure. It seems more likely that other mechanisms are involved in the UV-induced impairment of splenic APC function.

One potential mechanism for the induction of systemic immune defects following exposure to UV radiation is the release of immunomodulatory cytokines by UV-irradiated epidermal cells (9). One of the cytokines produced by activated keratinocytes is IL-10 (10, 11). Recently, we provided evidence for the essential role of keratinocyte-derived IL-10 in the systemic suppression of delayed-type

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[3] Abbreviations used in this paper: SAC, splenic adherent cells; DTH, delayed-type hypersensitivity; HEL, hen egg lysozyme; SN, supernatant protein.

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hypersensitivity (DTH) following UV exposure (11). Because of the well-documented effects of IL-10 on APC function (12–16), the release of IL-10 by keratinocytes also appears to provide a plausible explanation for the impairment of splenic APC function found following in vivo UV exposure. In this study, we test the hypothesis that keratinocyte-derived IL-10 modulates splenic APC function.

Materials and Methods

Animals

Specific pathogen-free female C3H/HeNCr (MTV−) and BALB/c AnNCr mice (8- to 12-wk-old) were purchased from the National Cancer Institute-Frederick Cancer Research Facility Animal Production Area (Frederick, MD). Animals are maintained in facilities approved by the American Association for Accreditation of Laboratory Animal Care and in accordance with current United States Department of Agriculture, Department of Health and Human Services, and National Institutes of Health regulations and standards. All animal procedures were approved by the Institutional Animal Care and Use Committee. Within each experiment, all mice were age and sex matched. The mice received NIH-31 open formula mouse chow and sterile water ad libitum. Ambient light was controlled to provide regular cycles of 12 h of light and 12 h of darkness.

Cell lines, antibodies, and reagents

Pam 212, a spontaneous transformed murine keratinocyte cell line, was provided by Dr. Stuart Yuspa (National Cancer Institute, Bethesda, MD) (17). HDK-1, a KLH-specific Th1 clone (18), and SXC-1, a hybridoma producing rat anti-mouse-IL-10 (IgM isotype) (19), were provided by Dr. Timothy Mosmann (University of Alberta, Edmonton, Canada). 3A9, a hen egg lysozyme (HEL, Sigma Chemical Co., St. Louis, MO) specific T cell hybridoma, was provided by Dr. Emil Unanue (University of Washington, St. Louis, MO). D10.G4.1, a conalbumin-specific Th2 clone, was purchased from American Type Culture Collection (Rockville, MD). Monoclonal anti-CD3 was purchased from PharMingen (San Diego, CA). The production of IFN-γ, IL-2, or IL-10 was measured by ELISA with kits purchased from Genzyme (Boston, MA, IFN-γ, sensitivity 125 pg/ml), PharMingen (IL-2; sensitivity 0.5 U/ml), or Endogen (Cambridge, MA, IL-10, sensitivity 0.14 U/ml) and performed according to the manufacturer's instructions. Murine rIL-10 was purchased from Pepro-Tech Inc. (Princeton, NJ). Tissue culture medium and supplements were purchased from GIBCO BRL (Grand Island, NY). FCS was purchased from HyClone Laboratories, Inc. (Logan, UT).

Radiation sources

A bank of 6 FS-40 sun lamps (Westinghouse, Bloomfield, NJ) was used to treat mice with UV radiation. These lamps emit a continuous spectrum from 270 to 390 nm; with a peak emission at 313 nm; approximately 65% of the radiation emitted by these lamps is within the UVB range (280 to 320 nm). The irradiance of the source averaged 10 J/m^2/s, as measured by an IL-700 radiometer, using a SEE 240 UVB detector equipped with an A127 quartz diffuser (International Light, Inc., Newburyport, MA). Because of shielding by the cage lids, the incident dose received by the animals was approximately 4.5 J/m^2/s. The total dose of UVB received was approximately 15 kJ/m^2. Before irradiation, the dorsal hair of the mice was removed with electric clippers. Keratinocyte cultures were irradiated with a single FS-40 bulb. The output of this lamp was 1.43 J/m^2/s, at a tube-to-target distance of 23 cm.

