A Critical Role for Fas Ligand in the Active Suppression of Systemic Immune Responses by Ultraviolet Radiation

By Laurie L. Hill, Vijay K. Shreedhar, Margaret L. Kripke, and Laurie B. Owen-Schaub

From the Department of Immunology, University of Texas M.D. Anderson Cancer Center, Houston, Texas 77030

Summary

Induction of antigen-specific suppression elicited by environmental insults, such as ultraviolet (UV)-B radiation in sunlight, can inhibit an effective immune response in vivo and may contribute to the outgrowth of UV-induced skin cancer. Although UV-induced DNA damage is known to be an initiating event in the immune suppression of most antigen responses, the underlying mechanism(s) of such suppression remain undefined. In this report, we document that Fas ligand (FasL) is critical for UV-induced systemic immune suppression. Normal mice acutely exposed to UV exhibit a profound suppression of both contact hypersensitivity and delayed type hypersensitivity (DTH) reactions and the development of transferable antigen-specific suppressor cells. FasL-deficient mice exposed to UV lack both transferable suppressor cell activity and primary suppression to all antigens tested, with the exception of the DTH response to allogeneic spleen cells. Interestingly, suppression of this response is also known to occur independently of UV-induced DNA damage. Delivery of alloantigen as protein, rather than intact cells, restored the requirement for FasL in UV-induced immune suppression of this response. These results substantiate that FasL/Fas interactions are essential for systemic UV-induced suppression of immune responses that involve host antigen presentation and suggest an interrelationship between UV-induced DNA damage and FasL in this phenomenon. Collectively, our results suggest a model whereby UV-induced DNA damage disarms the immune system in a manner similar to that observed in immunologically privileged sites.

Key words: Fas ligand • ultraviolet radiation • immunosuppression • CD95 • T suppressor cells

Exposure to UV-B radiation from sunlight is a significant worldwide environmental hazard. UV exposure induces the most common malignancy in humans, skin cancer, with an estimated 1,000,000 cases per year in the United States (1). UV exposure also significantly down-modulates immune responses, exacerbating a number of infectious diseases and permitting the outgrowth of highly antigenic UV-induced skin tumors in mice (1, 2). The effects of UV on the immune system have been clinically exploited to attenuate pathophysiological responses associated with autoimmune disease in the skin (e.g., psoriasis), transfusion-associated graft-versus-host disease, and tumor growth (e.g., cutaneous T cell lymphoma) (3–5). Elucidating the mechanisms underlying UV-induced immunosuppression may permit significant advances in the therapeutic use of UV light and define a target for intervention in infectious diseases adversely affected by UV exposure. Moreover, the contribution of UV-induced immune suppression to skin carcinogenesis could be more accurately defined.

A large body of evidence supports the concept that exposure to UV radiation can suppress cellular immune responses in animals and humans to antigens applied both locally (at the site of irradiation) and distally (at a nonirradiated [NR] site) (6). Acute exposure to UV-B radiation alters the local site such that epicutaneous application of hapten fails to induce contact hypersensitivity (CHS) and instead induces tolerance in UV-B-susceptible mice (7, 8). Acute UV-B exposure can also result in diminished CHS and delayed type hypersensitivity (DTH) responses when antigens are applied epicutaneously or subcutaneously, respectively, at NR sites (6, 9, 10). Suppression of immune responses to antigens administered at distant, NR sites is known as systemic immune suppression and is characterized by the generation of antigen-specific splenic T suppressor cells (11–13). Although the phenotype and mode of action of the T suppressor cell population remain largely uncharacterized,
previous studies have documented that U V -induced immu
mune suppression can be transferred to naive, N R animals
by C D 4 + cells from U V -irradiated (U V R ), antigen-primed
mice (14, 15). Suppressor cells cannot be elicited by either U V R or antigen alone but require the introduction of anti-
gen at a critical time after irradiation (16, 17). Suppressor
cell activity appears to be targeted to the Th population
and requires I L -10 for both induction and function (18–20).
Data from a number of laboratories suggest that one conse-
quence of U V -B exposure is a shift from a Th1- to a Th2-
type immune response, resulting in the suppression of CH S and D T H induction and the generation and maintenance of
suppressor cells (19–24).
DNA damage (in the form of cyclobutane pyrimidine
dimers) has been identified as the initial photobiological
event triggering U V -induced systemic immune suppression
to most antigens in the mouse (25, 26). Application of lipo-
somes containing bacteriophage T 4 endonuclease V (an ex-
cision repair enzyme specific for pyrimidine dimers) to U V -irradiated skin can repair the U V -induced DNA damage
in the skin, diminish U V -induced Th2-type cytokine
production, reverse U V -induced systemic suppression, and
prevent the generation of U V -induced suppressor cells
(27). Conversely, inducing DNA damage in the skin using liposomas containing H indIII can result in the production of
Th2-type cytokines and the systemic suppression of CH S and D T H responses (28, 29). These observations point to
DNA damage as a central mechanism underlying U V -induced
immune suppression and suggest a sequence of events in
which U V -initiated DNA damage triggers immunomodu-
ulatory cytokine production, the generation of C D 4 + sup-
pressor cells, and immune perturbations leading to dimin-
ished CH S and D T H responses. Considering that primary
immunosuppression and the generation of suppressor cells
are dissociable events and that immunomodulatory cyto-
kine production is necessary, but not sufficient, to recapitu-
late the effects of U V R on the immune response, the exist-
ence of additional molecular linkages between DNA
damage and immune suppression is likely.
Recent evidence has documented the interactions of Fas and
Fas ligand (Fas l ) in the control of specific T cell-medi-
ated immune responses (reviewed in 30). T his complemen-
tary receptor-ligand pair initiates apoptosis in activated
lymphocytes (31, 32) and is required for the maintenance of
peripheral tolerance (33) and immune privilege (34, 35).
Several lines of circumstantial evidence led us to query
whether Fas/Fas l interactions were involved in systemic
U V -induced immune suppression. First, Fas l is inducible
by DNA damage (36) and is upregulated in normal skin
following exposure to U V irradiation (3); second, Fas/Fas l
interactions have been shown to mediate antigen-specific
immune suppression (35, 37); third, C D 4 + T cells have
been documented to upregulate Fas l expression and in-
duce autocrine, paracrine, or juxtacrine cell death (38–41);
and finally, the preferential elimination of Th1 cells by Fas l
has been reported (42). T o test the hypothesis that Fas and
Fas l are involved in the immunomodulatory effects of U V R,
we investigated the biological consequences of Fas and
Fas l .

