A Cytokine Cascade Including Prostaglandin E₂, IL-4, and IL-10 Is Responsible for UV-Induced Systemic Immune Suppression

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A Cytokine Cascade Including Prostaglandin E\textsubscript{2}, IL-4, and IL-10 Is Responsible for UV-Induced Systemic Immune Suppression\textsuperscript{1}

Vijay Shreedhar, Todd Giese, Victor W. Sung, and Stephen E. Ullrich\textsuperscript{2}

Even though all of the energy contained with the UV wavelengths of solar radiation is absorbed within the epidermis and upper layers of the dermis, UV irradiation can suppress immune responses to Ag introduced at distant nonirradiated sites. In addition, data from a number of laboratories have suggested that one consequence of UV exposure is suppressed Th1 cell activation with normal or enhanced Th2 cell activation, resulting in a shift to a Th2-like phenotype. Cytokines secreted by UV-irradiated keratinocytes, particularly IL-10, have been shown to play a major role in the induction of systemic immune suppression and differential activation of T helper cell subsets. Although IL-10 can influence Th1 cell activation by altering Ag presentation and suppressing IFN-\textgamma secretion, the major signal for the development of a Th2 response is IL-4. Here we tested the hypothesis that UV irradiation induces IL-4 secretion. UV irradiation induced serum IL-4 in a dose-dependent fashion. Injecting UV-irradiated mice with anti-IL-4 blocked immune suppression. We could find no evidence, however, supporting secretion of IL-4 by UV-irradiated keratinocytes. Rather, we suggest that prostaglandins released by irradiated keratinocytes induce serum IL-4 since UV-irradiated mice with anti-IL-4 suppressed serum IL-4 levels. Moreover, we found that treating UV-irradiated mice with cyclooxygenase-2 inhibitor blocked its production. We could find no evidence, however, supporting secretion of IL-4 by UV-irradiated keratinocytes. Rather, we suggest that prostaglandins released by irradiated keratinocytes induce serum IL-4 since treating UV-irradiated mice with a cyclooxygenase-2 inhibitor blocked its production. Moreover, we found that treating UV-irradiated mice with anti-IL-4 suppressed serum IL-4 levels. In addition, injecting normal mice with PGE\textsubscript{2} induced serum IL-4 and IL-10. We suggest that UV exposure activates a cytokine cascade (PGE\textsubscript{2} \rightarrow IL-4 \rightarrow IL-10) that ultimately results in systemic immune suppression. The Journal of Immunology, 1998, 160: 3783–3789.

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\textsuperscript{3} Abbreviations used in this paper: DTH, delayed type hypersensitivity; COX-2, cyclooxygenase-2.
function, as measured by cytokine secretion by the responder cells, was significantly enhanced. Administration of monoclonal anti-IL-10 to the UV-irradiated mice that were the source of the APCs reversed these effects and restored the ability of APC from the UV-irradiated mice to present to Th1 cells (15).

Additional findings support the hypothesis that UV irradiation may preferentially alter the balance between Th1 and Th2 cell activation. First, the Ag-specific suppressor T cells found in the spleens of UV-irradiated mice appear to be Th2-like cells because blocking IL-4 and/or IL-10 production by these cells abrogates their suppressive activity (13). Moreover, Yagi et al. reported that a cloned suppressor T cell line isolated from UV-irradiated mice secretes IL-4 and IL-10, but not IFN-γ, suggesting it is a Th2 cell (16). Second, UV exposure affects the production of Ig isotypes that are helped by different subsets of T helper cells. In the mouse, Th1 cells provide the help necessary for the secretion of IgG2a and 2b, whereas Th2 cells provide help for IgG1 secretion. UV irradiation was found to suppress the production of IgG2a and IgG2b in mice infected with Borrelia burgdorferi. In addition, a small but significant increase in IgG1 Ab production was seen in response to UV exposure. Administration of monoclonal anti-IL-10 to the UV-irradiated mice returned the amount of B. burgdorferi-specific IgG2a and IgG2b to levels that were indistinguishable from the controls, suggesting that UV-induced IL-10 is altering the activation of Th1 and Th2 cells in vivo (17). Finally, injecting UV-irradiated mice with IL-12 reversed the induction of immune suppression as well as blocked the induction of, and activity of, the UV-induced suppressor T cells (18). Because IL-12 is reported to enhance the production of Th1 cells (19) and to block the activation/differentiation of Th2 cells in vivo (20, 21), these findings provide further support for the hypothesis that UV exposure blocks Th1 cell function while allowing Th2 cell stimulation to proceed.

