IL-10 Contributes to the Inhibition of Contact Hypersensitivity in Mice Treated with Photodynamic Therapy

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We have explored the effect of photodynamic therapy (PDT) with verteporfin on the induction and expression of contact hypersensitivity (CHS) to 2,4-dinitrofluorobenzene (DNFB) in normal mice and IL-10-deficient mice. Our results indicate that DNFB sensitized mice given PDT with verteporfin and whole body red light irradiation exhibited a significant reduction in CHS compared with control animals. Administration of rIL-12 reversed the effect(s) of PDT as did treatment of mice with anti-IL-10-neutralizing Ab. Knockout mice deficient in IL-10 were found to be resistant to the inhibitory effects of PDT. In vitro proliferative responses using spleen cells from DNFB-sensitized and PDT-treated mice showed a significantly lower response to DNBS as compared with cells from DNFB-sensitized mice or DNFB and PDT-treated IL-10-deficient mice. Finally, naive mice exposed to PDT exhibited an increase in skin IL-10 levels, which peaked between 72 and 120 h post-PDT. Together these data support the role of IL-10 as a key modulator in the inhibition of the CHS response by whole body PDT. ***The Journal of Immunology,*** **2000, 164: 2457–2462.**

Photodynamic therapy (PDT)[2] is an approved cancer treatment based upon the preferential localization of a light-absorbing compound within rapidly dividing/activated cells (1). Subsequent illumination of the afflicted region with a sufficient amount of light generates reactive oxygen intermediates and other radical species, which trigger complex biochemical processes resulting in tissue damage (2). Tumor necrosis results by direct cytotoxicity and concomitant microvascular occlusion that compromises blood supply to the area (3). It has also become evident that whole body PDT combining certain photosensitizers and light irradiation at subphototoxic, suberythematous levels has immune modulatory effects. The photodynamic treatment of normal mice with the porphyrin photosensitizers haematoporphyrin derivative (HpD) (4) or Photofrin (5) impaired the immunologically mediated contact hypersensitivity (CHS) response to the hapten 2,4-dinitrofluorobenzene (DNFB). Suppression of the CHS response induced by PDT was adoptively transferable by splenocytes, and it was suggested that the cells responsible for the effect belong to the macrophage lineage (5). A further indication that PDT could alter the immune status of the skin was provided by studies showing that pretreatment of murine skin grafts with HpD and light prolonged their survival on immunocompetent allogeneic recipients (6). Moreover, PDT with HpD of the host promoted skin allograft survival, a situation associated with peritoneal lymphocyte inactivation and macrophage stimulation (7).

Benzoporphyrin derivative monoacid ring A (BPD-MA, verteporfin) (8–10), a lipophilic chlorin-like photosensitizer with a maximum light absorption peak at 690 nm, is currently undergoing clinical evaluation for the treatment of a number of pathological conditions including age-related macular degeneration, skin cancer, psoriasis, and rheumatoid arthritis (11, 12). Pretreatment of skin grafts with subtoxic levels of verteporfin and light prolonged their acceptance on allogeneic recipients (13). Mice given verteporfin exhibited reduced CHS responses to DNFB under ambient light conditions as well as following whole body light irradiation, but not when protected from light (14). Immunologic reactivity to DNFB does develop in mice treated with subtoxic PDT, albeit at reduced levels. The reduction in reactivity to topically applied DNFB produced by verteporfin and light is transient, lasting 7–10 days, and the formation of immune reactivity to an unrelated hapten (oxazolone) proceeds normally 7 days after PDT (G. O. Simkin, unpublished observations). Furthermore, animals given whole body subtoxic levels of photosensitizer and light exhibit no evidence of infection indicating that antimicrobial immunity is retained.