Materials and Methods
Mice. Specific pathogen-free C 3 H / H e j , B a l b / c , and C 5 7 B l / 6
male mice were purchased from the National Cancer Institute-
Frederick Cancer Research Facility Animal Production Area.
C 5 7 B l / 6 g l d / g l d and C 5 7 B l / 6 l p r / l p r male mice were purchased
from The Jackson Laboratory. C 3 H / H e j g l d / g l d male mice were
generated from a breeder colony maintained in our facility and
used between 8 and 14 wk of age. Mice were housed in a patho-
gen-free barrier facility accredited by the American Association
for Accreditation of Laboratory Animal Care, in accordance with
current U.S. Department of Agriculture, Department of Health
e and Human Services, and N ational Institutes of Health regula-
tions and standards. All animal procedures were approved by the
Institutional Animal Care and Use Committee.
U V -B Radiation Source and Irradiation Procedure. A bank of six
Westinghouse FS40 sunlamps was used as a source of U V radia-
tion as described (25).
D T H Responses to C antida albicans and A lloantigen. D T H
responses were assessed as previously described (9). In brief, mice
were shaved and exposed to U V -B radiation (2–5 and 15 kJ/m2
for C. albicans and alloantigen, respectively). 3 d later, mice were
sensitized by subcutaneous injection of antigen (10 7 formalin-
fixed C. albicans or 5 × 10 7 Balb/c spleen cells or cell equiva-
 rents). 6–10 d after antigen sensitization, mice were challenged by
injecting either purified C. albicans protein (Allercheck, Inc.) or
10 6 Balb/c spleen cells in the footpad. 24 h later, footpad swelling
was quantitated using a spring loaded micrometer (Swiss Preci-
sion Instruments). Specific footpad swelling ( Δswelling) was
determined by subtracting the footpad swelling in mice that were
challenged but not sensitized from that observed in mice that
were sensitized and challenged. Percent suppression was calcu-
lated as % suppression = 1 − [T − N/P − N] × 100, where N =
negative control (response of unsensitized mice to challenge), P =
positive control (response of sensitized mice to challenge), and
T = test group (response of mice given U V irradiation before
sensitization and challenge). Treatment groups consisted of 3–6
(typically 5) mice; both hind footpads were measured.
CH S Response to FITC. CH S responses were determined as
previously described (43). In brief, for FITC responses, the ab-
dominal hair of mice was shaved, their ears protected with elec-
trical tape, and the animals exposed to U V -B radiation (2 kJ/m2).
3 d later, the dorsal hair was shaved and the animals sensitized by
epicutaneous application of 400 μl of 0.5% FITC (Isomer I, Al-
drich Chemical Co.) in acetone-dibutylphthalate (1:1, vol/vol).
5–7 d later, the mice were challenged by applying either 10 μl
0.5% FITC to the ventral and dorsal surfaces of both ears. Ear
swelling ( Δswelling) was quantitated 24 h later using a spring
loaded micrometer and specific ear swelling determined by sub-
tracting the ear swelling in mice challenged but not sensitized
from that observed in mice that had been sensitized and chal-
enged; percent suppression was calculated as described for D T H
responses.
Transfer of Spleen Cells. For transfer of splenic suppressor cell
populations, mice were killed, spleens harvested, and single cell
suspensions prepared immediately following D T H or CH S anal-
ysis. Approximately 10 5 spleen cells were injected into the tail
veins of N R mice, naive recipient mice and the animals immedi-
ately sensitized by subcutaneous injection (10 7 formalin-fixed C. albi-
To explore the effects of diminished Fas function on UVR-induced immune suppression, we next examined the DTH response of wild-type B6 and Fas-deficient C57Bl/6 lpr/lpr (B6/lpr) mice acutely exposed to a single dose of UVR and immunized with C. albicans. Results from a representative experiment are shown in Fig. 1. Unlike mice containing the gld mutation, DTH induction was significantly suppressed in both UVR-treated B6/lpr and wild-type B6 mice compared with NR control mice in each group. Notably, UVR-induced suppression in lpr mice was only half that observed in wild-type B6 mice with intact Fas expression (40% suppression in lpr mice compared with 83% in wild-type mice). The observation that B6/lpr mice have intermediate sensitivity to UVR-induced immunosuppression is not likely the result of differences in genetic background between the two mouse strains, as B6/gld mice also exhibited a complete absence of UVR-induced immune suppression (data not shown). Instead, these findings likely reflect the leaky nature of the lpr mutation and, moreover, suggest that the induction of Fas after UV exposure may reestablish the capacity to induce systemic immune suppression. Indeed, Fas upregulation has been recently reported to occur in lpr mice exposed to gamma irradiation (52). Taken together, these results point toward a critical requirement for Fas/FasL interactions in primary, UVR-induced systemic suppression of CHS and DTH induction.

