

Neutrophils as inflammatory and immune effectors in photodynamic therapy-treated mouse SCCVII tumours

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Neutrophils have become recognised as important contributors to the effectiveness of tumour eradication by photodynamic therapy (PDT). In this study, we have used the mouse SCCVII squamous cell carcinoma model to investigate the activity of neutrophils in tumours treated by PDT. Tumour levels of neutrophilic myeloperoxidase (MPO) demonstrated not only a massive and sustained sequestration of these cells in PDT-treated tumours but also revealed their activated state evidenced by the presence of released MPO. Among the adhesion molecules expressed on tumour vascular endothelium, ICAM-1 appears to be of primary importance in the invasion of neutrophils into PDT-treated tumours, because its functional blocking with monoclonal antibodies reduced the tumour cure rate. A marked upregulation of its ligands CD11b/CD18 and CD11c/CD18 found on neutrophils associated with PDT-treated tumours supports this assumption. To evaluate the role of inflammatory cytokines regulating neutrophil activity, neutralising antibodies were given to mice before PDT treatment. The results suggest that IL-1 β activity is critical for the therapeutic outcome, since its neutralisation diminished the cure rates of PDT-treated tumours. No significant effect was observed with anti-IL-6 and anti-TNF- α treatment. Further flow cytometry-based examination of neutrophils found in PDT-treated tumours revealed that these cells express MHC class II molecules, which suggests their engagement as antigen-presenting cells and involvement in the development of antitumour immune response.

Introduction

The inflammatory response elicited by photodynamic therapy (PDT) treatment of solid tumours is receiving increased attention, because it has become apparent that the engagement of effector systems for inflammatory tissue destruction has a major role in the eventual outcome of this anti-cancer modality.^{1,2} Initial PDT injury causes a massive release of inflammatory mediators from the treated tumour,³ prompting the mobilization of host defence mechanisms for resolving the perturbed homeostasis at the affected site.² The inflammatory events such as the activation of complement together with inter-related coagulation and kinin plasma cascade systems, ischemia-reperfusion insult and poly (ADP-ribose)polymerase (PARP) activation were suggested to play important roles in the eradication of PDT-treated tumour tissue.²⁻⁴ Their effects are to a large extent mediated through the action of neutrophils and other inflammatory cells.

The importance of neutrophils for the curative outcome of PDT has been demonstrated following their depletion from the hosts bearing PDT-treated tumours, which diminishes the effect of this therapy.^{5,6} On the other hand, the thrombocyte antiserum administration that causes an increased neutrophil accumulation in PDT-treated tumours improved tumour response.⁷ It was also shown that tumour-targeted PDT induces a strong acute phase response dominated by a marked elevation in peripheral blood neutrophil levels (neutrophilia) in host mice and rats.^{8,9} This is due to the recruitment of these cells from their storage/marginated pools and accelerated maturation of their progenitors in the bone marrow.⁸ We have recently identified multiple mediators whose regulated action is responsible for the PDT-induced systemic mobilization and activation of neutrophils.³ Some of these mediators are further investigated in this study, in which we continue to examine the

activity of neutrophils in PDT-treated tumours together with the role of inflammatory adhesion molecules and inflammatory cytokines responsible for regulating neutrophil engagement.