In the past decade, a considerable amount of information has been accumulated to define the existence of functionally polarized immune responses driven by Th cells, comprised of Th1 and Th2 subsets, each producing a distinct array of cytokines (15). Th1-like immune responses, characterized by the dominance of pro-inflammatory cytokines including IFN-γ and TNF-α, favor the formation of cell-mediated immunity, delayed-type hypersensitivity, and macrophage activation (16). Th2-like immune responses, characterized by the production of IL-4, IL-5, IL-6, IL-10, and IL-13 promote humoral responses, production of IgE and IgA, as well as activation of eosinophils, mast cells, and basophils (15, 16). We suggested that subtoxic PDT with verteporfin might negatively modulate the CHS response (14, 17), a Th1-like immune response, by stimulating the production of Th2-type cytokines. Splenocytes and draining lymph node cells from PDT-treated mice painted with DNFB released higher amounts of IL-10 than lymphoid cells from control DNFB-sensitized animals (17). When rIL-12 was administered, the inhibitory effect of PDT on CHS was not evident (17). IL-10 levels were increased in skin of mice exposed to PDT with Photofrin (18). IL-10 regulates cutaneous inflammatory responses

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[2] Abbreviations used in this paper: PDT, photodynamic therapy; BPD-MA, benzoporphyrin derivative monoacid ring A, verteporfin; CHS, contact hypersensitivity; DC, dendritic cell; DNFB, 2,4-dinitrofluorobenzene; BrdU, 5-bromo-2′-deoxyuridine; KO, knock out; LC, Langerhans cells.

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(19) and participates in the induction and elicitation phases of the CHS response (20, 21).

BALB/c and C57BL/6 (B6) mouse strains have been extensively utilized to study the regulation of Th cell responses. These two strains form dissimilar T cell responses to *Leishmania major* (22). BALB/c mice generate a Th2-like immune response and are susceptible to Leishmaniasis, whereas B6 mice develop a Th1-like response and are resistant to infection with this protozoan (22). Furthermore, B6 mice are more sensitive than BALB/c mice to the inhibitory effect of UV-B light on the development of the CHS response, a model for Th1-like immunity (23). In this study we examined the contribution of IL-10 to the inhibition of the CHS response by PDT, utilizing wild-type BALB/c and B6 mice as well as B6 animals rendered genetically deficient for IL-10.

Materials and Methods

Animals

Females, 8–10 wk of age, BALB/cJ, wild-type CB57BL/6 (B6), and IL-10-deficient CB57BL/6-IL-10^{tmlCgn} (IL-10-KO B6) mice were purchased from The Jackson Laboratory (Bar Harbor, ME) and housed under fluorescent light for 12 h per day. Mice were maintained in compliance with the Canadian Council on Animal Care and given rodent chow and acidified water ad libitum.

Sensitization and elicitation of CHS

Mice were sensitized and ear challenged to elicit CHS responses to DNFB as described (14, 17). Briefly, CHS was induced on day 0 by applying 35 μl of a DNFB (Sigma, St. Louis, MO) solution (0.5% DNFB in a 4:1 mixture of acetone and olive oil) with a micropipette to the inguinal region (14). The area was shaved before DNFB application. Six days later, the hapten solution (10 μl of 0.25% DNFB in delivery vehicle) was applied to the dorsal surface of the right ear. To gauge toxic effects, the solvent solution was applied to the left ear. Nonsensitized mice were evaluated in parallel to determine the skin irritant component of the DNFB challenge solution. CHS responses were determined in a blinded manner 24 h after DNFB application by measuring ear thickness with a dial caliper (model no. 7309, Mitutoyo, Kanagawa, Japan). The magnitude of ear swelling was calculated as the difference in ear thickness between the pre- and postchallenge measurements and expressed as the mean ($\pm$SD) for each group of animals or as a percentage of the positive control response (100%).

PDT, cytokine, and Ab treatments

PDT. Lipid-formulated clinical grade verteporfin (Verteporfin for Injection, QLT PhotoTherapeutics, Vancouver, BC, Canada) was reconstituted in sterile distilled water. Further dilution was with 5% dextrose injection United States Pharmacopoeia (Baxter, Toronto, Ontario, Canada). Whole body PDT with verteporfin was delivered as follows: animals received verteporfin (1 mg/kg, i.v.) and then placed in the dark for 1 h. For light treatments, mice were placed in clear plexiglass containers, and 15 J/cm^2 of red light (692 $\pm$ 12 nm) at 12.5 mW/cm^2 was delivered from a pair of light emitting diodes (LED) panels (Hewlett Packard, San Jose, CA) positioned above and below the subject. PDT was given either 24 h before or 24 h after DNFB application, treatment times associated with strongly reduced CHS responses to DNFB (14).