Earlier work in our laboratory demonstrated the presence of DNA damage (cyclobutane pyrimidine dimers) in skin-derived dendritic cells up to 1 wk after a single acute exposure of UVR (27). As DNA damage can induce FasL expression, a potential mechanism for the requirement for FasL in UV-mediated suppression might be the inappropriate expression of FasL in the draining lymph nodes after UVR. To explore this possibility, the skin-draining lymph

**Figure 1.** UV-induced systemic suppression of antigen-specific responses to FITC and C. albicans. Mice were exposed to UVR, sensitized with antigen, and challenged, and DTH response was determined 24 h later as described in Materials and Methods. Values shown represent mean ± SE for five mice per group using measurements from two footpads per mouse. *, negative (challenge only); +, positive (sensitized and challenged); and UV (UVR, sensitized, and challenged). **P < 0.0001 vs. positive control. *P < 0.05 vs. positive control.
nodes from wild-type mice were removed 3 d after UVR and FasL expression determined by RT-PCR. As shown in Fig. 2, FasL mRNA was markedly induced in the lymph nodes of mice that received 15 kJ/m² UVR but was undetectable in untreated control animals. Specificity was demonstrated using L929 murine fibroblasts and unactivated lpr/lpr splenocytes as negative and positive controls for FasL expression, respectively (53). FasL induction in the skin-draining lymph nodes after UVR points to an interrelationship with DNA damage and suggests a potential scenario in which inappropriate FasL expression eliminates antigen-responsive T cells (54) or serves to clonally expand a suppressor cell population (55). Experiments are currently underway to test these possibilities.

UV-induced Systemic Suppression of DTH to Alloantigen Does Not Require FasL. UVR-induced suppression of the DTH response to whole alloantigen differs from that of FITC or C. albicans in that it is mediated by photoisomerization of cis-urocanic acid and is UVR-induced DNA damage-independent (Kripke, M.L., unpublished observations). Such findings suggest that UVR-induced suppression of the DTH response to alloantigen differs mechanistically from that of C. albicans. To evaluate the role of FasL in UVR-induced immune suppression of DTH responses to whole alloantigen, C3H, C3H/gld, B6, and B6/gld mice were exposed to a single dose of UVR and immunized with intact Babc/c spleen cells, and DTH responses were measured. In three independent experiments, UVR exposure potently suppressed DTH responses in both strains of FasL-deficient mice (Fig. 3) at levels comparable to those of matched, wild-type control mice. These results demonstrate that, in contrast to other antigen systems tested (FITC and C. albicans), primary UVR-induced suppression of DTH to whole alloantigen can proceed in a manner independent of host-derived FasL.

