Reduced Ultraviolet-Induced Carcinogenesis in Mice with a Functional Disruption in B7-Mediated Costimulation

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Reduced Ultraviolet-Induced Carcinogenesis in Mice with a Functional Disruption in B7-Mediated Costimulation

Stefan Beissert,* Jeffrey A. Bluestone, ‡ Iris Mindt,* Maik Voskort,* Dieter Metze,* Annette Mehling,* Thomas A. Luger,* Thomas Schwarz,* and Stephan Grabbe*

Immunosuppression by UV light contributes significantly to the induction of skin cancer by suppressing the cell-mediated immune responses which control the development of carcinogenesis. The B7/CD28-CTLA-4 signaling pathway provides costimulatory signals essential for Ag-specific T cell activation. To investigate the role of this pathway in photocarcinogenesis, we utilized transgenic (Tg) mice which constitutively express CTLA-4Ig, a high-affinity CD28/CTLA-4 antagonist that binds to both B7-1 and B7-2. The transgene is driven by a skin-specific promoter yielding high levels of CTLA-4Ig in the skin and serum. Chronic UV exposure of CTLA-4Ig Tg mice resulted in significantly reduced numbers of skin tumors, when compared to control mice. In addition, Tg mice were resistant to UV-induced suppression of delayed-type hypersensitivity responses to alloantigens. Most importantly, upon stimulation with mitogens and alloantigens, T cells isolated from CTLA-4Ig Tg mice produced significantly less IL-4 but more IFN-γ compared to control T cells, suggesting an impaired Th2 response and a relative increase of Th1-type immunity. Together, these data show that overall B7 engagement directs immune responses toward the Th2 pathway. Moreover, they point out the crucial role of Th1 immune reactions in the protection against photocarcinogenesis. The Journal of Immunology, 1999, 163: 6725–6731.

Ultraviolet irradiation (UVR) is the most important risk factor for the induction of nonmelanoma skin cancer. Although generally nonfatal, UV-induced skin tumors such as squamous cell carcinomas and basal cell carcinomas currently exhibit the most rapidly rising incidence of all human tumors with an estimated rate of 600,000–800,000 new cases per year in the U.S. alone (1, 2). Despite the proven protective effects of topical sunscreens and extensive information campaigns, the incidence of sunlight-induced skin cancer is expected to increase further in the future due to the cumulative nature of factors inducing photocarcinogenesis in combination with increasing life expectancy and participation in recreational outdoor activities and to decreasing atmospheric ozone levels (3). Within the skin, the development of tumors appears to be controlled by the immune system. Data to substantiate this hypothesis derive from reports investigating the incidence of skin tumors in therapeutically immunosuppressed organ transplant patients, 14–17% of whom develop skin tumors (a 30- to 40-fold increase) (4, 5). Indeed, several lines of evidence exist for an involvement of the T cell immune system in the development of UV-induced tumors. First, UV-induced skin cancers are generally highly antigenic and are rejected in normal syngeneic hosts, whereas they grow progressively in immunosuppressed or UV-irradiated mice (6, 7). Second, transferable regulatory T cells promote tumor growth in UV-irradiated animals, whereas effector T cells mediate tumor rejection in unirradiated mice (8). Third, UVR inhibits the ability of APC to induce protective antitumor immunity (9–11).