Murine rIL-12 was kindly supplied by the Genetics Institute (Cambridge, MA). rIL-12 was diluted with PBS and 1 μg in 50 μl was administered i.m. in the flank 3 h following PDT.

Treatment with anti-IL-10 neutralizing mAb. Wild-type B6 mice sensitized with DNFB on day 0 and treated PDT on day +1 were administered purified rat IgG1 anti-mouse IL-10 (clone JES5-2A5, PharMingen, San Diego, CA), 0.2 mg/daily, for 5 consecutive days (days 1–5). Control animals received purified rat IgG (Sigma).

In vitro proliferation assays: 5-bromo-2′-deoxyuridine (BrdU) uptake

In vitro proliferation assays were performed to evaluate the impact of PDT on cellular immune responses to DNFB and to a mixture of anti-CD3 and anti-CD28 Abs. Spleen cell suspensions were prepared 6 days after the initial DNFB application. Cells from animals within each group were pooled and resuspended in culture medium (RPMI 1640 medium containing 5% heat-inactivated FCS, 100 U/ml penicillin, 100 μg/ml streptomycin, 1 mM sodium pyruvate, 0.02 M HEPES, 5×10^{-2} mM 2-ME, and 2 mM glutamine (all from Life Technologies, Burlington, Ontario, Canada), and cell numbers were adjusted to 2.5×10^6 cells/ml. Quadruplicates of 100 μl (2.5×10^5 cells/well) were added to 96-well plates. Splenocytes were cultured with an optimal concentration of DNBS (Sigma), 90 μg/ml in culture medium (24), or with soluble anti-CD3 (rat anti-mouse CD3ϵ, clone 145-2c11) and anti-CD28 (rat anti-mouse CD28, clone 37.51) (no sodium azide and low endotoxin content, PharMingen) each at 2 μg/ml. Cultures were incubated for 96 h in 5% CO_2 at 37°C. For the final 6 h of incubation, 10 μl (110 μM) of BrdU (Boehringer Mannheim, Mannheim, Germany) in culture medium was added to each well. Plates were centrifuged at 2000 rpm for 5 min, supernatants removed, and cells fixed with ethanol containing 0.5 M HCl at −20°C for 30 min. An ELISA kit for the determination of BrdU was utilized according to the manufacturer's (Boehringer Mannheim) instructions. Proliferation corresponded to the mean OD $\pm$ SD for stimulated cells minus the result obtained for cells not exposed to the stimulus.

Preparation of protein extracts from skin at various times after PDT

Untreated BALB/cJ mice (control group) or mice given PDT or PBS and 15 J/cm^2 red light alone (sham PDT) were sacrificed and shaved from 6 to 144 h after PDT. Shaved ventral and trunk skin samples (~6 cm^2) were collected. Subcutaneous tissue was removed, and the remaining skin was cut in small pieces and placed into tubes with lysis buffer (1 mM MOPS, pH 7.2, 5 mM EGTA, 1% (w/v) Nonidet P-40, 1 mM DTT, 75 mM β-glycerol phosphate, 1 mM Na_3VO_4, and 1 mM PMSF (all from Sigma-Aldrich Canada, Oakville, Ontario, Canada) in ice until homogenization. Samples were disrupted with a homogenizer (Polytron PT 3100, Kinematica, Luzern, Switzerland). Samples were initially centrifuged at 3500 rpm at 4°C. The supernatants obtained were centrifuged at 50,000 rpm at 4°C with an Optima, TLX ultracentrifuge (Beckman, Fullerton, CA). Supernatants were collected, aliquoted, and kept at −70°C until required. Total protein levels were determined using the Coomassie Brilliant Blue G-250 dye-binding assay (Bio-Rad, Hercules, CA).

