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Original Article

Photodynamic therapy induces interleukin secretion from dendritic cells

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Abstract: Dendritic cells (DC) pulsed with tumor-derived peptides, proteins, genes, or lysates have been studied as therapeutic cancer vaccines. However, the overall therapeutic efficacy of this approach has been limited, indicating a need to either enhance its potency or combine it with other treatment modalities. Photodynamic therapy (PDT) process consists of injecting a photosensitizer, which selectively accumulates at the lesion site, followed by local illumination of the tumor with a laser of the appropriate wavelength to activate the specific drug. PDT has the potential to create an environment at the tumor site that favors both tumor antigen loading and activation of DCs, key requirements for induction of antitumor immunity. Here, we report that PDT can induce IL-1 and IL-6 and reduce TNF-α expression from DCs. This finding has potentially broad clinical implications since these changes are mechanistically involved in the observed effects of PDT on host immune responses. Not all tumors are amenable to PDT, either because of size or location, and one could conceive of an adjuvant use for PDT vaccines in conjunction with other cancer modalities that do not enhance the host antitumor immune response.

Keywords: Photodynamic therapy, dendritic cells, tumor immunity, interleukin

Introduction

Dendritic cells (DC) are the most potent antigen-presenting cells. DCs pulsed with tumor-derived peptides, proteins, genes, or lysates, as well as DCs fused with tumor cells, have been studied as therapeutic cancer vaccines [1-9]. Although the methods are complex and costly to implement, promising results have been obtained in clinical trials in patients with advanced malignancies. These trials have shown DC-based vaccination to be well tolerated and capable of inducing tumor-specific T-cell responses and regression of metastatic disease. On the other hand, the overall therapeutic efficacy of this approach has been limited, indicating a need to either enhance its potency or combine it with other treatment modalities. Among the modalities that might be combined with DC-based immunotherapy are systemically administered antitumor drugs as well as locally targeted therapies such as radiation, radiofrequency ablation, and photodynamic therapy (PDT).

PDT has been approved in many countries as an anticancer therapy, mainly for the palliative treatment of surgically inaccessible tumors. The PDT process consists of injecting a photosensitizer, which selectively accumulates at the lesion site, followed by local illumination of the tumor with a laser of the appropriate wavelength to activate the specific photosensitizer [10]. Irradiation with light of the proper wavelength can lead to two outcomes. First, activation of the photosensitizer can transform the drug from its ground state to an excited singlet state; from this state, the drug may decay directly back to the ground state by emitting fluorescence, a property that can be used clinically for photodetection. Second, to generate a therapeutic photodynamic effect, the photosensitizer must undergo electron spin conversion to a triplet state. In the presence of oxygen, this excited molecule can react directly with its substrate, by

proton or electron transfer, to form radicals or radical ions that interact with oxygen to produce oxygenated products (a type I reaction). Alternatively, the energy of the excited photosensitizer can be directly transferred to oxygen to form a singlet oxygen (a type II reaction), which is the most damaging species generated during PDT [12]. Tumor destruction after PDT results from direct cytotoxic effects as well as from the induction of a local inflammatory response [13]. Thus, preclinical studies have shown that PDT not only mediates apoptotic and necrotic killing of tumor cells but also alters the tumor microenvironment through the release of cytokines and interleukins. On the basis of its unique mechanism of tumor destruction, PDT has the potential to create an environment at the tumor site that favors both tumor antigen loading and activation of DCs, key requirements for induction of antitumor immunity [14]. Here, we report that PDT can induce the expression of IL-1 and IL-6 from DCs and propose that these changes are mechanistically involved in the observed effects of PDT on the host immune response.

Materials and methods

PDT was performed as reported previously [15]. For stationary culture, mouse Lewis lung carcinoma (LLC) cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM) (Sigma-Aldrich Inc., MO) with 10% fetal calf serum (FCS) in a Black with Clear Bottom 96-well Microtest™ Optilux™ Plate (BD bioscience Inc., CA) (1×10^4 cells/well). Twenty-four hours later, when the cells had adhered to the plate, the medium was removed and the cultures were washed three times with phosphate-buffered saline solution (PBS, pH 7.4, Sigma). Talaporfin sodium (Mono-L-aspartyl chlorin e6, Laserphyrin®, provided by Meiji Seika Co., Ltd., Tokyo, Japan) dissolved in FCS free DMEM was added to each well (50 μg/mL). After 2 hrs, the supernatant was removed and the cells were thoroughly washed three times with PBS. FCS free DMEM was added, and the cells were subjected to laser irradiation (wavelength; 664 nm, power density; 60 mW/cm^2) for 30 sec at a dose equivalent to the lethal dose (LD_{100}). Immediately after laser irradiation, the culture medium was replaced with complete growth medium. Twenty-four hours after PDT, cells and supernatants were collected and spun to clear cell debris (PDT-generated lysates). In parallel, freeze/thaw lysates were generated by subjecting LLC cells (FCS free DMEM) to three freeze/thaw cycles in liquid nitrogen and 37°C water bath, followed by centrifugation to remove the cell debris (freeze/thaw-generated lysates). Stationary culture media of LLC cells (FCS free DMEM) was used as a control.