Presentation of Alloantigen Determines the Requirement for Host-derived FasL in UVR-induced Immune Suppression. To resolve the differences in FITC, C. albicans, and alloantigen requirements for FasL in the mediation of UVR-induced immune suppression, a more detailed analysis of allogeneic FasL expression was undertaken. Whereas FITC and formalin-fixed C. albicans require processing and presentation by host APC, whole alloantogenic splenocytes may bypass such a requirement through direct antigen presentation on the various cell populations in the sensitizing inoculum. To this end, we queried whether FasL was required for UVR-induced suppression when alloantigenic splenocytes were disrupted before immunization. UVR-induced suppression of DTH responses to intact and disrupted alloantigen (C3H and C3H/gld) mice by exposing mice to a single dose of UVR, immunizing 3 d later with either intact or disrupted alloantigen Babc/c spleen cells, and challenging 4–10 d later with intact spleen cells to elicit a DTH response. Disrupted alloantigenic spleen cells were prepared by several freeze–thaw cycles and sonication to insure uniform dispersion. R results from one of two consistent experiments are shown in Fig. 4. DTH responses were suppressed in UVR-treated wild-type mice whether intact or disrupted alloantigen was used for sensitization (76 and 89% suppression, respectively, compared with positive control mice; P < 0.0001). In contrast to the potent suppression of DTH observed in UVR-treated C3H/gld mice immunized with intact allogeneic spleen cells (62% suppression; P < 0.0001), UVR-treated C3H/gld mice immunized with disrupted allogeneic spleen cells failed to demonstrate such suppression. The lack of primary, UV-induced immunosuppression in FasL-deficient mice immunized with disrupted alloantigen is in accordance with our findings using FITC and C. albicans (Fig. 1), suggesting that such suppression is dependent upon FasL and host-derived APC for antigen presentation.

Transfer of UV-induced Suppression Requires Donor-derived FasL. Antigen-specific T suppressor cells capable of transferring antigen-specific suppression to a naïve host exist in the spleens of mice exposed to UVR and sensitized to anti-
sequent generation of transferable suppressor cells. Although primary, U VR-mediated suppression was absent in FasL-deficient mice, it remained uncertain whether transferable suppressor cell function was also absent in such mice. To address this premise, we compared suppressor cell activity in adoptively transferred spleen cells from immunized, U VR-exposed wild-type and gld donors. These experiments were carried out by exposing C3H and C3H/gld mice to a single dose of U VR followed by immunization and challenge with FITC, C. albicans, or alloantigen. On the day that CHS and DTH responses were measured, spleens from U VR-exposed animals and NR control animals were harvested and transferred into naïve recipient mice. Recipient mice were immediately sensitized with the indicated antigen and received antigenic challenge 6–10 d later. Mice receiving NR, antigen-primed spleen cells from either C3H or C3H/gld donors showed potent CHS and DTH responses to FITC and C. albicans, respectively. Animals that received spleen cells from U VR-exposed, antigen-primed C3H mice demonstrated a suppression of both CHS and DTH responses, confirming the presence of U V-induced splenic suppressor cells as expected (Fig. 5). In contrast, adoptively transferred U VR-exposed spleen cells from C3H/gld mice primed with either FITC or C. albicans failed to inhibit antigen sensitization in naïve mice. Although these experiments suggest a loss of transferable suppressor cell activity in the absence of donor-derived FasL, they are not definitive, as such mice also lack primary U VR-mediated suppression, which may be essential for the subsequent generation of transferable suppressor cells.

To this end, transferable suppression was also evaluated in C3H/gld mice immunized with allogeneic spleen cells in which primary, U VR-mediated immune suppression was intact. For these experiments, spleen cells from U VR-exposed, FasL-deficient C3H/gld (data not shown) or B6/gld mice immunized with allogeneic spleen cells were transferred to naïve, matched wild-type recipients (Fig. 5). As expected, spleen cells from wild-type mice that received U VR before antigen priming showed potent suppressor cell activity when transferred into matched wild-type recipients (Fig. 4). Consistent with our previous experiments, spleen cells from FasL-deficient mice that received U VR before antigen priming showed no such transferable suppressor cell activity. That C3H/gld and B6/gld mice exhibited potent U VR-induced primary suppression but not splenic suppressor cell activity when immunized with allogeneic whole spleen cells (Fig. 3) suggests that these events are mechanistically dissimilar and that transferable suppression is strictly dependent upon FasL expression on the donor population. In further support of this premise, wild-type mice that received U VR before immunization with disrupted allogeneic spleen cells exhibited transferable suppressor cell activity comparable to that of C3H mice immunized with intact allogeneic spleen cells. Spleen cells from FasL-deficient mice that received U VR before immunization with disrupted allogeneic, however, again demonstrated no such transferable suppressor cell activity (data not shown). These results substantiate that donor-derived FasL expression is uniformly required for the generation and/or effector activity of U V-induced transferable suppressor cells for all antigen systems tested.