Enzyme immunoassays for mouse IL-10

IL-10 levels in skin extracts were determined by an "Ag capture" ELISA developed using an Ab pair and mouse rIL-10 standard (PharMingen). Maxisorp F16 multiwell strips (Nunc, Roskilde, Denmark) were coated with capture Ab (rat anti-mouse IL-10, JES5-2A5, at 4 μg/ml) in 0.1 M $NaHCO_3$, pH 8.6, 100 μl/well, overnight at 4°C. Plates were washed three times with 0.05% Tween 20 in PBS and blocked for 1 h at room temperature with 10% FCS in PBS (blocking and diluent buffer). Duplicate samples (100 μg of total protein) or standards in diluent buffer were incubated for 2 h at room temperature. Plates were washed three times and incubated with biotinylated rat anti-mouse IL-10 (JES5-16E3) at 2 μg/ml for 1 h at room temperature. Plates were extensively washed and a 1/2000 dilution of streptavidin-HRP (PharMingen) was added for 45 min at room temperature. Plates were again washed and 0.5 mg/ml ABTS substrate (2,2′-azino-di[3-ethylbenzthiazoline sulfonate(6)]diammonium salt) in ABTS buffer (Boehringer Mannheim) was added. Color development was terminated adding 50 μl of 0.2% (w/v) SDS (Sigma) after 35 min incubation at room temperature. Absorbance was read at 405 nm with a MRX microplate reader (Dynatech, Hamilton, VA). The assay detection limit was 10 pg/ml.

Statistical analysis

Statistical analysis of results was performed using one-way ANOVA with Bonferroni's *t* test for multiple comparisons among the means. A difference between means was regarded as statistically significant when $p < 0.05$. Mean values with SDs are presented.

Results

Influence of PDT on the CHS response of DNFB-painted BALB/cJ and B6 mice

BALB/c and B6 mice painted with DNFB, treated with verteporfin, and given whole body light irradiation exhibited significantly lower ear-swelling responses than DNFB-treated mice injected with PBS and exposed to the same amount of light. DNFB-treated mice of both strains given PDT and rIL-12 displayed ear-swelling responses statistically no different from those of light-treated positive control animals, but significantly ($p < 0.01$) different from mice given only PDT (Table I).

Table I. *rIL-12 reverses the PDT-induced inhibition of the CHS responses of BALB/c and B6 mice*

	Ear Swelling[a]			
	BALB/cJ		B6	
Treatment	$\times 10^{-2}$ mm	Suppression (%)	$\times 10^{-2}$ mm	Suppression (%)
Light	20.2 ± 3.5	0	23.6 ± 3	0
PDT	9.7 ± 1.4*	52	12.3 ± 2.0	48‡
PDT + rIL-12	19.1 ± 2.2	5	21 ± 2.5	11

[a] Ear swelling responses were determined for BALB/cJ and B6 mice painted with DNFB on day 0 and treated with light (positive control), verteporfin (1 mg/kg), and light (PDT) or PDT and rIL-12 (1 μg) 24 h afterward. Mice were challenged with DNFB on day +5 and ear-swelling responses recorded 24 h later. Data represent the mean ± SD values of 5–10 mice per group from one experiment. Irritant controls for BALB/c and B6 mice exhibited an increase in ear thickness of 1.2 ± 0.4 × 10^{-2} mm and 4.0 ± 1.5 × 10^{-2} mm, respectively. This experiment was performed twice with similar findings.

*, ‡, $p < 0.01$, mice given PDT differ from mice treated with light only or PDT plus rIL-12.