Langerhans cells (LC), potent APC within the epidermis, are able to present tumor Ag of UV-induced tumors necessary for the induction as well as for the elicitation of protective antitumor immune responses in murine systems (9). Several reports have demonstrated that UVR inhibits the capacity of LC to present tumor Ag both directly via UV-induced LC cytotoxicity and indirectly via the release of inhibitory cytokines by keratinocytes (10-12). B7-1 (CD80) and B7-2 (CD86) expressed on APC bind to their counterreceptors CD28 and CTLA-4, respectively, on T cells. Previous data suggested that an overall blockade of all B7-mediated signals appears to inhibit T cell-mediated immune responses. In this respect, it has been shown that a soluble form of CTLA-4, CTLA-4Ig, which binds to B7 (CD80/86) with 20-fold higher avidity than CD28, effectively blocks all B7-mediated signaling to T cells and functions to inhibit the induction of antitumoral immune responses, the rejection of transplanted organs, autoimmune responses, and cutaneous immune responses in rodents (13–16). Moreover, several groups have demonstrated that transfection of tumor cell lines with CD80 or CD86 enhances host cellular antitumoral immunity, leading to the destruction of the tumor itself and generating protection against subsequent challenge with wild-type tumor cells (17–19). Thus, at face value, it would appear that B7-mediated costimulation supports the generation of effective cellular immunity. However, recent studies suggest a more complicated role for CD80 and CD86 in the immune response, most likely due to differential effects of CD28 and CTLA-4 ligation. Whereas CD28 ligation clearly provides a costimulatory signal for T cell activation (15, 20), whether a possible preference of CD28...
to stimulate Th2 vs Th1 responses exists and the functional relevance of CTLA-4 engagement remain controversial. In this respect, CD28/B7 engagement has been shown to increase IL-4 production leading to an enhancement of Th2 T cell development and a suppression of pro-inflammatory Th1 T cell responses (21). Moreover, engagement of B7 with activation-induced CTLA-4 down-regulates T cell function (22). In fact, a selective blockade of the B7-mediated signaling by treatment with anti-CTLA-4 Ab enhances the generation of antitumor immunity and the rejection of preexisting tumors (23). Thus, the inhibition of CD80/86 interactions may have unanticipated effects on the immune surveillance of tumorigenic stimuli.

For this reason, the role of the B7/CD28-CTLA-4 pathway was examined in a model of UV-induced carcinogenesis, because photocarcinogenesis is highly influenced by the immune system. Using a CTLA-4Ig transgenic (Tg) mouse model, we demonstrate that expression of high amounts of CTLA-4Ig in the skin and in the systemic circulation can significantly suppress skin tumor formation in Tg mice chronically irradiated with UV. Moreover, blockade of B7/CD28-CTLA-4 interactions in the Tg mice also mitigates UV-induced immunosuppression and significantly alters the Th1/Th2 balance in favor of Th1 responses. Therefore, interference with the B7/CD28-CTLA-4 pathway appears to counteract UV-induced immunosuppression by counteracting UV-induced suppression of Th1 responses, resulting in reduction of photocarcinogenesis.

### Materials and Methods

#### Mice

CTLA-4 Tg mice were generated as described (24). The murine CTLA-4/ IgG2aFc hybrid gene was cloned into a human keratin 14 (K14) promoter cassette to generate the Tg construct that was microinjected into C57/HeNe background oocytes. Founder mice expressed the soluble murine CTLA-4Ig predominantly in the skin and also had significant CTLA-4Ig serum concentrations (10 –30 μg/ml). CTLA-4Ig Tg and C3H/HeN mice were shaved on the back and immunized before challenge, or only challenged without prior immunization. (Mitutoyo, Tokyo, Japan) as a measure of delayed-type hypersensitivity (DTH) responses. Groups of control mice were either irradiated but not immunized before challenge, or only challenged without prior immunization.

#### Generation and culture of bone-marrow derived dendritic cells

Bone marrow-derived dendritic cells (BmDC) were generated by culture of bone marrow (BM) cells in the presence of GM-CSF and IL-4, as described by Inaba et al. (28) with modifications. Briefly, BM was collected from tibias and femurs of BALB/c mice using PBS and a syringe with a 25-gauge needle, and suspended by vigorous pipetting. Erythrocytes were lysed by incubating cells in lysing buffer (Ortho, Neckargmünd, Germany) for 2 min. Remaining cells were passed through nylon mesh to remove small pieces of bone and debris. The cells were washed twice with cold PBS, resuspended in RPMI 1640 medium (5% FCS, 2 mM l-glutamine, 0.1 mM essential and nonessential amino acids, 20 μM 2-mercaptoethanol, 20 μg/ml gentamicin), and cultured in petri dishes (Becton Dickinson, Heidelberg, Germany) at a density of 0.5 × 10^6 cells/ml for 4 h. Nonadherent cells were collected, then 1 × 10^6 cells were placed in 24-well plates (Becton Dickinson) and adjusted to 1 ml with BM medium supplemented with 150 U/ml GM-CSF (R&D Systems, Wiesbaden, Germany) and 75 U/ml IL-4 (PharMingen, Hamburg, Germany). After 2 days of incubation (37°C, 5% CO2), 600 μl of medium containing the same volume of fresh BM medium containing 150 U/ml GM-CSF and 75 U/ml IL-4 was added. Cells were incubated for an additional 3 days, and nonadherent cells were harvested by pipetting and subsequently subcultured in 6-well plates in medium containing 150 U/ml GM-CSF and 75 U/ml IL-4. After 2 days of incubation, more than 70% of the nonadherent cells in culture had acquired typical dendritic morphology. These cells were harvested and used as a source of BmDC in subsequent experiments.

