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Antigen specific and nonspecific modulation of the immune response by aminolevulinic acid based photodynamic therapy

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Abstract

Photosensitizers used normally in treating cancers have considerable potential for treatment of other diseases. One such photosensitizer is the endogenously synthesized photosensitizer protoporphyrin IX (PpIX). To better understand how protoporphyrin might be used in transplantation or in treating autoimmune diseases, information must be obtained on how the photosensitizer affects all immune cells. We used a combination of flow cytometry and in vitro activation assays (recall assays and mixed-lymphocyte reactions) to examine the effects of PpIX on the antigen specific component, lymphocytes and the non-antigen specific component, the macrophages/monocytes and dendritic cells of the immune system. Whereas, lymphocytes accumulate PpIX only when activated, both macrophages and dendritic cells accumulated PpIX immediately, without in vitro activation, as measured by flow cytometry. ALA–PDT (aminolevulenic acid–photodynamic therapy) treated adherent cells in the recall assay had a decreased capability to activate lymphocytes. By increasing the light dose in the recall assay, antigen primed lymphocytes were selectively eliminated from a population of cells. Stimulator cells in an MLR had a decreased stimulatory capacity following ALA–PDT treatment. Functional alterations are seen in both the antigen specific and nonspecific immune components.

Keywords: Aminolevulinic acid (ALA); Antigen presenting cells; Dendritic cells; Immunomodulation; Photodynamic therapy (PDT); Human; Mixed lymphocyte reaction (MLR)

1. Introduction

Research with photosensitizers has shown that activated lymphocytes can be targeted following photodynamic therapy (PDT) treatment (North et al., 1993; Rittenhouse-Diakun et al., 1995; Hryhorenko

Abbreviations: δ-Aminolevulinic acid, ALA; *R*-phycoerythrin-cyanine 5 conjugated goat anti-mouse, TC-GAM; One-way-mixed lymphocyte reaction, MLR; Peripheral blood mononuclear cells, PBMC; Photodynamic therapy, PDT; Phytohemagglutinin, PHA; Protoporphyrin IX, PpIX

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et al., 1998). The ability of immune cells to accumulate photosensitizers has expanded the utility of PDT to areas other than cancer therapy. Studies using PDT for immunological diseases included treatment of adjuvant-enhanced arthritis (Ratkay et al., 1994; Chowdhary et al., 1994) and treatment of experimental autoimmune encephalomyelitis (Leong et al., 1996). Although the foundations are being laid to advance the clinical usefulness of PpIX-PDT in immunotherapy additional studies must be performed to determine how the therapy affects antigen specific and nonspecific immune functions.

The photosensitizer, protoporphyrin IX (PpIX), is an endogenously synthesized photosensitizer produced as a precursor to heme in the heme synthesis pathway. The pathway is normally negatively regulated by negative feedback of heme on the enzyme aminolevulinic synthase but can be bypassed by the addition of exogenous aminolevulinic acid (ALA). Lymphocytes, the antigen specific cells of the immune system, have been shown to accumulate PpIX when activated. In addition antigen activated, but not resting lymphocytes were shown to be killed by ALA–PDT treatment (Hryhorenko et al., 1998).

Antigen presenting cells (APC) such as macrophages/monocytes, and dendritic cells are an integral part of most immune responses. They process and present antigen and provide important costimulatory signals for the generation of an efficient immune response. Research using the photosensitizer hematoporphyrin derivative (HpD) has shown that peritoneal application of HpD–PDT elevated the phagocytic potential of mouse macrophages (Qin et al., 1993). Experiments using dihematoporphyrin ether (DHE) on human monocytes have shown that following PDT treatment FcγRI receptors (responsible for binding the Fc region of antibodies) are structurally altered rather than lost from the cell surface (Krutmann et al., 1989). Murine skin transplantation studies following benzoporphyrin derivative (BPD) and low dose PDT significantly extended time to allograft rejection (Obochi et al., 1997). In the latter studies it was determined that Langerhans cells were not eliminated from the graft, but their cell surface receptors such as the major histocompatibility complex and costimulatory B7 molecules were reduced. How PpIX affects antigen presenting cells is relatively unknown.

