# The role of IL-4, IL-10, and TNF-α in the immune suppression induced by ultraviolet radiation

Jorge M. Rivas and Stephen E. Ullrich
*Department of Immunology, University of Texas, M.D. Anderson Cancer Center Houston, Texas*

**Abstract: Cytokines produced by keratinocytes play an essential role in the induction of immune suppression following ultraviolet (UV) exposure. Using antibodies specific for either interleukin-10 (IL-10) or tumor necrosis factor α (TNF-α), we present evidence indicating that IL-10 suppresses delayed-type hypersensitivity (DTH) but not contact hypersensitivity (CHS), whereas TNF-α suppresses CHS but not DTH following UV exposure. UV exposure also activates antigen-specific suppressor T cells. To determine whether the antigen-specific CD4⁺ T cells that transfer suppression in this system mediate their suppressive effect by releasing IL-4 or IL-10, we transferred UV Ts into normal mice that were then injected with either anti-IL-4 or anti-IL-10 antibody. Both anti-IL-4 and anti-IL-10 blocked the ability of UV Ts cells to suppress DTH in the recipient animals. When UV Ts that suppress CHS were transferred into normal recipients, however, neither antibody was able to block the UV Ts activity. These findings suggest that UV Ts suppress DTH by secreting IL-4 and IL-10 and appear to act like Th2 cells. Because anti-IL-4 and anti-IL-10 did not block the activity of the UV Ts that regulate contact hypersensitivity, their effects appear to be mediated by a different mechanism. *J. Leukoc. Biol.* 56: 769-775; 1994.**

***Key Words:*** *cytokines • suppressor cells • Th2 cells • DTH • CHS*

## INTRODUCTION

Exposure to ultraviolet (UV) radiation, a major environmental carcinogen, induces systemic immune suppression. Following UV irradiation, the ability to generate T cell-mediated immune reactions, such as the rejection of UV-induced skin cancers, delayed-type hypersensitivity (DTH), and contact hypersensitivity (CHS) is severely suppressed in both experimental animals and humans [1-7]. In fact, the immunosuppressive effects of UV radiation and its carcinogenic potential appear to be linked. Studies by Kripke and colleagues, with mice, demonstrated that UV exposure induces antigen-specific suppressor T (Ts) cells that play a role in the induction of skin cancer in the primary host and interfere with tumor rejection when transferred to a secondary recipient [2, 8]. More recent studies, with biopsy-proven skin cancer patients, have also indicated that UV-induced immune suppression is a risk factor for skin cancer development in humans [9].

Although it is not entirely clear how UV exposure induces systemic immune suppression, most of the evidence to date supports a role for soluble photoproducts produced by UV-irradiated keratinocytes. Injecting supernatants from UV-irradiated keratinocyte cultures mimics many of the immune defects induced by total-body UV exposure. The induction of DTH is suppressed after injecting supernatants from UV-irradiated keratinocytes and antigen-specific suppressor T cells are found in the spleens of the factor-injected mice [10]. UV-irradiated keratinocytes secrete biologically active interleukin (IL)-10, and the IL-10 present in the culture supernatant is responsible for suppressing DTH. Injecting anti-IL-10 into UV-irradiated mice overcomes the UV-induced suppression of DTH, indicating the essential role of IL-10 in the induction of immune suppression following UV irradiation [11].

In all probability, UV-induced, keratinocyte-derived IL-10 down-regulates DTH in vivo by preventing the secretion of IL-2 and interferon (IFN)-γ by T helper 1 cells (Th1) [12]. Another consequence of the release of IL-10 and down-regulation of IFN-γ production following UV exposure may be the preferential expansion of T helper 2 cells (Th2). We reported that compared to normal controls, spleen cells from UV-irradiated mice do not present antigen to Th1 cells and their ability to present antigen to Th2 clones is enhanced. Moreover, injecting UV-irradiated animals with anti-IL-10 reversed these effects [13]. Because the UV Ts that suppress tumor rejection, DTH, and CHS are $CD4^+$, $CD8^-$ T cells [14, 15], we suggest that UV Ts are Th2 cells, which suppress cellular immune reactions by producing cytokines such as IL-4 and IL-10.

The purpose of this study was to test the hypothesis that UV Ts down-regulate delayed hypersensitivity reactions through the release of IL-4 or IL-10. Spleen cells from mice exposed to UV radiation and then immunized with alloantigen (DTH) or sensitized with oxazolone (CHS) were transferred into recipient mice, and the effect of injected anti-IL-4 or anti-IL-10 on immune suppression was measured.

## MATERIALS AND METHODS

### Animals

Specific pathogen-free female C3H/HeNCr (MTV-) and BALB/c AnNCr mice (8 to 12 weeks old) were purchased from the National Cancer Institute-Frederick Cancer Research Facility Animal Production Area (Frederick, MD). The 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

Abbreviations: APC, antigen-presenting cell; CHS, contact hypersensitivity; DTH, delayed-type hypersensitivity; IFN, interferon; IL, interleukin; Th1, T helper 1 cell; Th2, T helper 2 cell; TNF-α, tumor necrosis factor α; Ts, suppressor T cell; UV, ultraviolet

Reprint requests: Stephen E. Ullrich, Department of Immunology (Box 178), M.D. Anderson Cancer Center, 1515 Holcombe Boulevard, Houston, TX 77030.

Received May 31, 1994; accepted July 28, 1994.