Influence of PDT on the CHS response of DNFB-sensitized wild-type B6 and IL-10-KO B6 mice

B6 and IL-10-KO B6 mice were utilized to evaluate the role of IL-10 as a mediator of the inhibitory impact of PDT on the CHS response (Fig. 1). DNFB-painted mice of both strains treated with PBS and light and challenged with the hapten on day +5 developed strong ear-swelling responses of a similar magnitude. Wild-type B6 mice treated with PDT either on day −1 or day +1 exhibited significantly lower CHS responses ($p < 0.01$) than the positive control mice. However, DNFB-painted IL-10-KO B6 mice treated with PDT either on day −1 or on day +1 developed ear-swelling responses no different from their respective positive control animals. Naive wild-type B6 and IL-10-KO B6 mice exhibited marked ear irritant reactions following exposure to the DNFB challenge solution, eliciting responses corresponding to 19% and 45% of that of the positive controls animals, respectively. In comparison to wild-type B6 mice, IL-10-KO B6 animals generated stronger CHS and irritant responses. This feature has been reported by others (25).

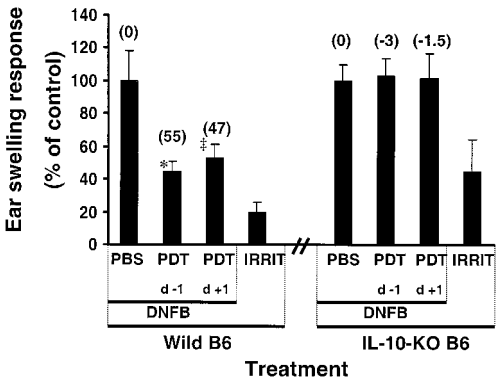

FIGURE 1. PDT with verteporfin does not impair the CHS response of DNFB-treated IL-10-KO mice. Wild-type B6 and IL-10-KO B6 mice were painted with DNFB and treated with PBS (100% control result), verteporfin, and light 24 h before (PDT d −1) or 24 h later (PDT d 1). Naive (irritant control, IRRIT) and DNFB-painted animals were challenged with the hapten on day +5 of the experiment and ear-swelling responses recorded 24 h later. For the B6 and IL-10-KO B6-positive control mice, the specific anti-DNFB response corresponded to an increase of ear thickness of 22.3 ± 3.0 and 20.5 ± 2.5 × 10^{-2} mm, respectively. The percentage of suppression produced by each treatment is given in parentheses. Each treatment consisted of 6–10 animals. * and ‡, $p < 0.01$, different from 100% control group.

Proliferative responses of splenocytes

Spleen cells from all DNFB-sensitized animals generated a proliferative response in the presence of DNBS (Fig. 2). However, splenocytes from B6 mice treated with PDT exhibited a significantly ($p < 0.01$) lower proliferative response to DNBS than cells from the positive control animals. Splenocytes from IL-10-KO B6 mice given PBS or PDT generated strong proliferative responses to DNBS (Fig. 2*A*). The proliferative response of splenocytes to anti-CD3 and anti-CD28 Abs was of a similar magnitude for all groups,

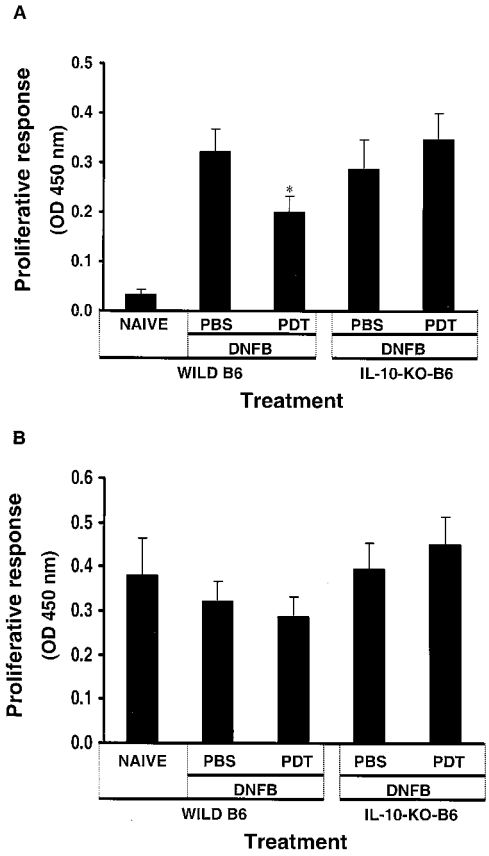

FIGURE 2. The proliferative response of splenocytes to DNBS or anti-CD3 plus anti-CD28 Abs was assessed. Spleen cell suspensions were prepared 6 days after the initial exposure to DNFB and cultured with DNBS (*A*) or a mixture of anti-CD3 + anti-CD28 Abs (*B*). Cell proliferation was measured by BrdU uptake as describe in *Materials and Methods*. *, $p < 0.01$, the proliferative response is different as compared to other groups.

