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Photodynamic therapy and the immune system in experimental oncology

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Introduction

Photodynamic therapy (PDT), a newly established treatment for solid tumors, involves the systemic administration of a tumor localizing photosensitizer that is only activated when exposed to light of appropriate wavelength. Photoactivation of the photosensitizer in the presence of oxygen results in the generation of highly cytotoxic molecular species.[1] The PDT-mediated antitumor effect is oxygen dependent and is the consequence of direct cytotoxicity and an antivascular effect which impairs blood supply to the area.[2] Furthermore, the development of a generalised inflammatory state in the treatment region and immunological mechanism may contribute to tumor regression. The most widely studied PDT drugs both in experimental and clinical trials have been hematoporphyrin derivative and Photofrin (a complex mixture of monomeric and oligomeric porphyrins). While encouraging results have been obtained with these drugs, their documented limitations have led to the search for second generation photosensitizers in the hope of increasing the efficacy of the treatment (clearly definable molecular structure, activation at longer, more penetrating wavelengths of light and a more rapid clearance from the subject). Second generation photosensitizers that are currently under clinical evaluation include tin etiopurpurin dichloride (SnET2), lutetium texaphyrin, *meso*-tetrahydroxyphenylchlorin (*m*-THPC), phthalocyanines and benzoporphyrin derivatives, monoacid ring A (BPD-MA, Verteporfin) and ALA (5-aminolevulinic acid).

Immune system and PDT

PDT can have immune-modifying actions at dose levels below those that cause skin inflammation or erithema. This form of PDT takes advantage of differential photosensitizer uptake by different cell types and their relative susceptibility to photodynamic killing. Furthermore at sub-lethal levels, the treatment may alter the function of immune and non-immune cell types, a change that may influence the underlying immune-mediated condition. The intracellular sites of photosensitizer localisation influence subsequent photodynamic effects within cells.[3] Damage to membranes and different organelles has been described following PDT. A continuum of dose-related events is demonstrable for PDT-treated cells. With low intensity PDT, viability may be maintained while cell traits (cell signalling events, cytokine formation, surface receptor expression) may be modified. At more intensive PDT levels, the cells may rapidly undergo the distinct death process termed apoptosis (programmed cell death), morphologically characterised by chromatin condensation, nuclear dissolution and membrane blebbing. Apoptosis is of importance in development and homeostasis of tissues and the organism, and occurs in response to extracellular, regulating or damaging factors. Growth factors, hormones, irradiation, heat, drugs and PDT may induce or prevent apoptosis, often in a cell-specific manner.[4] At high photosensitization levels, PDT may kill cells by causing an immediate disruption of the outer membrane or organelles such as lysosomes. This less controlled cell death, designated necrosis, may contribute to the formation of an inflammatory state within the treated tissues with the involvement of the **immune system**.

Photodynamically induced changes in the plasma membrane and membranes of cellular organelles, which represent the most abundant damage with the majority of photosensitizers used for PDT, can trigger events with far-reaching consequences. One process initiated at the membrane level involves signal transduction pathways. These include enhanced expression of stress proteins and early response genes,[5] activation of genes regulating the process of apoptotic cell death and possibly the up-regulation of some cytokine genes.[6] Due to their role in cell adhesion and antigen presentation, some of the PDT-induced stress proteins may participate in the development of an inflammatory/immune response manifested by this therapy. A strong inflammatory reaction is a central event in the mechanism of PDT-mediated tumor destruction with the release of a wide variety of potent mediators like vasoactive substances, components of the complement cascades, cytokines (IL-6, IL-1β, IL-2, tumor necrosis factor-α and granulocyte colony-stimulating factor), growth factors and other immunoregulators.[7] Furthermore some photosensitizers, shown to stimulate hematopoiesis in treated mice, may induce cytokine or growth factors independently of light treatment.[8]

Antitumor immunity and PDT

With cancer, various features may blunt the achievement of antitumor immunity. These include tumor cells' production of factors that impair immune cell function, deficient tumor cell major histocompatibility complex (MHC) and costimulatory molecule expression, a low capacity of tumor cells to present tumor-specific antigens to T-cells and loss of tumor-specific antigen expression. A growing body of evidence indicates that complete tumor resolution with PDT requires the involvement of the immune system.[9,10] Further, PDT may promote the formation of immunity against tumors that are normally weakly or non-immunogenic. The intense localised inflammatory effect of PDT may be an initiating event for the formation of effective antitumor immunity.