In vitro UV irradiation of keratinocytes

Five million keratinocytes were added to 100-mm tissue-culture dishes in 5 ml of MEM supplemented with 10% FCS and cultured overnight. The medium was removed, and the keratinocyte monolayers were washed three times with PBS and overlaid with PBS. The monolayers were then exposed to 200 J/m^2 of UV radiation. Only a single dose of UV radiation was used in the present study because results from previous studies had indicated that 200 J/m^2 caused maximal release of the suppressive cytokine without greatly affecting keratinocyte survival and cell viability (20). After irradiation, the cells were resuspended in serum-free MEM. Twenty-four hours later, the supernatant fluid was removed. The protein concentration was determined by the Bradford assay (Bio-Rad Laboratories, Rockville Centre, NY). Approximately 20 μg of protein was injected into each mouse. Control supernatants were obtained from keratinocytes handled in a similar manner but not exposed to UV radiation (mock-irradiated cells). Endotoxin contamination, as determined by the *Limulus* amebocyte lysate assay (Cape Cod Associates, Woods Hole, MA), was below the limit of detection (0.125 ng/ml).

Neutralization of IL-10 activity

One hundred micrograms of protein from UV-irradiated or mock-irradiated keratinocytes was precleared for 1 h at 37°C with protein A-/G agarose (Pierce Immuno-chemicals, Rockford, IL) beads. The material was centrifuged, and the supernatant was transferred to a new microcentrifuge tube. The samples were then incubated overnight at 4°C with 10 μg/ml of rat anti-mouse IL-10 (SXC-1), isotype-matched control Ab (RA3-2C2/1), or normal rat serum. The samples were then treated for 90 min at 37°C with goat anti-rat IgM (IgG isotype). All samples were then incubated at 37°C for 60 min with protein A/G-agarose beads. The samples were centrifuged, and the supernatant was injected into mice.

Determination of APC function

Spleen cells were isolated from normal mice, mice injected with supernatants from UV (UV-SN) or mock-irradiated keratinocytes (NR-SN), or mice exposed to UV radiation and used to present Ag to T cell clones or T cell hybridomas. The production of IL-2 by HEL-specific T cell hybridoma 3A9 was measured according to the procedure described by Allen and Unanue (21). Spleens were removed from the treated mice, single-cell suspensions were prepared, and contaminating erythrocytes were lysed with ammonium chloride. The remaining cells were resuspended in complete RPMI 1640 medium and incubated on plastic for 1 h at 37°C. The nonadherent cells were removed, the monolayers washed three times with warm medium, and then adherent cells removed from the plastic by a 15-min incubation with 12 mM lidocaine (Astra Pharmaceutical Products, Worcester, MA). The cells were washed three times in HBSS, resuspended in complete RPMI medium, and counted. Approximately 1×10^5 SAC were cultured with an equal number of 3A9 cells and 100 μg/ml of HEL in 96-well tissue culture dishes. After 24 to 36 h, the production of IL-2 by the 3A9 cells was measured by ELISA.

In some experiments, spleen cells from mice injected with supernatants from the UV-irradiated keratinocyte cultures or from mice exposed to UV radiation were used to present Ag to Th1 or Th2 T cell clones. The procedures described by Cherwinski et al. (HDK-1;Th1) (18) or Kaye et al. (D10G4.1;Th2) (22) were used. Spleen cells were isolated from BALB/c (HDK-1) or C3H/HeN mice, (D10G4.1), single-cell suspensions prepared, and erythrocytes lysed as described above. Approximately 1×10^6 whole spleen cells were mixed with 1×10^5 T cells and the appropriate Ag in 96-well dishes and cultured at 37°C for 24 to 36 h. At the end of the culture period, IFN-γ production by the Th1 cells and IL-10 production by the Th2 cells was measured by ELISA.