We examined the ability of macrophages and dendritic cells to accumulate PpIX by flow cytometry. The stimulatory capacity of these cells following ALA-based PDT was assessed by recall assays and mixed lymphocyte reactions. The mixed lymphocyte reaction determines the level of alloreactivity between recipient and donor skin transplants. Van Voorhis et al. showed that both macrophages and dendritic cells have important stimulatory roles in an MLR with dendritic cells being 10 times more stimulatory then macrophages (Van Voorhis et al., 1982). MHC class II antigens on the donor APCs are the antigens to which recipient lymphocytes respond. The MLR is not a 'classical' immunological response in that processing of antigens is not required (although processing can occur in an MLR response). Recipient T cells can respond to allogenic donor MHC class II antigen directly without presentation of antigen by self MHC and become activated (Paul, 1993; Wright-Browne et al., 1997). The results show that PpIX accumulates differently in APC compared to lymphocytes.

By elucidating the effects of PpIX–PDT on APC function(s) a clearer understanding of how this therapy affects immune responses can be gained. Such information may help to optimize the role of host immune responses in treatment of malignancies, and also may provide a basis for PpIX–PDT in the treatment of autoimmune diseases or organ transplants.

2. Materials and methods

2.1. Cells and media

Human peripheral blood mononuclear cells (PBMC) were isolated using density centrifugation over a Ficoll–Paque gradient (Pharmacia, Piscataway, NJ). Following two washes of the buffy coat (250 g, 5 min) with sterile PBS (pH 7.2, without Ca^{2+} and Mg^{2+}) the PBMC were counted and resuspended in RPMI 1640 (Life Technologies, Grand Island, NY) containing 10% heat inactivated pooled AB human serum (Pel-Freez, Brown Deer, WI), supplemented with 2.0 mM L-glutamine and 0.1 mM non-essential amino acids (Life Technologies, Grand Island, NY)(10% medium). Assays that incor-

porated PDT treatments used 1% serum during ALA incubations (1% medium). Cells were incubated at 37°C with 5% CO_2 unless stated otherwise.

2.2. ALA and light treatment

Prior to incubation with ALA, PBMC were washed twice with sterile PBS (250 g, 5 min) and resuspended in 1% medium. Cells were incubated with 1.0 mM ALA (Sigma, St. Louis, MO) for 4 h in a 37°C incubator. ALA treated cells were irradiated with either 20 or 50 J/cm^2 of white light (400–700 nm) from a light table fitted with two 500 W quartz halogen bulbs (K mart) and infrared and dichroic filters.

2.3. Surface staining

The staining procedure was a modification of that used by Stewart and Stewart (Stewart and Stewart, 1994a,b); all steps were carried out in dim light to avoid photobleaching the PpIX. In brief, following ALA incubation, PBMC were washed twice with PBS and cellular Fc receptors were blocked with mouse IgG (10 microliters of a 1 mg/ml, Caltag, San Francisco, CA). The cells were incubated with antibodies to monocytes/macrophages, CD14-TC (Caltag), or peripheral blood dendritic cells, HB-15a (CD83 a IgG_{2b} antibody kindly provided by Dr. Tom Tedder; Zhou et al., 1992; Zhou and Tedder, 1995a,b, 1996) for 15 min at 4°C. A secondary antibody with a TRI-COLOR (TC) (*R*-phycoerythrin-cyanine 5) conjugate (TC labeled-goat anti-mouse [TC-GAM], Caltag) was used to detect the antibody to CD83. Experiments performed using a secondary labeled goat anti-mouse reagent used goat IgG as a Fc receptor block (10 microliters of a 1 mg/ml, Caltag). Following staining, cells were washed, fixed in 1% ultrapure formaldehyde in PBS and immediately analyzed using a flow cytometer modified FACScan [model FC](Becton Dickinson Immunocytometry Systems [BDIS], San Jose, CA). Ten thousand cells (events) were analyzed per sample. Isotype and secondary antibody alone controls (Caltag) were run to determine levels of nonspecific binding, which were subtracted from the samples. Data were acquired as described previously (Hryhorenko et al., 1998), using LYSYS II software (BDIS). The excitation light (argon laser 15 mW) was 488 nm. Bandpass filters used to detect fluorescence were as follows: FL1 (FITC), 530 ± 15 nm; FL3 (TC), 680 ± 10 nm; and FL4 (PpIX), 630 ± 11 nm.