Transfer of U V-induced Suppression Does Not Require Recipient FasL. In the experimental models above, we have shown a stringent requirement for donor-derived FasL in U V-mediated transferable suppression. To discern whether FasL was also required in recipient mice, we evaluated U V-induced transferable suppression in FasL-deficient recipients. Such experiments were carried out by transferring spleen cells from wild-type mice that received U VR before immunization with C. albicans into either wild-type or FasL-deficient recipient mice (C3H or C3H/gld). Results from one such experiment are shown in Fig. 6. Consistent with our previous findings (Fig. 5), spleen cells from U VR-exposed, C. albicans-immunized C3H donor mice suppressed subsequent antigen responsiveness in naïve C3H recipients (58% suppression relative to positive control mice; P < 0.0001). Similarly, U VR-exposed, C. albicans-immunized C3H donor spleen cells markedly suppressed subsequent antigen responsiveness in C3H/gld recipients (53% suppression relative to positive control mice; P < 0.0001), ruling out a requirement for recipient-derived FasL in transferable suppression induced by U VR. No suppression was observed when NR, C. albicans-immunized C3H splenocytes were transferred to either C3H or C3H/gld recipients, confirming a requirement for both U VR and antigen exposure in the generation of suppressor cell activity (16, 17).

### Discussion

Fas and FasL are complementary receptor–ligand proteins that induce apoptosis in many cell types (30). Fas is

|        | FITC | Candida | Alloantigen |
|--------|------|---------|-------------|
| C3H    | ![C3H FITC](image1) | ![C3H Candida](image2) | ![C3H Alloantigen](image3) |
| gld    | ![gld FITC](image4) | ![gld Candida](image5) | ![gld Alloantigen](image6) |

**Figure 5.** Adoptive transfer of U V-induced suppression by spleen cells requires FasL. 10⁸ spleen cells from indicated donors were injected intravenously into naïve hosts, sensitized, and challenged with FITC, C. albicans, and B6 or C. albicans spleen cells as described in Materials and Methods. Values shown represent mean ± SE for five mice per group using measurements from two footpads per mouse.-, negative (challenge only); +, positive (sensitized and challenged); NR, recipient of spleen cells from NR sensitized donors, sensitized, and challenged; and U V, recipient of spleen cells from U V-irradiated sensitized donors, sensitized, and challenged. **P < 0.0001 vs. positive control.**
constitutively expressed in numerous tissues (56) and is rapidly upregulated in activated lymphocytes (31, 32). Although constitutive FasL expression is restricted to only a few tissues (57), transient FasL upregulation has been observed in a variety of cell types after genotoxic damage or cellular injury (3, 36, 58). Activated Fas+ lymphocytes can upregulate FasL upon T cell receptor engagement (39–41). Fas/FasL-induced apoptosis has been shown to play a critical role in the control of lymphocyte proliferation, peripheral tolerance, and specific immune responses occurring in discrete organ environments (35, 59). The effect of UVR on immune function shares several commonalities with Fas/ FasL-driven immunoregulation. UVR (6, 10), like FasL (35, 37), can mediate antigen-specific immune suppression. Moreover, UVR (19–23), like FasL (42), has been shown to shift the activation of T cells from a Th1- to a Th2-type immune response. Therefore, we were prompted to examine the role of Fas/FasL interactions in UVR-induced immune suppression.

Our observations are important in that they identify FasL as a fundamental constituent of both primary and transferable antigen-specific suppression induced by UVR (Table I). The central role of FasL in UVR-induced systemic suppression of CHS and DTH responses varies from that of Th2-like immunomodulatory cytokines and cis-urocanic acid, which are not shared conjointly in the suppression of CHS and DTH responses (19–21; Kripke, M.L., unpublished observations). For example, IL-10 appears to be essential for systemic UVR-induced suppression of DTH responses (20), whereas TNF-α is essential for CHS suppression (19, 60). The selective requirement of FasL for UVR-mediated suppression of CHS responses to FITC and DTH responses to Candida, but not alloantigen, is reminiscent of our previous finding that repair of UV-induced DNA damage could restore immune responses to FITC and Candida but not alloantigen (25–27; Kripke, M.L., unpublished observations). Taken with the recent report that DNA damage can activate the FasL promoter and upregulate FasL expression (61), our findings raise the interesting possibility that UVR-induced DNA damage and FasL are interrelated in the induction of UVR-induced immune suppression. The potent induction of FasL mRNA in skin-draining lymph nodes after UVR lends additional credence to this premise (Fig. 2).