Results

Supernatants from UV-irradiated keratinocyte cultures depress splenic Ag-presenting cell function

Previously, we reported that injecting 20 μg of SN from UV-irradiated keratinocytes could suppress DTH to alloantigen (20). Therefore, the first series of experiments presented here were designed to determine if injecting a similar amount of SN could affect splenic APC function. Mice were injected intravenously with supernatants from UV- or mock-irradiated keratinocytes. Five days later, SAC were isolated from these mice and from a group of normal controls. Data from such an experiment is found in

Table I. *Supernatants from UV-irradiated keratinocytes impair APC function of splenic adherent cells*

Source of APC[a]	Cytokine Production (U/ml ± SD)[b] Experiment 1	Experiment 2
Normal mice	7.6 ± 2.0	20.0 ± 0.7
Mice injected with UV-SN	< 1	< 1
Mice injected with NR-SN	9.8 ± 1.4	26.0 ± 1.0

[a] Spleen cells were isolated from mice injected with 20 μg supernatant protein from UV-irradiated keratinocytes (UV-SN) or mock-irradiated keratinocytes (NR-SN), 5 days after injection. SAC were isolated by adherence.
[b] 3A9 cells (1×10^5) were cultured with an equal number of SAC and HEL (100 μg/ml) for 24 to 36 h. At the end of the culture period, IL-2 production was determined by ELISA.

Table I. Whereas SAC isolated from normal mice or from mice injected with supernatants from the mock-irradiated cells did present Ag to the 3A9 cells, SAC isolated from mice injected with supernatants from the UV-irradiated keratinocytes had little or no APC function.

Monoclonal anti-IL-10 blocks the suppressive effects of supernatants from UV-irradiated keratinocytes

The next experiment tested the hypothesis that IL-10 was responsible for the suppression of APC function seen after supernatants from UV-irradiated keratinocytes were injected into mice. Supernatants from UV-irradiated keratinocytes were first treated with monoclonal anti-IL-10 (SXC-1) or isotype-matched control Ab (anti-B220, Clone RA3-2X2/1, ATCC). Goat anti-rat IgM was then added, and the immune complexes were precipitated with protein A/G-coated agarose beads. The remaining supernatants were injected into mice, and 5 days later, SAC were isolated and their ability to present Ag to 3A9 cells was tested. Data from such an experiment is found in Figure 1. Between 5 to 7 U/ml of IL-2 was generated when HEL was presented to 3A9 cells by SAC from normal mice or mice injected with supernatants from mock-irradiated cells. When the SAC were isolated from mice injected with supernatants from the UV-irradiated keratinocytes, almost no IL-2 was produced ($p < 0.05$; UV-SN vs normal; Student's *t*-test). Treating the supernatants with anti-IL-10 depleted all suppressive activity. ($p > 0.05$; UV-SN + anti-IL-10 vs normal) No depletion of suppressive activity was noted when the supernatants were treated with isotype-matched control Ab, suggesting that the IL-10 present in the supernatant was responsible for depressing splenic APC function.

rIL-10 suppresses splenic APC activity

Next, we determined if murine rIL-10 similarly depresses splenic APC function. By ELISA, we determined that approximately 10 U of IL-10 was injected into each mouse. Therefore, an experiment was performed in which various doses of murine rIL-10 were injected into the tail veins of normal mice. Five days later, SAC were prepared and used to present HEL to 3A9 cells. The data from this experiment is shown in Table II. Injecting mice with rIL-10 did depress splenic APC function. We found that maximal suppression was observed when the animals were injected with between 10 and 20 U of IL-10.

The kinetics of this effect were also studied (Fig. 2). SAC were removed at various times after injecting mice i.v. with supernatants from UV-irradiated keratinocytes or rIL-10 (10 U/mouse). At 1 day after injection, there was no difference in the APC function of SAC isolated from normal mice or from mice injected with supernatants from UV-irradiated keratinocytes or rIL-10. When the SAC were removed from mice injected with UV-SN or rIL-10 3 days after injection, there was a reduction in their ability