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

SXC-1, a hybridoma producing rat anti-mouse-IL-10 (IgM isotype) [16], was provided by Dr. Timothy Mosmann, University of Alberta, Edmonton, Canada. 11B11, a hybridoma producing rat anti-mouse IL-4 (IgG isotype) was purchased from ATCC (Rockville, MD). Anti-tumor necrosis factor $\alpha$ (TNF-$\alpha$) was purchased from Genzyme (Cambridge, MA). Serum levels of IL-10 were measured by ELISA with reagents purchased from PharMingen (San Diego, CA) and performed according to the manufacturer's instructions; sensitivity of the ELISA is approximately 15 pg/ml. Tissue culture medium and supplements were purchased from GIBCO (Grand Island, NY). Fetal calf serum 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 270–390 nm, with a peak emission at 313 nm. Approximately 65% of the radiation emitted by these lamps is within the UVB range (280–320 nm), with 35% in the UVA region and less than 1% in the UVC (240–280) range. The irradiance of the source averaged 10 J/m²/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 of UVB received by the animals was approximately 4.5 J/m²/s.

### Effect of monoclonal antibody administration on the UV-induced suppression of contact hypersensitivity

The dorsal hair of the mice was shaved with electric clippers, their ears were protected with electrical tape, and the animals were exposed to 15 kJ/m² of UVB radiation. Four and 24 h later the mice were injected intraperitoneally with either 100 $\mu$g of monoclonal anti-IL-10, 100 $\mu$g of monoclonal anti-IL-4, rabbit anti-TNF-$\alpha$ polyclonal antiserum ($1 \times 10^4$ neutralizing units/injection), rabbit anti-BSA polyclonal anti-serum, or control normal rat serum. Five days later, the abdominal hair of the mice was shaved with a razor blade and the animals were sensitized by epicutaneous application of 100 $\mu$l of a 3% (w/v) solution of oxazolone (4-ethoxymethylene-2-phenyloxalo-5-one; Sigma Chemical Co., St. Louis, MO) in 95% ethanol. Six days after sensitization, the ears of the mice were measured with a spring loaded micrometer, and then were challenged by applying 5 $\mu$l of a 3% solution of oxazolone on each ear surface. Twenty-four h later the ears were measured again, and the specific ear swelling was determined by subtracting the ear swelling response found in mice that were not sensitized but were challenged from that seen in mice that were sensitized and challenged. Control animals had their dorsal hair shaved and their ears taped, but were not exposed to UV radiation.

In some experiments the effect of antibody treatment on the activity of suppressor cells was determined. Suppressor cells were generated by exposing mice to 15 kJ/m² of UVB radiation, followed by sensitization with oxazolone as described above. Seven days after sensitization, the animals were killed, their spleens removed and single cell suspensions prepared. Similarly, control lymphocytes from mice that were not exposed to UV, but were sensitized with oxazolone (NR cells) were prepared. Approximately $1 \times 10^8$ cells were injected into the tail veins of normal recipient mice. The recipient animals were then immediately sensitized with a 3% solution of oxazolone on the shaved abdomen. Four and 24 h after adoptive transfer of suppressor cells, the mice were injected intraperitoneally with either 100 $\mu$g of monoclonal anti-IL-10, 100 $\mu$g of monoclonal anti-IL-4, or control normal rat serum. Six days after sensitization the ears were measured and challenged and ear swelling was determined 24 h later.

### Effect of monoclonal antibody administration on the UV-induced suppression of delayed-type hypersensitivity

The dorsal hair of the mice was shaved with electric clippers and the animals were exposed to 15 kJ/m² of UVB radiation. Four and 24 h later the mice were injected intraperitoneally with either 100 $\mu$g of monoclonal anti-IL-10, 100 $\mu$g of monoclonal anti-IL-4, rabbit anti-TNF-$\alpha$ polyclonal antiserum ($1 \times 10^4$ neutralizing units/injection), rabbit anti-BSA polyclonal anti-serum, or control normal rat serum. Five days later, the mice were immunized by subcutaneous injection of $5 \times 10^7$ allogeneic spleen cells. Six days after sensitization, the hind feet of the mice were first measured with a spring loaded micrometer, and then were challenged by injected $10^7$ allogeneic spleen cells suspended in 50 $\mu$l of PBS. Twenty-four h later the feet were measured again, and the specific foot swelling was determined by subtracting the footpad swelling response found in mice that were not sensitized but were challenged from that seen in mice that were sensitized and challenged. Control animals had their dorsal hair shaved, but were not exposed to UV radiation.

In some experiments the effect of antibody treatment on the activity of suppressor cells was determined. Suppressor cells were generated by exposing mice to 15 kJ/m² of UVB radiation, followed by immunization with alloantigen, as described above. Seven days after immunization, the animals were killed, their spleens removed and single cell suspensions prepared. Similarly, control lymphocytes from mice that were not exposed to UV, but were immunized with alloantigen (NR cells) were prepared. Approximately $1 \times 10^8$ cells were injected into the tail veins of normal recipient mice. The recipient animals were then immediately immunized with $5 \times 10^7$ allogeneic spleen cells. Four and 24 h after adoptive transfer of suppressor cells, the mice were injected intraperitoneally with either 100 $\mu$g of monoclonal anti-IL-10, 100 $\mu$g of monoclonal anti-IL-4, or control normal rat serum. Six days after sensitization the hind feet were measured and challenged and footpad swelling was determined 24 h later.