Materials and methods

Tumour model and PDT treatment

Subcutaneous SCCVII squamous cell carcinomas¹⁰ were inoculated in syngeneic female C3H/HeN mice by implanting one million cells per mouse in the lower dorsal region. The tumours were used for experiments when they reached 7–8 mm in largest diameter. For PDT treatment, mice were given an iv injection of either Photofrin (10 mg kg⁻¹) or mTHPC [tetrakis(*m*-hydroxyphenyl)chlorin; 0.1 mg kg⁻¹] and 24 hours later the tumours were exposed to photodynamic light treatment while the mice were held unanesthetized in restraining holders. The photosensitizers Photofrin (porfimer sodium) and mTHPC (foscan) were kindly provided by Axan Pharma Inc. and Scotia Pharmaceuticals Ltd., respectively. The light for Photofrin-PDT was generated by a high throughput fiber illuminator (Sciencetech Inc., London, Ontario, Canada) equipped with a 150 W QTH lamp with integrated ellipsoidal reflector and 630 \pm 10 nm interference filter. The 652 \pm 1 nm light for mTHPC-PDT was from a 0.25 W laser diode model SDL-7422-H1 (Spectra Diode Labs, San Jose, CA). In both cases the light was delivered through an 8 mm core diameter liquid light guide, model 77638 (Oriel Instruments, Stratford, CT). The fluence rates achieved by monodirectional superficial transillumination of the tumour and \sim 1 mm of surrounding normal tissue for Photofrin- and mTHPC-PDT were 110–120 and 120–130 mW cm⁻², respectively. When the experimental endpoint was tumour cure or re-growth, the mice (8 per treatment group) were examined three times per week after PDT for

signs of tumour growth. No sign of recurrence at 90 days post PDT qualified as a tumour cure. All animal work was approved by The Animal Ethics Committee of the University of British Columbia.

***in vivo* Blocking protocols**

Monoclonal antibodies 3E2 and 10E9.6 raised against mouse ICAM-1 (intercellular cell adhesion molecule-1, CD54) and E-selectin (CD62E), respectively, which block the adhesion function of these molecules, were purchased from BD PharMingen (BD Biosciences Canada, Mississauga, Ontario, Canada). They were injected intravenously at 30 µg per mouse immediately after the termination of photodynamic light treatment. The same treatment was done with the immunoglobulin isotype controls (hamster IgG, group 1, κ and rat IgG2a for ICAM-1 and ELAM-1, respectively) that were also obtained from BD PharMingen. The functional blocking of mouse cytokines IL-1β and IL-6 was done using monoclonal antibodies 1400.24.17 and 20F3, respectively, purchased from Endogen Inc. (Woburn, MA), while TNF-α was blocked using rabbit polyclonal antibodies raised against that cytokine (PeproTech Inc., Rocky Hill, NJ). All these cytokine antibodies and their respective immunoglobulin isotype controls were administered at 30 minutes before the onset of photodynamic light treatment by an intraperitoneal injection at 50 µg per mouse.

Myeloperoxidase activity

Tumour-localized neutrophil sequestration was quantified using a modification of a spectrophotometric assay for myeloperoxidase (MPO), an enzyme characteristically expressed by neutrophils.¹¹ Briefly, solid tumours were excised, and their homogenates analyzed for myeloperoxidase by measuring the peroxidation of the substrate 3,3',5,5'-tetramethylbenzidine at 630 nm in the presence of hydrogen peroxide in phosphate buffer. In addition to this measurement of total MPO present in tumour tissue, fragments of the excised tumour tissue were used for determining the levels of cell-free MPO released from neutrophils. For this, tumour fragments were chopped thoroughly, and incubated for 1 hour at 37 °C in serum-free minimum essential medium (Sigma Chemical Co., St. Louis, MO). Samples of the spun tumour supernatant were then analyzed for MPO activity. Blood samples from the tail vein of tumour hosts were also taken and used for the determination of neutrophil MPO content (neutrophil blood levels were derived from the combined total and differential leukocyte counts). MPO activity was in all cases expressed in enzyme units defined as the amount of enzyme that produces an increase of optical absorbance per unit time, using an enzyme standard of known activity for calibration. The results are expressed as units of MPO activity per 100 mg tumour (wet) weight with four tumour-bearing mice used per data point.

