Ultraviolet Light Exposure Suppresses Contact Hypersensitivity by Abrogating Endothelial Intercellular Adhesion Molecule-1 Up-Regulation at the Elicitation Site

Kazuhiro Komura, Minoru Hasegawa, Yasuhito Hamaguchi, Eriko Saito, Yuko Kaburagi, Koichi Yanaba, Shigeru Kawara, Kazuhiko Takehara, Makoto Seki, Douglas A. Steeber, Thomas F. Tedder and Shinichi Sato

*J Immunol* 2003; 171:2855-2862; doi: 10.4049/jimmunol.171.6.2855
http://www.jimmunol.org/content/171/6/2855

References
This article cites 42 articles, 18 of which you can access for free at:
http://www.jimmunol.org/content/171/6/2855.full#ref-list-1

Subscription
Information about subscribing to *The Journal of Immunology* is online at:
http://jimmunol.org/subscription

Permissions
Submit copyright permission requests at:
http://www.aai.org/About/Publications/JI/copyright.html

Email Alerts
Receive free email-alerts when new articles cite this article. Sign up at:
http://jimmunol.org/alerts
Ultraviolet Light Exposure Suppresses Contact Hypersensitivity by Abrogating Endothelial Intercellular Adhesion Molecule-1 Up-Regulation at the Elicitation Site

Kazuhiro Komura,* Minoru Hasegawa,* Yasuhito Hamaguchi,* Eriko Saito,* Yuko Kaburagi,* Koichi Yanaba,* Shigeru Kawara,* Kazuhiro Takehara,* Makoto Seki, † Douglas A. Steeber, ‡ Thomas F. Tedder, § and Shinichi Sato2*

Hapten sensitization through UV-exposed skin induces systemic immune suppression, which is experimentally demonstrated by inhibition of contact hypersensitivity (CHS). Although this UV-induced effect has been shown to be mediated by inhibition of the afferent phase of the CHS, the UV effects on the efferent (elicitation) phase remain unknown. In this study, UV effects on endothelial ICAM-1 expression at elicitation sites were first examined. Mice were sensitized by hapten application onto UV-exposed back skin, and ears were challenged 5 days later. ICAM-1 up-regulation at nonirradiated elicitation sites following hapten challenge was eliminated by UV exposure on sensitization sites distant from elicitation sites. To assess whether loss of the ICAM-1 up-regulation at elicitation sites contributed to UV-induced immunosuppression, we examined CHS responses in UV-exposed ICAM-1-deficient (ICAM-1−/−) mice that genetically lacked the ICAM-1 up-regulation. ICAM-1−/− mice exhibited reduced CHS responses without UV exposure, but UV exposure did not further reduce CHS responses in ICAM-1−/− mice. Furthermore, ICAM-1 deficiency did not affect the afferent limb, because ICAM-1−/− mice had normal generation of hapten-specific suppressor and effector T cells. This UV-induced immunosuppression was associated with a lack of TNF-α production after Ag challenge at elicitation sites. Local TNF-α injection before elicitation abrogated the UV-induced CHS inhibition with increased endothelial ICAM-1 expression. TNF-α production at elicitation sites was down-regulated by IL-10, a possible mediator produced by hapten-specific suppressor T cells that are generated by UV exposure. These results indicate that UV exposure inhibits CHS by abrogating up-regulation of endothelial ICAM-1 expression after Ag challenge at elicitation sites. The Journal of Immunology, 2003, 171: 2855–2862.

Exposure to UV light-B radiation has the potential to affect humans. As an environmental hazard, UV exposure can induce skin cancer, promote premature aging of the skin, and cause ocular damage (1). UV radiation induces flares of both systemic and cutaneous diseases in some autoimmune disorders (2). One of the most significant effects of UV radiation is systemic immune suppression that exacerbates a number of infectious diseases and permits the outgrowth of skin tumors (1, 3–6). The suppression effects of UV on the immune system have been clinically applied to attenuate cutaneous disorders, such as psoriasis. Elucidation of the mechanisms underlying UV-induced immune suppression contributes not only to the understanding of biological effects of UV but also to clarifying of fundamental mechanisms of the immune system.

UV-induced immunosuppression can be experimentally demonstrated by the inhibition of cellular immune responses such as contact hypersensitivity (CHS).3 Specifically, hapten sensitization of skin previously irradiated by low-dose cutaneous UV results in the inhibition of CHS to the same hapten (4–7). Direct UV radiation causes a profound depletion of Langerhans cells and inhibits expression of costimulatory cell surface molecules on Langerhans cells, such as ICAM-1 and CD80/CD86 (1, 4, 8). Furthermore, UV-induced release of several cytokines, including TNF-α and IL-10 from keratinocytes, is able to functionally suppress Langerhans cells (1, 4). These findings suggest that UV exposure modulates Langerhans cell function, which results in impaired sensitization and reduced CHS responses. In addition to defective sensitization by UV, the suppressive effect of UV is characterized by the generation of hapten-specific suppressor T cells, because suppression can be transferred by injecting splenocytes from UV-irradiated mice into naive mice (4–7). Suppressor T cells have been suggested to inhibit the activation and generation of effector T cells in the regional lymph nodes (LN) (7). Thus, most previous studies on the immunosuppressive properties of UV have focused on the afferent limb of CHS responses such as sensitization or appropriate generation of effector cells.