The JAWSII mouse DC line was cultured in complete growth medium containing Minimum Essential Medium (MEM) alpha with ribonucleosides, deoxyribonucleosides, 4 mM L-glutamine, and 1 mM sodium pyruvate supplemented with 10% FCS and 5 ng/mL murine recombinant GM-CSF. JAWSII cells were incubated overnight in a 96-well microplate (1×10^4 cells/well), and the culture media was replaced with PDT-generated lysates or freeze/thaw-generated lysates (100 μL/well). After 24 hrs, the supernatants were collected and subjected to ELISA assay. The IL-1α, IL-1β, IL-2, IL-6, IL-10, IL-12, Macrophage Inflammatory Protein (MIP) 1α, Transforming Growth Factor (TGF) β, Tumor Necrosis Factor (TNF) α, and Vascular Endothelial Growth Factor (VEGF) levels in the supernatant were quantified from each sample using a Quantikine ELISA kit (R&D Systems, Minneapolis, MN).

All data are expressed as the relative ratio against the control (stationary LLC cultured media) and as the means ± standard derivation (n=6-12). Statistical significance (defined as *P* values <0.01) was evaluated using the unpaired Student's *t* test (two-tailed).

Results and discussion

PDT has been shown to enhance the host antitumor immune response [13]. To determine whether this enhancement was at least in part a consequence of the effects of PDT on tumor cells, we tested the immunogenicity of tumor cell lysates generated by *in vitro* PDT treatment. To do so, PDT-generated tumor cell lysates were added to DC cultures and IL-1α, IL-1β, IL-2, IL-6, IL-10, IL-12, MIP-1α, TGF-β, TNF-α, and VEGF levels were measured. Before the experiments, we performed the array test by using Proteome Profiler™ Array kit (R&D Systems, Minneapolis, MN) and picked up the cytokines or growth factors that seemed to be the differences between experimental group and negative control group (data not shown). IL-1α, IL-1β, and IL-6 were the most markedly increased, and TNF-α was decreased in DC culture supernatants following this treatment (**Figure 1A**). These cytokines