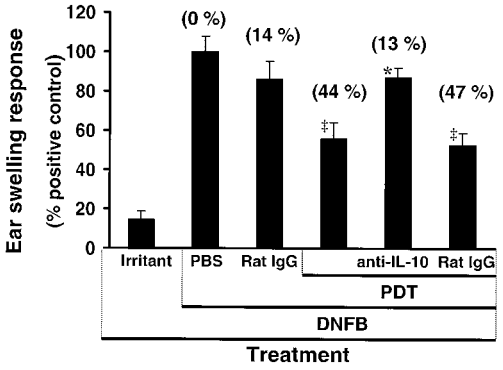

FIGURE 3. Administration of a neutralizing anti-IL-10 Ab to DNFB-painted and PDT-treated B6 mice eliminates the inhibitory effect of PDT on the CHS response. For the positive control mice (100% control result) the specific anti-DNFB response corresponded to an increase of ear thickness of $27.6 \pm 3.0 \times 10^{-2}$ mm (mean ± SD). The percent suppression produced by each treatment is given in parentheses. Each treatment group consisted of 5–10 animals. *, $p < 0/01$, different as compared to PDT treated mice; ‡, $p < 0.01$, different as compared to positive control mice.

although splenocytes from PDT-treated animals apparently exhibited lower proliferative responses (Fig. 2*B*).

Administration of anti-IL-10-neutralizing Abs blocked the inhibitory effect of PDT on the CHS response

To further evaluate whether IL-10 is involved in the inhibition of the CHS response with PDT, B6 mice were administered the neutralizing rat anti-mouse IL-10 mAb JES5-2A5 (Fig. 3). Animals treated with PDT and anti-IL-10 Ab developed ear-swelling responses of a similar magnitude as the positive control mice. Control animals given rat IgG developed normal CHS responses to DNFB. However, mice treated with DNFB, PDT, and given rat IgG exhibited deficient ear-swelling responses.

PDT increases IL-10 expression in skin

Naive BALB/c animals were treated with verteporfin or PBS and whole body light irradiation (sham PDT). Skin extracts were prepared at various times after PDT. Skin IL-10 protein levels progressively increased following PDT as determined by ELISA, with a maximum expression at 72–120 h. (Fig. 4). Red light alone (sham PDT) did not induce elevation of IL-10 in skin extracts prepared at 24, 72, and 120 h after light exposure, and IL-10 protein levels were not different from those of the naive control group (data not shown).

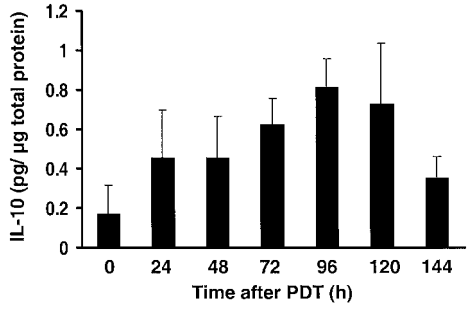

FIGURE 4. ELISA determinations of IL-10 protein for skin extracts prepared at various times after exposing naive BALB/c mice to whole body PFT with verteporfin. Base line values (0 h) represent untreated animals. IL-10 protein levels for skin extracts prepared at 24, 72, and 120 h after exposing mice to red light alone (sham PDT) were comparable to levels of IL-10 of untreated mice (data not shown). Data represent the mean ± SD values of five individual mice per group from one experiment. This experiment was performed twice ($n = 3$–5) with similar results.