#### BmDC-lymphocyte alloreaction

BmDC were incubated in RPMI 1640 supplemented with 10% heat-inactivated FCS (PAA, Linz, Austria), 100 U/ml penicillin, 100 μg/ml streptomycin, 0.1 mM essential and nonessential amino acids, 2 mM l-glutamine, 1 mM sodium pyruvate, and 0.01 M HEPES buffer (complete medium); and applied in serial dilutions to 2 × 10^6 allogeneic T cells from CTLA-4Ig Tg or wild-type mice in 96-well round bottom plates. T cells were obtained from spleen cells of mice by nylon-wool purification. After 4 days, T cell proliferation was measured by adding 1 μCi [¹⁵]H-thymidine; incorporated [¹⁵]H-thymidine was quantified, and supernatants were taken for cytokine quantification.

#### T cell stimulation and cytokine quantification

Nylon-wool-enriched splenic T cells were used for proliferation experiments. Stimulation of T cells was induced with PMA (3 ng/ml) and ionomycin (300 ng/ml) (both from Sigma, Deisenhofen, Germany). Supernatants were harvested at 48 h for cytokine quantification. IL-4 concentrations were measured using the CT.4S cell line as described (29). IFN-γ and IL-10 were quantitated using a commercially available ELISA (R&D Systems). The limits of detection were 10 pg/ml IFN-γ and 10 pg/ml IL-10.

For quantification of serum IL-10 concentrations induced by UV exposure, CTLA-4Ig Tg and C3/HeN mice were shaved on the back and exposed to 30 kJ/m² UV. Serum was harvested at 0, 1, 2, 3, and 4 days and IL-10 content was determined using an IL-10 ELISA (R&D Systems). The limits of detection were 10 pg/ml IFN-γ and 10 pg/ml IL-10.

#### Immunohistochemistry

Dorsal skin of CTLA-4Ig Tg and C3/HeN mice was shaved and exposed to 30 kJ/m² UV. The skin was biopsied at 0, 1, 2, 3, and 4 days after UV treatment and snap frozen in liquid nitrogen. Cryostat sections were fixed on aminoalkysilane-treated slides (Sigma-Aldrich, Steinheim, Germany). The sections of interest were stained with hematoxylin and eosin, and documented by a video-computer-assisted digital image-processing technique (DISKUS version 3.99 for Windows 95, Hitlers, Königswinter, Germany).

#### Modulation of the induction of delayed-type hypersensitivity responses by UVR

A commonly used high-dose UVR protocol was employed, as described elsewhere (12, 27), in which mice were exposed to 30 kJ/m² UVR on the shaved back and immunized to alloantigens 5 days later by s.c. injection of 1 × 10^6 allogeneic (BALB/c, C57BL/6) nucleated spleen cells at the non-irradiated abdominal site. These mice were challenged 5 days later by injection of 1 × 10^6 allogeneic (BALB/c, C57BL/6) spleen cells into one hind footpad. Footpad swelling was assessed at 24 h with a micrometer (Mitutoyo, Tokyo, Japan) as a measure of delayed-type hypersensitivity (DTH) responses. Groups of control mice were either irradiated but not immunized before challenge, or only challenged without prior immunization.

#### Evaluation of LC, dendritic epidermal T cells, and sunburn cells

Epidermal sheets were prepared from the ears of CTLA-4Ig Tg and wild-type control mice by separation with EDTA 20 mM (pH 7.3). LC in these
epidermal sheets were stained indirectly with a monoclonal anti-I-A Ab (from clone M5/114; American Type Culture Collection, Manassas, VA) and fluorescein-conjugated goat anti-rat IgG (PharMingen). Dendritic epidermal T cells were visualized indirectly by staining with anti-CD3 Ab (from clone 145-2C11; American Type Culture Collection) and fluorescein-conjugated goat anti-rat IgG. Positively stained cells were counted using a net micrometer grid. Five 0.04 mm² sections were counted and averaged on each sample. Samples from three mice of each strain were counted.

