Impaired Immunosuppressive Response to Ultraviolet Radiation in Interleukin-10–Deficient Mice

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Exposure to mid-range ultraviolet radiation (UVR) [280–320 nm, ultraviolet B (UVB) radiation] inhibits the acquisition of delayed-type hypersensitivity in mice and contact hypersensitivity in rodents and humans. Intraperitoneal administration of interleukin 10 (IL-10) inhibits the sensitization of mice to alloantigens for a delayed-type hypersensitivity reaction and administration of neutralizing antibodies to IL-10 largely, but not totally, blocks the UVR-mediated suppression of the ability to sensitize mice. This suggests that these inhibitory effects of UVB radiation may be mediated by release of IL-10. To test this hypothesis directly, IL-10 gene-targeted (IL-10T) mice lacking expression of IL-10 were examined for the ability of UVB radiation to suppress induction of delayed-type hypersensitivity to alloantigens. IL-10T mice were completely resistant to UVB-induced immunosuppression in this system. Interestingly, UVB radiation could suppress in IL-10T mice the induction of contact hypersensitivity to a hapten applied to the skin at a site distant of irradiation, supporting the concept that regulation pathways of delayed-type hypersensitivity and contact hypersensitivity responses by UVR differ. These data provide additional understanding of the mechanisms of immunosuppression induced by UVR and suggest that IL-10 release subsequent to UVB radiation may play a role in the growth of immunogenic UVB-induced cutaneous malignancies in the primary host. ***Key words: immunology/delayed-type hypersensitivity/contact hypersensitivity. J Invest Dermatol 107:553–557, 1996***

Exposure of mice to relatively large doses of mid-range ultraviolet radiation (UVR) [ultraviolet B (UVB), 280–320 nm] inhibits the acquisition of contact hypersensitivity (CHS) by application of haptens to nonirradiated sites as well as the induction of delayed-type hypersensitivity (DTH) by injection of allogeneic cells subcutaneously (Ullrich, 1986; Moledijk *et al*, 1987). The magnitude of suppression of DTH induction was found to reach its maximum 4 d after UVR and lasted 3 wk (Moledijk *et al*, 1987). These observations have been interpreted as suggesting that UVB-mediated immunosuppression plays a role in the development of cutaneous malignancies by chronic UVB exposure. It has also been demonstrated that humans can be suppressed for induction of CHS responses, at least locally, by UVB radiation (Yoshikawa *et al*, 1990). Interestingly, there is some evidence that the ability of humans to be suppressed in this manner is heterogeneous and that individuals more susceptible to such immunosuppression are at greater risk for development of skin cancer (Yoshikawa *et al*, 1990).

The mechanisms by which UVB irradiation causes immunosuppression are under intense investigation. Evidence exists both for DNA and urocanic acid as photoreceptors (Tan and Stoughton, 1969; De Fabo and Noonan, 1983; Wolf *et al*, 1993) initiating a cascade of events resulting in the functional effects described above. Recently, it was shown that intraperitoneal administration of interleukin 10 (IL-10) to mice inhibits their ability to be sensitized to trinitrophenyl-coupled spleen cells for a DTH response (Schwarz *et al*, 1994). Furthermore, administration of neutralizing antibodies to IL-10 largely, but not totally, inhibited the ability of UVB irradiation to suppress sensitization to alloantigens (Rivas and Ullrich, 1994) or sheep erythrocytes (S.E. Ullrich, personal communication) in mice. Together with the observation that exposure of a transformed murine keratinocyte line (PAM 212 cells) to UVB radiation results in production of biologically relevant concentrations of IL-10 (Rivas and Ullrich, 1992), these data suggest a role for IL-10 in UVB-induced immunosuppression. In order to directly address the question of the relevance and importance of IL-10 in UVB-induced immunosuppression, we have utilized IL-10–deficient, gene-targeted (IL-10T) mice. We find that IL-10T mice can not be suppressed by UVB irradiation for induction of DTH but are normally suppressed by UVB irradiation for induction of CHS. Examination of serum for IL-10 from irradiated wild-type control mice revealed peak IL-10 levels at 4 d, coinciding with the same time course for immunosuppression after UVB irradiation as reported previously (Moledijk *et al*, 1987). Freshly explanted epidermal cells from IL-10T mice demonstrated enhanced presentation of alloantigen *in vitro* to unprimed T cells, consistent with a regulatory role for epidermal-derived IL-10.