Flow cytometry analysis

Cell suspensions from PDT-treated and untreated SCCVII tumours were obtained by mincing the tissues and dissociating them using enzymatic digestion.¹² These cells and blood leukocyte samples (erythrocytes removed by lysis) were then prepared for a standard three-colour (FITC-PE-CyChrome) flow cytometry analysis by staining with monoclonal antibodies raised against murine surface antigens that were conjugated to fluorescent labels. Antimouse CD11b and CD11c were used for the analysis of the expression of these integrin molecules on blood neutrophils (identified by antibodies against myeloid differentiation antigen Gr1) and tumour-associated neutrophils (Gr1++ cells stained negatively for macrophage-specific antigen F4/80). Antibodies against I-A^d/I-E^d were used for examining the expression of class II major histocompatibility antigen (MHC_{II}) on tumour-associated neutrophils. The antibodies

were obtained from BD PharMingen, except anti-F4/80 (Cedarlane Laboratories Ltd., Hornby, Ontario, Canada). The flow cytometry analysis was performed using a Coulter Epics Elite ESP (Coulter Electronics, Hialeah, FL).

Statistical analysis

Log-rank test was used for the tumour response data evaluation, while the unpaired Student's *t*-test was applied to test the difference between means for the data from the MPO measurement and flow cytometry. The difference with *p* < 0.05 was considered statistically significant.

Results

1 Myeloperoxidase activity in PDT-treated tumours

The influx of host neutrophils into solid tumours following PDT has been documented by detecting their percentage in cell suspensions obtained from disaggregated tumour tissue.^{13,14} A quantitatively more reliable method for determining neutrophil levels is based on the activity of myeloperoxidase (MPO), a neutrophil-specific enzyme.^{10,15} Neutrophil granules contain large amounts of this enzyme (up to 5% of a cell's dry weight) which is released into the extracellular compartment upon cell activation.¹⁶ In the present study, we used the MPO method to determine the numbers of neutrophils in PDT-treated tumours (from tumour tissue homogenates), as well as the portion of MPO released from neutrophils (from cell-free tumour supernatants). Following Photofrin-based PDT, the total MPO content in treated SCCVII tumours has risen from the pre-therapy levels of around 0.15 MPO units to close to 27 MPO units per 100 mg of tumour tissue at its peak at 13 hours post PDT (Fig. 1a). The increase in tumour MPO levels was already evident at 2 hours post PDT, but the most dramatic rise was detected at 8 hours post PDT. The highest amounts of released MPO were found at 8 and 13 hours post PDT, which reached close to ¼ of total MPO at the 8 hour time point.

The information on MPO content in neutrophils obtained from blood samples can be used for converting the total MPO values in tumour tissue into the numbers of neutrophils contained in that tissue. However, the neutrophil MPO content changes with the age of these cells, and is known to be significantly higher in younger neutrophils.¹⁷ The evidence obtained in our earlier studies revealed that PDT induces a massive release of neutrophils from the bone marrow accompanied by the accelerated maturation of these cells from their progenitors.⁸ Thus, it was necessary to register the MPO content exhibited by neutrophil populations at the post PDT time intervals chosen for the measurement of tumour MPO. Indeed, during the first 18 hour post therapy period we found a small but significant rise in the MPO content of blood neutrophils collected from mice bearing PDT-treated tumours (Fig. 1b). While there were 1.85 ± 0.13 (SD) MPO units per million circulating neutrophils in untreated mice, this rose to 2.88 ± 0.54 (SD) MPO units at 13 hours post PDT. These MPO values were used separately for each time point to derive (from the total MPO levels) the numbers of neutrophils contained in PDT-treated tumours during the first 18 hours following therapy, as shown in Fig. 1b. These data reveal that over 9 million neutrophils had accumulated per 100 mg of PDT-treated tumour tissue at the peak time point (13 hours post therapy), which is over 100-fold increase compared to neutrophil numbers in untreated SCCVII tumours.