Accumulating evidence indicates that the afferent limb or elicitation phase of CHS is regulated by various factors including cytokines and expression of costimulatory molecules and cell adhesion molecules (9). Resident skin cells, mainly keratinocytes, are likely to initiate the elicitation phase of the CHS responses by production of proinflammatory cytokines (9, 10). A stimulatory role is attributed primarily to TNF-α and IL-1 production, because

---

1 Department of Dermatology, Kanazawa University Graduate School of Medical Science, Kanazawa, Ishikawa, Japan; 2 Research Laboratory III (Immunology), Pharmaceutical Research Division, Mitsubishi Pharma Corporation, Yokohama, Japan; and 3 Department of Immunology, Duke University Medical Center, Durham, NC 27710

Received for publication December 19, 2002. Accepted for publication July 17, 2003.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

This work was supported by a grant-in-aid from the Ministry of Education, Culture, Sports, Science and Technology of Japan (to S.S.) and National Institutes of Health Grants CA54464 and CA81776 (to T.F.T.).

2 Address correspondence and reprint requests to Dr. Shinichi Sato, Department of Dermatology, Kanazawa University Graduate School of Medical Science, 13-1 Takaramachi, Kanazawa, Ishikawa 920-8641, Japan. E-mail address: s-sato@med.kanazawa-u.ac.jp

Copyright © 2003 by The American Association of Immunologists, Inc.

0022-1767/03/$02.00

---

3 Abbreviations used in this paper: CHS, contact hypersensitivity; LN, lymph node; DNFB, 2,4-dinitrofluorobenzene; DNBS, 2,4-dinitrobenzene sulfonic acid; BrdU, 5-bromo-2′-deoxyuridine.
intradermal injection of these cytokines results in CHS-like skin inflammation (9–11). Expression of costimulatory molecules is also involved in CHS elicitation, because mice injected with the Ig fusion protein CTLA-4-Ig, which blocks CD80/CD86 function, show the inhibited CHS elicitation (12). Expression of cell adhesion molecules significantly regulates the capacity to elicit CHS responses, because leukocyte recruitment into the inflammatory sites is achieved using distinct constitutive or inducible adhesion molecules (9, 13–15). L-selectin (CD62L) is constitutively expressed by most leukocytes, whereas P-selectin (CD62P) and E-selectin (CD62E) are expressed by activated endothelial cells (15). These selectins primarily mediate leukocyte capture and rolling on the endothelium (15). L-selectin-deficient (L-selectin−/−) mice exhibit decreased CHS responses, whereas E-selectin−/− mice and P-selectin−/− mice have normal CHS responses (16–18). ICAM-1 is constitutively, but weakly, expressed by endothelial cells and is rapidly up-regulated by local release of proinflammatory cytokines (13, 14). The interactions of ICAM-1 with β2 integrins on leukocytes enhance rolling, firm adhesion, and transmigration of leukocytes at sites of inflammation (13–15). ICAM-1 deficiency inhibits the elicitation phase of the CHS responses without affecting the sensitization phase (16).

Despite ample experimental evidence that CHS is regulated at the effenter limb, effects of UV radiation on the elicitation phase of CHS remain unknown. In the current study, up-regulation of endothelial ICAM-1 expression after hapten challenge at nonirradiated elicitation sites was completely abrogated by UV exposure on sensitization sites distant from elicitation sites. Further analysis using ICAM-1−/− mice revealed that the immunosuppressive effect of UV is mediated by loss of the ICAM-1 up-regulation at elicitation sites. Moreover, ICAM-1 deficiency did not affect the afferent limb of the CHS responses, because ICAM-1−/− mice were able to be sensitized to hapten and had normal generation of hapten-specific suppressor and effector T cells. The results of this study indicate that UV exposure suppresses the CHS response by abrogating endothelial ICAM-1 up-regulation following Ag challenge at sites of elicitation.

**Materials and Methods**

**Mice**

ICAM-1−/− (19), P-selectin−/− (20), and E-selectin−/− (21) mice were obtained from The Jackson Laboratory (Bar Harbor, ME). L-selectin−/− mice (22) and mice lacking both ICAM-1 and L-selectin (23) were generated as described previously. All mice were healthy and fertile, and did not display evidence of infection or disease. All mice were backcrossed between 5 and 10 generations onto the C57BL/6 genetic background. Mice used for experiments were 12–16 wk old. Age-matched wild-type littermates and C57BL/6 mice (The Jackson Laboratory) were used as controls with equivalent results, so all control results were pooled. All mice were housed in a specific pathogen-free barrier facility and screened regularly for pathogens. All studies and procedures were approved by the Committee on Animal Experimentation of Kanazawa University School of Medicine.

**CHS assay**

Mice were anesthetized with ether, and their backs were shaved and painted with 25 μl of 0.5% 2,4-dinitrofluorobenzene (DNFB; Sigma-Aldrich, St. Louis, MO) in 4:1 acetone:olive oil. Five days later, mice were challenged on the dorsal surface of the ears with 15 μl of 0.2% DNFB. Ear thickness was measured with a Peacock spring-loaded micrometer (Oraki, Tokyo, Japan) before and 48 h after Ag challenge. The ear-swelling reactions were expressed as the difference between the ear thickness before and after elicitation. Each ear lobe was measured three times at each time point, and the mean of those values was used for analysis.

**Low-dose UV-B irradiation**

UV-B irradiation was delivered by an unfiltered polychromatic fluorescent sun lamp (FS20T12-UVB; National Biological, Twinsburg, OH). This instrument emits wavelengths mainly between 280 and 320 nm, peaking at 313 nm. Three days before the sensitization, the shaved back skin was exposed to a single dose of 100 ml/cm² with an intensity of 0.5 mW/cm², as described elsewhere (24). The dose of UV-B was measured using an IL-1400A radiometer equipped with a SEL240/UVB 1/TD UV-B detector with a spectral sensitivity in the range of 280–320 nm (International Light, Montreal, Quebec, Canada). During exposure, the ears were shielded from UV-B with electrical tape.