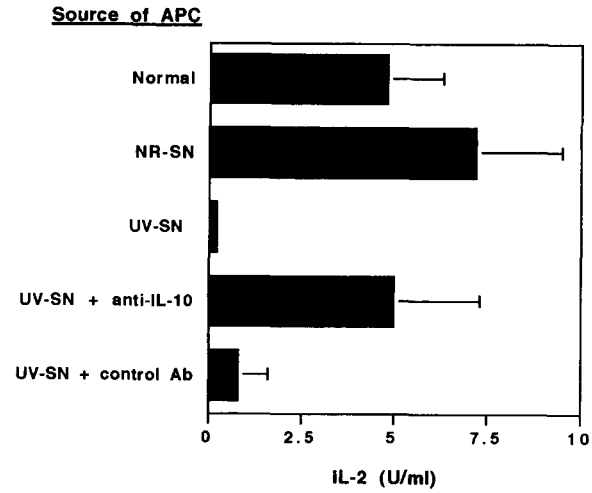

FIGURE 1. Treating supernatants from UV-irradiated keratinocytes with monoclonal anti-IL-10 reverses the suppressive activity. SAC were isolated from normal mice or from mice injected with supernatants from UV-irradiated keratinocytes (UV-SN) or mock-irradiated cells (NR-SN) and used to present Ag to 3A9 cells. The supernatants were treated with either anti-IL-10 or isotype-matched control Ab before injection.

Table II. *rIL-10 impairs APC function of splenic adherent cells*

Source of APC[a]	Cytokine Production (U/ml ± SD)[b] Experiment 1	Experiment 2
Normal mice	5.6 ± 0.73	8.1 ± 0.55
Mice injected with 0.1 U rIL-10	10.3 ± 1.9	7.9 ± 0.27
Mice injected with 1 U rIL-10	4.0 ± 0.36	9.5 ± 0.67
Mice injected with 5 U rIL-10	4.2 ± 0.8	6.3 ± 0.41
Mice injected with 10 U rIL-10	2.8 ± 0.19	4.0 ± 0.39
Mice injected with 20 U rIL-10	7.6 ± 0.13	4.7 ± 0.53

[a] SAC were isolated from normal mice or mice injected i.v. with rIL-10 (PeproTech, Inc.). In experiment 1, SAC were isolated 5 days after injection, in experiment 2, SAC were isolated 3 days after injection.
[b] 3A9 cells (1×10^5) were cultured with an equal number of SAC and HEL (100 μg/ml) for 24 to 36 h. At the end of the culture period, IL-2 production was determined by ELISA.

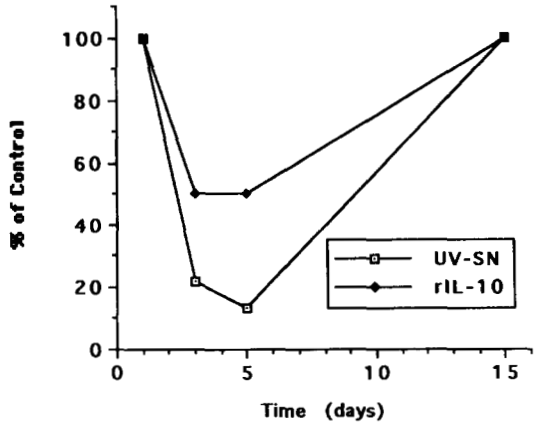

FIGURE 2. Kinetics of APC defect induced by UV-SN or rIL-10. Mice were injected with rIL-10 (10 U) or supernatants from UV-irradiated keratinocytes (UV-SN) containing ≈10 U of IL-10. At various times after injection, SAC were isolated and used to present Ag to 3A9 cells. Cytokine production by 3A9 cells cultured with normal SAC was considered to be 100%.

to present Ag. Similarly, at 5 days after injection, the ability of SAC from mice injected with supernatants from UV-irradiated keratinocytes or rIL-10 was suppressed. By 15 days, there was no difference in IL-2 production by 3A9 cells, regardless of whether the Ag was presented by cells from normal- or factor-injected mice. The kinetics of this response is very similar to that which is observed following total-body UV exposure (23). However, it is of interest to note that the suppression induced by supernatants from the UV-irradiated keratinocytes was always greater than that seen with rIL-10, regardless of the fact that the relative amounts of IL-10, by ELISA, were the same.

Effect of anti-IL-10 mAb on the impairment of APC function found in UV-irradiated mice

As mentioned above, spleen cells from UV-irradiated mice have a defect in APC function. The data presented in Table I and Figure 1 suggest that keratinocyte-derived IL-10 is involved. To test this hypothesis directly, mice were first exposed to UV radiation, and then injected with monoclonal anti-IL-10 or normal rat serum. Five days later, we examined the ability of spleen cells from these mice to present Ag. Two different measures of APC function were examined: the requirement for accessory cell function in the activation of T cells by soluble anti-CD3 mAb (24) and the presentation of nominal Ag to T cell clones.