2 Role of adhesion molecules

Binding of circulating neutrophils to the endothelium of tumour vasculature is the first step in their tumour localization. This is facilitated by the upregulated expression of endothelial adhesion molecules induced by inflammatory stimuli^{17,18} that

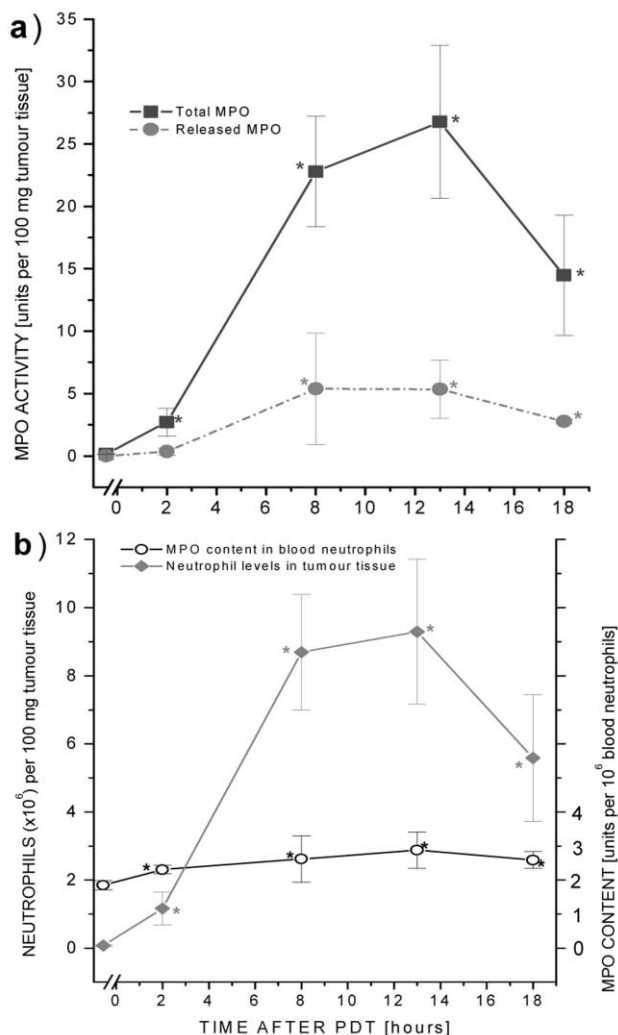


Fig. 1 Myeloperoxidase (MPO) activity in PDT-treated tumours. Subcutaneous SCCVII tumours growing in C3H/HeN mice were treated by PDT (Photofrin 10 mg kg⁻¹ followed 24 hours later by 150 J cm⁻²) and excised at various time intervals thereafter. Approximately half of the tumour tissue was homogenized and used for the determination of total MPO activity, while the other half was incubated *ex vivo* for 1 hour in minimum essential medium and the supernatants were used for the measurement of released MPO. Blood samples from tumour hosts were also taken immediately before their sacrifice and tumour excision for the determination of MPO levels in circulating neutrophils. The MPO activity was calculated per 100 mg of tumour tissue (a), and from these values the number of neutrophils in tumour tissue was derived by normalizing with the blood neutrophil MPO content for each time point (b). Bars are SD, * = *p* < 0.05 compared to non-treated controls.

was suggested to occur following PDT treatment.^{2,3} The effect on the tumour PDT response of blocking the function of the two endothelial adhesion molecules that have a major role in this process, ICAM-1 and E-selectin, is shown in Fig. 2. The contribution of ICAM-1 to the outcome of PDT was evaluated with two photosensitizers, Photofrin and mTHPC. The doses of PDT used with both photosensitizers were effective in producing a complete initial ablation of treated tumours, but over 30% of tumours treated with Photofrin-PDT showed subsequent recurrence, while mTHPC-based PDT was curative for all treated tumours. The injection into mice of antibodies blocking the ICAM-1 function immediately after PDT markedly reduced the cure rate of tumours treated by both Photofrin-based PDT (Fig. 2a) and by mTHPC-based PDT (Fig. 2b). No effect was detectable following the same treatment with the same type of hamster IgG raised against an unrelated epitope (trinitrophenol). The blocking of E-selectin produced only a minor, statistically insignificant decrease in the cures of