## RESULTS

### Different cytokines are involved in the systemic suppression of DTH and CHS following UV radiation

Data we presented previously demonstrated that UV-irradiated keratinocytes secrete IL-10 and that the injection of anti-IL-10 into UV-irradiated mice blocks the induction of systemic immune suppression [11]. Yoshikawa and Streilein, however, have suggested a role for keratinocyte-derived TNF-$\alpha$ in the UV-induced suppression of CHS following UV exposure, because anti-TNF-$\alpha$ blocks the induction of immune suppression [17]. Since the experimental protocols differ greatly (UV dose, application of hapten at the site of irradia-

tion versus a distant site, DTH versus CHS), we sought to determine the effect of injection of antibodies against IL-10 and TNF-α on the development of systemic immune suppression in a single experimental system. We therefore exposed groups of C3H/HeN mice to 15 kJ/m² of UV radiation and 4 and 24 h later injected them with either anti-IL-10 (100 μg/injection, i.p.) or anti-TNF-α ($1 \times 10^4$ neutralizing units/injection, i.p.). Five days after irradiation the mice were immunized at a distant non-irradiated site with allogeneic (BALB/c) spleen cells, and DTH was measured 7 days after immunization. The data from such an experiment are presented in **Table 1.** Compared to the positive control, exposure to UV radiation resulted in a significant suppression of DTH. As reported previously, injecting the UV-irradiated mice with anti-IL-10 blocked the induction of immune suppression, and injecting the UV-irradiated mice with normal rat serum did not block the induction of suppression. Similarly, injecting anti-TNF-α or control anti-BSA antibody into the UV-irradiated mice did not overcome the immune suppression, as a significant difference between the footpad swelling observed in these mice and the positive control was noted ($P < 0.004$). Similar results were obtained when BALB/c mice were exposed to UV radiation and their response to alloantigen was measured (data not shown).

Next we measured the effect of injecting anti-IL-10 or anti-TNF-α on the UV-induced systemic suppression of CHS. Mice were exposed to 15 kJ/m² of UV radiation, and 4 and 24 h later were injected with anti-IL-10 or anti-TNF-α. Five days after irradiation, the mice were sensitized with oxazolone at a distant, non-irradiated site, and CHS was measured 7 days post-sensitization. Data from this experiment are presented in **Table 2.** Compared to the positive control, UV exposure results in a significant suppression of CHS. Injecting the UV-irradiated mice with anti-IL-10 or normal rat serum had no effect on the induction of systemic immune suppression. Injecting UV-irradiated mice with anti-TNF-α, however, reversed the induction of immune suppression, as we found no significant difference between the ear swelling observed in the positive control and in mice exposed to UV and injected with anti-TNF-α. Injecting UV-irradiated mice with anti-BSA did not ameliorate the induction of systemic immune suppression.

TABLE 1. Antibodies to IL-10 but Not TNF-α Block UV-induced Suppression of DTH

| Treatment[a] | Δ Footpad Swelling (± SD)[b] | Specific Swelling[c] | % Suppression[d] | P[e] |
|---|---|---|---|---|
| Negative control | 4 ± 3 | – | | |
| Positive control | 19 ± 6 | 15 | – | |
| UV | 10 ± 6 | 6 | 60 | .01 |
| UV + anti-IL-10 | 14 ± 6 | 10 | 33 | .1 |
| UV + normal rat serum | 5 ± 4 | 1 | 93 | .001 |
| UV + anti-TNF-α | 8 ± 3 | 4 | 94 | .001 |
| UV + anti-BSA | 9 ± 6 | 5 | 60 | .004 |

[a] C3H mice were exposed to UV radiation (15 kJ/m²) and injected with anti-IL-10 or anti-TNF-α, 4 and 24 h after exposure. Five days later they were immunized with allogeneic spleen cells. DTH was determined 7 days after immunization. Negative control refers to mice that were not immunized but were challenged. Positive control refers to mice that were immunized and challenged.

[b] Units = mm × $10^{-2}$ (ten mice per group).

[c] Background swelling (negative control) was subtracted from that found in the immunized groups.

[d] % suppression = [1-(specific footpad swelling in experimental groups/specific footpad swelling in the positive control)] × 100.

[e] P values determined by two-tailed Student's t-test, positive control versus experimental groups. $P > 0.05$ = no significant difference from the positive control. A representative experiment is shown: this experiment was repeated 3 times with similar results.

TABLE 2. Antibodies to TNF-α but Not IL-10 Block UV-induced suppression of CHS

| Treatmemt[a] | Δ Ear Swelling (± SD)[b] | Specific Swelling[c] | % Suppression[d] | P[e] |
|---|---|---|---|---|
| Negative control | 4 ± 1 | – | | |
| Positive control | 12 ± 3 | 8 | – | |
| UV | 4 ± 2 | 0 | 100 | .001 |
| UV + anti-IL-10 | 4 ± 2 | 0 | 100 | .001 |
| UV + normal rat serum | 3 ± 2 | 0 | 100 | .001 |
| UV + anti-TNF-α | 9 ± 5 | 5 | 38 | .12 |
| UV + anti-BSA | 6 ± 3 | 2 | 75 | .001 |

[a] C3H mice were exposed to UV radiation (15 kJ/m²) and injected with anti-IL-10 or anti-TNF-α, 4 and 24 h after exposure. Five days later they were sensitized with a 3% (w/v) solution of oxazolone in ethanol. CHS was determined 7 days after sensitization. Negative control refers to mice that were not sensitized but were challenged. Positive control refers to mice that were sensitized and challenged.

[b] Units = mm × $10^{-2}$ (ten mice per group).

[c] Background swelling (negative control) was subtracted from that found in the sensitized groups.

[d] % suppression = [1-(specific ear swelling in experimental groups/specific ear swelling in the positive control)] × 100.

[e] P values determined by two-tailed Student's t-test, positive control versus experimental groups. $P > 0.05$ = no significant difference from the positive control. A representative experiment is shown: this experiment was repeated 3 times with similar results.