It is clear, however, that, while IL-10 can influence Th1 cell activation by altering Ag presentation and suppressing IFN-γ secretion (22), the major signal for the development of a Th2 response is IL-4 (23). Although a number of immune modulatory factors, including TNF-α, IL-1, IL-6, IL-10, and PGE2, are secreted by UV-irradiated keratinocytes and some are found in the serum of UV-irradiated mice (24), it is not known if UV exposure also upregulates IL-4 production. Because available evidence supports the hypothesis that UV exposure shifts the immune response to one predominated by Th2 cells, and because IL-4 is a critical mediator in the development of a Th2 immune response, the purposes of the experiments presented here were to determine whether IL-4 is induced following UV exposure and to determine whether IL-4 is involved in the induction of immune suppression following UV exposure.

Materials and Methods

Abs, cell lines, and reagents

The spontaneously transformed keratinocyte cell line PAM 212 was obtained from Dr. Stuart Yuspa (National Cancer Institute, Bethesda, MD) and grown in complete MEM as described previously (12). The hybridoma secreting anti-IL-4 (11B11; rat IgG1) was purchased from the American Type Culture Collection (Rockville, MD). The hybridoma secreting anti-IL-10 (JE55-2A5;11, rat IgG1) was kindly provided to us by Dr. Anne O’Garra (DNAX Research Institute, Palo Alto, CA). The hybridoma cells were grown in RPMI 1640 tissue culture medium (Life Technologies, Grand Island, NY) supplemented with 10% newborn calf serum (HyClone Laboratories, Logan, UT). The supernatants were collected, the IgG fraction was enriched by 33% ammonium sulfate precipitation, and IgG was purified by passage over protein A/G agarose columns (Pierce Immunoch- emicals, Rockford, IL). Protein concentration was determined by use of bicinchoninic acid (BCA protein assay kit, Pierce). Control rat IgG was purchased from Sigma Chemical (St. Louis, MO). The IL-2/IL-4-depen- dent T cell line HT-2 was purchased from ATCC. PGE2 was purchased from Cayman Chemical (Ann Arbor, MD). The selective cyclooxygenase (COX)-2 inhibitor SC236 was kindly provided by Dr. Peter C. Isakson (G. D. Searle & Co., St. Louis, MO). It was diluted in PBS and injected i.p.

Mice

Specific-pathogen-free female C3H/HeNcr (MTV−) and BALB/cAnCr mice were obtained from the National Cancer Institute Frederick Cancer Research Facility Animal Production Area (Frederick, MD). The animals were maintained in facilities approved by the Association for Assessment and Accreditation of Laboratory Animal Care International, in accordance with current regulations and standards of the U.S. Department of Agriculture, the Department of Health and Human Services, and National Institutes of Health. All animal procedures were reviewed and approved by the Institutional Animal Care and Use Committee. Within each experiment, all mice were age and sex matched. The mice were 8 to 10 wk old at the start of each experiment.

Radiation sources

A bank of six FS-40 sunlamps (National Biological, Twinsburg, OH) was used to irradiate the mice. These lamps emit a continuous spectrum from 270 to 390 nm, with peak emission at 313 nm; approximately 65% of the irradiation is within the UVB range (280–320 nm) of the solar spectrum. The irradiance of the six bulbs averaged 10 J/m2/s, as measured by an IL-100 research radiometer, using an SEE 240 UVB detector equipped with an A127 quartz diffuser (International Light, Newburyport, MA). Because of shielding by the cage lids, the incident dose received by the mice was approximately 4.5 J/m2/s. Keratinocyte cultures were irradiated with a single FS-40 bulb as described previously (12). The output of this lamp was 1.43 J/m2/s at a tube-to-target distance of 23 cm.