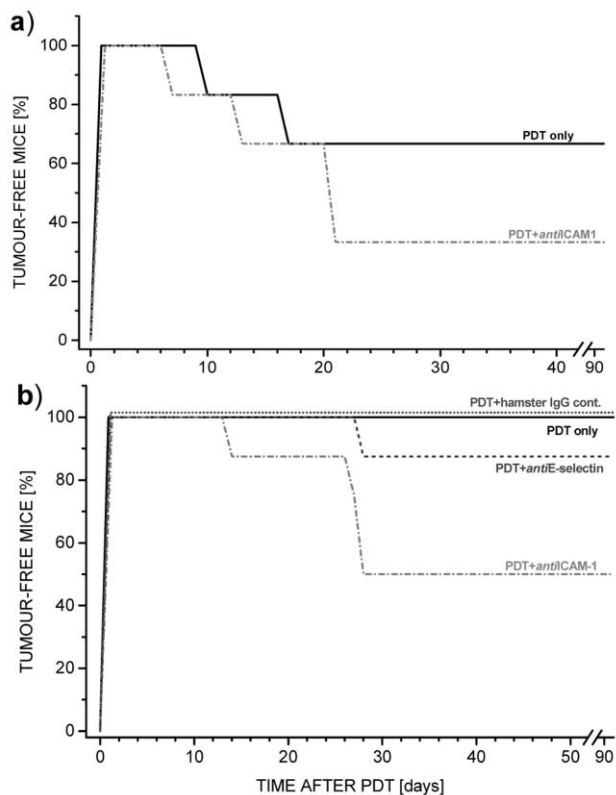


Fig. 2 The effect of blocking the function of ICAM-1 and E-selectin adhesion molecules on PDT response of SCCVII tumours. Subcutaneous SCCVII tumours growing in C3H/HeN mice were treated with either a) Photofrin-based PDT (Photofrin 10 mg kg⁻¹ plus 180 J cm⁻²), or b) mTHPC-based PDT (mTHPC 0.2 mg kg⁻¹ plus 60 J cm⁻²) with tumour-localized light treatment given in both cases 24 hours after the photosensitizer injection. Immediately after photodynamic light delivery, the mice were intravenously injected with monoclonal antibodies specifically blocking the function of ICAM-1 or E-selectin, or with isotype control antibodies (30 µg per mouse in all cases). The mice were thereafter monitored for 90 days for signs of tumour growth. The difference in response between PDT only and PDT plus anti-ICAM-1 groups in both graphs is statistically significant (*p* < 0.05).

PDT-treated tumours (Fig. 2b). The anti-ICAM-1 treatment produced no obvious effect on the growth of PDT-non-treated tumours.

The ICAM-1 ligand on neutrophils is the integrin molecule CD11b/CD18 (Mac-1). The results of flow cytometry-based examination of its expression on neutrophils collected from peripheral blood of non-treated and PDT-treated SCCVII tumour-bearing mice and neutrophils found in PDT-treated tumours excised at 30 minutes post PDT are shown in Fig. 3. The CD11b/CD18 expression, which was faint and insignificant on circulating neutrophils both before and after PDT, increased dramatically on neutrophils invading the PDT-treated tumours. A similar upregulation was found for the integrin CD11c/CD18, which as a lower affinity ligand also mediates leukocyte binding to ICAM-1¹⁹ (Fig. 3). It should be noted that both CD11b/CD18 and CD11c/CD18 serve as receptors for complement proteins, which is of significance in light of the recently demonstrated complement activation in PDT-treated tumours.³

3 Contribution of inflammatory cytokines to tumour cures

The three major inflammatory cytokines, IL-1β, IL-6 and TNF-α, which are known to be the key regulators of neutrophil migration and activity, have already been implicated as important participants in the PDT tumour response.^{3,9,14,20} The impact of their blocking on the response of SCCVII tumours to mTHPC-based PDT is depicted in Fig. 4. The chosen PDT dose produced close to 40% of tumour cures and the blockage of