For the generation of sunburn cells, CTLA-4Ig Tg and control mice (n = 3) were UVB-irradiated on their shaved backs with 1 kJ/m². Twenty-four hours later skin biopsies were obtained, fixed in buffered formaldehyde, embedded in paraffin, sectioned (7 μm), and stained with hematoxylin and eosin. Sunburn cells were defined as apoptotic cells within the epidermis exhibiting an eosinophilic cytoplasm and a dense nucleus. Sunburn cells were counted per mm length of the epithelium.

Statistical analysis

The method of Kaplan and Meier was used to describe the probability of tumor development in the carcinogenesis study. This is a life table analysis and also takes into account animals that die before developing a tumor. Statistical differences for the development of tumors between the two strains of mice were determined using a logrank test by Peto et al. (30). The differences in tumor latent periods were analyzed by a Mann-Whitney U test for independent samples. The data from the DTH experiments were analyzed using the Student t test for independent samples.

Results

Reduced incidence of UV-induced skin tumors in CTLA-4Ig Tg mice

Although the importance of the B7/CD28-CTLA-4 signaling pathway in the generation of Ag-specific immune responses is well established, the relevance of this mechanism for tumor-specific immune responses is still controversial. To investigate the role of the B7/CD28-CTLA-4 pathway in the development of UV-induced skin cancer in vivo, CTLA-4Ig Tg and littermate control mice were chronically irradiated with UV and skin tumors were documented over time. CTLA-4Ig Tg mice are healthy, breed well, and developed tumors and the overall number of tumors observed was lower compared to wild-type control mice (p < 0.04). Groups of wild-type (n = 15) and CTLA-4Ig Tg mice (n = 15) that were not irradiated but were otherwise treated identically did not develop any skin tumors. These results suggest that functional disruption of the B7/CD28-CTLA-4 pathway leads to reduced photocarcinogenesis.

The data depicted in Table II demonstrate that of 20 UV-irradiated wild-type mice, 16 developed a total of 21 skin tumors (4 died without a tumor). In the CTLA-4Ig Tg group, 7 mice of 20 developed a total of 8 skin tumors (3 mice died without a tumor). Remarkably, none of the wild-type mice but 50% of the CTLA-4Ig Tg mice remained free of tumors 1 year after the onset of the experiment. A total of 76% of the tumors occurring in wild-type mice and 88% of the tumors in the CTLA-4Ig Tg group were located on the ears and the back which, in accordance with other reports, are the primary sites of UV-induced tumor development in mice (7).

CTLA-4Ig Tg mice develop fewer poorly differentiated UV-induced skin tumors

Histopathological examination revealed that the tumors arose uniformly from the epidermis and showed typical features of squamous cell carcinoma in both the CTLA-4Ig Tg mice and the wild-type controls. The differentiation state of UV-induced skin tumors was assessed using three categories: 1) a carcinoma was interpreted as well-differentiated if regular endo-exophytic proliferation of squamous cells with mild cytoplasmic atypia and central orthokeratotic keratinization was noted; 2) a moderately differentiated carcinoma was defined by irregular dermal nests of pleomorphic keratinocytes within the epidermis that become apoptotic after UVR. They can be used as an indicator for UV damage of the epidermis after light exposure. However, as seen in Fig. 1, the period of time between the first UVR and the appearance of the first visible skin tumor was significantly different in the two groups. Although both Tg and wild-type mice were susceptible to photocarcinogenesis, UV-induced tumor development was delayed in CTLA-4Ig Tg mice. Furthermore, the incidence of UV-induced skin tumors was significantly reduced in the CTLA-4Ig Tg group; fewer mice developed tumors and the overall number of tumors observed was lower compared to wild-type control mice (p < 0.04). Groups of wild-type (n = 15) and CTLA-4Ig Tg mice (n = 15) that were not irradiated but

### Table I. Numbers of LC, dendritic epidermal T cells, and sunburn cells in CTLA-4Ig Tg and wild-type mice

<table>
<thead>
<tr>
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<th>Wild-Type Mice</th>
<th>CTLA-4Ig Tg Mice</th>
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<tbody>
<tr>
<td>Langerhans cells (LC)</td>
<td>1040 ± 84*</td>
<td>980 ± 93</td>
</tr>
<tr>
<td>Dendritic epidermal T cells (DETC)</td>
<td>603 ± 30</td>
<td>640 ± 22</td>
</tr>
<tr>
<td>Sunburn cells (SC)²</td>
<td>8 ± 2</td>
<td>9 ± 3</td>
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</tbody>
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* Numbers represent cells per mm². 
² Sunburn cells per mm length of epidermis. The p value is NS for LC, DETC, and SC wild-type vs CTLA-4Ig Tg mice.