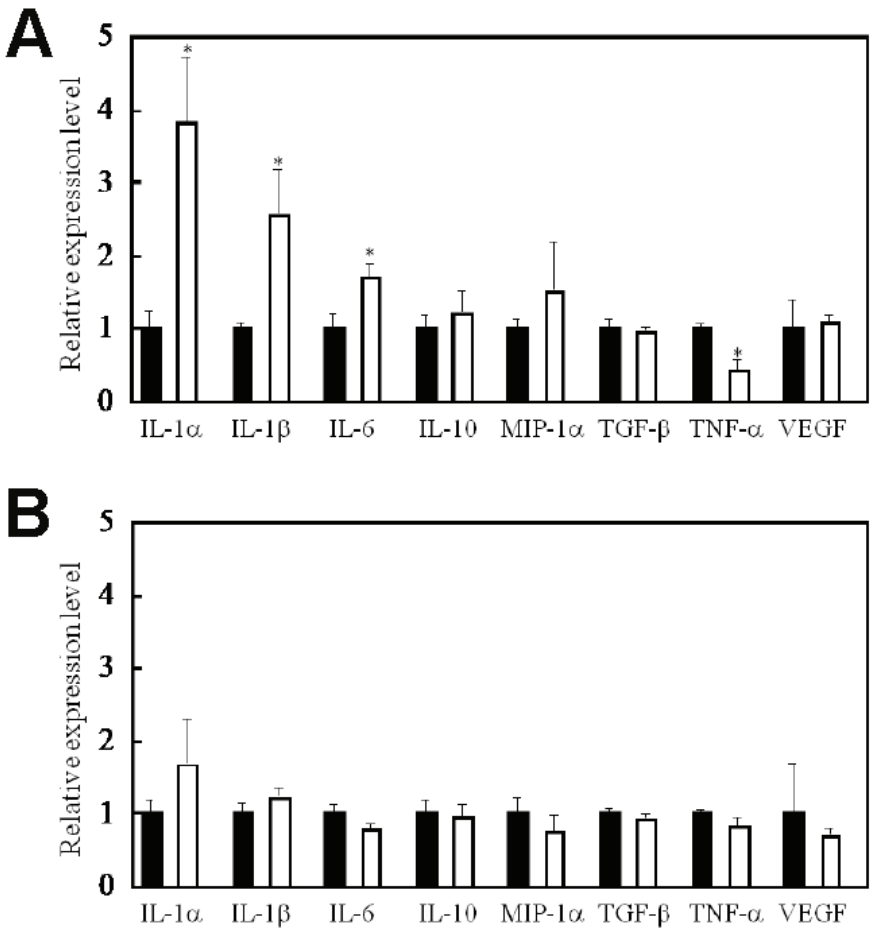

Figure 1. PDT-generated cell lysates activate DCs. IL-1α, IL-1β, and IL-6 were most markedly increased and TNF-α was decreased following the addition of PDT-generated lysates to DC cultures (a). In contrast, cytokine levels did not change after addition of freeze/thaw-generated tumor cell lysates to DC cultures (b). IL-2 and IL-12 secretion levels were below the detection limit of ELISA. *, $p<0.01$: significant difference between the addition of PDT-generated lysates and addition of stationary culture media from LLC cells.

must have been secreted from DCs because they were not detected in the tumor cell lysates. The concentrations of other cytokines, with the exception of IL-2 and IL-12, which were below the detection limit of ELISA, were not changed compared with those of control cells. In parallel, cytokine levels were also examined in the supernatants of DC cultures treated with freeze/thaw-generated tumor cell lysates (**Figure 1B**). In these experiments, the levels of cytokines and growth factors secreted to the supernatant were unchanged after treatment with the freeze/thaw -generated lysates.

IL-1α, IL-1β, and TNF-α were investigated in parallel because they are recognized IL-6 inducers and act synergistically with IL-6 to induce antitumor responses in mice [16,17]. We confirmed the enhancement of IL-6 secretion from cells after PDT *in vitro* described earlier by Kick et al. [18]. Further, as suggested by Kick et al., TNF-α does not seem to play a role in IL-6 induction by PDT because the changes in IL-6 are neither preceded nor accompanied by similar changes in TNF-α. PDT induces TNF-α in murine peritoneal macrophages *in vitro* [19], and a recent study by Anderson et al. [20] has demonstrated up-regulation of TNF-α in keratinocytes *in vitro* by PDT using a phthalocyanine-derived photosensitizer. The decreased levels of TNF-α observed in our study might be related to the DCs used, as the regulatory region of the TNF-α gene

has been shown to have allelic differences [21].

It remains to be determined whether the enhanced generation of IL-6 plays a role in the PDT tumor response. Intratumoral injection of IL-6 or transduction of the IL-6 gene into tumor cells can enhance tumor immunogenicity and inhibit tumor growth in experimental murine tumor systems [17,22,23]. Thus, PDT may enhance local antitumor immunity by up-regulating IL-6 production in DCs. The mechanisms by which this is achieved are not yet clear. Dougherty et al. [23] have suggested that IL-6 may further the recruitment of tumoricidal macrophages into the tumor bed. On the other hand, Mule et al. [17] have shown that IL-6-mediated tumor regression could be abrogated by *in vivo* depletion of either $CD4^+$ or $CD8^+$ T-cell subsets. Although this study did not examine T-cell responses, changes in T-cell function might occur, and we are presently analyzing this using co-culture methods. Luna et al. [24] have shown in murine RIF cells *in vitro* that the early-response genes c-fos and c-jun are induced by Photofrin; these gene products form the AP-1 transcription factor, which induces IL-6 expression [16,18,25].

Gollnick et al. [26] reported that vaccination with PDT-generated tumor cell lysates elicits a tumor-specific immune response as demonstrated by protection against subsequent tumor inoculation, induction of tumoricidal activity in the spleen, and increased numbers of IFN-g-secreting splenic cells. These studies demonstrate that PDT is able to enhance the inherent immunogenicity of at least some tumor cells. The nature of the "activation" factor in PDT-generated tumor cell lysates is unknown, although there are several promising candidates. Although recent studies have focused on the use of genetically engineered cancer vaccines or tumor-associated antigen-primed DCs [27,28], there is no convincing evidence that these vaccines have an overwhelming advantage over crude vaccines [27]. The finding that PDT-generated tumor cell lysates were effective antitumor vaccines has potentially broad clinical implications. Not all tumors are amenable to PDT, either because of size or location, and one could conceive of an adjuvant use for PDT vaccines in conjunction with other cancer modalities that do not enhance the host antitumor immune response, such as surgery and/or chemotherapy.

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