Characteristics of the Immunosuppression Induced by Cutaneous Photodynamic Therapy: Persistence, Antigen Specificity and Cell Type Involved

David A. Musser* and Allan R. Oseroff
Department of Dermatology, Roswell Park Cancer Institute, Buffalo, NY

Received 13 November 2000; accepted 16 February 2001

ABSTRACT

Relatively little is known about the immunosuppression induced in mice which have received cutaneous photodynamic therapy (PDT). Consequently, experiments were undertaken using mice which received dorsal PDT using Photofrin® as the photosensitizer in an attempt to characterize the overall nature of the immunosuppression. Photoradiation of mice at various times after injection indicated there was no correlation between photosensitivity and immunosuppression. The suppression was found to be adoptively transferable and antigen specific suggesting the generation of suppressor cells. Selective cell deletions prior to adoptive transfer indicated a CD4⁺ T cell to be responsible for the immunosuppression. Interestingly, using allogeneic spleen cells, no effect on the delayed type hypersensitivity (DTH) response was found. The results indicate that the suppression induced by cutaneous PDT, with the exception of the lack of DTH suppression, is similar to that induced by UVB irradiation but unlike that reported using laser PDT of the peritoneal cavity. This suggests that not only the type of photoradiation but also the site of photoradiation might determine the character of the induced immuno-suppression.

INTRODUCTION

The most common side effect noted in people receiving photodynamic therapy (PDT) is prolonged cutaneous photosensitivity. Animal studies have indicated that in addition systemic immunosuppression occurs following cutaneous light exposure (1). Relatively little is known about this induced immunosuppression in animal models and virtually no information is available concerning human subjects. Other studies in mice have found that intraperitoneal PDT also elicits immunosuppression (2,3). Whether the observed suppression could have an impact on the therapeutic efficacy of PDT is not known, but it seems likely since PDT has an acknowledged immunologic component associated with it (4-6).

Although PDT is used principally as an anticancer therapy, some recent work is directed at the application of PDT to nononcologic disease states such as arthritis (7,8) or for purposes such as the enhancement of skin grafts (9,10). The rationale, formulated mostly from in vitro studies, for the use of PDT to treat arthritis is the observation that activated lymphocytes take up more photosensitizer than do nonactivated cells and can be selectively destroyed (11,12). Prolonged skin graft survival is attributed to a PDT-induced modification of an antigen-presenting cell, presumably by altering its surface molecules (13,14). The use of PDT to treat cutaneous T-cell lymphoma is also under investigation (A. Oseroff, unpublished). These applications, termed transdermal PDT (11), all use light of relatively low intensity spread over a large area, a situation not unlike the use of dorsal/cutaneous photoradiation used in mice to elicit immunosuppression.

Since immunosuppression is thought to be one aspect of UV-induced carcinogenesis, it was of interest to determine if cutaneous PDT-induced immunosuppression mimics that of UVB-induced immunosuppression at the cellular level. It was also of interest to determine if the nature of the immunosuppression elicited by cutaneous PDT is similar to that observed following intraperitoneal PDT administered via laser.

MATERIALS AND METHODS

Animals. Pathogen-free BALB/cAnNCr and C3H/HeNCr MTB- female mice were purchased from the NCI and housed in our institute's animal facility. The mice received food and acidified water ad libitum and were used when 8-10 weeks of age.

Chemicals and antibodies. 2,4-dinitrofluorobenzene (DNFB) and 4 ethoxymethylene-2-phenyl-2-oxazolin-5-one (oxazoline) were purchased from Sigma Chemical Co. (St. Louis, MO). Rat anti-mouse Thy-1.2 (clone 5a-8) and Low-Tox M rabbit complement were obtained from Cedarlane Laboratories Ltd. (Hornby, Ontario, Canada). Goat anti-rat IgG was purchased from Kirkegaard and Perry Laboratories, Inc. (Gaithersburg, MD). Rat anti-mouse CD4 (clone CT-CD4) and anti-CD8a (clone CT-CD8a) were purchased from Caltag laboratories (Burlingame, CA).
RESULTS