**Adaptive transfer of spleen cells**

Donor mice were exposed to UV on the shaved back and, 3 days later, sensitized with DNFB through the UV-exposed skin. Seven days after sensitization, mice were killed, spleens were harvested, and single-cell suspensions were prepared. Viable spleen cells (1 × 10⁶) were injected into the tail veins of naive recipient mice (25). Recipients were sensitized 24 h later by epicutaneous application of DNFB on the shaved back. Five days later, mice were challenged on the ear, and ear swelling was measured 48 h later. Mice injected with identical numbers of cells obtained from non-UV-irradiated and sensitized mice served as controls.

**Isolation of regional LN cells and in vitro proliferative response to hapten**

Mice were immunized with DNFB onto the UV-exposed or nonirradiated back. Single-cell suspensions from draining regional (inguinal, brachial, and axillary) LNs were isolated 72 h after sensitization. Total cell numbers were counted to assess overall proliferation of LN cells following sensitization. To assess in vitro proliferative responses to hapten, single-cell suspensions from regional LNs were collected 5 days after immunization. Cells (2 × 10⁶ cells/well in 0.2 ml) were cultured in RPMI 1640 medium (Sigma-Aldrich) containing 10% FCS with or without 0.1 mM 2,4-dinitrofluorobenzene (DNBS; a water-soluble analog of DNFB; Tokyo Kasei, Tokyo, Japan). Cellular proliferation was quantified by the addition of 10 μM 5-bromo-2′-deoxyuridine (Brdu) (Roche Diagnostics, Mannheim, Germany) during the last 18 h of a 5-day culture, and Brdu incorporation was assayed by ELISA (Roche Diagnostics), according to the manufacturer’s instructions.

**Histology and immunohistochemical staining**

Twelve hours after Ag challenge, a central strip of the ear was harvested, fixed in 3.5% paraformaldehyde, and then paraffin embedded. Six-micrometer sections were stained using H&E for general histological evaluation. For immunohistochemistry, frozen tissue sections of ear biopsies were aceton eted and then incubated with 10% normal rabbit serum in PBS (10 min, 37°C) to block nonspecific binding. Sections were then incubated with rat mAb specific for mouse ICAM-1 (Coulter, Miami, FL) as described previously (26). Rat IgG (Southern Biotechnology Associates, Birmingham, AL) was used as a control for nonspecific staining. Sections were then incubated sequentially (20 min, 37°C) with a biotinylated rabbit anti-rat IgG secondary Ab (Vectastain ABC kit; Vector Laboratories, Burlingame, CA) and then HRP-conjugated avidin-biotin complexes (Vectastain ABC kit; Vector Laboratories). Sections were developed with 3,3-diaminobenzidine tetrahydrochloride and hydrogen peroxide, and then counterstained with methyl green.

**Treatment with TNF-α or anti-IL-10 mAb**

Immediately before Ag challenge, 40 μl of TNF-α (0.05 ng/ml in PBS; R&D Systems, Minneapolis, MN) or 40 μl of anti-IL-10 mAb (JES5.2A5; 1 mg/ml in PBS; BD PharMingen, San Diego, CA) was injected intradermally into the ear of wild-type mice using a 29-gauge needle under ether anesthesia. Ear swelling was measured 48 h after Ag challenge. Injection of an equal volume of PBS (or rat polyclonal IgG [1 mg/ml in PBS; Sigma-Aldrich]) into the ears served as controls for treatment with TNF-α and anti-IL-10 mAb, respectively.

**RT-PCR**

RT-PCR was performed as previously described (27). Total RNA isolated from frozen ear specimens was reversely transcribed into cDNA and amplified. Amplification was performed in a PCR thermal cycler MP (Takara, Kusatsu, Japan) for 40 cycles of denaturation at 94°C for 30 s, annealing at 60°C for 45 s, and extension at 72°C for 60 s. The final extension was performed for 10 min, and then for 5 min at 5°C. The sense primer for mouse TNF-α was 5′-AGG CCA CGT AGC AAA CCA CCA A-3′, and the antisense primer was 5′-ACA CCC ATT CCC TCC ACA GAG CAA T-3′. The sense primer for β-actin was 5′-GGT GGG CGC CCC AGG CAC CA-3′, and the antisense primer was 5′-GCT CCG CCG TGG TGG TGA AGC-3′ (all from Bex, Tokyo, Japan).
Cotransfer of sensitized spleen cells and UV-irradiated spleen cells

Two panels of donors were prepared: one panel was the UV-treated donor that was exposed to UV on the shaved back, and 3 days later, sensitized with DNBFB through UV-exposed skin, whereas another panel was the non-UV-irradiated donor that was nonirradiated and sensitized with DNBFB on the shaved back. Seven days after sensitization, both panels of donor mice were killed, spleens were harvested, and single-cell suspensions were prepared. Naïve wild-type recipients i.v. received a mixture of $1 \times 10^8$ spleen cells from nonirradiated and sensitized donors and $1 \times 10^8$ spleen cells from UV-exposed and sensitized donors. Recipients were challenged on the ear without sensitization, and ear swelling was measured 48 h later.

Statistical analysis

The Mann-Whitney $U$ test was used for determining the level of significance of differences in sample means, and Bonferroni’s test was used for multiple comparisons.