## Serum levels of IL-10 following UV exposure

We determined next whether the serum of UV-irradiated mice contained IL-10. Mice were irradiated with 15 kJ/m² of UV radiation and, at various time points after exposure, we obtained serum samples and measured IL-10 levels by ELISA. The data, presented in **Figure 1,** indicate that IL-10 is found in the serum of UV-irradiated mice. It is first detec-

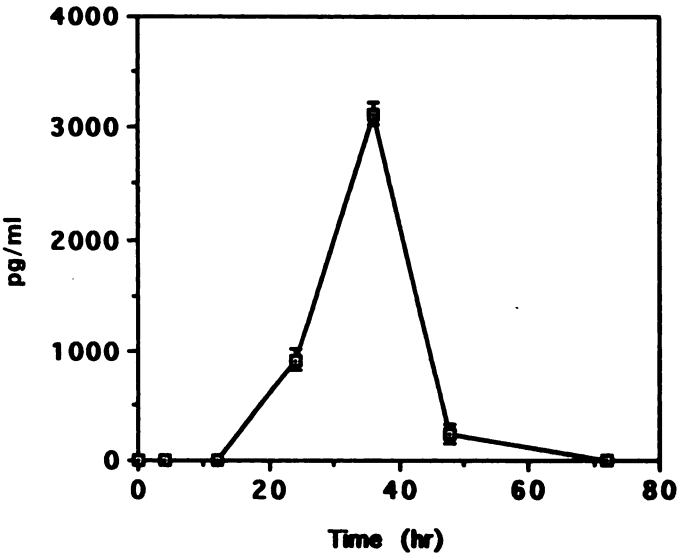


**Fig. 1.** Serum levels of IL-10 following UV exposure. After mice were exposed to 15 kJ/m² of UV radiation, serum samples were obtained at various times. Serum IL-10 was determined by ELISA. No IL-10 was found in normal mouse serum.

table at 24 h post-irradiation, peaks at 36 h, and is cleared by 72 h. No IL-10 was found in the serum of normal non-irradiated animals.

### Effect of anti-IL-4 and anti-IL-10 on the activity of UV-induced Ts that regulate DTH

Previous studies have demonstrated that immune suppression can be transferred in this system with antigen-specific $CD3^+$, $CD4^+$, $CD8^-$ Ts [15]. Moreover, our recent findings, indicated that antigen-presenting cells (APCs) from UV-irradiated mice preferentially stimulate Th2 cells [13]. To determine whether the suppressor cells induced by UV radiation are Th2-like cells, we measured the effect of antibodies against IL-4 and IL-10 on the suppressive activity of these cells. Spleen cells from UV-irradiated C3H mice immunized to alloantigen (BALB/c), were injected into syngeneic recipients, which were immediately immunized with BALB/c spleen cells. At 4 and 24 h later the recipient mice were injected with either anti-IL-4 or anti-IL-10; DTH was read 7 days after immunization. Data from such an experiment are shown in **Figure 2.** Compared to the positive control, significant ($P < 0.01$) immune suppression was observed in mice injected with UV-induced suppressor cells (UV SC). The suppressive activity of these cells was reversed when the recipient mice were injected with either anti-IL-10 (Fig. 2, panel A) or anti-IL-4 (Fig. 2, panel B). Injection of UV SC and normal rat serum did not overcome the activity of the UV Ts. An important control for this experiment consists of injecting recipient mice with an equal number of T cells from donors that were not exposed to UV radiation but were immunized with BALB/c spleen cells (NR). Injection with NR cells did not suppress the induction of the immune response. Moreover, injecting anti-IL-10 or anti-IL-4 into mice that received NR cells had no adverse effect, since their response was identical to that seen in either the positive or NR controls. These findings indicate that the suppressive activity of the UV Ts can be overcome by injecting the recipient mice with anti-IL-10 or anti-IL-4.