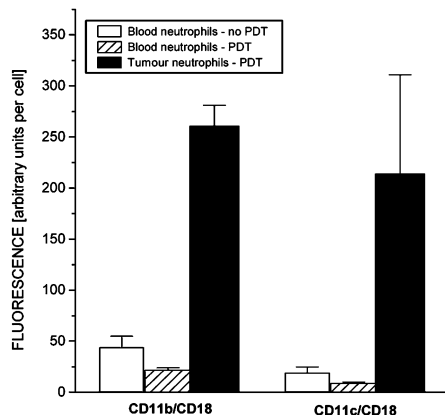


Fig. 3 Expression of CD11b and CD11c molecules on blood neutrophils and neutrophils invading PDT-treated SCCVII tumours. Subcutaneous SCCVII tumours growing in C3H/HeN mice were treated with Photofrin-based PDT as described in Fig. 1. Blood samples were collected and mice sacrificed at 30 minutes after PDT light treatment. The tumours were excised and dissociated into single cell suspensions. Blood samples were also taken from mice bearing PDT-non-treated tumours. The expression of CD11b and CD11c on neutrophils (GR1-positive cells) in these samples was analyzed by flow cytometry. The difference in the levels of both molecules between tumour and blood neutrophils is statistically significant. Bars are SD, $n = 4$.

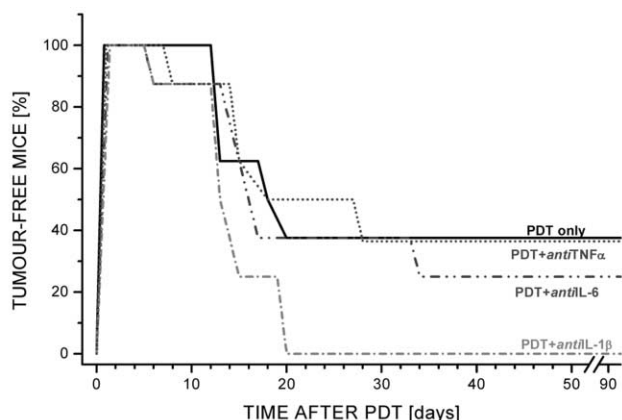


Fig. 4 The effect of blocking IL-1 β , IL-6 or TNF- α activity on the PDT response of SCCVII tumours. Subcutaneous SCCVII tumours growing in C3H/HeN mice were treated by mTHPC-based PDT (mTHPC 0.15 mg kg⁻¹ followed 24 hours later by 80 J cm⁻²). Antibodies neutralizing the activity of specific cytokines or immunoglobulin isotype controls were administered intraperitoneally (50 μ g per mouse) at 30 minutes before the onset of photodynamic light treatment. The mice were thereafter monitored as described in Fig. 2. The difference in the response between PDT only and PDT plus anti-IL-1 β groups is statistically significant ($p < 0.05$).

TNF- α in the host mice exerted no obvious influence on the tumour response rate. A small reduction in PDT-mediated tumour cures was observed with the antibodies blocking IL-6, but this effect was not statistically significant. The same anti-TNF- α and anti-IL-6 antibodies at the same doses were effective in inhibiting the PDT-induced neutrophilia.³ In contrast, the activity of IL-1 β appears to make a substantial contribution to the efficacy of PDT treatment of SCCVII tumours, since the administration of antibodies that block this cytokine completely abolished the curative effect (Fig. 4). The equivalent treatment with the immunoglobulin isotype control antibodies produced no detectable effect on the PDT response of SCCVII tumours, while in the absence of PDT the anti-IL-1 β treatment had no obvious effect (not shown).