Results

**UV exposure eliminated up-regulation of ICAM-1 expression at the elicitation site**

ICAM-1 expression is up-regulated on endothelial cells during the elicitation phase of CHS (28). To assess the effect of UV exposure on ICAM-1 expression at the elicitation site, ICAM-1 expression was examined following Ag challenge in UV-exposed wild-type mice. Mice were sensitized by topical application of the contact sensitizer DNBFB onto low-dose UV-exposed or nonirradiated back skin. Five days later, ears were challenged with DNBFB, and ICAM-1 expression in ear biopsies was immunohistochemically assessed 12 h after Ag challenge. In normal ears, ICAM-1 expression was detected only at low levels on endothelial cells and not at all on keratinocytes and fibroblasts (data not shown). When mice were challenged without prior sensitization, endothelial ICAM-1 expression remained weak (Fig. 1A). By contrast, endothelial ICAM-1 expression was significantly augmented 12 h after Ag administration in sensitized mice, and ICAM-1 remained expressed exclusively on endothelial cells during the CHS response (Fig. 1B). Remarkably, UV exposure before sensitization eliminated up-regulation of endothelial ICAM-1 expression in the ear pinnae after Ag challenge (Fig. 1C). This lack of ICAM-1 up-regulation following UV exposure was not due to direct effects of UV exposure, because the ears were protected from UV irradiation during the treatment. Thus, although the elicitation site was distant from the UV-exposed sensitization site, UV radiation eliminated up-regulation of endothelial ICAM-1 expression after Ag challenge.

**CHS was not suppressed by UV radiation in ICAM-1$^{−/−}$ mice**

To assess whether loss of the ICAM-1 up-regulation at the elicitation site contributed to UV-induced immune suppression, we examined the CHS responses in UV-exposed ICAM-1$^{−/−}$ mice. The up-regulation of ICAM-1 expression following hapten challenge occurred only in wild-type mice, but not in ICAM-1$^{−/−}$ mice. Therefore, it would be predicted that UV-induced immune suppression was not observed in ICAM-1$^{−/−}$ mice if the suppressive effect by UV was mediated primarily by the removal of the ICAM-1 up-regulation. Mice were sensitized by DNBFB application onto UV-exposed or nonirradiated back skin. Five days later, ears were challenged with DNBFB, and ear swelling was measured after 48 h. In wild-type littersmates, UV exposure significantly suppressed the CHS response compared with that observed without UV exposure (by 43%; $p < 0.05$; Fig. 2A). Histopathology of the ear sections demonstrated that inhibited ear swelling was associated with a reduction in both edema and leukocyte infiltration (Fig. 2B). CHS responses without UV exposure were reduced in ICAM-1$^{−/−}$ mice relative to wild-type littersmates (48% decrease; $p < 0.05$; Fig. 2A) as previously reported (16, 29). Remarkably, UV exposure did not further reduce the CHS responses in ICAM-1$^{−/−}$ mice (Fig. 2). These results suggest that the effect by ICAM-1 deficiency was equivalent to that by UV irradiation, because both lacked the up-regulation of ICAM-1 expression following hapten challenge. Consistently, there was the same amount of ear swelling

**FIGURE 1.** ICAM-1 expression 12 h after Ag challenge at the elicitation site. Mice were sensitized with DNBFB on UV-exposed (UV+) or nonirradiated (UV−) back skin. The ears were protected from UV irradiation with electrical tape. Five days later, mice were challenged on the ear. After 12 h, ICAM-1 expression was assessed immunohistochemically in the ear biopsies of nonsensitized (control) (A), sensitized, but nonirradiated (B), and UV-exposed and sensitized (C) mice. These results represent those obtained with five mice of each group. Arrows indicate endothelium. Magnification, $×300$.

**FIGURE 2.** The effect of adhesion molecule deficiency on CHS suppression mediated by UV exposure. A, UV effect on the CHS response in each adhesion molecule-deficient and wild-type littersmates. Mice were sensitized with DNBFB on UV-exposed (+) or nonirradiated (−) back skin and challenged on the ear after 5 days. Ear swelling was measured 48 h after Ag challenge. All values represent the means ± SD of results obtained with five mice in each group. *, $p < 0.05$; **, $p < 0.001$. B, Histologic sections of the ear pinnae from ICAM-1$^{−/−}$ and wild-type littersmates 48 h after Ag challenge. Mice were treated as described above. UV+ and UV− represent UV exposure and nonirradiation before sensitization, respectively. Wild-type littersmates that were challenged without prior sensitization served as controls. The sections were stained with H&E. Magnification, $×40$. 


in challenged, UV-treated wild-type mice as there was in challenged, nonirradiated ICAM-1$^{-/-}$ mice.

UV-induced inhibition of CHS was further analyzed in mice lacking expression of each selectin or both L-selectin and ICAM-1. The CHS responses without UV exposure were diminished in L-selectin$^{-/-}$ (42% decrease; $p < 0.05$) and L-selectin/ICAM-1$^{-/-}$ (80%; $p < 0.003$) mice relative to wild-type littermates, whereas CHS responses were normal in P-selectin$^{-/-}$ and E-selectin$^{-/-}$ mice (Fig. 2A). UV exposure significantly suppressed CHS responses in L-selectin$^{-/-}$ (77% decrease; $p < 0.001$), P-selectin$^{-/-}$ (52%; $p < 0.001$), and E-selectin$^{-/-}$ (56%; $p < 0.001$) mice compared with nonirradiated littermates, respectively. By contrast, like ICAM-1$^{-/-}$ mice, UV exposure did not further reduce the CHS responses in L-selectin/ICAM-1$^{-/-}$ mice. Furthermore, the CHS responses in UV-treated L-selectin$^{-/-}$ mice were suppressed to the same level as those observed in nonirradiated L-selectin/ICAM-1$^{-/-}$ mice. These results suggest that the immunosuppressive effect by UV was mediated by the loss of the ICAM-1 up-regulation at elicitation sites, but not by altered expression of other adhesion molecules.