2.4. Tritiated thymidine measurements

Proliferation of lymphocytes was measured by tritiated thymidine incorporation assays. Eighteen hours before the completion of an experiment, 20 μl of medium containing 1 μCi tritiated thymidine (ICN) was added to each well. Background wells consisted of medium in the absence of cells. Eighteen hours later cells were harvested using a Skatron semiautomated cell harvester (Skatron Ins., Sterling, VA). Filters containing the radiolabeled samples were transferred to scintillation tubes (Skatron) followed by the addition of 3.0 ml of Ecoscint A (National Diagnostics, Atlanta, GA). Counts per minute (cpm) were determined using a Packard liquid scintillation counter (Packard, Downers Grove, IL).

2.5. Separation of cells

Adherent cells were separated from nonadherent cells on six well plates (Linbro, Hamden, CT). Plates were incubated with 1.0 ml of 10% medium in each well at 37°C for 1 h. Following the incubation, 1.0 ml of 1×10^7 cells/ml was added to each well and incubated for 1 h at 37°C. Nonadherent cells were recovered from the wells by tilting the plates and gently aspirating the cells. The plates were washed to remove additional nonadherent cells by gently pipetting medium over the wells. Following a 10 min incubation with EDTA treated PBS (0.02g EDTA/100 ml PBS) adherent cells were removed using a plastic scraper (Falcon, Lincoln Par, NJ), then washed to remove the EDTA. Adherent cells used in the antigen recall assay were irradiated with 4000 rads of gamma irradiation (^{137}Cesium). Adherent cells and nonadherent cells used in the MLR were treated with ALA and light, controls were untreated. Following PDT treatment cells were washed with PBS, centrifuged ($250 \times g$, 5 min), and resuspended in 10% medium at 1×10^6 cells/ml. Both adherent and nonadherent populations of cells were exposed to 4000 rads to inactivate contaminating lymphocytes and added (in various combinations in a 1:1

ratio with responder cells) at a total amount of 1×10^5 cells/well to the MLR (see below).

2.6. Antigen recall assay

PBMC from healthy donors were plated in multiples of 12 for each sample in 96 well round bottom plates (Falcon) at a concentration of 2.5×10^5 cells/well. Tetanus toxoid (Connaught) was added at a final dilution of 1:500 (previously determined to be optimal for activation). Total volume including cells and antigen was 200 μl per well. Plates were incubated for 4 days, after which 100 μl per well of medium was carefully removed and discarded. Added back to the wells was 100 μl of fresh 10% medium, tetanus antigen and 1 unit human recombinant IL-2 (Life Technologies), controls received no IL-2. Addition of 1 unit of IL-2 permitted longer incubations of the lymphocytes without being mitogenic to the cells (Mookerjee and Pauly, 1990). Plates were incubated for 2 additional days and then treated with ALA and light (see Section 2.2). Control samples consisted of cells with light only and cells incubated with ALA but no light.

Following treatment, plates were washed with PBS, and fresh medium was added for recall stimulation. Fresh medium consisted of medium alone, medium containing 1 unit of IL-2, or medium containing both IL-2 and either tetanus antigen (1:500) or mumps skin test antigen (Connaught, Swiftwater, PA), 1:1000 dilution of mumps antigen was previously found to be optimal for activation. Plates were incubated for 6 days, with a change of medium (100 μl replaced by 100 μl of fresh medium) occurring half way through the incubation (day 9). Proliferation was measured using a tritiated thymidine uptake assay (see above). In some experiments gamma irradiated adherent cells (1×10^5 cells/well) were added following ALA–PDT treatment of cells. Adherent cells were isolated as described above.

2.7. Mixed lymphocyte reaction

PBMC were isolated from two healthy donors as described above. Cells designated as responders were resuspended in 10% medium at 1×10^6 cells/ml. 100 μl aliquots were transferred to 96 well round bottom plates in replicates of six. Stimulators were resuspended to 5×10^6 cells/ml in 1% medium and plated in 6 well plates. Wells were then treated with ALA (1 mM) or medium (controls), incubated for 4 h and treated with light. Stimulators were then washed, gamma irradiated and resuspended to 1×10^6 cells/ml in 10% medium. 100 μl aliquots were added to the responders already in the 96 well plates. Plates were incubated for 6 days and proliferation measured by the tritiated thymidine uptake assay. In the experiments in which adherent and nonadherent cells were separated prior to use as stimulators, 50 μl of each (50 μl adherent + 50 μl nonadherent) cell type (0.5×10^5/50 μl) was added to the responders (1.0×10^5/well) in the 96 well plates.