Accessory cell function for anti-CD3-induced T cell activation was determined by taking whole spleen cells from normal mice, mice exposed to UV radiation, UV-irradiated mice injected with anti-IL-10 Ab, or UV-irradiated mice injected with normal rat serum. The cells were incubated with anti-CD-3 (10 μg/ml) and 200 U/ml of mouse rIL-2. Twenty-four to 36 h later, IFN-γ production by the

Table III. *Injecting monoclonal anti-IL-10 blocks UV-induced impairment of splenic accessory cell function*

Source of Spleen Cells[a]	IFN-γ Production (U/ml ± SD)[b]
Normal mice	380 ± 8
UV-irradiated	< 1
UV + anti-IL-10	337 ± 17
UV + normal rat serum	< 1

[a] Spleen cells were isolated from normal mice, mice exposed to UV radiation (15 kJ/m²), exposed to UV, and injected with anti-IL-10 (100 μg/mouse, i.p. 4 and 24 h after exposure), or mice exposed to UV and injected with normal rat serum.

[b] Whole spleen cells were cultured with anti-CD-3 (10 μg/ml) and 200 U of murine rIL-2. 24 to 36 h later IFN-γ production was measured by ELISA.

Table IV. *Injecting monoclonal anti-IL-10 restores UV-induced impairment of splenic APC function*

	Cytokine Production (U/ml ± SD) Responder Cells[b]		
Source of APC[a]	3A9	HDK-1	D10G4.1
Normal mice	68 ± 11	410 ± 10	30 ± 2
UV-irradiated	33 ± 10[c]	170 ± 40[d]	398 ± 92[d]
UV + anti-IL-10	54 ± 8[e]	537 ± 130[e]	20 ± 4[e]
UV + normal rat serum	31 ± 6[c]	200 ± 38[d]	358 ± 14[d]

[a] Spleen cells were isolated from normal mice, mice exposed to UV radiation (15 kJ/m²), exposed to UV and injected with anti-IL-10 (100 μg/mouse, i.p. 4 and 24 h after exposure), or mice exposed to UV and injected with normal rat serum.

[b] 1×10^6 whole spleen cells were cultured with 1×10^5 3A9, HDK-1 or D10G4.1 cells and Ag (100 μg/ml) for 36 h, cytokine production was determined by ELISA (3A9; IL-2; HDK-1; IFN-γ; D10G4.1; IL-10).

[c] $p < 0.05$ vs cytokine production using normal APC (two-tailed Student's *t*-test).

[d] $p < 0.001$ vs cytokine production using normal APC.

[e] Not significantly different vs cytokine production using normal APC ($p > 0.05$).

CD-3-activated cells was determined by ELISA. As is shown in Table III, IFN-γ was secreted when cells from normal mice were stimulated with anti-CD3 Ab. Anti-CD3 treatment was ineffective at stimulating cells isolated from UV-irradiated animals, as no IFN-γ was secreted by these cultures. Injecting anti-IL-10 immediately following exposure to UV radiation restored accessory cell function, whereas IFN-γ was not produced by cells isolated from UV-irradiated mice injected with normal rat serum.

The ability of spleen cells from UV-irradiated mice to present nominal Ag to T cell clones was also examined (Table IV). Three different responder cell populations were used in this experiment: 3A9, a T cell hybridoma; HDK-1, a Th1 clone; and D10G4.1, a Th2 clone. Spleen cells from normal mice or from mice exposed to UV radiation were used to present Ag to the T cells. (Spleen cells were used in these studies because, in preliminary experiments, we found better APC function when spleen cells were used instead of SAC. This may reflect the fact that the Th1 and Th2 clones were originally derived using whole spleen cells.) Cytokine production by the 3A9 and HDK-1 cells was lower than that of positive controls when

the Ag was presented by APC from UV-irradiated animals. APC function was restored by injecting the UV-irradiated mice with anti-IL-10 antibody; injecting the mice with normal rat serum had no effect.