Discussion

Our laboratory has evaluated PDT using the photosensitizer verteporfin in a number of immunologic test systems (8, 10). Prolonged skin allograft acceptance (13), impaired adoptive transfer of autoimmune encephalomyelitis (26), prevention of adjuvant-enhanced arthritis (27), and inhibition of CHS (14) have been described for verteporfin-mediated PDT. Deficient CHS responses ensued when whole body PDT with verteporfin was delivered at a time between 48 h prior and up to 72 h after DNFB application (14). Significantly, the magnitude of CHS response was unaffected if PDT was given 24 h before DNFB prechallenge (14). These results suggested that the sensitization but not the effector arm of the immune response to DNFB was susceptible to the effects of PDT. Viable Langerhans cells (LC) isolated from mouse skin treated with verteporfin and light ex vivo had lower levels of MHC Ags as well as CD80 and CD86 costimulatory molecules (13). Correspondingly, LC isolated from PDT-treated skin were deficient in their ability to stimulate the proliferation of alloreactive T cells (13). Mouse splenic dendritic cells (DC) treated with PDT in vitro retained viability but exhibited reduced levels of MHC, costimulatory and adhesion molecules, and a reduced capacity to stimulate the proliferation of alloreactive T cells (28). Blockade of CD80/86-CD28/CTLA-4 or CD40-CD40 ligand (CD40L) costimulatory pathways, concomitant with the sensitization phase, inhibited the murine CHS response to DNFB (29, 30). Interaction of CD40L on T cells with CD40 on macrophages and DC is critical for IL-12 production by these APC types (31, 32). It is evident that PDT can modify APC function and interference of APC-T cell interaction can inhibit the formation of CHS responses. However, evidence that PDT with verteporfin inhibits the CHS response by acting at this level remains circumstantial.

The Th1 and Th2 cytokine formation patterns represent the polarities of immune responses mediated by Th cells (15, 16). CHS induced by the hapten DNFB is considered a prototypic Th1-type immune response in the skin (33). Both $CD4^+$ and $CD8^+$ hapten-specific T cells participate in the CHS response, while MHC class II restricted $CD4^+$ Ag-specific T cells mediate the delayed-type hypersensitivity (DTH) response (i.e., tuberculin reaction) (34, 35). Studies aimed at defining the role of T cell subsets in the CHS reaction have yielded conflicting data. $CD4^+$ and $CD8^+$ hapten-specific T cells are capable of mediating this inflammatory response. Purified murine $CD4^+$ T cells transferred hapten-specific CHS reactivity to naive syngeneic recipients (36) and Ab-mediated $CD4^+$ T cell depletion impeded the transfer of CHS responsiveness (36). Experiments utilizing cell depletion and adoptive transfer techniques as well as MHC class I or MHC class II-deficient mice showed that $CD4^+$ T cells act to limit CHS responses (37–39). $CD8^+$ T cells appear necessary and sufficient for the expression of the CHS inflammatory reaction, whereas $CD4^+$ T cells act to down-regulate this response (37, 39). The $CD8^+$ T cells that mediate CHS activity elaborate Th1 cytokines, whereas the regulatory $CD4^+$ T cells produce Th2-type cytokines (25). How PDT with verteporfin influences the T cell subsets that participate in the CHS response is unclear. DNFB-treated mice given verteporfin and whole body red light irradiation developed fully hyperplastic draining LN despite exhibiting weak ear-swelling responses to the hapten upon antigenic challenge (data not shown). This suggests that immune sensitization to DNFB does occur in mice given PDT.

However, the diminished effector response to DNFB in PDT-treated mice may result from a modification of the cytokine milieu in which hapten-specific T cell immunity develops.