**ICAM-1 deficiency did not affect the sensitization phase of CHS**

Because ICAM-1 can provide costimulatory signals for T cell activation (30), we assessed whether loss of UV-induced immunosuppression in ICAM-1$^{-/-}$ mice resulted from impaired sensitization and generation of effector cells during the CHS response. First, the total numbers of lymphocytes isolated from draining regional LNs were determined 3 days after sensitization. Sensitized wild-type littermates exhibited a significant increase in the total number of lymphocytes within LNs compared with nonimmunized littermates (by 6.3-fold; $p < 0.005$; Table I). A similar significant increase was observed in sensitized ICAM-1$^{-/-}$ mice (7.3-fold increase; $p < 0.005$). UV exposure did not affect the cellularity of LNs after sensitization in either the ICAM-1$^{-/-}$ or wild-type littermates. Second, the hapten-specific T cell responses to DNBS, a water-soluble analog of DNF, were analyzed. Five days after sensitization, lymphocytes from the draining regional LNs were isolated and cultured in vitro with DNBS for 5 days. In the non-UV-exposed animals, significant T cell proliferation was similarly observed in both the ICAM-1$^{-/-}$ and wild-type littermates compared with nonsensitized littermates (8.1- and 11.2-fold increase, respectively; $p < 0.001$; Table II). UV exposure resulted in a modest decrease in T cell proliferation that was similar for ICAM-1$^{-/-}$ (32% decrease; $p < 0.05$) and wild-type (24%; $p < 0.05$) littermates. Thus, ICAM-1 deficiency did not affect the sensitization or generation of effector cells during a CHS response.

**ICAM-1 was not required for the generation and function of suppressor T cells**

UV exposure induces the generation of T suppressor activity that can be transferred to naive, nonirradiated mice by adoptive transfer of T cells from UV-exposed and sensitized mice (4-7). To assess the effect of ICAM-1 deficiency on the generation and function of these suppressor T cells, adoptive transfer experiments were performed. Seven days after DNFB sensitization through UV-exposed skin, spleen cells from donor mice were transferred into naive recipients that were subsequently immunized with DNFB. After 5 days, mice were challenged on the ear, and ear swelling was measured 48 h later. Wild-type littermates receiving spleen cells from nonirradiated, but sensitized wild-type or ICAM-1$^{-/-}$ donormice exhibited normal CHS responses (Fig. 3A). The transfer of splenocytes from UV-exposed wild-type donors into wild-type recipients resulted in a significantly inhibited CHS response (by 45%; $p < 0.05$ vs nonirradiated donors of the same genotype).

Table I. Total cell numbers obtained from regional LNs after sensitization in ICAM-1$^{-/-}$ and wild-type littersates

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Control</th>
<th>S sensitization</th>
<th>UV + sensitization</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wild type</td>
<td>15 ± 1.2</td>
<td>95 ± 11.0*</td>
<td>92 ± 8.6*</td>
</tr>
<tr>
<td>ICAM-1$^{-/-}$</td>
<td>12 ± 1.5</td>
<td>88 ± 6.4*</td>
<td>83 ± 4.5*</td>
</tr>
</tbody>
</table>

a Draining regional (inguinal, brachial, and axillary) LNs were harvested 72 h after sensitization onto the nonirradiated (sensitization) or UV-exposed (UV + sensitization) back skin, and total cell numbers were determined using a hemocytometer. Values represent the mean number (±SEM) from five mice of each group.

Table II. In vitro hapten-specific T cell proliferative responses in ICAM-1$^{-/-}$ and wild-type littersates

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Cultured With</th>
<th>Proliferative Responses (OD)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Wild type</td>
<td>ICAM-1$^{-/-}$</td>
</tr>
<tr>
<td>Control*</td>
<td>0.21 ± 0.06</td>
<td>0.31 ± 0.06</td>
</tr>
<tr>
<td>Sensitization</td>
<td>0.25 ± 0.02</td>
<td>0.23 ± 0.03</td>
</tr>
<tr>
<td>UV + sensitization</td>
<td>1.53 ± 0.12*</td>
<td>1.46 ± 0.14*</td>
</tr>
<tr>
<td>UV</td>
<td>0.22 ± 0.08</td>
<td>0.25 ± 0.04</td>
</tr>
<tr>
<td>DNBS</td>
<td>1.17 ± 0.03**</td>
<td>1.00 ± 0.08***</td>
</tr>
</tbody>
</table>

a Mice were sensitized with DNF through the nonirradiated (sensitization) or UV-exposed (UV + sensitization) back skin. Five days after sensitization, lymphocytes from draining LNs were isolated and cultured in vitro with or without DNBS for 5 days. BrdU was added during the last 18 h of culture, and BrdU incorporation was measured by ELISA. Values represent the mean OD (±SEM) obtained with five mice in each group.