How might DNA damage, FasL, and immunomodulatory cytokines interrelate in the induction of UVR-induced immune suppression? First, DNA damage by a variety of agents, including UVR light, has been shown to induce the expression of both Fas and FasL (3, 36, 61). Our laboratory has previously demonstrated that cyclobutane pyrimidine dimer–containing APC are present in the lymph nodes of UVR-exposed mice (27). Such APC may upregulate FasL and eliminate responding Fas+ T cells as has been recently reported for dendritic cells in vitro (42, 54). Second, aberrant FasL upregulation coupled with alterations in Fas sensitivity as a result of UVR-induced immunomodulatory cytokine production may contribute to the termination of immune responses by inappropriate apoptosis of T cells and/or APC (38, 40, 41). Third, suppressor T cells responding to antigen in the context of the UVR cytokine milieu may differentiate along a novel pathway requiring FasL as a growth factor (55, 62). Published observations, along with this report, collectively favor a model in which UVR-induced changes in APC phenotype/function are pivotal in the induction of antigen-specific immune suppression. Our data involving intact and disrupted alloantigen point toward a requirement for FasL on UVR-exposed host APC. While such APC are required for the antigen presentation of FITC, C. albicans, and disrupted alloantigen, intact allogeneic spleen cells may circumvent this requirement by providing a source of FasL while acting as their own APC. These results suggest that the nature of the antigen (and thus the APC involved) critically determines the requirement for host-derived FasL in primary UVR-induced immune suppression.

What then might be the requirement for FasL in the generation and activity of transferable suppressor cells? Considering the low penetrance of UVR in the skin, it ap-
appears unlikely that direct UVR exposure and UV-induced DNA damage occurs on T cells. It is conceivable, however, that DNA-damaged APC may influence the development of such T cells. In this regard, we have previously shown that DNA-damaged APC cluster with suppressor T cells in the draining lymph nodes after UVR and antigen exposure (27, 43, 63). Taken with the observation that Fas ligation can induce proliferation in some T cells (62), it is possible that FasL on DNA-damaged APC may act as a growth factor for UV-induced T suppressor cells in the context of the UVR-treated animal. Interestingly, Groux et al. (64) have recently described an IL-10 driven, antigen-specific CD4+ T cell that can potently suppress antigen-specific immune responses in vivo. Such findings suggest that UV-induced immunoregulatory Th2 cytokines such as IL-10 (18–20) may also participate in the differentiation and maintenance of the suppressor cell population. On the other hand, FasL may be required for effector activity of the UV-induced suppressor cells, perhaps by inducing apoptosis in the responding recipient T cell population. Experiments are currently in progress to test these possibilities.

Recent studies highlight the complexity of the immunomodulatory effects of UV in vivo. Hart et al. have documented mast cell–derived histamine as a component of the UV-induced systemic immune suppression of DTH responses to alloantigen (65). In contrast to our studies on UV-induced systemic immune suppression, Schwartz et al. have shown a nonessential role for FasL in UV-induced local immune suppression (66). The local model of UV-induced immune suppression differs markedly from the systemic model in both the route of administration (antigen is administered through the UV-irradiated site), specific cytokine involvement, and the requirement for FasL. For example, UV-induced suppression of local responses is TNF-α dependent and IL-10 independent and involves the production of cis-urocacidic acid (67). In contrast, UV-induced systemic immune suppression is independent of both TNF-α and cis-urocacidic acid but dependent upon IL-10 production (19, 20; Kripke, M.L., unpublished observations). Collectively, these findings emphasize mechanistic differences between UV-mediated local and systemic suppression and suggest the existence of at least two nonoverlapping pathways in the generation of systemic UV-induced immune suppression. One pathway requires FasL on host-derived APC and is sensitive to reversal by the repair of UV-induced DNA damage (25–27; Kripke, M.L., unpublished observations); the other requires histamine (65) and is independent of host-derived FasL. Interestingly, both pathways require FasL for the generation of transferable suppression but may differ in their requirement for FasL in the host (66).

In summary, our experiments document that Fas/FasL interactions are essential for UV-induced systemic suppression of CHS and DTH responses to antigens presented by host-derived APC (Table I). The requirement for Fas/FasL in UV-induced immune suppression can be eliminated if antigen presentation bypasses the requirement for host-derived APC (intact alloantigen). Moreover, host-derived, but not recipient-derived, FasL expression is critically required for the generation and/or function of UV-induced suppressor cells. The crucial role of FasL in both systemic primary and transferable UV-induced immune suppression suggests that the dysregulation of Fas-mediated apoptosis may ultimately underlie both processes.

We would like to thank Lauri Eichler and Katherine Rath for expert technical assistance and Dr. Kathleen M. McAveney for critical scientific discussions.