Determination of cytokine levels

For the most part ELISA assays were used to determine cytokine levels. The capture Abs and biotinylated detecting Abs were purchased from PharMingen (San Diego, CA) and used according to the manufacturer’s instructions (2 μg/ml) in a “sandwich” ELISA procedure. Recombinant cytokines were used to generate standard curves; the concentration of each cytokine was determined using the linear portion of the curve. The background response was determined by replacing the recombinant cytokines with PBS, tissue culture medium, or normal mouse serum. The mean ODs (±SD) and SD of triplicate samples was calculated, and a positive response was defined as one having an OD at least three SDs above background. Generally the limit of detection for IL-4 was between 2 and 10 pg/ml and for IL-10 was 15 to 25 pg/ml. Statistical differences between groups were determined by use of a two-tailed Student t test, with a probability of <0.05 considered significant.

Alternatively IL-4 levels were determined by bioassay. Serum samples (in triplicate) from UV-irradiated or nonirradiated control mice were cultured with 5 × 105 HT-2 cells in 96-well microtiter dishes for 18 to 24 h. Background responses were determined by culturing the cells with medium. A standard curve was generated by culturing the cells with different concentrations of IL-4 (PharMingen). During the last 6 h of culture, 1 μCi of tritiated thymidine (ICN Radiochemical, Irvine, CA) was added to each well. The radioactivity incorporated by the cells was determined by harvesting the cell lysate onto glass fiber filters followed by liquid scintillation counting. The proliferation of the HT-2 cells cultured with serum from the UV-irradiated mice was reduced to background by adding monoclonal anti-IL-4 (11B11; 10 μg/ml) to the wells. Statistical differences between groups were determined by use of a two-tailed Student t test, with a probability of <0.05 considered significant.

Delayed type hypersensitivity (DTH)

The dorsal hair of the C3H/HeN mice was removed with electric clippers, and the mice were exposed to 15 kJ/m2 of UVB radiation. Control mice were shaved but not exposed to UV. Within 2 to 4 h, the mice were injected (i.p.) with 100 μg of anti-IL-4, anti-IL-10, or rat IgG. Five days later the animals were immunized by injecting 2.5 × 106 BALB/c spleen cells into each footpad as described previously (25). Six days later, each hind footpad was measured with an engineer’s micrometer (Swiss Precision Instruments, Los angles, CA), the size recorded, and the animals were challenged by injecting 106 BALB/c spleen cells into each hind footpad. Footpad thickness was measured again 18 to 24 h later and the swelling determined. The specific footpad swelling was calculated by subtracting the response found in control mice that were not immunized but were challenged from the swelling seen in mice that were both immunized and challenged. There were at least five mice per group; the data are expressed as specific footpad swelling.
swelling ± the SD. Statistical differences between the controls and experimental groups were determined by use of a two-tailed Student's $t$ test, with a probability of $<0.05$ considered significant.

**Results**

**Induction of serum IL-4 by UV exposure**

The first series of experiments was designed to determine whether IL-4 could be found in the serum of UV-irradiated mice. At various times after UV exposure (15 kJ/m$^2$), a dose of UV that routinely induces $>50\%$ immune suppression, mice were bled, serum was obtained, and the IL-4 level was determined by ELISA. Control samples were simultaneously obtained from mice that were shaved but not exposed to UV radiation (Fig. 1). Although no IL-4 was found in the serum of the shaved nonirradiated control mice, IL-4 was found in the serum of the UV-irradiated animals ($p < 0.0001$; UV vs shaved nonirradiated mice, Mann-Whitney $U$ test). The peak response was seen 24 h postirradiation, and by 48 h all the IL-4 was cleared from the serum.

We next determined the effect of changing the dose of UV radiation on serum IL-4 levels. Mice were shaved and irradiated with various doses of UV, and at 24 h the animals were bled and serum IL-4 measured. As before, the control mice were shaved but not irradiated and, as before, no IL-4 was found in the serum of these mice (Fig. 2). IL-4 was found in the serum of the UV-irradiated mice, and, as the dose of UV used to irradiate the animals was increased, higher concentrations of IL-4 were found in the serum.