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Abbreviations: CHS, contact hypersensitivity; IL-10T, IL-10 transgenic mice homozygous for a targeted disruption of the IL-10 gene.

MATERIALS AND METHODS

Mice IL-10T mice were generated by Kühn *et al* on a C57BL/6 background as previously described (Kühn *et al*, 1993). Briefly, codons 5–55 of the first exon of the murine IL-10 gene were replaced by a linker containing a termination codon and a *neo* gene. Additionally, a stop codon was introduced into exon 3 by mutagenesis of the *Eco*RI site. Mice heterozygous for this mutation were bred, and litters were typed by Southern blotting. Animals were housed under conventional conditions and received water and dry food pellets *ad libitum*. IL-10T mice started to develop an inflammatory bowel disease after 8–9 wk of age. The animals used in the experiments were all age-matched litters. BALB/c and C57BL/6 mice were obtained from Jackson Laboratories (Bar Harbor, ME).

Effects of UVB Radiation on Induction of DTH and CHS Responses The most commonly used high-dose UVB radiation procedure was employed as described elsewhere (Ullrich, 1986; Moledijk *et al*, 1987, Rivas and Ullrich, 1994). Groups of IL-10T mice, heterozygous littermates, and wild-type control mice were exposed to 3×10^4 joules (J) UVB radiation per m^2 on the shaved dorsum and, 5 days later, immunized by subcutaneous injection of 10^8 allogeneic (BALB/c) nucleated spleen cells at the nonirradiated abdominal site. Five days later these mice were challenged by injection of 10^7 BALB/c spleen cells in a hind footpad, and footpad swelling was assessed at 24 and 48 h with a micrometer (Mitutoyo, Tokyo, Japan) as a measure of DTH response. Groups of control mice were either irradiated but not immunized before challenge or only challenged without prior immunization. This experiment was performed twice.

For studying the effects of UVR on the induction of CHS, several groups of IL-10T mice were irradiated as above, but their ears were protected with vinyl electrical tape (3M Electrical Products Division, Austin, TX). Five days after irradiation, 100 μl of a 5% solution of oxazolone (4-ethoxymethylene-2-phenyloxazoline-5-one, Sigma Chemical Co., St. Louis, MO) in acetone:corn oil (4:1) were applied to their shaved, nonirradiated abdomens. Five days after immunization, these mice and a group of nonimmunized mice (negative control) were challenged to the hapten by epicutaneously painting 5 μl of 1% oxazolone solution onto each side of each ear. Twenty-four and 48 h later, ear swelling, as a measure of CHS, was assessed with a micrometer as described above. In another experiment, trinitrochlorobenzene was used as a hapten in the same experimental fashion with a similar result (data not shown).

Serum IL-10 Levels After Irradiation To examine the serum concentration of IL-10 after UVB irradiation, C57BL/6 mice were exposed to 3×10^4 J UVB per m^2 on the shaved dorsum, and serum was obtained from three mice at various timepoints. Subsequently, this serum was tested for IL-10 content with a mIL-10 Cytoscreen ELISA-Kit (Biosource International, Camorillo, CA).