This work was supported by a grant from the Skin Cancer Foundation Research Program, University of Texas M.D. Anderson Cancer Center (to L. Owen-Schaub), American Cancer Society grant C1M-88929 (to L. Owen-Schaub), National Institutes of Health (N.I.H.) grant CA52457 (to M.L. Kripke), and institutional core grant CA16672 from the National Cancer Institute. L.L. Hill is the recipient of an N.I.H. postdoctoral fellowship and a Cockrell Foundation University Cancer Fighters Scientific Achievement Fellowship.

Address correspondence to Laurie B. Owen-Schaub, Department of Immunology, Box 178, University of Texas M.D. Anderson Cancer Center, 1515 Holcombe Blvd., Houston, TX 77030. Phone: 713-792-8735; Fax: 713-745-1633; E-mail: lowensch@mdanderson.org

V.K. Shreedhar’s current address is the Department of Medicine, Harvard Medical School, 300 Longwood Ave., Elders 1220, Boston, MA 02115.

Received for publication 2 December 1998 and in revised form 18 February 1999.

References

1. Whittaker, S. 1996. Sun and skin cancer. Br. J. Hosp. Med. 56:515–518.

2. Fisher, M.S., and M.L. Kripke. 1977. Systemic alteration induced in mice by ultraviolet light irradiation and its relationship to ultraviolet carcinogenesis. Proc. Natl. Acad. Sci. U.S.A. 74:1688–1692.

3. Gutierrez-Steil, C., T. Wrone-Smith, X. Sun, J.G. Krueger, T. Coven, and B. Nickoloff. 1998. Sunlight-induced basal cell carcinoma tumor cells and ultraviolet-B-irradiated psoriatic plaques express Fas ligand (CD95L). J. Clin. Invest. 101:33–39.

4. Orlin, J.B., and M.H. Ellis. 1997. Transfusion-associated
11. Elmets, C.A., P.R. Bergstresser, R.E. Tigelaar, P.J. Wood, and D.B. Yarosh. 1992. Pyrimidine dimers in DNA initiate systemic immunosuppression in UV-irradiated mice. Proc. Natl. Acad. Sci. USA. 89:7516–7520.

12. Noonan, F.P., E.C. DeFabo, and M.L. Kripke. 1981. Suppressor cells on T-cell activity. J. Immunol. 127:252–258.

13. Denkins, Y., I.J. Fidler, and M.L. Kripke. 1989. Exposure of mice to UV-B radiation suppresses delayed hypersensitivity to Candida albicans. Photochem. Photobiol. 54:6102–6105.

14. Ullrich, S.E. 1985. Suppression of lymphoproliferation by ultraviolet radiation. J. Immunol. 133:2786–2790.

15. Ullrich, S.E., and M.J. Magee. 1988. Specific suppression of allograft rejection after treatment of recipient mice with UV radiation and allogeneic spleen cells. Transplantation. 46:115–119.

16. Kripke, M.L., W.L. Morison, and J.A. Parrish. 1983. Systemic suppression of contact hypersensitivity in mice by psoralen plus UVA (PUVA). J. Invest. Dermatol. 80:87–102.

17. Yoshikawa, T., and J.W. Streilein. 1990. Genetic basis of the ultraviolet-B-induced suppression of tumor immunity. Photochem. Photobiol. 53:683–689.

18. Fisher, M.S., and M.L. Kripke. 1982. Suppressor T lymphocytes control the development of primary skin cancers in ultraviolet-irradiated mice. Science. 216:1133–1134.

19. Kripke, M.L. 1984. Immunological unresponsiveness induced by ultraviolet radiation. Immunol. Rev. 80:87–102.

20. Kripke, M.L., W.L. Morison, and J.A. Parrish. 1983. Analysis of the mechanism of unresponsiveness produced by haptens painted on the skin exposed to low dose ultraviolet radiation. J. Exp. Med. 158:781–794.

21. Noonan, F.P., E.C. DeFabo, and M.L. Kripke. 1981. Suppression of contact hypersensitivity by UV radiation and its relation to UV-induced suppression of tumor immunity. Photochem. Photobiol. 34:683–689.

22. Kripke, M.L. 1984. Ultraviolet radiation and immunology: something new under the sun—Presidential Address. Cancer Res. 44:1990.
B. Falk, K.A. Schooley, R.G. Goodwin, C.A. Smith, F. Ramsdell, and D.H. Lynch. 1995. Fas ligand mediates activation-induced cell death in human T lymphocytes. J. Exp. Med. 181:71–77.

40. Brunner, T., R.J. Mogil, D. LaFace, N.J. Yoo, A. Mahboubi, E. Echeverri, S.J. Martin, W.R. Force, D.H. Lynch, C.F. Ware, and D.R. Green. 1995. Cell-autonomous Fas (CD95)/Fas-ligand interaction mediates activation-induced apoptosis in T-cell hybridomas. Nature 373:441–444.