**FIGURE 3.** Adoptive transfer of UV-induced CHS suppression by spleen cells. Donor mice were sensitized with DNF through UV-exposed (UV+) or nonirradiated (UV−) back skin. After 7 days, 1 × 10⁸ spleen cells were isolated from the indicated donor and injected i.v. into a naive wild-type (A) or ICAM-1$^{-/-}$ (B) recipient that was subsequently sensitized with DNF on the shaved back. Five days later, recipient mice were challenged on the ear, and ear swelling was measured 48 h later. Wild-type littermates receiving spleen cells from nonirradiated, but sensitized wild-type or ICAM-1$^{-/-}$ donors exhibited normal CHS responses (Fig. 3A). The transfer of splenocytes from UV-exposed wild-type donors into wild-type recipients resulted in a significantly inhibited CHS response (by 45%; $p < 0.05$ vs nonirradiated donors of the same genotype).
Similarly, adoptively transferred spleen cells from UV-irradiated ICAM-1−/− donors suppressed the CHS response in wild-type recipients (by 48%; \( p < 0.005 \)). Thus, the generation and function of suppressor activity did not require ICAM-1 expression. The transfer of spleen cells from nonirradiated and sensitized wild-type donors into ICAM-1−/− recipients reduced the CHS response compared with the transfer of similarly treated wild-type donors into wild-type recipients (by 45%; \( p < 0.01 \); Fig. 3). Moreover, the transfer of splenocytes from UV-exposed wild-type donors into ICAM-1−/− recipients did not result in more significant suppression of the CHS response relative to the transfer of cells from nonirradiated wild-type donors into ICAM-1−/− recipients. Therefore, UV-induced suppression of the CHS response requires ICAM-1 expression in recipients.

**TNF-α injection at the elicitation site overcomes UV-induced CHS suppression**

Up-regulation of endothelial ICAM-1 expression during the elicitation phase of a CHS response is primarily due to local TNF-α release (28). Keratinocytes and mast cells were suggested to be the major source of TNF-α (10, 28). Therefore, a lack of up-regulated endothelial ICAM-1 expression at the challenge site of UV-exposed mice may result from reduced TNF-α production. To assess this possibility, TNF-α mRNA levels were examined in ear biopsies 2 h after elicitation by RT-PCR. TNF-α production was not detected in the normal ear or in the challenged ear of nonsensitized wild-type mice (Fig. 4A). TNF-α mRNA levels were up-regulated in ear biopsies from sensitized wild-type mice 2 h after Ag challenge. By contrast, TNF-α mRNA was not detected in UV-exposed and sensitized wild-type mice following Ag challenge. Thus, up-regulation of TNF-α mRNA after Ag challenge was eliminated at the elicitation site in UV-irradiated wild-type mice.

The effects of local TNF-α injection on UV-induced suppression of CHS were examined. Immediately before Ag challenge, TNF-α was injected intradermally into the ear pinnae of mice sensitized through UV-exposed back skin. TNF-α injection followed immediately by Ag challenge induced a potent CHS response that was 2.1-fold higher than that observed with PBS injection (\( p < 0.05 \); Fig. 4B). Furthermore, TNF-α treatment in UV-exposed mice augmented the CHS response to a level comparable with normal CHS responses in nonirradiated mice (Fig. 4B). TNF-α injection without Ag challenge induced only mild ear swelling that was 67% lower than that induced by TNF-α injection with Ag challenge (\( p < 0.005 \)). In addition, TNF-α injection up-regulated ICAM-1 expression on the endothelium 12 h after Ag administration (Fig. 4C). Thus, local TNF-α injection at the elicitation site restored the UV-suppressed CHS responses through endothelial ICAM-1 up-regulation.

**Anti-IL-10 mAb treatment at the elicitation site abrogated UV-induced CHS suppression**

Keratinocytes are one of the major sources of TNF-α, and IL-10 down-regulates TNF-α production by keratinocytes (10, 28, 31). IL-10 is involved in mediating tolerance induced by low-dose UV (32). Furthermore, suppressor T cells from UV-irradiated mice produce IL-10, but no IL-4 (33, 34). Therefore, UV-induced production of IL-10 may be responsible for decreased TNF-α production that results in a lack of up-regulated endothelial ICAM-1 expression. To assess this possibility, the effect of anti-IL-10 mAb injection immediately before Ag challenge on UV-induced inhibition of CHS was investigated in wild-type mice. Intradermal injection of a control rat polyclonal IgG before Ag administration in the ear pinnae of UV-exposed and sensitized mice did not affect suppression of CHS by UV (Fig. 5A). By contrast, intradermal anti-IL-10 mAb injection in the ear pinnae of similarly treated mice restored CHS responses to a level similar to that of nonirradiated mice. Furthermore, the enhanced CHS response generated by anti-IL-10 mAb treatment was associated with up-regulation of TNF-α mRNA levels (Fig. 5B) and endothelial ICAM-1 expression (C) after Ag challenge. Thus, anti-IL-10 mAb injection at the elicitation site eliminated UV-induced suppression of CHS responses with up-regulated ICAM-1 expression.

**UV-induced suppressor cells inhibited the elicitation phase of CHS**

Because suppressor T cells from UV-irradiated mice produce IL-10 (33, 34), the present finding that IL-10 production at the elicitation sites mediated UV-induced suppression of CHS responses suggested the presence of suppressor T cells acting on the efferent limb. To assess whether UV-induced suppressor cells affected the elicitation phase and whether their generation was influenced by ICAM-1 deficiency, an additional adoptive transfer...
compared with spleen cells from only nonirradiated and sensitized donors significantly suppressed the efferent limb of CHS responses, along with spleen cells from nonirradiated and sensitized wild-type donors into naive recipients (Fig. 6). Cotransferred suppressor cells as well as effector T cells acting on the efferent limb.

FIGURE 5. Effects of IL-10 on UV-induced CHS suppression at the elicitation site. A, Effect of anti-IL-10 mAb injection at the elicitation site following Ag challenge (Fig. 1). Furthermore, normal UV-induced inhibition of the CHS response in wild-type recipients. The CHS responses were measured 48 h later. Challenged wild-type recipients that received spleen cells from nonirradiated and sensitized wild-type donors containing suppressor T cells as well as effector cells acting on the efferent limb.