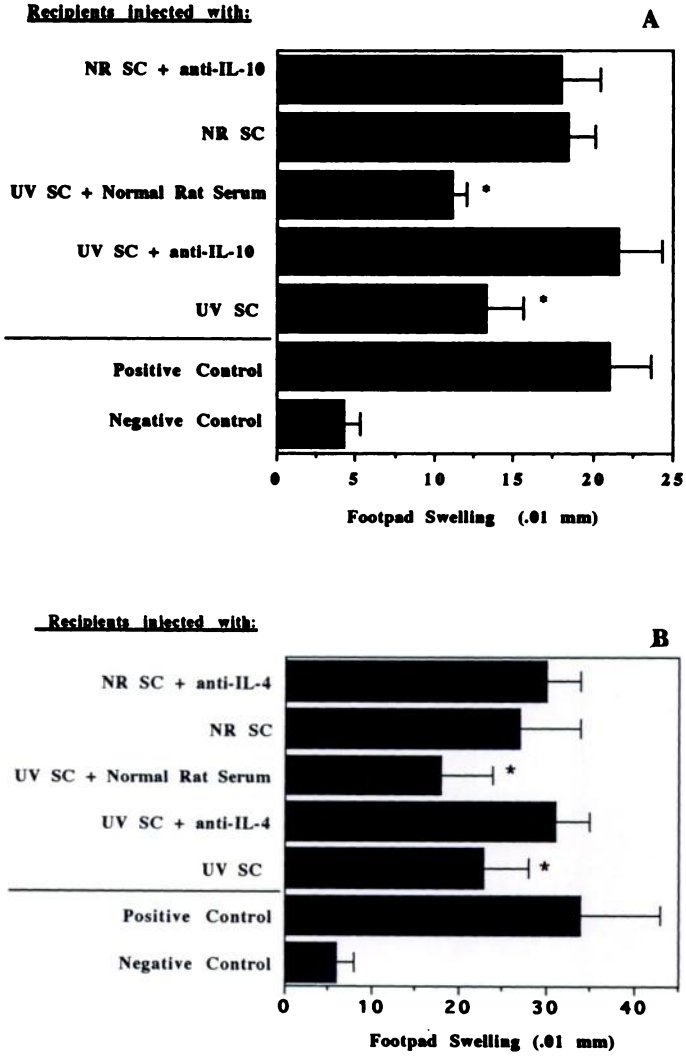


**Fig. 2.** Antibodies to IL-10 (panel A) or IL-4 (panel B) block the activity of UV-induced cells that suppress DTH. Suppressor cells were induced in donor mice exposed to 15 kJ/m² of UV radiation and immunized with alloantigen (UV SC). Control cells were isolated from non-irradiated mice that were immunized with alloantigen (NR SC). Seven days after immunization, $10^8$ whole spleen cells from the UV-irradiated and control mice were injected i.v. into normal recipient mice, which were immediately immunized with alloantigen. These mice were injected with 100 μg of anti-IL-10, anti-IL-4, or normal rat serum 4 and 24 h after transfer. DTH in the recipients was measured 7 days following adoptive transfer. *indicates significant difference ($P < 0.05$) from the positive control.

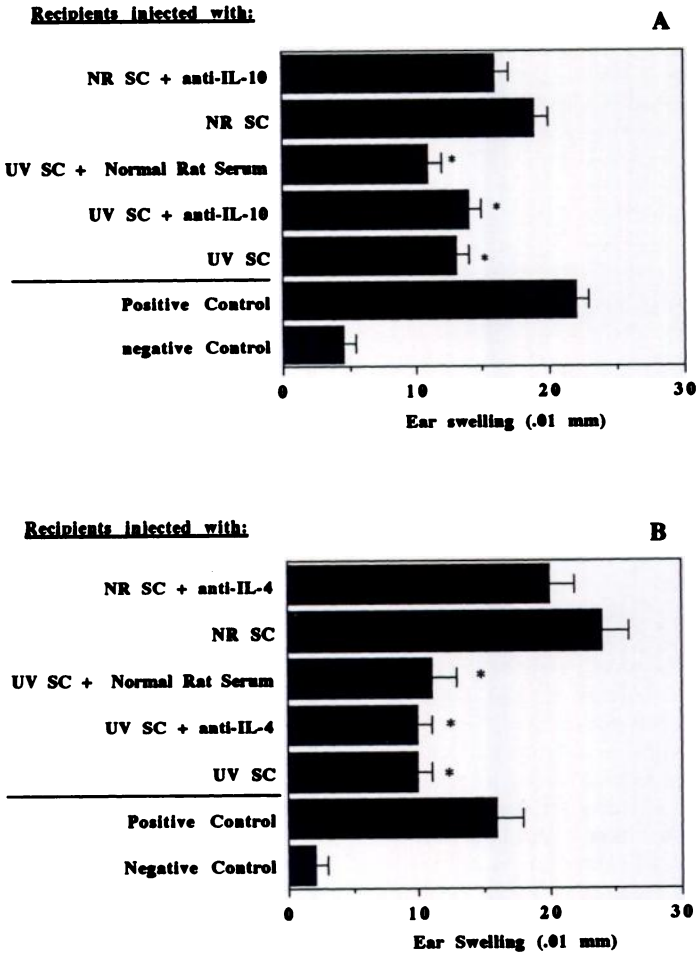


**Fig. 3.** Antibodies to IL-10 (panel A) or IL-4 (panel B) do not block the activity of UV-induced cells that suppress CHS. Suppressor cells were induced in donor mice exposed to 15 kJ/m² of UV radiation and sensitized with oxazolone (UV SC). Control cells were isolated from non-irradiated mice sensitized with oxazolone (NR SC). Seven days after immunization, $10^8$ whole spleen cells from the UV-irradiated and control mice were injected i.v. into normal recipient mice, which were immediately sensitized with oxazolone. These mice were injected with 100 μg of anti-IL-10, anti-IL-4, or normal rat serum 4 and 24 h after transfer. CHS in the recipients was measured 7 days following adoptive transfer. *indicates significant difference ($P < 0.05$) from the positive control.

### Effect of anti-IL-4 and anti-IL-10 on the activity of UV-induced Ts that regulate CHS.

In the next series of experiments we wished to determine whether the $CD4^+$, $CD8^-$ UV Ts that suppress CHS also mediate their suppressive effects through the release of IL-4 or IL-10. UV Ts that suppress CHS were generated and injected into recipient mice that were immediately sensitized with oxazolone, and 4 and 24 h later were injected with either anti-IL-4 or anti-IL-10. CHS was read 7 days post-sensitization. Data from such an experiment are shown in **Figure 3.** Compared to the positive control, injection of spleen cells from UV-irradiated, oxazolone-sensitized mice suppressed the generation of CHS in the recipient animals ($P < 0.01$). Unlike the situation described above, injection of either anti-IL-10 or anti-IL-4 into the recipient mice did not reverse the suppressive effect. These findings suggest that the activity of UV Ts that suppress CHS cannot be overcome by anti-IL-10 or anti-IL-4.