41. Dhein, J., H. Walczak, C. Bäumler, K.M. Debatin, and P.H. Krammer. 1995. Autocrine T-cell suicide mediated by APO-1/(Fas/CD95). Nature 373:438–441.

42. Zhang, X., T. Brunner, L. Carter, R.W. Dutton, P. Rogers, L. Bradley, T. Sato, J.C. Reed, D. Green, and S.L. Swain. 1997. Unequal death in T helper (Th)1 but not Th2 effectors undergo rapid Fas/Fas-ligand-mediated apoptosis. J. Exp. Med. 185:1837–1849.

43. Saio, S., C.D. Bucana, K.M. Rameriz, P.A. Cox, M.L. Kripke, and F.M. Strickland. 1995. Deficient antigen presentation and T induction are separate effects of ultraviolet irradiation. Cell. Immunol. 164:189–202.

44. Wu, J., T. Zhou, J. He, and J.D. Mountz. 1993. Autoimmune disease in mice due to integration of an endogenous retrovirus in an apoptosis gene. J. Exp. Med. 178:461–468.

45. Watanabe-Fukunaga, R., C.I. Brannan, N.G. Copeland, N.A. Jenkins, and S. Nagata. 1992. Lymphoproliferation disorder in mice explained by defects in Fas antigen that mediates apoptosis. Nature 356:314–317.

46. Chu, J.-L., J. Drappa, A. Parnassa, and K.B. Elkon. 1993. The defect in Fas mRNA expression in MRL/lpr mice is associated with insertion of the retrotransposon, 5′ETn. J. Exp. Med. 178:723–730.

47. Adachi, M., R. Watanabe-Fukunaga, and S. Nagata. 1993. A small transposon, insertion of the retrotransposon, 5′ETn element in an intron of the Fas antigen gene of lpr mice. Proc. Natl. Acad. Sci. USA. 90:1756–1760.

48. Mariani, S.M., B. Matiba, E.A. Armandola, and P.H. Cohen. 1994. Constitutive activation of the Fas ligand gene in mouse lymphoproliferative disorders. Immunity 1:543–551.

49. Tschopp, J., E.A. Reap, K. Roof, M. Maynor, M. Borrello, J. Booker, and P.L. Cohen. 1997. Radiation and stress-induced apoptosis a role for Fas/Fas ligand interactions. Proc. Natl. Acad. Sci. USA. 94:5750–5755.

50. Griffith, T.S., X. Yu, J.M. Herndon, D.R. Green, and T.A. Ferguson. 1996. CD95-induced apoptosis of lymphocytes in an immune privileged site induces immunological tolerance. Immunity. 5:7–16.

51. Yoshikawa, T., and J.W. Streilein. 1990. Tumor necrosis factor-alpha and ultraviolet light have similar effects on contact hypersensitivity in mice. Reg. Immunol. 3:139–144.

52. Kasibhatla, S., T. Brunner, L. Genestier, F. Echeverri, A. Mahboubi, and D.R. Green. 1998. DNA damaging agents induce expression of Fas ligand and subsequent apoptosis in T lymphocytes via the activation of NF-κB and AP-1. Mol. Cell. Biol. 1:543–551.

53. Alderson, M.R., R.J. Armitage, E. Marakovsky, T.W. Tough, E. Roux, K. Schooley, F. Ramsdell, and D.H. Lynch. 1993. Fas transduces activation signals in normal human T lymphocytes. J. Exp. Med. 178:2231–2235.

54. O’kamoto, H., and M.L. Kripke. 1987. Effector and suppressor circuits of the immune response are activated in vivo by different mechanisms. Proc. Natl. Acad. Sci. USA. 84:3841–3845.

55. Groux, H., A. O’Garra, M. Bigler, M. Rouleau, S. Antonenkon, J.E. de Vries, and M.G. R. Oncorato. 1997. A CD4+ T Cell subset inhibits antigen-specific T cell responses and prevents colitis. Nature 389:737–742.

56. Hart, P.H., M.A. Grimbaldeston, G.J. Swift, A. Jakse, F.P. Noonan, and J.J. Finlay-Jones. 1998. Dermal mast cells determine susceptibility to ultraviolet B-induced systemic suppression of contact hypersensitivity responses in mice. J. Exp. Med. 187:2045–2053.

57. Schwartz, A., S. Grabbe, K. Grosse-Heitmeyer, B. Roters, H. Römisch, T.A. Lugner, G. Trinchieri, and T. Schwartz. 1998. Ultraviolet light-induced immune tolerance is mediated via the Fas/Fas ligand system. J. Immunol. 160:4262–4270.

58. Niizuki, H., and J.W. Streilein. 1997. Hapten-specific tolerance induced by acute, low-dose ultraviolet B radiation of skin is mediated via interleukin-10. J. Invest. Dermatol. 109:25–30.