### Table II. Site and histological type of skin tumor produced by chronic UVR

<table>
<thead>
<tr>
<th>Tumor Bearsers</th>
<th>Site</th>
<th>No. of Tumors</th>
<th>Histological Differentiation</th>
</tr>
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<tbody>
<tr>
<td></td>
<td></td>
<td>Poorly</td>
<td>Moderately</td>
</tr>
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</table>

| Wild-type mice |        |        |        |       |       |       |
| n = 20         |        |        |        |       |       |       |
| Ear            | 16/20  | 7      | 4      | 2      | 1      |
| Back           |        |        |        |       |       |       |
| Foot           |        |        |        |       |       |       |
| Eye            |        |        |        |       |       |       |

| CTLA-4Ig Tg mice |        |        |        |       |       |       |
| n = 20          |        |        |        |       |       |       |
| Ear             | 7/20   | 5      | 2      | 1      | 2      |
| Back            |        |        |        |       |       |       |
| Foot            |        |        |        |       |       |       |
| Eye             |        |        |        |       |       |       |

FIGURE 1. Reduced photocarcinogenesis in CTLA-4Ig Tg mice compared with wild-type controls. Rate of UV-induced skin tumor development in CTLA-4Ig Tg and wild-type control mice. Mice were initially treated with 2.5 kJ/m² UVR for 4 wk, then with 5 kJ/m² UVB for 4 wk, and then with 10 kJ/m² UVR for 6 mo. Their dorsal hair was shaved weekly. This experiment was performed once. For both groups n = 20, * p < 0.002 for wild-type vs CTLA-4Ig Tg mice.
epithelia with individual cell keratinization and horn pearl formation; and 3) a poorly differentiated carcinoma was composed of highly mitotic spindle cells with large vesicular, pleomorphic nuclei and scanty eosinophilic cytoplasmas. Based on this classification, 37.5% of the tumors seen in the Tg mice were determined to be well differentiated, 12.5% moderately differentiated, and 50% poorly differentiated squamous cell carcinomas. The wild-type controls developed 19% well differentiated, 14.3% moderately differentiated, and 66.7% poorly differentiated squamous cell carcinomas. Normalized to tumor numbers, this result translated into a 3.5-fold decrease in poorly differentiated carcinomas (n = 4) in Tg mice compared with wild-type controls (n = 14). The proportion of well differentiated, usually less aggressive tumors vs poorly differentiated, more malignant carcinomas was increased in the CTLA-4Ig Tg group, though this difference was less striking. Although there was a trend toward later tumor onset in the CTLA-4Ig Tg group, differences in the latencies for the development of poorly, moderately, or well differentiated skin tumors between wild-type and Tg mice were not statistically significant.

**UV-induced immunosuppression is reduced in CTLA-4Ig Tg mice**

Besides TCR-MHC I and/or II interactions, costimulation via the B7/CD28-CTLA-4 pathway seems to be important for the induction of optimal immune responses, including antitumor immunity. Thus, it appeared surprising that functional blocking of this pathway by soluble CTLA-4Ig resulted in reduced rather than enhanced UV-induced skin tumor development. Because UVR has been shown to induce systemic immunosuppression, we were interested in investigating the effects of CD28/CTLA-4 blockade on UV-induced suppression of DTH responses to alloantigens. Wild-type and CTLA-4Ig Tg mice were exposed to a single dose of 30 kJ/m² UVR on the shaved back before sensitization by s.c. injection of allogeneic spleen cells. Control groups of mice were sensitized but not UV-exposed. Five days after sensitization, all groups of mice were challenged at one hind footpad by injection of allogeneic spleen cells (H-2ª). Footpad swelling was assessed 24 h later as a measure of DTH response to alloantigens. Wild-type mice that were immunized and challenged developed a significant footpad swelling response (Fig. 2A). Exposure of wild-type mice to UVR before sensitization suppressed the DTH response to alloantigens by 50%, indicating that UVR suppressed the induction of DTH. In contrast, UV-treated CTLA-4Ig Tg mice were still able to mount a significant DTH response to alloantigens, suggesting that these mice were resistant to UV-induced suppression of DTH responses (Fig. 2A). In another experiment CTLA-4Ig Tg mice as well as littermates were immunized to alloantigen (H-2ª) and challenged 5 days later at one hind footpad. The data in Fig. 2A show an enhanced footpad swelling response in the CTLA-4Ig Tg mice compared to controls, suggesting a hyperresponsiveness of the CTLA-4 Tg mice to alloantigens.