Persistence of immunosuppressive effects

Elmets and Bowen (1) found, using hematoporphyrin derivative (HPD), that sensitization and challenge at different times after PDT resulted in immunosuppression suggesting a prolonged effect. To determine if PII also manifests a prolonged effect, mice were injected with PII, and at later times photoirradiated and their CHS response evaluated. In addition, cutaneous photosensitivity studies were done to determine if any correlation exists between skin photosensitivity and immunosuppression. The results shown in Fig. 1a indicate that cutaneous photosensitivity drops off rapidly as seen by Bellnier and Dougherty (16). Figure 1b, however, indicates that the immunosuppressive effect did not diminish throughout the 21 day observation period. We have also assessed immunosuppression using the protocol of Elmets and Bowen (1) in which both sensitization and challenge are done at various times after PDT. The immunosuppressive effect was found to substantially increase if sensitization occurred greater than 3 days after PDT and extended through 28 days (unpublished). This finding is in agreement with our previous work (15) indicating that at least 3 days are needed between PDT and sensitization if immunosuppression is to become manifest using PII. These results indicate that not only is the PDT-induced immunosuppression long lived (\(\approx 21\) days) but also no correlation exists between cutaneous photosensitivity and immunosuppression.

Surface area studies

Having determined the persistence of the immunosuppressive effect, we next sought to determine if the suppression was correlated with the size of the photoirradiated area. Our standard protocol calls for photoirradiation of the shaved back, an area encompassing approximately \(2.54 \times 5.08\) cm\(^2\) (12.9 cm\(^2\)). To determine if any correlation exists, the shaved backs of mice were covered with aluminum foil in which rectangular areas of various sizes were cut out, followed by photoirradiation and assessment of the CHS response as described. As shown in Fig. 2, a surprisingly small area of exposure resulted in immunosuppression. Using the formula for surface area \(A = K \times W^{2/3}\) (17) and assuming a mouse
weight of 20 g and the constant \( K \approx 10 \) we get a total surface area of 72.2 cm\(^2\). The photoirradiated area (3.22 cm\(^2\)) at which suppression appeared is then approximately 4.45% of the total body surface. It is likely that both the dose of PII given and the time of photoirradiation after injection dictates the minimal area of photoirradiation necessary to elicit immunosuppression.

**Effect on the DTH response**

It is well known that dorsally applied UVB irradiation suppresses both the CHS and the DTH responses (18–20) presumably via different mechanisms. It was therefore of interest to determine if dorsal PDT did likewise. The results of the DTH studies, shown in Fig. 3, using allogeneic C3H/HeNCr MTV- spleen cells indicate no apparent effect of dorsal PDT on the DTH response \((P = 0.492)\).

**Adoptive transfer studies**

The immunosuppressive effect noted by Elmets and Bowen (1) was adaptively transferable suggesting the generation of suppressor cells. We therefore undertook a series of experiments to determine if (1) the PII-induced immunosuppres-

![Figure 1](image1.png)

**Figure 1.** Persistence of: (a) cutaneous photosensitivity; and (b) immunosuppression in PII-injected mice. Mice received an i.v. injection of 10 mg/kg PII on day 0 and at various times after injection received either irradiation to the right ear (a) or dorsal photoirradiation (b) followed by an evaluation of either cutaneous photosensitivity or immunosuppression. The points represent the mean ± SD of five mice evaluated at the indicated times. The (+) control was both sensitized and challenged and the (-) control challenged only. Light controls received light but no drug. Dark controls received drug but no light.

![Figure 2](image2.png)

**Figure 2.** Effect of surface area photoirradiated on immunosuppression. Mice were injected with PII and 6 h later photoirradiated on their backs which were covered with aluminum foil containing rectangular cutouts of different dimensions. The CHS response was then evaluated as described. Values are the mean ± SD of five mice per group. The (+) control was both sensitized and challenged. The (-) control was challenged only. The numbers in brackets represent the % suppression relative to the (+) control.

![Figure 3](image3.png)

**Figure 3.** Effect of dorsal PDT on the DTH response. Photoirradiated and/or immunized mice were injected with \( 10^5 \) allogeneic spleen cells into the right footpad following a preliminary measurement of thickness. Twenty-four hours later the footpad thickness was again measured. The results are the means ± SD of three experiments using 4–5 mice per group. (--): not immunized; PDT-treated = PDT + immunization; untreated = immunized only.
the mice were subsequently sensitized and challenged with DNB-F. Conversely, sensitization and challenge with oxazalone elicited suppression. A similar situation was noted using photoreirradiated/DNB-F spleen cells followed by oxazalone sensitization and challenge. These results indicate that the observed immunosuppression is clearly antigen specific.