**Abs to IL-4 block UV-induced immune suppression**

Next we wanted to determine whether the UV-induced serum IL-4 plays any role in the induction of systemic immune suppression. C3H/HeN mice were exposed to 15 kJ/m$^2$ of UV radiation, and within 4 h were injected with control rat IgG, anti-IL-4, or anti-IL-10. Five days later the mice were immunized with alloantigen (BALB/c spleen cells), and the resulting DTH response was measured 1 wk later. Data from such an experiment are presented in Table I. Injecting the nonirradiated control mice with rat IgG, anti-

![FIGURE 1. Induction of IL-4 by UV irradiation. Serum was obtained from UV-irradiated mice at various times postirradiation, and IL-4 levels were determined. Each point represents the response of an individual mouse (serum samples measured in triplicate by ELISA). The data presented here are pooled from four separate experiments in which there were three mice per group. The bar represents the mean IL-4 level for all 12 mice. For the sake of clarity, only one point represents serum IL-4 in all 12 individual mice that were measured 12, 48, and 72 h post UV irradiation. No IL-4 was found in the serum of any of the shaved nonirradiated mice.](http://www.jimmunol.org/)

![FIGURE 2. Increasing the dose of UV results in increasing serum IL-4 levels. Mice were exposed to different doses of UV radiation, and 24 h later IL-4 in the serum of the irradiated mice was determined. Each serum sample was tested in triplicate. The data represent the means and SEM of serum samples obtained from three individual mice. This experiment was repeated three times with similar results.](http://www.jimmunol.org/)
accomplish this, we measured cytokine production by UV-irradiated keratinocytes in vitro. The spontaneously transformed keratinocyte cell line Pam 212 was cultured in 100-mm tissue culture dishes and exposed to various doses of UV radiation. Eighteen to 24 h later, the supernatant fluid was recovered and the IL-4 content was determined by ELISA. As a positive control, the IL-10 content of the keratinocyte supernatant was also measured. Data from this experiment are found in Table II. As expected, UV irradiation of the keratinocytes resulted in IL-10 secretion, and the amount of IL-10 found in the supernatant fluid increased as the dose of UV used to irradiate the cells increased. No IL-4, however, was found in the supernatants of the UV-irradiated keratinocytes, regardless of the dose of UV radiation applied. Because we found a significant and substantial decrease in the viability of the keratinocytes when doses higher than 200 J/m² were used, we did not attempt to determine whether irradiation with higher doses would result in IL-4 secretion. This experiment has been repeated three times, and in no case was any IL-4 found in the supernatant of the UV-irradiated keratinocyte cell line.

If the IL-4 is not secreted by keratinocytes, what then is the source of the IL-4 found in the serum of the UV-irradiated mice? We began to focus on the potential role of PGE₂ in UV-induced IL-4 production because UV-irradiation is a potent inducer of PGE₂ (26). PGE₂ is found in the serum of UV-irradiated mice (27), and others have shown that PGE₂ up-regulates IL-4 production (28, 29). To examine the role of PGE₂ in UV-induced IL-4 production, we decided to employ a selective COX-2 inhibitor to block inducible PGE₂ production in vivo (30) and to determine its effect on serum IL-4 levels. Mice were injected with various doses of the selective COX-2 inhibitor SC236 and, within 2 h, exposed to UV radiation. We used a relatively high dose of UV (30 kJ/m²) in this experiment to maximize the amount of IL-4 found in the serum. Twenty-four hours later the serum was collected, and the IL-4 content determined. Data from this experiment are presented in Table II. As shown previously, IL-4 was not present in the serum of the normal mice, and injecting SC236 did not cause IL-4 release into the serum. UV irradiation did cause an up-regulation of serum IL-4 levels. This UV-induced up-regulation of serum IL-4 was suppressed by injecting UV-irradiated mice with the selective COX-2 inhibitor. The suppression was dependent on the dose of SC236 injected, and complete inhibition of serum IL-4 was found when 0.2 μg of the inhibitor was injected per mouse. These findings suggest that the IL-4 found in the serum of UV-irradiated mice is induced by PGE₂.

Modulation of serum IL-10 by IL-4

From the data presented in Table I, it is clear that neutralizing either IL-4 or IL-10 blocked UV-induced immune suppression to an equivalent degree. One potential explanation for this finding is to suggest that a cascade of events is occurring and that IL-4, which peaks 24 h after UV exposure (Fig. 1), is acting to up-regulate the production of serum IL-10, which is found maximally 36 to 48 h post UV (13, 14), and that blocking the activity of either blocks the induction of immune suppression. To test this hypothesis, the following experiment was performed. Mice were exposed to UV radiation (15 kJ/m²) and then immediately injected with rat IgG or anti-IL-4. Twenty-four to 72 h later the serum from these mice was collected and serum IL-10 levels were determined. These data are presented in Table IV. As demonstrated previously, serum samples from mice exposed to UV and injected with control IgG were positive for IL-10. Interestingly, treating the UV-irradiated mice with anti-IL-4 totally blocked the induction of serum IL-10 (p < 0.05), suggesting that IL-4 contributes the appearance of IL-10 in the serum of UV-irradiated mice.