The opposite was seen when spleen cells from UV-irradiated mice were used to present Ag to Th2 cells. Presentation of Ag by normal spleen cells resulted in little stimulation of the D10G4.1 cells. However, when the Ag was presented by spleen cells from UV-irradiated animals, a significant increase in cytokine production was noted. Injecting the UV-irradiated mice with anti-IL-10 abolished the enhancement of cytokine production, whereas injecting normal rat serum did not. Thus, these data suggest that IL-10, presumably released by UV-irradiated keratinocytes, suppresses accessory cell function in UV-irradiated animals. Furthermore, the data suggest that although exposure to UV radiation impairs APC function for Th1 clones, it enhances Ag presentation for Th2 clones, and injecting monoclonal anti-IL-10 can reverse these effects on APC.

Discussion

Although almost all of the energy contained within the UVB region of the solar spectrum is absorbed by the epidermis and upper layers of the dermis, exposure to UV radiation has systemic effects on the immune system. It is important to study the mechanisms involved in the induction of systemic immune suppression following UV exposure because of the link between the immunosuppressive and carcinogenic activities of UV radiation (4, 5). Recently, we provided evidence for the essential role of IL-10 in suppressing DTH following exposure to UV radiation (11). The data reported here indicate that keratinocyte-derived IL-10 also plays a prominent role in the modulation of splenic APC function. This conclusion is based on the observations that injecting supernatants containing IL-10 depresses the ability of spleen cells to present Ag to T cells. Treating the supernatants with anti-IL-10 reverses suppressive activity. In addition, spleen cells from UV-irradiated mice do not present Ag to T cells, and injecting UV-irradiated mice with anti-IL-10 mAb overcomes the suppressive effect. Thus, these findings suggest that the APC defect found in vivo after whole-body UV irradiation is due to the release of IL-10 by UV-irradiated keratinocytes.

As mentioned above, the effects of IL-10 on APC function are well documented (12–16). In all probability, keratinocyte-derived IL-10 down-regulates APC function in vivo by similar mechanisms. For example, murine IL-10 has been shown to suppress IFN-γ secretion by Th1 cells through its effect on monokine production by the relevant APC (15). Moreover, recent studies by Ding et al. (25) suggest that IL-10 prevents the up-regulation of the costimulatory molecule B7/BB1 on macrophages. It seems reasonable to suggest that keratinocyte-derived IL-10 similarly affects monokine production and/or B7/BB1 expression in vivo following UV exposure; studies are currently in progress to test these hypotheses.

The depressed ability of spleen cells from UV-treated mice to present Ag to Th1 cells, with enhanced presentation to Th2 clones, as illustrated in Table III is reminiscent of the findings of Simon et al. (26). In their study, FACS "purified" Langerhans cells were used to present Ag to Th1 and Th2 clones. Although normal Langerhans cells could present to both Th subsets, UV-irradiated Langerhans cells were unable to present to Th1 cells, while retaining their ability to present to Th2 cells. We see a similar situation in vivo following UV exposure. Spleen cells from UV-irradiated mice were relatively inefficient at presenting Ag to Th1 cells (Tables II and III), but were more efficient at presenting Ag to Th2 cells (Table III). In addition, IL-10 appears to be critical in modulating APC function in vivo since injecting anti-IL-10 reverses the UV-induced effects.

Taken together, these findings suggest that there are at least two different mechanisms by which exposure to UV radiation modulates APC function. Simon et al. (26) suggested that direct UV irradiation of APC down-modulates the expression of costimulatory molecules that were necessary for the activation of Th1 cells. Data to support this hypothesis was recently presented by Young and colleagues (27), who found that the expression of the costimulatory molecules B7/BB1 and ICAM-1 was inhibited by UV light. The data presented in this paper suggests a second indirect mechanism may be operational: inhibition of systemic APC function by keratinocyte-derived IL-10. Moreover, it is entirely possible that in UV-irradiated skin the two mechanisms overlap. Exposure to UV radiation could be down-regulating the expression of costimulatory molecules on Langerhans cells and, at the same time, inducing neighboring keratinocytes to produce IL-10 that further modulates local APC function. The observation of Enk et al. (28) demonstrating that Langerhans cells APC function is sensitive to the inhibitory effects of IL-10 would support this hypothesis.