3. Results

3.1. The endogenously synthesized photosensitizer, PpIX, accumulates in CD14+ and CD83+ human peripheral blood cells

To determine if antigen presenting cells, monocytes and dendritic cells, accumulated the photosensitizer, human peripheral blood cells were isolated from healthy donors and incubated immediately with aminolevulinic acid (ALA). Using flow cytometry, freshly isolated monocytes (CD14+) and dendritic cells (CD83+) were shown to accumulate PpIX following the 4 h incubation (Fig. 1). Approximately 98% of CD14+ (B) and 83% of CD83+ cells (D) were PpIX+ ($N = 3$). These results show that monocytes and dendritic cells behave differently from previous studies on lymphocytes. Lymphocytes must be activated to accumulate PpIX (Hryhorenko et al., 1998), whereas monocytes and dendritic cells accumulate the photosensitizer without requiring in vitro activation.

3.2. Adherent antigen presenting cells are affected by ALA–PDT treatment

To examine the effects of ALA-based photodynamic therapy on antigen presenting cells and lymphocytes a recall assay using tetanus and mumps antigens was performed. The recall assay measures antigen specific and nonspecific responses by measuring the degree of lymphocyte proliferation after

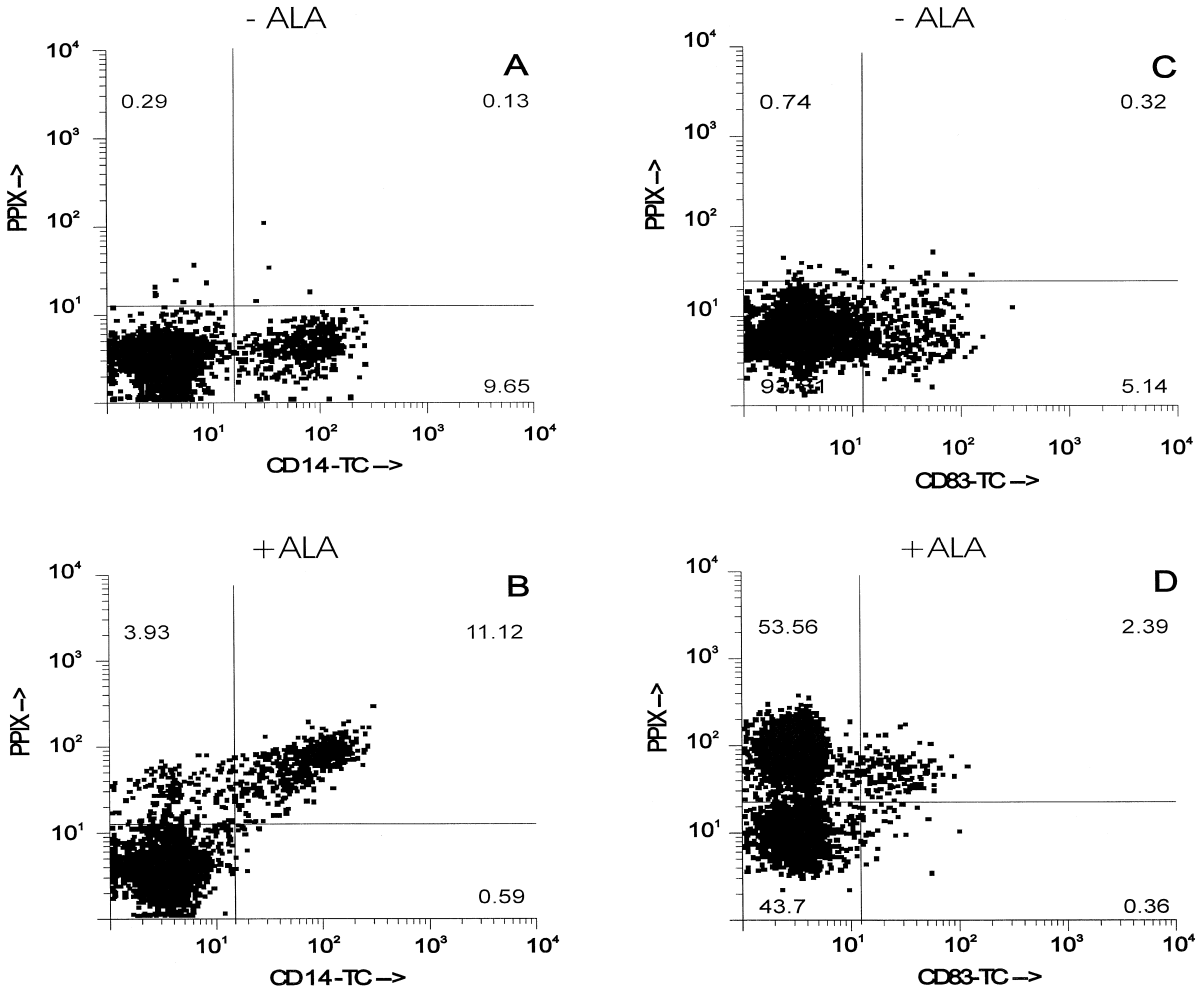

Fig. 1. PpIX accumulation in macrophages (CD14 +) and dendritic cells (CD83 +). Human peripheral blood mononuclear cells were isolated from whole blood. Samples were immediately incubated with (B, D) or without ALA (A, C)(1 mM, 4 h). Monocytes/macrophages were stained with anti-CD14 TC (A, B) and dendritic cells were stained with anti-CD83 (C, D) (TC-GAM was used as a secondary antibody to anti-CD83). Flow cytometry was used to analyzed fluorochrome fluorescence as well as PpIX fluorescence, 10,000 events were taken for each sample. The numbers in the corners are percent cells in a particular region. The dot plots shown are representative of a typical experiment.