## DISUCSSION

Exposing mice to UV radiation results in a specific, selective suppression of the immune response [18]. The suppressed state can be transferred to non-immune animals by $CD4^+$ antigen-specific suppressor T cells, which have been shown to interfere with Th cell function [15, 19]. How UV exposure induces systemic immune suppression and activates splenic suppressor cells is an intriguing question. The inability of UV radiation to penetrate far past the dermal-epidermal junction suggests that indirect mechanisms are involved, and data from a number of laboratories have supported the hypothesis that keratinocyte-derived cytokines are involved in the induction of systemic immune suppression [20]. Our previous findings have indicated a role for IL-10 in the immune suppression induced by UV irradiation. UV exposure impairs the ability of splenic APC to present antigen to Th1 cells and inhibits the induction of DTH. Moreover, UV exposure enhances the capacity of splenic APC to present antigen to Th2 clones [11, 13].

These observations led us to postulate that the $CD4^+$ suppressor cells found in the spleens of UV-irradiated mice may be antigen-activated Th2 cells, which mediate their immunosuppressive effects through the release of Th2 cytokines such as IL-4 and IL-10. To address this hypothesis, we initiated cell-transfer experiments and asked whether injecting the recipient mice with antibodies against IL-4 or IL-10 could reverse the activity of UV-induced suppressor cells. In UV-induced suppression of DTH, this appears to be the case. As expected, the transfer of cells from UV-irradiated mice, which were immunized with alloantigen, into normal animals, suppressed the induction of DTH in the recipient mice. Injecting either anti-IL-4 or anti-IL-10 into the recipient animals blocked the activity of the suppressor cells, whereas the injection of control normal rat serum did not inhibit the induction of immune suppression. Furthermore, the transfer of control normal spleen cells into normal recipient mice did not suppress the induction of DTH. Similarly, injecting anti-IL-4 or anti-IL-10 into the mice transferred with control cells (NR SC + anti-IL-10) did not affect DTH. Thus, these data suggest that the transferred cells mediate their immunosuppressive effects by secreting IL-4 and IL-10. Since we have shown previously that the phenotype of these suppressor cells is $CD3^+$, $CD4^+$, and $CD8^-$ [10, 14], these findings suggest that the cells suppressing DTH in this system are Th2-like cells.

As mentioned above, all the work presented here concerns the systemic model of UV-induced immune suppression. In this model system, the antigen is applied, or injected, into a site distant and distinct from the site of irradiation. Generally we used high doses (10–30 $kJ/m^2$) of UV radiation and administered them during a single exposure. This differs from the "local" model of immune suppression, in which relatively small doses (1–2 $kJ/m^2$) of UV are given over a 4-day period, and the contact allergen is applied directly to the UV-irradiated skin. The mechanism of unresponsiveness in the local system appears to be the effect of UV radiation on epidermal Langerhans cells. UV exposure decreases the density of, and alters the morphology of epidermal Langerhans cells [21]. Injecting relatively pure populations of FACS-sorted, UV-irradiated, hapten-conjugated Langerhans cells into mice prevents the induction of an immune response [22]. In addition, Simon et al. observed that while normal Langerhans cells could present antigen to both Th1 and Th2 clones, UV-irradiated Langerhans cells were unable to present to Th1 clones, but retained their ability to present to Th2 cells [23]. Similarly, recent findings by Enk et al. demonstrated that IL-10 can also prevent the presentation of antigen to Th1 clones, while sparing presentation to Th2 cells [24]. We suggest that the key effect of UV radiation on the immune system is to modulate APC function so that Th2 cells are preferentially activated. This may occur directly, as is the case with UV-irradiated Langerhans cells, or indirectly, through the effect of keratinocyte-derived IL-10 on either Langerhans cells [24] or splenic APC [13]. In our model of systemic immune suppression, the net effect is the activation of $CD4^+$ Th2 cells that release IL-4 and IL-10 and suppress DTH. Whether the $CD4^+$, antigen-specific suppressor cells that are generated after sensitization through UV-irradiated skin (local immune suppression) [25] are also Th2 cells remains to be seen. We suggest, however, that a common mechanism (net effect of UV on APC function) may operate in both systems to induce antigen-specific T cells that suppress delayed hypersensitivity reactions.

Different mechanisms seem to be operating, however, during the UV-induced systemic suppression of CHS and DTH. First, different cytokines appear to be important in initiating the suppressive event following UV exposure. Our findings suggest that keratinocyte-derived IL-10 is essential for suppressing the induction of DTH, but not CHS, following UV exposure. Conversely, although keratinocyte-derived TNF-$\alpha$ is responsible for the UV-induced systemic suppression of CHS, an observation that agrees with the findings of Yoshikawa and Streilein [17], TNF-$\alpha$ does not seem to be involved in the UV-induced systemic suppression of DTH. Second, our data also suggests a basic difference in the mechanisms employed by UV-induced cells to suppress CHS and DTH. Although we have shown previously that UV-induced suppressor cells that inhibit CHS are $CD4^+$, $CD8^-$ T cells [14, 26], we were not able to block their suppressive activity with anti-IL-4 or anti-IL-10. These observations suggest that the $CD4^+$ T cells that suppress CHS differ from the cells that suppress DTH, and they do not appear to be Th2 cells. The findings presented here agree with previous data that suggested the involvement of different mechanisms in suppressing DTH and CHS following UV exposure [27, 28].