4 MHC_{II} expression on neutrophils following PDT

The PDT-treated SCCVII tumours were excised at various time intervals after therapy and the tumour tissue dissociated into

single cell suspensions. These cells were then stained with antibodies that enabled flow cytometry-based identification of neutrophils and the expression of MHC_{II} molecules. The results are presented as the intensity of MHC_{II} expression in tumour-associated neutrophils relative to the staining intensity of these molecules in neutrophils of untreated tumours (Fig. 5). The

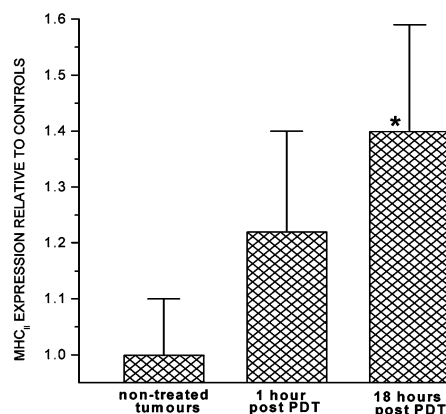


Fig. 5 The expression of MHC class II molecules on neutrophils accumulated in PDT-treated tumours. Subcutaneous SCCVII tumours growing in C3H/HeN mice were treated with Photofrin-based PDT as described in Fig. 1. The tumours were excised at 1 and 18 hours after PDT light treatment and dissociated into single cell suspensions. Following staining with specific antibodies conjugated to fluorescent labels, flow cytometry was used for the identification of neutrophils and recording of the extent of expression of MHC class II molecules on these cells. Bars are SD, $n = 4$; * = $p < 0.05$ compared to the levels on neutrophils in non-treated tumours.

average MHC_{II} expression in these cells (identified as GR1⁺⁺/F4/80⁻) appeared to increase already at 1 hour post PDT, but statistically this was not significant due to relatively large data scatter. However, the expression of MHC_{II} on neutrophils found in SCCVII tumours at 18 hours post PDT had increased even further, and this effect was statistically significant.

Discussion

The use of the MPO activity assay enabled us to verify previously reported observations of the massive accumulation of neutrophils in PDT-treated tumours^{13,14} and to quantify more reliably the extent of this neutrophil sequestration and its kinetics (Fig. 1). Moreover, the measurement of the extent of released MPO in relation to total MPO levels reveals the active engagement of invading neutrophils as the effectors of inflammatory destruction of PDT-treated tumour tissue, since the MPO release is an indicator of neutrophil-mediated cytotoxicity during inflammatory responses.²¹ Another manifestation of the activated status of these cells is the upregulation on their membranes of CD11b/CD18 and CD11c/CD18 molecules (Fig. 3), whose engagement is known to promote neutrophil granule release.²² However, we detected the elevated expression of these leukocyte integrins also on neutrophils localized in non-treated tumours (data not shown).

Vascular endothelium is one of the principal regulators of the inflammatory response, and the adhesion of neutrophils to endothelial cells is an early and requisite event in acute inflammation.^{23,24} The PDT-induced signals instigate a shift towards the pro-inflammatory state in the vasculature of treated tumours as evidenced by the involvement of endothelial adhesion molecules in the responses triggered by PDT treatment.² Our finding that blocking the function of ICAM-1 diminishes the extent of PDT-based tumour cures, and the demonstrated upregulation of its ligands CD11b/CD18 and CD11c/CD18 on neutrophils drawn into PDT-treated tumours, supports the role of these adhesion molecules in the process of neutrophil tumour invasion after PDT. The fact that ICAM-1

blocking produced similar results with both Photofrin- and mTHPC-based PDT (Fig. 2) suggests that PDT mediated by both of these photosensitizers induces a similar inflammatory response in treated tumours, which is in accordance with our findings from a number of related studies (*e.g.* ref. 8) indicating that in this particular aspect of tumour response Photofrin and mTHPC produce comparable effects. The same treatment with anti-ICAM-1 was shown earlier to attenuate the neutrophil trafficking in mice bearing PDT-treated tumours.³ In contrast to ICAM-1, blocking of E-selectin produced no significant effect on the response of PDT-treated tumours (except for a modest statistically insignificant inhibitory trend). At least in some types of inflammatory responses, E-selectin is known to play a role in later phases of neutrophil endothelial adhesion than ICAM-1.²⁵ This could pertain to the inflammatory events in PDT-treated SCCVII tumours, with more critical damage exerted by neutrophils in the early stages post treatment when ICAM-1 is instrumental in the invasion process. The importance of various adhesion molecules may also differ depending on tumour type and/or photosensitizer used for PDT treatment.