Mixed Epidermal Cell–Lymphocyte Reaction Epidermal cells were prepared as described (Grabbe *et al*, 1994) from truncal skins of shaved and chemically depilated (Neet, Whitehall Laboratories, New York, NY) IL-10T and wild-type (WT) mice. Subcutaneous fat and panniculus carnosus were removed, after which the skins were floated dermis side down on 0.5 U dispase per ml and 0.4% trypsin in Ca^{2+}/Mg^{2+}-free phosphate-buffered saline for 40 min at 37°C. Epidermal sheets were collected and dissociated by gentle stirring for 20 min. The resulting epidermal cells (EC) were filtered through nylon gauze (Nitex, Tecto, Elmsford, NJ) and washed. Thy 1.2^+-bearing cells were deleted by incubation in anti-Thy 1.2 monoclonal antibodies (Sigma Chemical Co.) for 30 min on ice, followed by washing and subsequent incubation in low-toxicity rabbit complement (Cedarlane, Hornby, Ontario, Canada) for 30 min at 37°C. Dead cells were removed by treatment with 0.05% trypsin and 80 μg DNase per ml in Ca^{2+}/Mg^{2+}-free phosphate-buffered saline for 5 min at room temperature.

The primary mixed epidermal cell–lymphocyte reaction was performed as described (Grabbe *et al*, 1994). Briefly, 2×10^5 nylon-wool–enriched BALB/c splenic T cells were cocultured with varying numbers of freshly prepared EC from IL-10T and WT mice in RPMI-1640 (Cellgro, Washington, DC) supplemented with 1.5% mouse serum and 5 μg indomethacin per ml (Sigma Chemical Co.), and 5×10^{-5} M 2-mercaptoethanol (MELR-medium). Cells were cultured for 6 d at 37°C in round-bottom 96-well plates and then pulse-labeled with 1 μCi [^{3}H]thymidine per ml for 24 h before harvesting. Incorporation of radioactivity was assessed by liquid scintillation counting to evaluate response.

Statistical Analysis The significance of differences among groups was examined by the two-tailed Student's t test for independent events.

RESULTS

DTH Response to Alloantigen After High-Dose UVB Radiation in IL-10T Mice Is Not Suppressed In the first set of experiments, groups of age-matched IL-10T, heterozygous littermates, and wild-type control mice were irradiated with 3×10^4 J UVB radiation per m^2. Five days later, these mice were sensitized to allogeneic spleen cells at the nonirradiated abdominal site. Five days after that, these mice as well as control mice treated identically except not immunized were challenged by injection of allogeneic spleen cells into a hind footpad. UVB irradiation suppressed the induction of immunity as described in *Materials and Methods* in wild-type and heterozygous animals by >80% whereas IL-10T mice failed to exhibit significant suppression **(Fig 1)**. Also, the magnitude of the response was higher in IL-10T mice compared with the control groups.

IL-10 Serum Levels in C57BL/6 Mice After High-Dose UV Irradiation Examination of serum from C57BL/6 mice exposed to 3×10^4 J UVB radiation per m^2 demonstrated a rise in IL-10 content detectable by 72 h and maximal at 4 d **(Fig 2)**. This time course is in agreement with reports of others (Rivas and Ullrich, 1994) and coincides with the maximum UVB-induced suppression of the sensitization phase for DTH responses as demonstrated previously (Moledijk *et al*, 1987). These investigators found maximal systemic immunosuppression 4 d after high-dose UVB irradiation, which lasted 3 wk. Immunization shortly after irradiation (24 or 48 h) yielded normal levels of sensitivity responses (Moledijk *et al*, 1987).

Inhibition of CHS Responses to Contact Allergen by UVR in IL-10T Mice Regulation of DTH and CHS responses by UVR can be clearly distinguished in IL-10T mice. For induction of CHS, mice were immunized 5 days after UVB irradiation by painting hapten epicutaneously on the nonirradiated abdomen. Control mice were treated identically but were not irradiated. Five days later these mice were challenged. Unlike the situation with DTH responses, UVB radiation significantly and substantially inhibited the induction of CHS in IL-10T mice **(Fig 3)**. These results support previous data suggesting that the regulation of DTH and CHS by UVR is mediated by different cytokine signals (Kim *et al*, 1990; Yoshikawa *et al*, 1990; Rivas and Ullrich, 1994). CHS responses were higher in IL-10T mice in some experiments, as previously observed (Berg *et al*, 1995), but were lower in others (such as the one shown in **Fig 3**). Experiments are underway to determine whether this variance depends on the age of the mice, the degree of inflammatory bowel disease present or, perhaps, the hapten employed.