A massive regulated invasion of neutrophils, mast cells and monocytes/macrophages during and after photodynamic light treatment has been documented in studies using murine tumor models.[11] There is increasing evidence that these cells have a profound impact on PDT-mediated destruction of cancerous cells. Neutrophils can remain within tumor blood vessels and be a key contributor to the infliction of endothelial damage releasing chemotactic substances that will attract a new wave of immune cell invasion. Another class of non-specific immune effector cells whose activation substantially contributes to the antitumor effects of PDT is monocytes/macrophages. The tumoricidal activity of these cells was found to be potentiated by PDT *in vivo* and *in vitro*.[12]

There have been substantial advances in the understanding of the PDT-induced tumor specific immune reaction. This effect may not be relevant to the initial tumor excision, but may be important in attaining long-term tumor control. Tumor sensitized lymphocytes can, under reduced tumor burden, eliminate small foci of viable cancer cells that have escaped from PDT. PDT induced antitumor immunity has similarities to the immune reaction induced by tumor inflammation caused by bacterial vaccines or some cytokines. Tumor-associated macrophages and/or dendritic cells serving as antigen presenting cells[13] are likely to mediate the initial critical step of tumor-specific immune development. These cells are prompted to phagocytize large numbers of cancer cells killed or damaged by PDT. Directed by powerful inflammation associated signalling, the antigen presenting cells will process tumor-specific peptides and present them on their membranes in the context of major histocompatibility class II molecules. Presentation of tumor peptides, accompanied by intense accessory signals, creates conditions for the recognition of tumor antigens by helper T lymphocytes. These lymphocytes become activated and in turn sensitized cytotoxic T cells to tumor specific epitopes. The activity of tumor sensitized lymphocytes is not limited to the original PDT treated site but can include disseminated and metastatic lesions of the same cancer. Thus, although the PDT treatment is localized to the tumor site, its effect can have systemic attributes due to the induction of an immune reaction. PDT generated tumor sensitized lymphocytes can be recovered from distant lymphoid tissues (spleen, lymphonodes) at protracted times after light treatment. Therefore, these lymphocyte populations consisting of immune memory cells were also evident.[13] The induction of immunity against a weekly immunogenic murine fibrosarcoma MS-2 by aluminium phthalocyanine-based PDT was also described.[14] Thus antitumor immunity fostered by PDT has a strong dependence on the activity of **cytotoxic T cells**. Immunosuppression effects, as in the case with PDT, frequently accompany inflammation. The PDT-induced immunosuppression was detected primarily as a transient reduction in the delayed-type contact hypersensitivity response, which appears to be mediated by antigen nonspecific suppressor cells.[15] Cellular events provoked by PDT may unveil previously hidden tumor-associated antigens in forms that are recognisable by the immune system. This process may lead to the development of specific cellular antitumor immunity. The capacity of PDT to indirectly or directly induce tumor cell apoptosis may provide a source of antigenic material that can be effectively presented by APC (antigen presenting cells) to tumor specific T cells. Tumor-sensitized T cells may eliminate tumor cell foci that have escaped the direct action of PDT. The administration of cytokines that activated DC (dendritic cells) and/or increased the production of this cell type may contribute to cancer cures and/or limit metastatic disease in PDT-treated subjects.

In conclusion, due to its inflammatory/immune character, PDT can be successfully combined with various immunotherapy protocols for achieving substantial gains in long-term tumor controls. A common strategy to such a combination is to sustain and amplify the PDT-induced immunity against tumors. Its effectiveness has been demonstrated in a number of different murine tumor models (including poorly immunogenic tumors) using a wide variety of nonspecific or specific immunotherapy agents. These results demonstrate the generation of immune memory cells sensitized to PDT treated tumor and suggest that PDT may be particularly suitable for a combined application with adoptive immunotherapy protocols.

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