initial stimulation of lymphocytes by an antigen followed by rechallenge with the same antigen or a disparate antigen. In our experiments, PBMC from healthy donors activated by the tetanus antigen (day 0) were treated with ALA–PDT 6 days after activation (1 mM ALA; 20 J/cm^2). Tetanus or mumps antigen was then used to restimulate the surviving cells. A lack of stimulation to the tetanus antigen, but stimulation to the mumps antigen would be indicative of a specific effect. In a purely T cell (antigen specific) response only tetanus activated T cells should have been eliminated in the ALA–PDT treatment leaving cells which respond to the mumps antigen. Conversely, a lack of proliferation to both antigens may indicate a nonspecific loss of function of the APC. The results in Fig. 2A and B, illustrate that following rechallenge there was a significant decrease in response to both antigens ($* p < 0.05$). The significant decrease in response to both antigens indicates an inability of the treated macrophages or

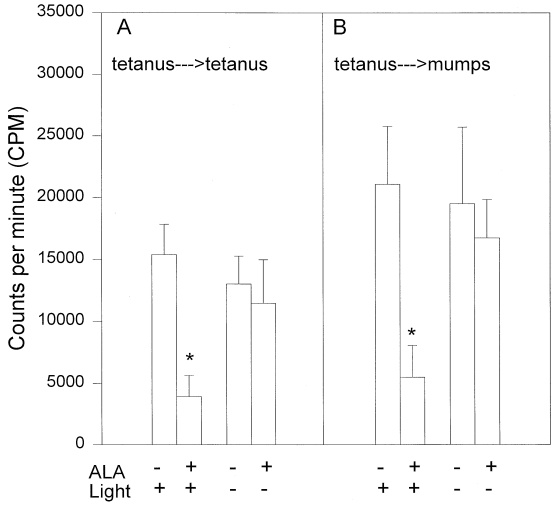

Fig. 2. Nonspecific effects of ALA–PDT in the antigen recall assay (20 J/cm^2). Peripheral blood cells from healthy human donors were activated in vitro using the tetanus antigen. Six days later samples were treated ± ALA (1 mM) ± light (20 J/cm^2) and then restimulated with either tetanus (A) or mumps antigen (B). Lymphocyte proliferation was measured 6 days later with a tritiated thymidine uptake assay. (* $p < 0.05$) samples that are significantly different (Student's *t*-test) from controls (cells without ALA treatment). One representative experiment is shown from a total of three analyzed.

dendritic cells to properly present antigens to T cells. The decrease in proliferation (Fig. 2) is also light dependent since cells treated with ALA but not exposed to light were not significantly different from controls (cells not treated with ALA).