Exposure to UVB light impairs the induction of CHS response to haptens topically applied to irradiated skin of mice and humans (40). This inhibition of CHS appears due to the development of T cells with hapten-specific suppressor activity (41). Both $CD8^+$ and $CD4^+$ T cells can mediate this suppressor function and which T cell subset mediates this inhibitory process depends on the experimental model utilized (40, 42). Administration of rIL-12 overcame UVB light induced hapten-specific tolerance (43, 44). Prevention of UVB light suppression of CHS with rIL-12 was explained through the inhibition of the development of suppressor $CD8^+$ T cells or by the activation $CD8^+$ effector T cells, rather than through an induction of $CD4^+$ effector T cells (42). Administration of rIL-12 prevented the inhibitory influence of PDT on the CHS response. The action of rIL-12 may be related to its well-defined role in promoting Th1 T cell responses by stimulating either $CD8^+$ or $CD4^+$ hapten-specific effector T cells (45). UVB light impairs immune responses by effects exerted at different levels including the generation of reactive oxygen species (46), direct DNA damage (46, 47) and the down-regulation of LC expression of MHC (48), ICAM-1 (49), CD80, and CD86 co-stimulatory (50) molecules. UVB light-irradiated LC anergize Th1 helper T cells while LC Ag presentation to Th2 T cells is preserved (51). Keratinocyte monolayers exposed to UVB light released IL-10 into the supernatant (52). When supernatants prepared in this fashion were administered to mice, a modest degree of systemic immune suppression was produced (52). Administration of neutralizing anti-IL-10 Abs partially inhibited the ability of UVB light irradiation to suppress the sensitization to alloantigens in mice (53). Impaired CHS responses for mice irradiated with UVB light and painted with DNFB was associated with skin infiltration of MHC class II^+/$CD11b^+$ monocyte/macrophage cells (54). $CD11b^+$ macrophages infiltrating human epidermis 72 h after UVB light exposure produce high levels of IL-10 (55). We have observed a macrophage-like dermal infiltration in BALB/c mice treated with PDT 36–48 h previously (data not shown). Whole body PDT and UVB light irradiation can inhibit CHS (4, 5, 14, 56). Common and distinct features of these two forms of phototherapy as well as how relatively low-intensity PDT modifies immune responses in the absence of overt tissue damage await further clarification.

IL-10, produced by a variety of cell types including Th2 type T cells, inhibits cell-mediated immune responses by down-regulating MHC Class II expression, lowering the costimulatory function of APC and the capacity of APC to secrete IL-12 (57–60). IL-10 is considered an endogenous suppressant of cutaneous inflammatory responses (19, 20) and can promote the formation of hapten-specific tolerance (21). Draining lymph node cells obtained from DNFB-painted, PDT-treated mice released higher amounts of IL-10 in culture than cells from mice exposed to DNFB but not given PDT (17). PDT might promote Th2-like immune responses by lowering the availability of IL-12 possibly by increasing IL-10 levels (14, 17).

BALB/c, B6, and IL-10-KO B6 mice form strong CHS responses to DNFB. BALB/c and B6 mice were susceptible to an impairment of the CHS response with PDT. In contrast, IL-10-KO B6 mice given the same PDT treatment developed full-fledged CHS responses. Administration of anti-IL-10 Ab to hapten-painted, PDT-treated B6 mice prevented PDT-induced inhibition of the CHS response. Spleen cells from DNFB-painted, PDT-treated wild-type B6 mice generated significantly lower proliferative response to DNBS in vitro than cells from DNFB-painted B6 mice. Importantly, splenocytes for all treatment groups from wild-type B6 mice exhibited comparable proliferative responses to the anti-CD3 and anti-CD28 Ab combination. These results indicate that PDT may have affected the priming process for DNFB during the sensitization phase (i.e., through the paracrine/exocrine influence of IL-10) rather than a general impairment of T cell responsiveness. Whole body PDT with the photosensitizer Photofrin combined with blue light irradiation increased skin IL-6 and IL-10 levels for BALB/c mice 72 to 120 h after treatment (18). Consistent with these results, verteporfin and red light irradiation elevated skin IL-10 levels that peaked between 72 and 120 h post-PDT. Overall, these studies indicate that IL-10 formation is up-regulated in mice treated with PDT. Application of local or whole body PDT is a distinct approach for modifying immune reactivity. PDT may be effective for the treatment of human immune conditions in which the action of Th1 cells is implicated in pathogenesis (61, 62).

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