These adhesive interactions between neutrophils and vascular endothelial cells will substantially increase their physical contact to enable the subsequent neutrophil transmigration from the vascular compartment. It may also prompt the adherent activated neutrophils to damage cells of the vessel wall and underlying basement membrane/matrix components by a synergistic release of oxygen metabolite products and proteolytic enzymes.^{26,27} The role of myeloperoxidase in such events is to process the oxygen metabolites generated by neutrophil membrane-associated NADPH oxidase into tissue destructive hypochlorous acid.²⁷ This action of neutrophils, as well as the reduced capillary perfusion caused by their massive adhesion to vessel walls, may represent a major contribution to the "anti-vascular" effect of PDT.

Among the cytokines that regulate inflammatory responses, our results identify IL-1 β as the mediator whose activity is critical for the therapeutic outcome of PDT with the SCCVII tumour model (Fig. 4). This cytokine is the key regulator of the acute inflammatory response whose role is particularly important in the initiation of early events in this process.^{28,29} It induces the synthesis of other inflammatory mediators (including the expression of adhesion molecules) and binds to receptors expressed on neutrophils and other cells to trigger the chemotaxis and activation of these cells. Other major inflammatory cytokines are IL-6 and TNF- α . They overlap in some inflammatory activities with IL-1 and share the ability of stimulating neutrophil migration and activation.²⁹⁻³¹ These three cytokines were shown to be induced following PDT^{2,9,14,20} and we have suggested that this phenomenon may result from the activation of the complement system in PDT-treated tumours.³ We have also demonstrated that IL-1 β , IL-6 and TNF- α participate in the induction of the early phase of post PDT neutrophilia in tumour-bearing hosts.³ However, in contrast to IL-1 β , the neutralization of IL-6 and TNF- α activity in mice bearing PDT-treated SCCVII tumours produced no demonstrable impact on the tumour cure-rate except for a statistically insignificant decrease in tumour response with anti-IL-6 (Fig. 4). This finding suggests that the activity of IL-6 and TNF- α may, in the events critical for the PDT response of SCCVII tumours, be compensated for by IL-1 β and/or the other engaged inflammatory mediators.³

Evidence of the expression of MHC class II molecules on neutrophils infiltrating PDT-treated SCCVII tumours (Fig. 5) demonstrates for the first time that neutrophils acquire the capacity to act as antigen-presenting cells following PDT. This indicates their direct involvement in the development of anti-tumour immune response that is known to be generated by PDT.^{1,2,32} We also observed a positive MHC class II staining on macrophages associated with SCCVII tumours that was not

significantly changed after PDT treatment (data not shown). Neutrophils normally do not express MHC class II molecules and were until recently considered to be incapable of functioning as antigen-presenting cells. However, there is now evidence that these cells can, under appropriate stimulatory conditions, display MHC class II molecules and present antigens to T cells,^{33,34} as well as produce a great variety of cytokines, chemokines and other inflammatory/immune mediators and thus influence immune functions of other leukocytes.³⁵ Neutrophils are now recognized to possess the capacity to direct both inflammatory and immune processes and regulate the interactions between innate resistance and adoptive immunity.^{35,36}

This work thus affirms the role of neutrophils as major effectors in the PDT-elicited inflammatory response and the ensuing development of antitumour immune response. Owing to an elaborate collection of inflammatory mediators and proteolytic agents in their armamentarium, neutrophils are well recognized primary mediators of inflammatory endothelial injury,^{26,27} which prompts their candidacy for major contributors to early vascular damage in PDT-treated tumours. The breakdown of the endothelial barrier after these early events facilitates even more massive accumulation and localization of activated neutrophils in perivascular parenchyma, where they have the capacity to directly destroy cancer cells.³⁷⁻³⁹

In conclusion, PDT inflicts a discrete traumatic injury in treated tumour tissues, and there is now compelling evidence for the fundamental inflammatory nature of the elicited response.²

Acknowledgements

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