We next attempted to determine what cell type might be responsible for the immunosuppression. Various spleen cell fractionation procedures such as nylon wool columns (T cell), sephadex G-10 chromatography (macrophage) and panning (B cell) did not result in sufficient enrichment of the desired cell types as determined by FACS. These preliminary studies did, however, indicate that T cells were probably responsible. Because of this observation and the large number of cells (≈50 × 10⁶) required for adoptive transfer, it was thought that negative selection using complement depletion of targeted populations would be more efficacious than magnetic separation. T cells were subsequently depleted using anti-Thy-1.2 antibody and rabbit complement and the remaining cells adoptively transferred. The results, Fig. 6, show that transfer of spleen cells lacking the T-cell population results in very little suppression, indicating that T cells are responsible. We next attempted to determine which T-cell subset (CD4⁺ or CD8⁺) might be responsible. CD4⁺ or CD8⁺ cells were removed by panning as described and the remaining cells adoptively transferred. As seen in Fig. 7, depletion of CD4⁺ cells negates the immunosuppressive effect indicating that (1) CD4⁺ cells are responsible for the suppression; and (2) CD8⁺ cells are the effector cell in the CHS response as previously found (21,22). The slightly

![Graph](image-url)
greater suppression noted in the −CD8− panel probably reflects the fact that not only are the suppressive CD4+ cells present but also that the CD8+ effector cell has been removed.

**DISCUSSION**

Bellnier and Dougherty (16) found that cutaneous photosensitivity when using PII, as measured using the murine ear swelling response, decreased rapidly with time after injection and concluded it was directly related to the PII concentration in the blood. It was therefore of interest to determine if any correlation exists between cutaneous photosensitivity and immunosuppression. The results clearly indicate no correlation exists and that immunosuppressive effects might well extend past the 21 day observation period. Bellnier et al. (23) also found PII to be retained in most tissues, including skin, for at least 75 days after injection and it is likely that this is responsible for the immunosuppression.

The relationship between photoirradiated surface area and immunosuppression proved surprising. It was anticipated that minimal immunosuppressive effects would be elicited if an area encompassing much less than the total back (≈18% of total body surface area) was photoirradiated and clearly this was not the case. It should be noted that our protocol involved a rather large dose (10 mg/kg) of drug and photoirradiation when skin values were highest. It would be of interest to determine if such a small photoirradiated area could elicit immunosuppression at longer time periods when the drug concentration in the skin is low.

Previous observations (9), using peritoneal PDT with HPD, indicating suppression of the CHS response and prolonged skin allograft survival in PDT-treated recipients suggested that in our system a DTH response might also be inhibited. The results, however, indicated that this is not the case and probably reflect differing mechanisms of immunosuppression caused by the different sites of photoirradiation and/or the use of differing light sources. Our findings are also unlike UVB photoirradiation which exerts suppressive effects on both the CHS and the DTH responses (18). Elmet and Bowen (1) noted their immunosuppression was adoptively transferable. Lynch et al. (3) not only found similar results but also found it was not antigen specific and concluded it was macrophage mediated. UVB photoirradiation is known to result in the generation of an antigen-specific T-cell suppressor population having a CD3+, CD4+, CD8− phenotype (19,20). Since UVB photoirradiation and dorsal PDT are perhaps more similar than is dorsal PDT and peritoneal PDT it was of interest to determine if the immunosuppression induced by dorsal PDT mimicked UVB-induced immunosuppression. The results of the adoptive transfer studies indicated that an antigen-specific suppression mediated by a CD4+ T cell was present which is similar to that found with UVB.

Regarding peritoneal PDT, other work in which a laser was used for flank photoirradiation resulted in our ability to adoptively transfer suppression using DNFB as the sensitizer, but our inability to transfer it when using either oxazolone or FITC as a sensitizer even though immunosuppression was evident in the photoirradiated donor. As a consequence, we were unable to definitively establish antigen specificity. Moreover, the suppression noted when using DNFB was T-cell mediated (D. Musser, unpublished). These results differ appreciably from the observations of Lynch et al. (3) and reaffirm the relationship between the site of photoirradiation and the characteristics of the induced immunosuppression.