Enhanced Mixed Epidermal Cell–Lymphocyte Reaction with EC from IL-10T Compared to EC from Wild-Type Mice In order to investigate the relevance of epidermal cell-derived IL-10 to an *in vitro* system, we explored the ability of EC to present alloantigen in the mixed epidermal cell-lymphocyte reaction. As shown in **Fig 4**, a greater response was seen with stimulation by IL-10T epidermal cells compared to the wild-type epidermal cells, consistent with an immunoregulatory role for epidermal-derived IL-10.

DISCUSSION

The finding that the induction of DTH in IL-10T mice cannot be suppressed by exposure to UVB radiation provides definitive evidence that IL-10 is a necessary mediator of UVB-induced suppression of the induction of DTH. In concert with the evidence that IL-10 inhibits the granulocyte-macrophage colony stimulating factor–induced ability of epidermal antigen-presenting cells (Langerhans cells) to effectively present tumor-associated antigens for induction of anti-tumor immunity (Beissert *et al*, 1995), these data suggest that IL-10 might play an important role in the induction of skin tumors in chronically UVB-exposed recipients.

These results confirm the findings of Rivas and Ullrich (1994), who used administration of neutralizing antisera to IL-10 to

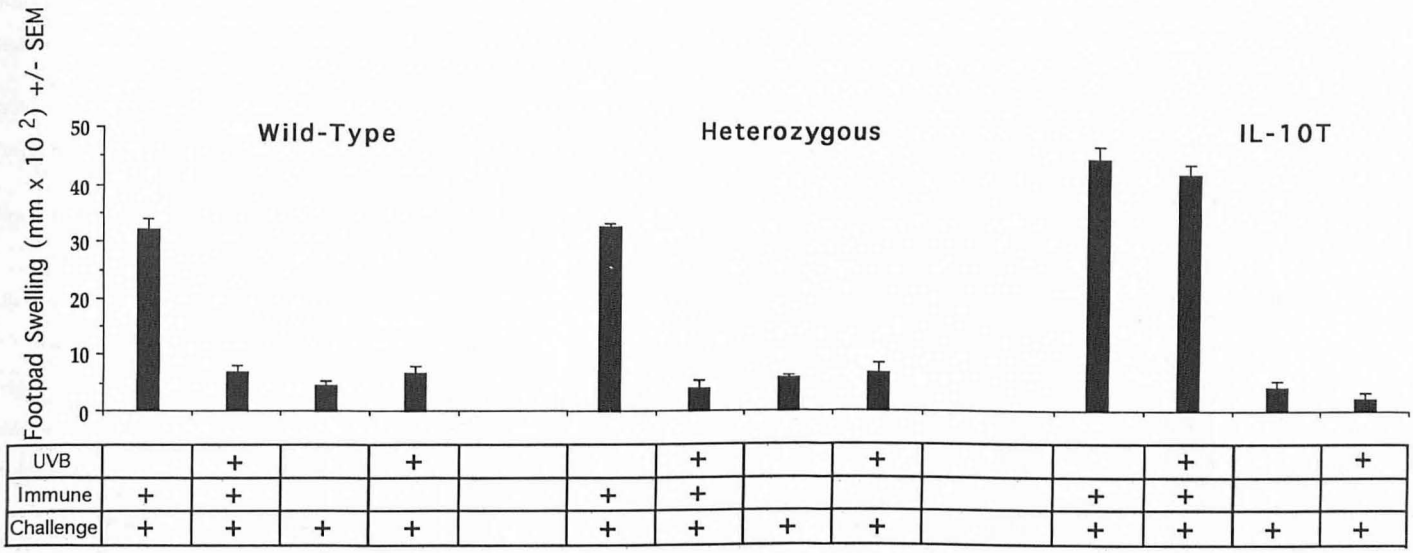

Figure 1. Exposure to UVB radiation systemically inhibits the induction of DTH to alloantigens in WT but not IL-10T mice. WT, heterozygous littermate, and IL-10T mice were exposed to 3×10^4 J UVB radiation per m^2 or not irradiated as indicated. Five days later, indicated groups were immunized to alloantigen by injection of 1×10^8 nucleated spleen cells from BALB/c mice. Five days later all mice were challenged at a hind footpad with 1×10^7 nucleated spleen cells from BALB/c mice. Footpad swelling was assessed 24 h later as the difference in thickness between the injected and noninjected footpad as a measure