B, Effect of anti-IL-10 mAb treatment on TNF-α mRNA expression. Wild-type mice were treated with either anti-IL-10 mAb or rat polyclonal IgG (CTL Ab) as described above, and 2 h after Ag challenge, TNF-α mRNA expression was evaluated in ear biopsies by RT-PCR. Representative mRNA expression of TNF-α and β-actin is shown. These results represent those obtained with five wild-type mice. C, Effect of anti-IL-10 mAb treatment on endothelial ICAM-1 expression. Wild-type mice were treated with either anti-IL-10 mAb or rat polyclonal IgG as described above, and 12 h after Ag challenge, ICAM-1 expression was assessed immunohistochemically in the ear biopsies. These results represent those obtained with five wild-type mice. Arrows indicate endothelium. Magnification, ×300.

FIGURE 6. UV-induced suppressor cells acting on the elicitation phase of the CHS response and the effect by ICAM-1 deficiency on their generation. The presence of effector suppressor cells and the effect by ICAM-1 loss on their generation were assessed by adoptive cotransfer of both effector and suppressor T cells into naive wild-type recipients. Wild-type or ICAM-1−/− donor mice were sensitized with DNPBR through UV-exposed (UV+) or nonirradiated (UV−) back skin. After 7 days, naive wild-type recipients i.v. received a mixture of spleen cells from nonirradiated and sensitized donors containing effector T cells (wild type or ICAM-1−/− UV−) and the same number of spleen cells from UV-exposed and sensitized donors containing suppressor T cells (wild type or ICAM-1−/− UV+). Recipients were challenged on the ear without sensitization, and ear swelling was measured 48 h later. Challenged wild-type recipients that received spleen cells from nonirradiated and sensitized wild-type or ICAM-1−/− donors (sensitized wild-type or ICAM-1−/− donor UV−) or spleen cells from nonirradiated and unsensitized wild-type donors (un- sensitized wild-type donor UV−) are also shown. Nonirradiated wild-type mice that were challenged with and without prior sensitization served as positive and negative controls for the CHS response, respectively. UV-irradiated and sensitized wild-type mice that were challenged were also used as controls (UV+ control, □□□□ and □□□□, UV exposure and nonexposure, respectively. All values represent the means ± SD for five mice per group. *, p < 0.05 vs nonirradiated but sensitized wild-type donors.

Discussion

In the current study, UV exposure on the sensitization site completely abrogated up-regulation of endothelial ICAM-1 expression at the elicitation site following Ag challenge (Fig. 1). Furthermore, ICAM-1−/− mice that genetically lacked the ICAM-1 up-regulation were resistant to UV-induced immunosuppression (Fig. 2). By contrast, normal UV-induced inhibition of the CHS response was
observed in the absence of either E-, P-, or L-selectin expression. These results indicate that the immunosuppressive effect by UV is mediated by loss of the ICAM-1 up-regulation at the elicitation site. Consistently, UV exposure in wild-type mice suppressed CHS responses to a level comparable with those found in nonirradiated ICAM-1−/− mice, indicating that the inhibitory effect of UV on the CHS is equivalent to the effect of ICAM-1 deficiency (Fig. 2). Furthermore, adoptive transfer experiments demonstrated that ICAM-1 expression on recipient endothelial cells was required for UV-induced suppression of the CHS response (Fig. 3). ICAM-1 deficiency did not affect the afferent limb of the CHS responses, because ICAM-1−/− mice were able to be sensitized to hapten and had normal generation of hapten-specific suppressor and effector T cells (Tables I and II; Figs. 3 and 6). The results of this study indicate that UV exposure suppresses the CHS response by abrogating endothelial ICAM-1 up-regulation at the elicitation site.

ICAM-1 not only mediates leukocyte recruitment into inflammatory sites but also functions as a costimulatory molecule on the surface of APCs (30). Direct UV radiation prevents up-regulation of ICAM-1 expression by Langerhans cells in culture, which may result in impaired sensitization and a reduced CHS response (8). This finding raises the possibility that the abrogated ICAM-1 up-regulation on Langerhans cells during the sensitization may result in the CHS inhibition by UV radiation, because in our experimental model, the sensitization was performed onto the UV-irradiated site. Moreover, the finding that UV radiation eliminated the ICAM-1 up-regulation at the nonirradiated challenge site was also consistent with the prior hypothesis that UV exposure suppresses the sensitization process, because the failure of the sensitization results in no induction of the elicitation, which is the same situation as observed in mice challenged without sensitization. However, this possibility is unlikely for several reasons. ICAM-1−/− mice exhibited a significant increase in LN cell number after sensitization and normal hapten-specific T cell proliferation (Tables I and II). In addition, adoptive transfer experiments revealed normal generation of hapten-specific suppressor T cells as well as effector T cells in ICAM-1−/− mice (Figs. 3A and 6). Consistent with these findings, a previous study has shown that ICAM-1 deficiency does not affect the generation of T cells able to mount in vitro proliferative responses to hapten, the generation of CTL responses, or humoral immune responses (16). Thus, the finding of the current study indicates that ICAM-1 loss in UV-induced immune suppression influences the effector limb of the CHS response, especially leukocyte migration during the elicitation, rather than the afferent limb including the sensitization or generation of effector cells.