Finally, we wished to determine whether injecting PGE₂ into normal mice would result in the up-regulation of serum IL-4 and IL-10 levels. Various concentrations of PGE₂ were injected (i.p.) into mice, and 24 h later the animals were bled and IL-4 and/or IL-10 levels determined by ELISA. As can be seen from the data presented in Figure 3, injecting PGE₂ into normal mice resulted in the induction of both IL-4 and IL-10. Thus, these findings suggest that, following UV exposure, a cascade of events occurs, PGE₂ → IL-4 → IL-10, and that this cascade ultimately results in systemic immune suppression.

### Table II. Effect of UV irradiation on keratinocyte cytokine production in vitro

<table>
<thead>
<tr>
<th>UV (J/m²)</th>
<th>IL-4 (pg/ml/10⁶ Cells)ᵃ</th>
<th>IL-10 (pg/ml/10⁶ Cells)ᵇ</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>BLDᵇ</td>
<td>BLDᵇ</td>
</tr>
<tr>
<td>50</td>
<td>19 ± 3</td>
<td>52 ± 7</td>
</tr>
<tr>
<td>100</td>
<td>313 ± 25</td>
<td>313 ± 25</td>
</tr>
</tbody>
</table>

ᵃ Supernatants were harvested 24 h post UV; the remaining cells were removed and counted. Generally the supernatants were concentrated 10-fold using Amicon microconcentrators, and the IL-4 or IL-10 content was determined by ELISA. The data is expressed as pg/ml of the original supernatant per 10⁶ viable cells recovered.

ᵇ Below the limit of detection of the assay.

### Table III. Inhibition of UV-induced serum IL-4 by a selective COX-2 inhibitorᵃ

<table>
<thead>
<tr>
<th>Treatment of Serum Donorsᵇ</th>
<th>SC 236 (μg/Mouse)ᶜ</th>
<th>Serum IL-4 (pg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>None</td>
<td>0.2</td>
<td>0</td>
</tr>
<tr>
<td>None</td>
<td>0.02</td>
<td>0</td>
</tr>
<tr>
<td>None</td>
<td>0.002</td>
<td>0</td>
</tr>
<tr>
<td>30 kJ/m²</td>
<td>0</td>
<td>194 ± 40</td>
</tr>
<tr>
<td>30 kJ/m²</td>
<td>0.2</td>
<td>0</td>
</tr>
<tr>
<td>30 kJ/m²</td>
<td>0.02</td>
<td>40 ± 11*</td>
</tr>
<tr>
<td>30 kJ/m²</td>
<td>0.002</td>
<td>90 ± 22*</td>
</tr>
</tbody>
</table>

ᵃ This experiment was repeated twice and identical results were obtained.

ᵇ Mice were shaved and exposed to UV radiation; 24 h later they were bled and IL-4 levels were determined by proliferation of HT-2 cells. Each serum sample was tested in triplicate. Each data point represents the mean ± SEM of three individual mice.

ᶜ SC236, a selective COX-2 inhibitor, was diluted in PBS and injected i.p. 2 h prior to UV irradiation.

⁷ p < 0.05 vs UV only, two-tailed Student’s t test.

### Table IV. Inhibition of UV-induced serum IL-10 by monoclonal anti-IL-4ᵃ

<table>
<thead>
<tr>
<th>Treatmentᵇ</th>
<th>Time Post UV</th>
<th>IL-10 (pg/ml ± SEM)ᶜ</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>24</td>
<td>0</td>
</tr>
<tr>
<td>UV + IgG</td>
<td>24</td>
<td>955 ± 56</td>
</tr>
<tr>
<td>UV + anti-IL-4</td>
<td>24</td>
<td>0</td>
</tr>
<tr>
<td>None</td>
<td>48</td>
<td>0</td>
</tr>
<tr>
<td>UV + IgG</td>
<td>48</td>
<td>899 ± 26</td>
</tr>
<tr>
<td>UV + anti-IL-4</td>
<td>48</td>
<td>0</td>
</tr>
<tr>
<td>None</td>
<td>72</td>
<td>0</td>
</tr>
<tr>
<td>UV + IgG</td>
<td>72</td>
<td>1702 ± 278</td>
</tr>
<tr>
<td>UV + anti-IL-4</td>
<td>72</td>
<td>0</td>
</tr>
</tbody>
</table>

ᵃ This experiment was repeated twice and identical results were obtained each time.