of DTH response to alloantigen. WT and heterozygote littermates showed highly significant suppression of the induction of DTH compared with nonirradiated controls ($p < 0.01$ for UVB group versus control group for both WT and heterozygous mice). IL-10T mice failed to exhibit suppression after UVB irradiation. Nonimmunized mice served as negative controls. n = 4 for all groups. *Error bars*, mean ± SEM.

demonstrate its role in UVB-induced suppression of the induction of DTH. The use of gene-targeted mice deficient in a single cytokine allowed for confirmation of these findings in a well-defined, definitive system.

Chronically UVB-irradiated mice develop highly immunogenic cutaneous malignancies that regress upon transfer to syngeneic recipients (Kripke, 1990; reviewed in Grabbe and Granstein, 1994). The development and growth of these tumors in the primary host depend on the development of specific downregulatory mechanisms including the appearance of splenic T suppressor cells that are capable of preventing the immune-mediated rejection of the tumor (Fisher and Kripke, 1982). The current data suggest that release of IL-10 subsequent to UVB radiation might play a role in non-

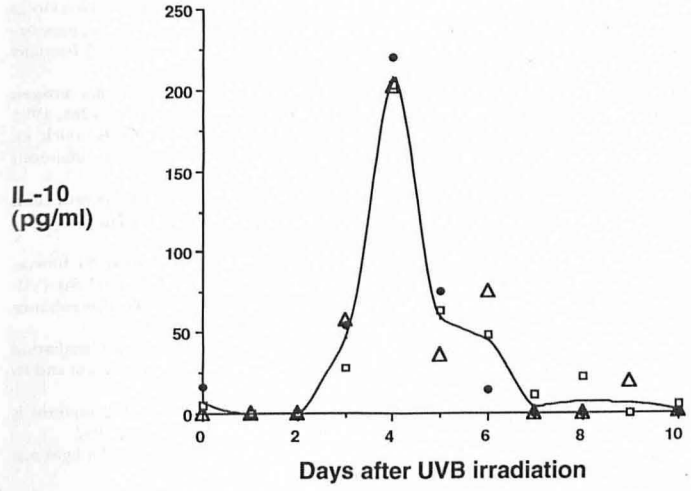

Figure 2. Serum IL-10 levels rise after UVB radiation exposure. C57BL/6 mice were irradiated with 3×10^4 J/m^2 at the shaved dorsum. At various time points, serum was harvested from three mice and IL-10 content was measured with an enzyme-linked immunosorbent assay. *Symbols* indicate three independent measurements at each timepoint.

recognition of these tumors. In addition, it has recently been reported that some skin cancer cell lines, including melanoma lines (Chen *et al*, 1994) and basal cell and squamous cell carcinomas (Kim *et al*, 1995), produce IL-10; such production may represent a mechanism by which these tumors can locally inhibit antigen presentation to escape immune recognition.