**Impaired Th2-type immune responses in CTLA-4Ig Tg mice**

The finding that CTLA-4Ig Tg mice have a predisposition toward Th1 responses might explain impaired photocarcinogenesis for several reasons. First, a competent Th1 response seems to be crucial for the induction of tumor immunity (31–33). Second, UV light impairs cellular immune reactions by inducing a shift toward Th2-type responses (12). This might also explain why CTLA-4Ig Tg mice are resistant, or at least less susceptible, to UV-induced suppression of DTH, a classical Th1 immune reaction. It has been suggested that UV light skews immunity toward Th2 by inducing the release of IL-4 and/or IL-10, because increased levels of IL-4 and IL-10 were detected in the sera of UV-exposed mice (12, 27, 34). In addition, treatment of animals with anti-IL-4 or anti-IL-10 mAb abrogates UV-induced immunosuppression. To address the issue that the differences in the tumor incidence and in the UV susceptibility of DTH in these Tg animals might be consequences of altered cytokine production, cytokine analysis was performed. Mitogen-stimulated splenic T cells from CTLA-4Ig Tg mice produced significantly less IL-4 compared to their wild-type counterparts (Fig. 3A). In contrast, IFN-γ secretion by mitogen-stimulated T cells from CTLA-4Ig Tg mice was enhanced compared to wild-type T cells (Fig. 3B). In addition, CTLA-4Ig Tg T cells that were stimulated by allogeneic BmDC produced significantly more IFN-γ and less IL-4 compared to wild-type T cells (Fig. 3C and
Additionally, CTLA-4Ig Tg T cells demonstrated an enhanced proliferative response to alloantigens compared to controls (Fig. 4). There was no difference in the mitogen- or alloantigen-induced production of IL-10 by splenic T cells from Tg and wild-type mice (data not shown). Because UVR itself is able to induce elevated serum levels of IL-10, we analyzed the serum of irradiated wild-type and Tg mice. No differences in the serum concentrations of IL-10 between the two groups of mice was observed (data not shown). Furthermore, immunohistochemical studies revealed equal staining of IL-10 in the skin of UV-treated Tg and wild-type mice (data not shown).

Discussion

Photocarcinogenesis is a classical experimental model for studying the development of tumors that are controlled by the immune system. Years ago, several studies had already demonstrated that UV-induced tumors are usually highly immunogenic and regress upon transfer into immunocompetent mice, whereas they grow in therapeutically immunosuppressed or UV-irradiated mice (6, 7, 25). These tumors have been shown to express tumor-specific Ag, which can lead to the generation of CTL (35). The progressive growth of these antigenic tumors in UV-irradiated hosts has been attributed to the generation of tumor-specific immunoregulatory T cells (suppressor cells) and/or to a shift from a Th1 to a Th2 immune response toward UV-induced tumors. This may be due to enhanced production of IL-10 and/or IL-4 after UVR (11, 12, 34). In addition, chronically UV-irradiated mice were found to have a tendency to generate Th2 rather than Th1 immune responses.