ᵇ Mice were exposed to 15 kJ/m² of UV radiation and within 4 h were injected with rat IgG or anti-IL-4 (100 μg/mouse, i.p.). At various times post UV irradiation, serum was obtained and IL-10 was measured.

ᶜ Each serum sample was tested in triplicate. Each data point represents the mean ± SEM of three individual mice.

ᵈ Below the limit of detection of the assay.
Discussion

Because of the link between the immunosuppressive and carcinogenic potential of UV radiation, we are very interested in determining the mechanism(s) by which UV-irradiation causes systemic immune suppression. Following a single acute exposure to UV radiation, contact and delayed type hypersensitivity reactions to Ags applied at distant nonirradiated sites are suppressed, the function of splenic APCs is suppressed, and Ag-specific suppressor T lymphocytes are found in the spleens of UV-irradiated animals (31). The mechanism that appears to best explain the induction of systemic immune suppression following UV irradiation is the release of immunomodulatory factors by UV-irradiated keratinocytes, including cis-urocanic acid, PGE₂, IL-10, and TNF-α (24). The data presented here indicate that we can now add IL-4 to the list, since a mAb specific for IL-4 blocks UV-induced suppression of DTH to alloantigen (Table I). These findings also illuminate the difficulties in trying to interpret and integrate all the diverse data published in the literature concerning the role of UV-induced cytokines in immune suppression. For example, if we know that IL-4 and IL-10 are both found in the serum of UV-irradiated mice, and if we neutralize the IL-4 with a specific mAb, why doesn’t the remaining IL-10 suppress DTH? Similarly, both PGE₂ and IL-10 can be found in the serum of UV-irradiated mice, so why if we neutralize IL-10 does not the remaining PGE₂ mediate immune suppression? Our data indicate that these UV-induced immune regulatory factors work in sequence, and not in parallel, to inhibit inflammatory immune reactions such as DTH. We suggest that PGE₂, which is released by UV-irradiated keratinocytes (26) and can be found in the serum of UV-irradiated mice (27), activates a cytokine cascade involving at least IL-4 and IL-10, a cascade that ultimately suppresses DTH.

Our examination into a potential role for IL-4 in the induction of immune suppression in UV-irradiated animals was based primarily on previously published data suggesting that Th2 cells are preferentially activated following UV-irradiation. Rivas and Ulrich found that the activity of the UV-induced suppressor T cells was blocked with Abs to IL-4 and IL-10, suggesting that they are Th2-like cells (13). This was subsequently confirmed by Yagi et al., who cloned a suppressor cell from the spleen of a UV-irradiated mouse that upon activation secreted IL-4 and IL-10, but not IFN-γ (16). Because IL-4 is the primary cytokine in driving the differentiation of Th2 cells, we wished to determine whether UV irradiation induces IL-4. Our data indicate that IL-4 is found in the serum of UV-irradiated mice and that neutralizing its biologic activity blocks the induction of immune suppression.

Our focus on PGE₂ as the initiator of the cytokine cascade was based on our failure to find any evidence supporting IL-4 secretion by UV-irradiated keratinocytes. This, coupled with the findings of others that PGE₂ induces IL-4 (28, 29), and in light of the well known ability of UV to induce PGE₂ (26, 27), led us to propose that PGE₂ could induce serum IL-4. To examine this question in vivo, we employed the selective COX-2 inhibitor SC236. The advantage in using a selective COX-2 inhibitor to block inducible PGE₂ synthesis is a significant reduction in the toxicity (gastric bleeding and kidney damage) usually associated with agents (i.e., indomethacin) that suppress constitutive and inducible prostaglandin production by inhibiting both the COX-1 and COX-2 pathways (30). The complete suppression of serum IL-4 levels in the presence of the COX-2 inhibitor indicates that PGE₂ is indeed inducing the serum IL-4 we find in UV-irradiated mice. This finding was supported by the observation that injecting PGE₂ into normal mice induces IL-4.