The questions remain, however, as to how photons initiate the production and secretion of IL-10 and what is the target cell. Since UVB radiation is almost entirely absorbed within the epidermis (Everett *et al*, 1965) and keratinocytes have been shown *in vitro* to produce both IL-10 mRNA and protein upon exposure (Enk and Katz, 1992; Rivas and Ullrich, 1992), they are a likely source of IL-10. Exposure to UVR and other DNA-damaging agents triggers a cascade of events that leads to what has been called the "UV response," which may serve to protect cells from damage. Among the earliest mammalian UV responses is the activation of Src tyrosine kinases, which then activates Ha-Ras and Raf-1 (Devary *et al*, 1992). Activation of Ras protein stimulates cytoplasmic protein kinases, which lead to increased AP-1 activity (Devary *et al*, 1992; Engelberg *et al*, 1994), and induce nuclear translocation of NFκB (Devary *et al*, 1993). The expression of both AP-1 complex components c-*jun* and c-*fos* are enhanced, and c-Jun is modified post-translationally (Radler-Pohl *et al*, 1993). The expression of several cytokines is modulated by AP-1 or NFκB, which would link their transcriptional regulation to a UVR-inducible pathway. Cytokines including tumor necrosis factor–α (Köck *et al*, 1990) and IL-10 (Rivas and Ullrich, 1992) have been shown to be UV-inducible and able to suppress cellular immune responses in some experimental systems. Surprisingly, the ability of UVR to activate this pathway is independent of DNA damage (Devary *et al*, 1993).

An intriguing question is why evolution would have selected for mechanisms of immunosuppression by UVR. Induction of suppressive cytokines by UVR may hypothetically serve to inhibit the development of autoimmune responses to novel molecular structures created by photo-rearrangement, such as cross-link formation, photoisomerization, or release of "hidden" antigens. In human skin following UV injury, macrophages with APC ability infiltrated the epidermis (Cooper *et al*, 1985). Those macrophages were found to stimulate autologous suppressor inducer T cells (Baadsgaard *et al*,