The present study indicates that CTLA-4Ig Tg mice are less susceptible to photocarcinogenesis. Because CTLA-4Ig specifically blocks B7-mediated costimulation, and because other effects of UVR on skin (e.g., epidermal hyperproliferation, erythema, changes in LC and dendritic epidermal T cell numbers, and sunburn cell formation) were unaffected by CTLA-4Ig overexpression, we conclude that the observed effects on photocarcinogenesis are due to the immunomodulatory effects of CTLA-4Ig. Two possibilities can be envisioned to explain these findings. First, a blockade of B7 interactions with CTLA-4 might prevent the generation of UV-induced immunosuppression. This may be due to CTLA-4 signaling, which has previously been shown to suppress T cell responses at least in certain situations. For instance, anti-CTLA-4 mAbs enhance antitumor immunity in a number of tumor systems (23). On the other hand, the disruption of CD28/B7-mediated signaling impairs costimulation (36), which would lead to the prediction that the systemic expression of CTLA-4Ig would result in a decreased capacity to generate tumor immunity. Second, a
blockade of costimulation via B7/CD28 might influence Th1/Th2 differentiation, resulting in an altered capacity to generate protective tumor immunity. Indeed, this view is supported by our data.

In this report we have demonstrated that, besides its well-documented overall immunosuppressive effect, a blockade of B7-mediated costimulation also leads to a shift of the remaining immune response toward Th1 or at least prevents UVR from inducing Th2-type immune responses. Surprisingly, it appears that this Th1 shift is more relevant than the overall impaired B7-mediated costimulation for in vivo tumor immune surveillance, resulting in reduced rather than enhanced generation of UV-induced skin tumors. In further support of this concept, UVR was also unable to inhibit a Th1-type DTH response to alloantigens in CTLA-4Ig Tg mice (Fig. 2A), suggesting that the capacity to mount Th1 immune responses is retained in these mice. Moreover, poorly differentiated skin tumors grow faster in vivo and in vitro and show signs of genetic instability that can lead to altered phenotypes. Due to this genetic instability, variants of the parental tumor gradually develop changes in their susceptibility to immune surveillance, which allows them to escape tumor immunity (26). Thus, enhanced immune surveillance (as evident in CTLA-4Ig Tg mice) would be expected to more effectively eliminate the less immunogenic poorly differentiated tumors. Consistent with this hypothesis, in our study we found that CTLA-4Ig Tg mice develop a greatly reduced number of poorly differentiated cutaneous squamous cell tumors compared with wild-type controls.

In addition, it has been reported before that B7-mediated costimulation affects Th1/Th2 differentiation (14, 24, 37). For example, CD28-deficient mice have an impaired Th2 immune response to Schistosoma mansoni infection, with less IL-4 and IL-5 being produced in CD28-deficient mice following infection (38). Similar results have been obtained in CD28-deficient nonobese diabetic mice in which less IL-4 and more IFN-γ was produced by the autoreactive T cells compared to wild-type nonobese diabetic controls (24). Moreover, in a murine experimental leishmaniasis model, treatment of susceptible mice with CTLA-4Ig converted them to a Leishmania-resistant phenotype with enhanced IFN-γ and reduced IL-4 transcripts in lymph node cells, suggesting a skewing toward Th1 responses by CTLA-4Ig treatment (39).

It has also been shown that CTLA-4Ig administration was able to block IL-4 but not IL-10 production during systemic in vivo immune responses (40). These findings are in agreement with our data demonstrating a relatively reduced IL-4 and an enhanced IFN-γ production by T cells from CTLA-4Ig Tg mice. Taken together, the results further support previous observations that functional disruption of the B7/CD28-CTLA-4 signaling pathway leads to impaired Th2 differentiation and a relative enhancement of Th1-mediated immune responses (24, 37, 39).

As a whole, our data indicate that a blockade of B7-mediated costimulation protects mice from developing UV-induced skin tumors. This decreased tumor incidence due to the disruption of the B7/CD28-CTLA-4 pathway correlates with the fact that these animals are prone to enhanced Th1 and decreased Th2 immune responses. Thus, these findings strongly support the concept that one of the major ways by which UV light compromises the immune system is by affecting the balance of Th1/Th2 reactions. This allows the speculation that UV causes immunomodulation by interfering with CD28 and/or CTLA-4 expression. This hypothesis is currently under investigation in our laboratory. Because CTLA-4Ig is available for human use and is currently being used in experimental clinical trials (e.g., for the treatment of psoriasis), it is interesting to speculate on potential pharmacologic intervention by manipulation of B7/CD28-CTLA-4 signaling as a means to modulate UV-induced skin cancer.

References
randomized clinical trials requiring prolonged observation of each patient.

II. Analysis and examples.