Data recently presented by Enk and colleagues demonstrating that administration of rIL-10 can suppress the induction of CHS [29] appear to be in conflict with the data presented here. However, it should be noted that UV-irradiated keratinocytes secrete a wide variety of biological response modifiers including IL-1$\alpha$, prostaglandin $E_2$ and TNF-$\alpha$, all of which have been shown to play a role in sup-

pressing CHS in UV-irradiated mice [30, 31]. We suggest that IL-10 is necessary, but not sufficient to suppress CHS in UV-irradiated mice. In our experiments, where monoclonal antibody is used to abrogate the function of IL-10, we suggest that the other keratinocyte-derived cytokines induce immune suppression. However, when the activity of TNF-$\alpha$ is removed in vivo, suppression of CHS is abolished. This would suggest that TNF-$\alpha$ is the main player in the suppression of CHS in UV-irradiated mice and that IL-10 plays a less important role. Conversely, in the suppression of DTH by UV radiation, we suggest that IL-10 may be the major player, with TNF-$\alpha$ playing a more minor role. Whether rTNF-$\alpha$ can suppress DTH like rIL-10 can suppress CHS remains to be seen.

In summary, our data support the hypothesis that the induction of systemic immune suppression following UV exposure is caused by the release of cytokines by UV-irradiated keratinocytes. IL-10 appears to be essential for the UV-induced suppression of DTH, whereas TNF-$\alpha$ is essential for the suppression of CHS. IL-10 is found in the serum of UV-irradiated mice, and we suggest that the effects of IL-10 on splenic APC function promotes the activation of Th2 cells, as described previously [13]. Moreover, our data suggest that the "suppressor" T cells present in the spleens of UV-irradiated mice are Th2 cells that mediate their suppressive effects through the release of IL-4 and IL-10. We suggest that UV exposure shifts the immune reaction to one that favors the activation of Th2 cells. This may be advantageous in certain situations, such as infection with *Nipostrongylus brasiliensis* [32] or *Plasmodium falciparum* [33], in which the induction of a Th2 response is protective. UV exposure, however, may exacerbate infections caused by such agents as *Mycobacterium avium, Listeria monocytogenes,* and *Leishmania major,* in which a Th1 response is protective. In addition, because recent studies suggest that the induction of a Th1 reaction is critical for immune function in HIV-infected individuals [34, 35], we suggest that UV exposure, by inducing Th2 cells, may exacerbate the immune complications associated with AIDS.

## ACKNOWLEDGMENTS

This work was supported by grant AR 40824 from the National Institute of Arthritis and Musculosketal and Skin Diseases. J.M.R. was supported by a grant from the NIAMS (AR 40824-S1) to promote the entry of underrepresented minorities into research. The animal facilities at the M.D. Anderson Cancer Center are supported in part by a grant (CA-16672) from the National Cancer Institute. The authors thank Anthony Apollo for his excellent technical assistance.

## REFERENCES

1. Kripke, M.L. (1974) Antigenicity of murine skin tumors induced by UV light. *J. Natl. Cancer Inst.* **53,** 1333-1336.
2. Fisher, M.S., Kripke, M.L. (1977) Systemic alteration induced in mice by ultraviolet light irradiation and its relationship to ultraviolet carcinogenesis. *Proc. Natl. Acad. Sci. USA* **74,** 1688-1692.
3. Noonan, F.P., Kripke, M.L., Pedersen, G.M., Greene, M.I. (1981) Suppression of contact hypersensitivity by UV radiation is associated with defective antigen presentation. *Immunology* **43,** 527-533.
4. Ullrich, S.E. (1986) Suppression of the immune response to allogeneic histocompatibility antigen by a single exposure to UV radiation. *Transplantation* **42,** 287-291.
5. Ullrich, S.E., Azizi, E., Kripke, M.L. (1986) Suppression of the induction of delayed hypersensitivity reactions in mice by a single exposure to UV radiation. *Photochem. Photobiol.* **43,** 633-638.
6. Cooper, K.D., Oberhelman, L., Hamilton, T.A., Baadsgaard, O., Terhune, M., Levee, G., Anderson, T., Koren, H. (1992) UV exposure reduces immunization rates and promotes tolerance to epicutaneous antigens in humans: Relationship to dose, $CD1a^-DR^+$ epidermal macrophage induction, and Langerhans cell depletion. *Proc. Natl. Acad. Sci. USA* **89,** 8497-8501.
7. Hersey, P., Haran, G., Hansic, E., Edwards, A. (1983) Alteration of T cell subsets and induction of suppressor T cell activity in normal subjects after exposure to sunlight. *J. Immunol.* **131,** 171-174.
8. Fisher, M.S., Kripke, M.L. (1982) Suppressor T lymphocytes control the development of primary skin cancers in UV-irradiated mice. *Science* **216,** 1133-1134.
9. Yoshikawa, T., Rae, V., Bruins-Slot, W., vand-den-Berg, J.W., Taylor, J.R., Streilein, J.W. (1990) Susceptibility to effects of UVB radiation on induction of contact hypersensitivity as a risk factor for skin cancer in humans. *J. Invest. Dermatol.* **95,** 530-536.
10. Ullrich, S.E., McIntyre, B.W., Rivas, J.M. (1990) Suppression of the immune response to alloantigen by factors released from ultraviolet-irradiated keratinocytes. *J. Immunol.* **145,** 489-498.
11. Rivas, J.M., Ullrich, S.E. (1992) Systemic suppression of DTH by supernatants from UV-irradiated keratinocytes: An essential role for interleukin 10. *J. Immunol.* **149,** 3865-3871.
12. Mosmann, T.R., Schumacher, J.H., Street, N.F., Buss, R., O'Garra, A., Fong, T.A.T., Bond, M.W., Moore, K.W., Sher, A., Fiorentino, D.F. (1991) Diversity of cytokine synthesis and function of mouse $CD4^+$ T cells. *Immunol. Rev.* **123,** 209-229.
13. Ullrich, S.E. (1994) 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. *J. Immunol.* **152,** 3410-3416.
14. Ullrich, S.E., Kripke, M.L. (1984) Mechanisms in the suppression of tumor rejection produced in mice by repeated UV irradiation. *J. Immunol.* **133,** 2786-2790.
15. Ullrich, S.E., Magee, M.J. (1988) Specific suppression of allograft rejection after treatment of recipient mice with UV radiation and allogeneic spleen cells. *Transplantation* **46,** 115-119.
16. Mosmann, T.R., Schumacher, J.H., Fiorentino, D.F., Leverah, J., Moore, K.W., Bond, M.W. (1990) Isolation of monoclonal antibodies specific for IL-4, IL-5, IL-6, and a new Th2-specific cytokine (IL-10), cytokine synthesis inhibitory factor, by using a solid phase radioimmunoadsorbent assay. *J. Immunol.* **145,** 2938-2945.
17. Yoshikawa, T., Streilein, J.W. (1990) Tumor necrosis factor-alpha and ultraviolet light have similar effects on contact hypersensitivity in mice. *Reg. Immunol.* **3,** 139-144.
18. Kripke, M.L. (1984) Immunologic unresponsiveness induced by UV radiation. *Immunol. Rev.* **80,** 87-102.
19. Ullrich, S.E. (1987) The effect of ultraviolet radiation-induced suppressor cells on T cell activity. *Immunology* **60,** 353-360.
20. Schwarz, T., Urbanski, A., Luger, T.A. (1994) Ultraviolet light and epidermal cell-derived cytokines. In *Epidermal Growth Factors and Cytokines* (T.A. Luger and T. Schwarz, eds.), Marcel Dekker, Inc., New York, 303-324.
21. Toews, G.B., Bergstresser, P.R., Streilein, J.W. (1980) Epidermal Langerhans cell density determines whether contact hypersensitivity or unresponsiveness follows skin painting with DNFB. *J. Immunol.* **124,** 445-449.
22. Cruz, P.D., Tigelaar, R.E., Bergstresser, P.R. (1990) Langerhans cells that migrate to skin after intravenous infusion regulate the induction of contact hypersensitivity. *J. Immunol.* **144,** 2486-2492.
23. Simon, J.C., Cruz, P.C., Bergstresser, P.R., Tigelaar, R.E. (1990) Low dose ultraviolet B-irradiated Langerhans cells preferentially activate $CD4^+$ cells of the T helper 2 subset. *J. Immunol.* **145,** 2087-2091.
24. Enk, A.H., Angeloni, V., Udey, M.C., Katz, S.I. (1993) Inhibition of Langerhans cell antigen-presenting function by IL-10: A role for IL-10 in induction of tolerance. *J. Immunol.* **151,** 2390-2398.