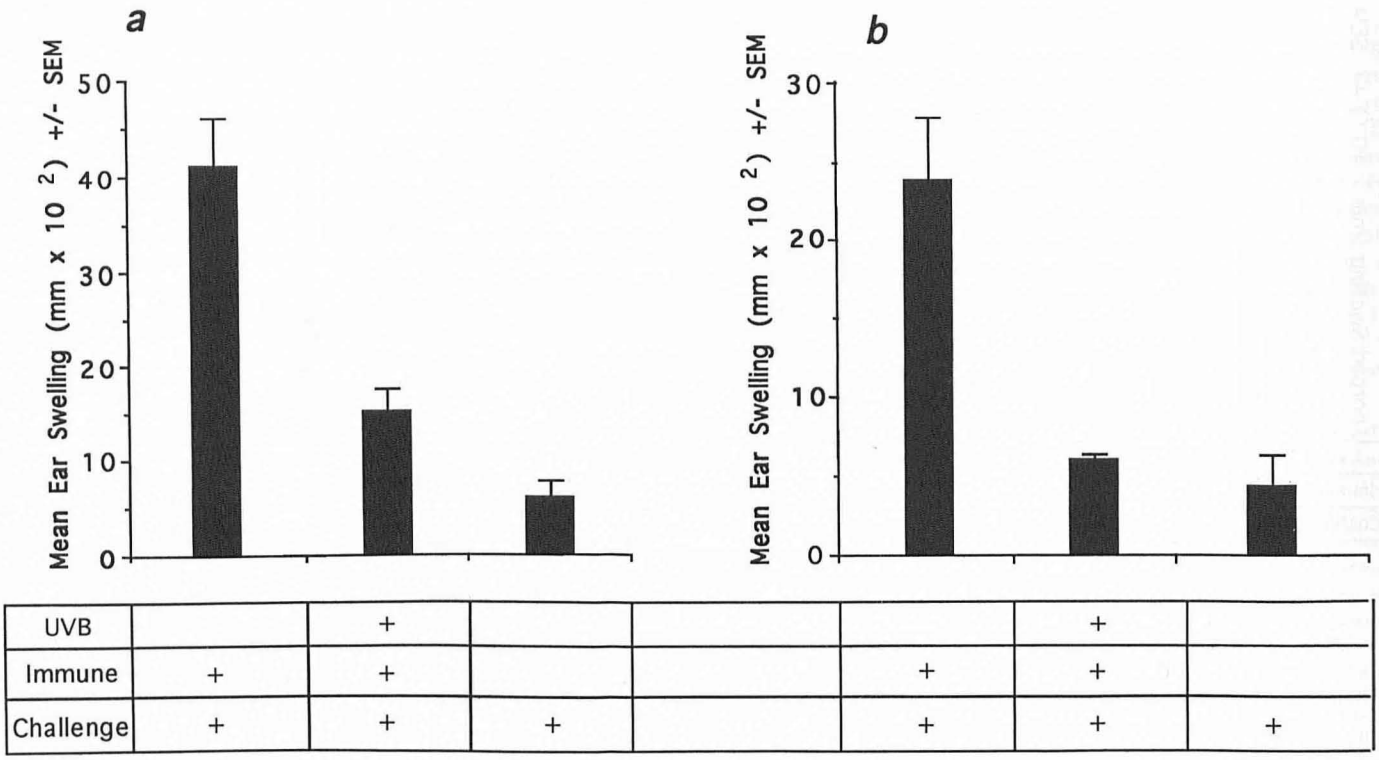

Figure 3. Exposure to UVB radiation systemically inhibits the induction of CHS to oxazolone in WT and IL-10T mice. WT *(a)* and IL-10T *(b)* mice were exposed to 3×10^4 J UVB radiation per m^2 as indicated in *Panel a*. Prior to UVR exposure, ears of all mice were covered with electrical tape. Five days later, indicated groups were immunized to oxazolone by painting 5% oxazolone (in acetone:corn oil, 4:1) onto the shaved nonirradiated abdomen. Five days after immunization, all mice were challenged with 1% oxazolone, and ear swelling was assessed 24 h later as a measure of CHS response. Both WT and IL-10T mice exposed to UVB radiation demonstrated highly significant suppression of the induction of CHS compared with nonirradiated controls ($p < 0.03$ for UVB group *versus* positive control group for both WT and IL-10T mice). Negative control groups were not immunized or irradiated. $n = 4$ for all groups except negative control, $n = 3$. *Error bars*, mean ± SEM.

1988). Multiple pathways for UV-induced immunosuppression appear to have been created in the course of evolution to provide organismic protection against the biologic consequences of high-dose UVR.

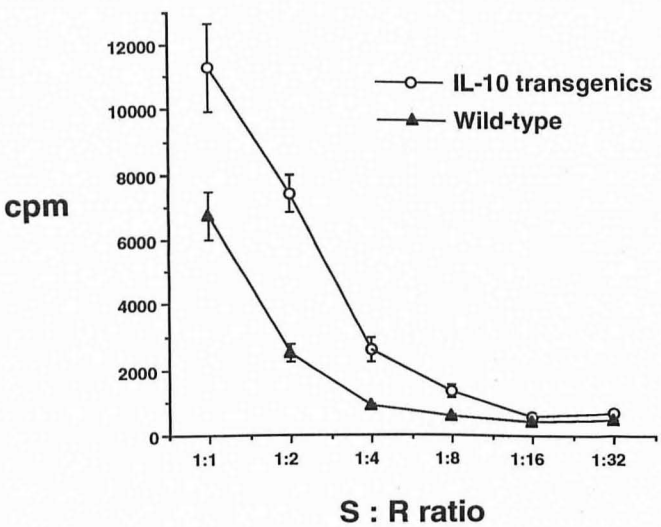

Figure 4. Epidermal cells from IL-10T mice are more effective at stimulating unprimed allogeneic T cells than WT control epidermal cells. Epidermal cells [stimulators (S)] were prepared from WT and IL-10T mice and mixed at various concentrations with nylon-wool–purified BALB/c splenic T cells as responders (R) for 6 d. Subsequently, [^{3}H]thymidine was added to culture wells, and 24 h later incorporation of radioactivity was measured. *Error bars*, mean ± SEM.

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