3.3. Nontreated adherent cells restore the ability of lymphocytes to proliferate to antigens

To show that the antigen presenting cells were affected by ALA–PDT, the recall experiment was repeated with fresh non-ALA treated but gamma irradiated adherent cells added following ALA–PDT treatment and rechallenged with antigen. Controls consisting of gamma irradiated adherent cells alone, were not stimulated by tetanus or the mumps antigen (data not shown), thus any proliferative response was due to T cells. The results (Fig. 3A and B) show that following addition of adherent cells, lymphocytes were able to respond well to the mumps antigen (Fig. 3B), but not to the tetanus antigen (Fig. 3A)(20 J/cm^2, 1 mM ALA). The tetanus stimulated ALA–PDT treated cells challenged with mumps (Fig. 3B) had proliferation values which reached control levels. Thus, the additional adherent cells restored the ability of lymphocytes to respond to mumps. The tetanus activated cells rechallenged with tetanus (Fig. 3A) had a significant ($p = 0.015$) decrease in proliferation compared to control levels, presumably due to the elimination of tetanus activated lymphocytes

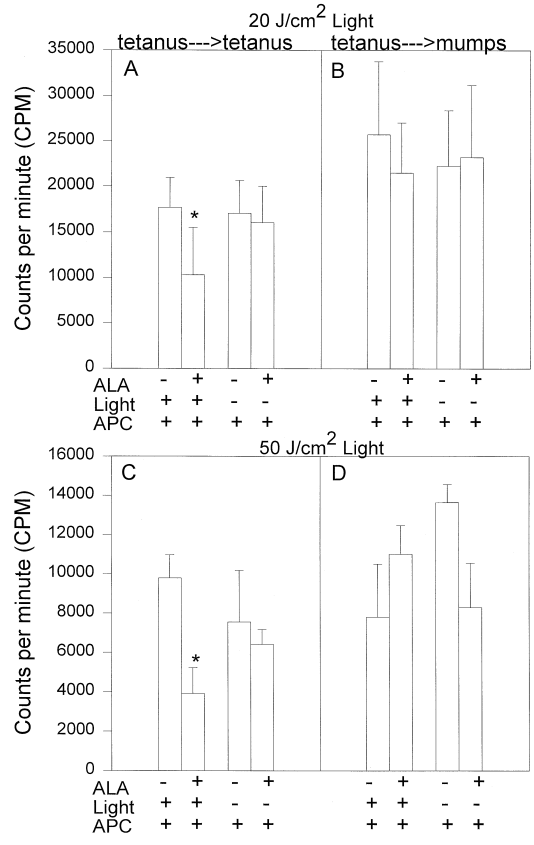

Fig. 3. APC add back eliminates nonspecific effects of ALA–PDT. Human peripheral blood mononuclear cells were activated by the antigen tetanus. Six days later cells were treated ± ALA (1 mM) ± light (20 J/cm^2) and restimulated with tetanus (A) or mumps (B). Gamma irradiated adherent cells (+APC) were then added (1×10^5/well) and cells incubated for 6 days. Tetanus activated cells were treated ± ALA (1 mM) ± light (50 J/cm^2) and restimulated with tetanus (C) or mumps (D). Following addition of gamma irradiated adherent cells plates were incubated for 6 days. Lymphocyte proliferation for all assays were measured by a tritiated thymidine uptake assay. (* $p < 0.05$) samples that are significantly different (Student's *t*-test) from controls (similarly activated cells without ALA treatment). All experiments were repeated three times. One representative experiment is shown.

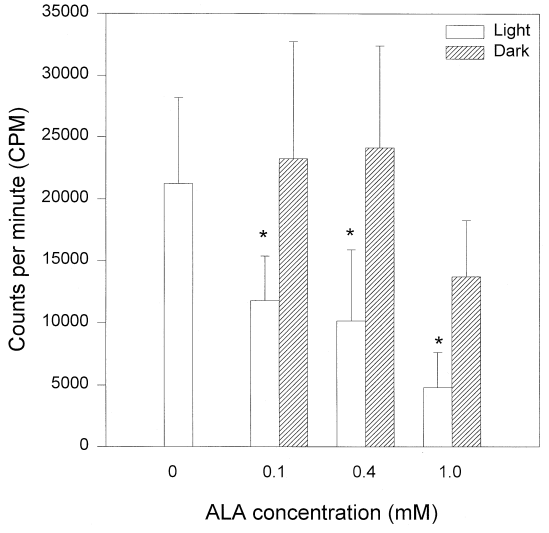

Fig. 4. ALA–PDT treated cells do not provide efficient stimulation in a MLR. Human peripheral blood mononuclear cells were isolated from two healthy donors. Cells designated as stimulators were gamma irradiated as well as treated ± ALA (0.1, 0.4, 1 mM) + light. Stimulators were then added to responders in a 1:1 ratio and cells incubated for 6 days. Lymphocyte proliferation was measured by a tritiated thymidine uptake assay. ($* p < 0.05$) samples that are significantly different (Student's *t*-test) from controls (similarly activated cells without ALA treatment).

in the initial challenge by ALA–PDT. The data in Figs. 2 and 3 suggest that activated lymphocytes were specifically eliminated by the ALA–PDT treatment.