We also noted total ablation of UV-induced serum IL-10 by injecting a mAb to IL-4. Unlike IL-4, there is ample evidence to suggest that both human and mouse keratinocytes secrete IL-10 following UV-irradiation (12, 32–34). Moreover, the secretion of biologically active IL-10 by keratinocytes is independent of PGE₂ secretion since it occurs in the presence of indomethacin (35) and because IL-10 secretion is not inhibited when UV-irradiated keratinocytes are treated with SC236 (data not shown). However, here we demonstrate that serum IL-10 production can be totally suppressed by neutralizing IL-4 activity. We suggest that UV exposure induces IL-10 production at two distinct levels. Direct irradiation of keratinocytes causes the release of epidermal IL-10 (a prostaglandin-independent event), which acts locally to suppress the function of Langerhans cells (11) and to induce tolerance to contact allergens applied directly to UV-irradiated skin (36). The data presented here suggest that keratinocyte-derived IL-10 is not entering into the circulation but rather the IL-10 we find in the serum is induced by a cascade involving at least PGE₂ and IL-4. We suggest that the UV-induced, serum-derived IL-10 is mediating the systemic suppression of DTH and impairment of APC function described earlier (12, 15, 17).

The source of the UV-induced serum IL-10, and IL-4 for that matter, is unknown. It is interesting to note, however, that in a study of the mechanism of enhanced sepsis following hemorrhage, Ayala and colleagues found that, when CD4⁺ T cells from hemorrhagic mice were cultured with PGE₂, they secreted IL-10, and the induction of IL-10 by PGE₂ could be suppressed by adding monoclonal anti-IL-4 to the cultures (29). Whether peripheral blood CD4⁺ T cells are producing IL-4 and IL-10 in response to PGE₂ in UV-irradiated mice remains to be seen.

Although we focused primarily on PGE₂ IL-4 and IL-10 in this study, we need to keep in mind that other mediators induced by UV radiation are undoubtedly involved in the induction of immune suppression. The most prominent is cis-urocanic acid. trans-Urocanic acid is found in the stratum corneum and, upon UV exposure, is isomerized to the immune suppressive cis isomer (37, 38). We believe that cis-urocanic acid may be inducing immune suppression by activating the cytokine cascade described here. Evidence to support this hypothesis comes from a number of studies. First, Hart et al. previously demonstrated that treating human peripheral blood monocytes with cis-urocanic acid induces them to release PGE₂ (39). Second, Moodycliffe et al. clearly demonstrated that cis-urocanic acid can be found in the serum of UV-irradiated mice (40), where it can presumably activate monocytes to secrete...
PGE₂. Finally, we showed recently that treating UV-irradiated mice with anti-cis-urocanic acid mAb modulates IL-10 production in vivo (41). We suggest, therefore, that cis-urocanic acid may induce immune suppression by activating the cytokine cascade identified here.

In addition to causing the release of immunosuppressive cytokines, UV-induced PGE₂ may also be contributing to immune suppression by blocking the production of essential immune stimulatory cytokines. For example, Tineke et al. have shown that PGE₂ is a potent inhibitor of IL-12 production by peripheral blood monocytes (42). Perhaps, in addition to inducing IL-4 and IL-10, PGE₂ may also down-regulate IL-12 production by monocytes in the UV-irradiated host. This may explain in part the ability of exogenous IL-12 to reverse the induction of immune suppression following UV irradiation (18, 43).

In summary, we present data indicating that a cascade of events occurs after UV irradiation of the skin that contributes to systemic immune suppression. We suggest that PGE₂ released by UV-irradiated keratinocytes induces peripheral blood leukocytes to produce IL-4, which then causes the secretion of IL-10. The UV-induced IL-10 then suppresses systemic APC function and the induction of DTH. This would explain why different experimental approaches, such as blocking prostaglandin production in UV-irradiated mice with indomethacin (27), monoclonal anti-IL-10 treatment (12, 17), and/or injecting monoclonal anti-IL-4 have all been used successfully to abrogate the induction of immune suppression following UV exposure. Furthermore, the findings presented here confirm a very recent observation by El-Ghorr and Norval (44) demonstrating no immune suppression of DTH in UV-irradiated IL-4-deficient mice, and suggest the inability to up-regulate serum IL-4 following PGE₂ release as a potential mechanism. We also suggest that cis-urocanic acid, by virtue of its ability to induce blood monocytes to secrete PGE₂, may also contribute to this cascade of events.

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