The exact role of the T cell in the CHS response is controversial. Traditionally it has been thought that the CHS response was mediated by CD4+ cells and downregulated by CD8− cells. This concept was derived from a series of experiments in which *in vivo* depletion of CD4+, but not CD8− T cells inhibited the CHS response to DNFB (24,25). It was also observed that *in vitro* depletion of CD4− but not CD8− cells from lymph nodes of sensitized mice resulted in a loss in the ability to transfer CHS (26) and lastly, CD4 knockout mice mount a reduced CHS response (27). Conversely, other studies suggest that CD8+ T cells are the effector cells in the CHS response. *In vivo* depletion of CD8− cells resulted in a reduction of the CHS responses to DNFB while depletion of CD4− cells led to an exaggerated CHS response to DNFB (21). More recently, Wang et al. (28) using both CD4+ and CD8− knockout mice found that mice lacking either phenotype exhibited a reduced CHS response. Subsequent *in vivo* depletion of CD4+ cells in the CD8− knockout or CD8+ cells in the CD4− knockout abolished the CHS response suggesting that both cell types function as effector cells. Our results, however, suggest that the effector cell is CD8−.

UVB irradiation can result in suppression of both the DTH and the CHS response (18–20) and in this regard differs from the results found in this study. One proposed mechanism of UV-induced suppression is that of an alteration of the antigen-presenting cell (APC). Both Grewe et al. (29) and Rivas and Ulrich (30) observed increased IL-10 production in UV-irradiated cultured keratinocytes. Rivas and Ulrich (30) subsequently found that injection into mice of the keratinocyte supernatants led to suppression of the DTH response. Additional studies by Ulrich (31) suggest that the secreted IL-10 can act on the APC resulting in its ability to present antigen to Th2 cells but an inability to present antigen to Th1 cells. A similar effect on the APCs following dorsal PDT does not appear to be present since splenic APCs from PDT-treated mice can present antigen equally well to both Th1 and Th2 cells (S. Gollnick, unpublished).

The nature of the T suppressor cell in PDT-induced immunosuppression is presently unknown but several possibilities exist. *In vitro* experiments indicate that T cells rendered anergic can act as suppressor cells that manifest their effect through APCs (32–34). This may take the form of competition for ligand or for cytokines such as IL-2 secreted by the APC. (33,34). Other proposed mechanisms are the modulation of the T-cell activating capacity of the APC or the inhibition of T cells recognizing their ligand in close proximity to a suppressor cell attached to the same APC (33).

It is also possible that the suppression is caused by a T regulatory (Tr) cell. Tr cells, a small subset of the T-cell population, have the ability to modify an immune response by affecting the activation of other T cells. Recent work indicates that IL-10 secreted by NKT cells plays a major role in both the generation of the Tr cell population and as its effector (35–39). It is conceivable that the IL-10 secreted by UVB irradiated keratinocytes might induce the expansion...
of a population of T regulatory cells via NKT cells and with their subsequent secretion of an immunosuppressive cytokine diminish Th1 responses. In support of such a possibility is a recent publication by Moodycliffe et al. (40) identifying the UV-induced suppressor T cell of DTH as a CD4+ natural killer T (NK1.1) IL-4 secreting cell which may by either direct or indirect means (Tr cells) cause immunosuppression. We have, however, not found IL-10 to play a major role in cutaneous PDT-induced immunosuppression suggesting that other mechanism(s) or cell types might be involved (S. Gollnick, unpublished).

In conclusion, the immunosuppression mediated by dorsal PDT using PII as a sensitizer was found to be long lived, requiring photoreirradiation to a much smaller surface area than anticipated for its development. It resembles the immunosuppression caused by UVB at the cellular level in that (1) it is adoptively transferable; (2) it is antigen specific; and (3) it is CD4+ T-cell mediated. However, at the subcellular level, substantial differences between UVB and dorsal PDT-induced immunosuppression have been found (D. Musser, unpublished). It differs principally from UVB-induced immunosuppression at the cellular level in that the DTH response is not suppressed. This might be considered fortuitous since an appreciable downregulation of the host’s response to tumor antigens which might be exposed/released during PDT would not then be expected. However, the use of allogeneic cells elicits a very strong antigenic response that might overwhelm the immunosuppressive effect. This may not be the case with weakly antigenic tumor antigens. Attempts to clarify the role of immunosuppression in the overall response to PDT are ongoing.

Acknowledgements—This work was supported by the Roswell Park Cancer Center Support Grant P30 CA 16056 and NIH Grant PO1 CA55791.

REFERENCES
Shahid, G. M. Shivji, T. W. Mak and D. N. Sauder (2000) CD4+ Th1 and CD8+ Type 1 cytotoxic T cells both play a crucial role in the full development of contact hypersensitivity. J. Immunol. 165, 6783–6790.