To confirm and strengthen the observation of a decreased proliferation of specifically activated lymphocytes after ALA–PDT treatment, the light dose was increased to 50 J/cm^2 (1 mM ALA). We hypothesized that the increased light dose should decrease the response of the ALA–PDT treated tetanus activated cells to tetanus but should have no effect on the response to mumps antigen resulting in an increased antigen specific effect. Fig. 3C and D, shows a significant ($p < 0.05$) decrease in the proliferation of ALA–PDT treated cells to tetanus antigen (Fig. 3C) after 50 J/cm^2 of light. The cells that were restimulated with mumps (Fig. 3D) gave proliferation values similar to controls. These data (Figs. 2–4) indicated that both the antigen specific (T lymphocytes) as well as nonantigen specific (APC) cell populations were affected by the ALA–PDT treatments.

3.4. APC effects measured in an MLR: changes in stimulatory capacity

To further explore the effects of ALA–PDT on antigen presenting cells ALA–PDT treatment of *stimulator cells* was performed prior to adding them to recipient lymphocytes in a one-way-MLR. A mixed lymphocyte reaction was chosen for the experiment because of the importance of APC in the reaction. If APCs were affected by ALA–PDT a decreased proliferative response to the alloantigens would be expected. Stimulator cells were incubated with various concentrations of ALA and then treated with 20 J/cm^2 of light. The results (Fig. 4) illustrate that following ALA (0.1, 0.4, and 1.0 mM) based PDT there was a significant decrease in the ability of stimulators to activate responders, compared to untreated controls (no ALA). In addition, as previously shown in Figs. 2 and 3, in the absence of light there was no significant difference from controls.

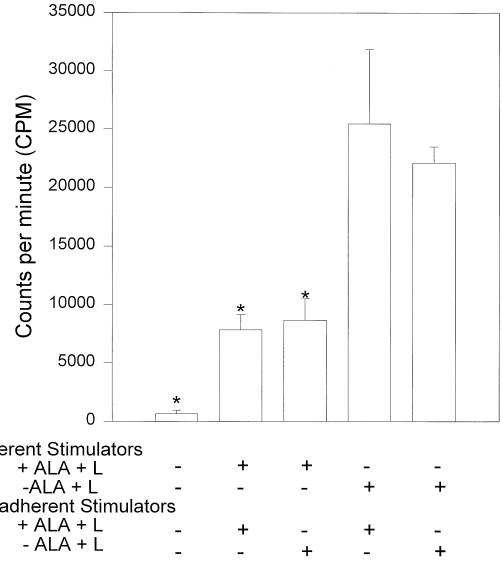

Fig. 5. ALA–PDT effect on stimulators are due to effects on adherent cells. Human PBMC were isolated from two healthy donors. Cells designated as stimulators were separated into adherent and nonadherent cells followed by treatment ± ALA (1 mM) + light. Various combinations of treated adherent and nonadherent cells were combined and then added to the responders. Proliferation was measured 6 days later using a tritiated thymidine incorporation assay. ($* p < 0.05$) samples that are significantly different (students t test) from controls (similarly activated cells without ALA treatment).

3.5. Partial characterization of treated stimulators in an MLR

To determine which population was responsible for the decrease in stimulatory capacity, MLR experiments were repeated, except that the stimulator cells were separated into nonadherent and adherent cell populations and added to responders in various combinations (Fig. 5). Only samples that contained ALA–PDT altered adherent cells had a significant decrease in proliferation. This indicated that the adherent and not nonadherent cells were stimulators in the MLR and contributed to the decrease in stimulatory capacity.

4. Discussion

The experiments performed show that macrophages and dendritic cells accumulate the photosensitizer, PpIX, without 'activation' and are functionally altered by ALA–PDT. The ability of these cells to accumulate a photosensitizer agrees with previous research with other photosensitizers used in PDT (Qin et al., 1993; Korbelik and Krosl, 1996a). Although phagocytosis was not measured in our experiments, the APC do appear to have a decreased potential to present antigens to lymphocytes following ALA–PDT. The significance of the above studies is to help to clarify the mechanism of ALA based PDT effects on an immune response.