On the basis of these observations, we propose that a major effect of UV radiation is that it modifies APC function so that only certain subclasses of T cells are activated. Our data suggests UV-induced, keratinocyte-derived IL-10 modulates splenic APC function so that Th2 cells are preferentially activated. We suggest that IL-10 interferes with the APC function of macrophages, thus inhibiting IFN-γ production by Th1 cells and limiting the induction of cellular immune responses. However, because the APC for Th1 and Th2 cells differ (29) and because IL-10 does not affect all APCs to the same degree (15, 16, 30), we suggest that keratinocyte-derived IL-10 enhances the activation of Th2 cells in vivo (as suggested by the findings reported in Table III). These activated Th2 cells, by virtue of producing cytokines such as IL-4 and IL-10, can further limit the induction of cellular immune responses. Furthermore, we

suggest that the $CD3^+$, $CD4^+$, $CD8^-$ suppressor cells found in the spleens of UV-irradiated animals (20, 31, 32) are Ag-activated Th2 cells. When transferred into recipient mice and stimulated by the proper Ag, these cells produce IL-4 and IL-10, which suppress the activation of Th1 cells and interfere with the development of DTH. We currently are testing this hypothesis by measuring the ability of monoclonal anti-IL-10 and anti-IL-4 to reverse the activity of these suppressor cells.

Although a variety of epidermal-derived factors have been implicated in the induction of systemic immune suppression following UV exposure (33), only a few have been shown to mimic what is seen after whole body exposure to UV radiation and suppress both the induction of delayed-in-time hypersensitivity reactions and APC function. In addition to IL-10, Contra-IL-1, and *cis*-urocanic acid have been found to suppress delayed or contact hypersensitivity and interfere with splenic APC function (34–37). Why have redundant mechanisms evolved to suppress the immune response following UV-irradiation? Undoubtedly, the mutagenic potential of UV radiation coupled with the need to maintain skin homeostasis resulted in the development of multiple suppressive mechanisms. One consequence of UV exposure may be the induction of new or altered "skin Ags" that elements of the immune system recognize as foreign (6). Perhaps the pressure to prevent antiskin autoimmune responses select for the development of UV-triggered immune suppression.

Our findings indicate that IL-10 is necessary to suppress splenic APC function, but our data also suggest that it may not be the only component involved. Activated keratinocytes release a wide variety of other immunomodulatory factors including, IL-1, 3, 6, 8, GM-CSF, TNF-α, TGF-β, and prostaglandin E_2 (9). Although we can reproducibly reverse the suppressive effects of UV radiation in vivo by injecting anti-IL-10 mAb, comparable levels of immune suppression were not induced following injection with rIL-10 or IL-10-containing supernatants from UV-irradiated keratinocytes (Table I vs Table II and Fig. 2). This would suggest that another factor released by the UV-irradiated keratinocytes is acting in concert with the IL-10 to promote immune suppression. It is not clear which of the many immunomodulatory factors released by UV-irradiated keratinocytes are involved, but it is interesting to note that prostaglandin E_2 inhibits the production of Th1 cytokines but not Th2 cytokines (38). Moreover, Araneo et al. (39) have indicated that UV exposure depresses Th1 cytokine production and enhances Th2 cytokine release through an IL-1-dependent mechanism. Whether keratinocyte-derived IL-10, prostaglandin E_2, and/or IL-1 are working in concert to suppress Th1 function and enhance Th2-like activity in this system remains to be seen.

In summary, the data reported here indicate that soluble factors released by UV-irradiated keratinocytes can depress splenic APC function. Treating the keratinocyte supernatant with monoclonal anti-IL-10 reverses the suppressive effect. In addition, spleen cells from UV-irradiated mice could not present Ag to Th1 cells, and this effect of UV radiation on systemic APC function was reversed by injecting monoclonal anti-IL-10. However, when spleen cells from UV-irradiated mice were used to present Ag to Th2 clones, it was observed that exposure to UV radiation enhanced APC function, and anti-IL-10 reversed this effect. On the basis of on these findings we suggest that IL-10, which presumably is released by UV-irradiated keratinocytes, plays an essential role in the induction of the systemic impairment of APC function found in UV-irradiated animals. Furthermore, we suggest that UV irradiation modulates splenic APC function to preferentially activate Th2 cells.

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