TNF-α plays a primary role in ICAM-1 up-regulation during CHS responses. Specifically, TNF-α mRNA is expressed by 30 min after Ag administration (10), and the early up-regulation of endothelial ICAM-1 expression is blocked by anti-TNF-α mAb, but not by anti-IL-1 mAb (28). In the current study, TNF-α production at the elicitation sites was eliminated by UV exposure, and TNF-α injection abrogated UV-induced immunosuppression with increased endothelial ICAM-1 expression (Fig. 4). These results indicate that a lack of augmented TNF-α production following Ag challenge is responsible for loss of ICAM-1 up-regulation. Because ICAM-1−/− mice exhibit a significantly decreased CHS responses (16) and treatment with mAb against ICAM-1 or its receptor LFA-1 (CD11a/CD18) before elicitation similarly reduces CHS responses (35), a lack of ICAM-1 up-regulation may lead to UV-induced suppression of CHS during the elicitation phase. Although TNF-α treatment before the elicitation abrogated UV-induced immunosuppression (Fig. 4B), a previous study has shown that TNF-α induces immune suppression by UV exposure when applied before sensitization (36). This may result from impaired function of Langerhans cells by TNF-α (1, 4). Thus, the effect of TNF-α on UV-induced immune suppression appears to be complex, depending upon the different phases of the CHS response. Nevertheless, the results of this study indicate that TNF-α plays a critical role in regulating UV-induced CHS suppression at the elicitation site by modulating endothelial ICAM-1 expression.

IL-10 is an important regulator of CHS, because IL-10 administration before Ag challenge diminishes the CHS response, whereas treatment with anti-IL-10 Ab augments the response (37). This effect of IL-10 appears to be mediated by TNF-α (37), because IL-10 down-regulates TNF-α production by keratinocytes (31), and prolonged treatment with anti-IL-10 mAb results in increased TNF-α production (38). Furthermore, IL-10 is a key mediator that induces tolerance by low-dose UV exposure, because i.p. injection of anti-IL-10 mAb prevents UV-induced tolerance (32). Treatment with anti-IL-10 mAb before Ag challenge eliminated UV-induced immunosuppression with up-regulation of TNF-α and ICAM-1 expression (Fig. 5). These results indicate that IL-10 contributes to UV-induced immune suppression by down-regulating TNF-α production at the elicitation site. Because the elicitation sites (i.e., ears) were distant from irradiated and sensitized sites (i.e., back) and were protected from UV irradiation, the inhibitory effect of UV on the nonirradiated elicitation sites suggests the presence of circulating mediators or cells that are generated in UV-exposed sites or their regional LNs. In this regard, suppressor T cells from mice irradiated by low-dose UV produce IL-10, but no IL-4 (33, 34). Furthermore, our adoptive cotransfer study of both effector and suppressor T cells without sensitization of recipients revealed that suppressor T cells inhibited the effluent (elicitation) limb of the CHS response (Fig. 6). Consistently, previous studies have shown suppressor T cells able to inhibit the effluent limb of CHS (39, 40). Furthermore, low-dose UV-induced suppression of delayed-type hypersensitivity to herpes virus is mediated by the suppressor T cells acting on the effluent limb (41). Therefore, it is possible that the effluent suppressor T cells generated by UV exposure produce IL-10 at the elicitation sites and thereby inhibit TNF-α production possibly by keratinocytes, which results in a lack of ICAM-1 up-regulation. However, the relationship between effluent suppressor T cells and IL-10 production was not examined in this study.

There are many potential mechanisms for the elimination of UV-induced suppression in ICAM-1−/− mice. ICAM-1−/− mice exhibited normal generation of suppressor cells and effector cells acting on the elicitation phase of CHS responses (Fig. 6). Therefore, it is unlikely that impaired function of effluent suppressor cells results in the abrogated UV-induced immunosuppression in ICAM-1−/− mice. However, in this study, it remains unknown whether the recruitment of suppressor cells into elicitation sites is influenced by ICAM-1 deficiency. Therefore, the two potential mechanisms are still possible. One possibility is that the recruitment of suppressor cells to the elicitation site is more dependent on ICAM-1 expression than is the recruitment of effector cells. The second possibility is that ICAM-1−/− mice possess a compensatory mechanism that selectively mediates the recruitment of the effector cells without providing a mechanism for the recruitment of suppressor cells to sites of elicitation. To assess these possibilities, additional experiments, in which UV-induced suppressor cells from ICAM-1−/− mice are directly injected into elicitation sites of CHS responses, will be needed.

L-selectin−/− mice exhibited the diminished CHS responses without UV exposure, but UV exposure further reduced the CHS response, which resulted in the much weaker residual CHS response than in mice lacking ICAM-1, E-, or P-selectin (Fig. 2A). The finding that UV exposure in L-selectin−/− mice decreased the
CHS response to a level similar to that found in L-selectin/ICAM-1−/− mice indicates that the reduced CHS responses by UV exposure in L-selectin−/− mice is dependent on ICAM-1 expression. Thus, the effect by L-selectin deficiency and the effect by UV exposure (i.e., ICAM-1 deficiency) appears to be additive. Consistent with this, previous studies using L-selectin/ICAM-1−/− mice have revealed that the loss of both L-selectin and ICAM-1 expression dramatically reduces leukocyte migration into sites of inflammation beyond what was observed with loss of either receptor alone (16, 26, 27, 42). Thus, L-selectin mediates the CHS response cooperatively with ICAM-1, which may be a target of UV-induced immunosuppression at sites of elicitation.

Although many previous studies have focused largely on the effects of UV exposure on the allergic limb of CHS, the current study demonstrates that UV exposure suppresses CHS at the effector limb in addition to the allergic limb. Why do redundant mechanisms exist to suppress the CHS response after UV exposure? UV exposure is known to induce or exacerbate some systemic autoimmune diseases, such as systemic lupus erythematosus and dermatomyositis (2). UV irradiation induces keratinocyte apoptosis, which leads to changes in the molecular context of self Ag. Indeed self Ag.

Acknowledgments

We thank M. Matsubara and Y. Yamada for technical assistance.

References