The ability of macrophages and dendritic cells to consistently accumulate PpIX contrasts with the requirement for activation of lymphocytes (PHA and MLR) before significant accumulation of PpIX (Rittenhouse-Diakun et al., 1995; Hryhorenko et al., 1998). The CD14 + and CD83 + cells analyzed in Fig. 1 were examined by flow cytometry after being removed from human donors only 6 h earlier. These cells were not activated although culturing monocytes on plastic has been shown to induce differentiation of cells into macrophages and this differentiation process might contribute to PpIX accumulation (Bodel et al., 1977; Hammerstrom, 1979; Newman et al., 1980). Dendritic cells can also proliferate in vitro following the addition of various cytokines (GM-CSF, IL-4, and TNF) (Romani et al., 1994; Thomas and Lipsky, 1996). However, cytokines were not added to these cultures so the dendritic cells appear to accumulate PpIX without in vitro activation.

In addition to accumulating PpIX the APC are functionally altered by PDT. In the recall experiments (Figs. 2 and 3) the antigen presenting qualities of the APC following ALA–PDT are altered as demonstrated by the decreased response of T cells to the antigens used (Fig. 2). However, adding back APC restored the T cell responsiveness (Fig. 3). One reason for the decreased ability of cells to present antigen may be due to alterations in MHC class II molecules. The MLR experiments, which rely heavily on the presence of *stimulator* MHC class II to stimulate responder lymphocytes, show decreased activation of responders following ALA–PDT treatment of stimulators (Figs. 4 and 5). The idea that MHC class II is affected by PDT is not novel. Grunner showed using hematoporphyrin derivative (HpD) plus visible light decreases HLA-DR positive cells 8 h after treatment (Grunner et al., 1986). Obochi et al. revealed that following low doses of benzoporphyrin derivative (BpD) and light, expression of surface MHC class II and class I molecules as well as costimulatory B7 molecules were decreased on murine Langerhans cells (Obochi et al., 1997). In addition, researchers have shown that UVA has an immunomodulatory effect upon APCs by decreasing costimulatory, adhesion and MHC molecule expression (Ullrich, 1995; Strickland and Kripke, 1997). Experiments are currently underway which examine the change of MHC class I and II expression on APCs following ALA–PDT treatment. The data which we obtained on whole adherent cells is indicative of the response of macrophage and dendritic cells. However, this population may also contain other cell types, so further experiments are being performed on cultured dendritic cells, as well as flow purified macrophages to confirm these studies. Experiments with cultured or flow sorted cells may involve cellular changes which can occur during culture and sorting, and these changes may affect the results, so both the experiments performed herein and future experiments on more purified cell populations are necessary to add to our understanding of the effects of ALA–PDT on macrophages and dendritic cells.

Down regulation of APC function by PDT does not occur in all systems. Yamamoto et al. working

with haematoporphyrin derivative (HPD) see enhancement of Fc receptor mediated phagocytosis with small amounts of HPD and light. This increased activation, was attributed by the authors to singlet oxygen peroxidation of lymphocyte membranes and subsequent activation of the macrophages. Substantiation for their theory included experiments which showed that the singlet oxygen quencher DABCO could block the photodynamic activation of the macrophages (Yamamoto et al., 1991).

We have shown that an antigen specific population was affected by ALA–PDT. Treatment of tetanus activated lymphocytes with ALA (1 mM) and 50 J/cm^2 of white light caused a significant decrease in the response to a rechallenge with tetanus but not to the mumps antigen (Fig. 3). The reason for this was that the tetanus primed T cells were eliminated from the reaction by ALA–PDT whereas the nonactivated T cells were still able to respond to a different antigen. Previous studies from our laboratory (Hryhorenko et al., 1998) and others (North et al., 1993) demonstrated that activated lymphocytes could be destroyed in an antigen specific manner. These results indicate that provided activated immune cells accumulate photosensitizer, a specific immune response can be modulated by selectively eliminating activated cells without harming resting cells.

It is well known that host immune responses are critical to clear tumors after PDT. Korbelik et al. used an adoptive transfer model with SCID, nude, and BALB/c mice to illustrate the importance of the host lymphoid population in delaying tumor recurrence in EMT6 murine mammary sarcoma following Photofrin based PDT (Korbelik et al., 1996b). Elucidating the effects of ALA–PDT on APC functions and understanding the underlying mechanisms may help to optimize the roles of host immune responses in treating malignancies. In addition, the information may provide a basis for the use of ALA–PDT for treating autoimmune diseases or organ transplants.

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