25. Elmets, C.A., Bergstresser, P.R., Tigelaar, R.E., Wood, P.J., Streilein, J.W. (1983) Analysis of the mechanism of unresponsiveness produced by haptens painted on skin to low dose UV radiation. *J. Exp. Med.* **158,** 781–794.
26. Ullrich, S.E., Yee, G.K., Kripke, M.L. (1986) Suppressor lymphocytes induced by epicutaneous sensitization of UV-irradiated mice control multiple immunological pathways. *Immunology* **58,** 185–190.
27. Kripke, M.L., Morison, W.L. (1986) Studies on the mechanism of systemic suppression of contact hypersensitivity by UVB radiation. II. Differences in the suppression of delayed and contact hypersensitivity in mice. *J. Invest. Dermatol.* **86,** 543–549.
28. Kim, T.-Y., Kripke, M.L., Ullrich, S.E. (1990) Immunosuppression by factors released from UV-irradiated epidermal cells: selective effects on the generation of contact and delayed hypersensitivity after exposure to UVA or UVB radiation. *J. Invest. Dermatol.* **94,** 26–32.
29. Enk, A.H., Saloga, J., Becker, D., Mohamadzadeh, M., Knop, J. (1994) Induction of hapten-specific tolerance by interleukin-10 in vivo. *J. Exp. Med.* **179,** 1397–1402.
30. Chung, H.-T., Burnham, D.K., Robertson, B., Roberts, L.K., Daynes, R.A. (1986) Involvement of prostaglandins in the immune alterations caused by the exposure of mice to ultraviolet radiation. *J. Immunol.* **137,** 2478–2484.
31. Robertson, B., Gahring, L., Newton, R., Daynes, R.A. (1987) In vivo administration of interleukin 1 to normal mice decreases their capacity to elicit contact hypersensitivity responses: Prostaglandins are involved in this modification of the immune response. *J. Invest. Dermatol.* **88,** 380–387.
32. Coffman, R.L., Seymore, B.W., Hudak, W.P., Jackson, J., Rennick, D. (1989) Antibody to Interleukin-5 inhibits helminth-induced eosinophilia in mice. *Science* **245,** 308–310.
33. Taylor-Robinson, A.W., Phillips, R.S., Severn, A., Moncada, S., Liew, F.Y. (1993) The role of Th1 and Th2 cells in a rodent malaria infection. *Science* **260,** 1931–1934.
34. Clerici, M., Lucey, D.R., Berzofsky, J.A., Pinto, L.A., Wynn, T.A., Blatt, S.P., Dolan, M.J., Hendrix, C.W., Wolf, S.F., Shearer, G.M. (1993) Restoration of HIV-specific cell-mediated immune responses by interleukin-12. *Science* **262,** 1721–1724.
35. Clerici, M., Shearer, G.M. (1993) A Th1 to Th2 switch is a critical step in the etiology of HIV infection. *Immunol. Today* **14,** 107–110.