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Activation of macrophages by Photofrin II during photodynamic therapy

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Abstract

In order to obtain information about the activation of macrophages (Mϕs) during photodynamic therapy (PDT), the influence of Photofrin II (Pf II) on the viability of thioglycollate-elicited murine Mϕs and the subsequent generation of superoxide anion was studied. Irradiations were performed at an energy density of 5 J cm^{-2}, a power density of 150 mW cm^{-2} and a wavelength of 405 nm. Viability of Mϕs was assessed using the acridine orange–ethidium bromide assay. Superoxide anion generation was determined using ferricytochrome *c* (cyt *c*) and nitroblue tetrazolium (NBT) reduction. Our results indicate that the Mϕs are highly susceptible to PDT as their viability is decreased to approximately 30% by 1 μg ml^{-1} Pf II at the energy density indicated above. Within the first 30 min of addition of the photosensitizer, a reducing agent is generated intracellularly by the stimulation of the Mϕs. An extracellular release of superoxide anion does not occur, as measured by the cyt *c* assay. Preincubation of the cells for 1 or 24 h with Pf II and a second challenge with phorbol myristate acetate (PMA) does not enhance the reduction of NBT. Thus, Pf II exerts an immediate effect on the Mϕs which could be interpreted as a first step for subsequent reactions.

1. Introduction

The cytotoxic activity of macrophages (Mϕs) can be stimulated by a variety of agents, *e.g.* phorbol myristate acetate (PMA) or serum-treated zymosan [1], lipopolysaccharide (LPS) [2] and the late complement components C5b-9 [3]. The antibacterial and cytocidal effects of Mϕs have been shown to depend on the generation of reactive oxygen intermediates [4] due to activation of the oxidative burst of Mϕs. During the course of the activation of the Mϕs, reduction of oxygen produces toxic agents such as superoxide anion (O_2^-), hydrogen peroxide (H_2O_2), the hydroxyl radical ($OH^\cdot$) and singlet molecular oxygen ($^1O_2$). As has been shown by Berton and Gordon [5] and Cohen *et al.* [6], the Mϕ most probably mediates tumor cell destruction by generating superoxide anion and hydrogen peroxide.

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In photodynamic therapy (PDT), a photosensitizing dye is applied systematically [7]. The dye preferentially accumulates in the tumor to be destroyed, and subsequent irradiation, at a particular wavelength which is absorbed by the photosensitizing dye, kills the tumor cells. The mechanism by which the photosensitizer exerts its toxicity has been attributed to the production of reactive oxygen intermediates, most probably singlet oxygen ($^1O_2$) [8]; the exact mechanisms, however, have not been established.

Most sarcomas and carcinomas contain resident Mϕs [9], so-called tumor-associated Mϕs (TAMs), at varying percentages. These cells can also take up and metabolize the photosensitizing dye. Recently, it has been shown that hematoporphyrin derivative (HpD; Photofrin), at very low concentrations, together with light, can enhance the phagocytic activity of peritoneal exudate cells (PECs) [10]. These PECs mostly consist of activated, inflammatory Mϕs. Photofrin II (Pf II) at 25 μg ml^{-1} in the presence of light induces the release of large amounts of prostaglandin E_2 (PGE_2) from peritoneal Mϕs [11]. A prerequisite for the release of PGE_2 is the activation of the oxidative burst of the Mϕs [12].

The aim of the present study was to investigate if the generation of superoxide anion by murine peritoneal Mϕs was affected when incubated with Pf II. Irradiation was conducted at 405 nm (emitted by a continuous wave (cw) krypton laser). This wavelength was chosen because of the strong absorption of Pf II (Soret band) [13]. The viability of the Mϕs was compared with the generation of superoxide anion to determine if sublethal or lethal concentrations of the photosensitizer were needed to activate the Mϕs.

Our results show that Pf II activated with 405 nm light has a lethal effect on the Mϕs, even at a concentration as low as 1 μg ml^{-1}. At sublethal concentrations, Pf II triggers the oxidative burst of these cells. As measured in the nitroblue tetrazolium (NBT) reduction assay, the generation of reactive oxygen intermediates occurs only intracellularly. The release of superoxide anion into the extracellular medium is not observed.

2. Materials and methods

2.1. Chemicals

Ferricytochrome *c* (cyt *c*), type VI, superoxide dismutase (SOD), nitroblue tetrazolium (NBT), iodoacetamide (IAc), phorbol-12-myristate-13-acetate (PMA), Hank's balanced salt solution (HBSS) and ethidium bromide were purchased from Sigma Chemical Co. (St. Louis, MO). The bicinchoninic acid (BCA) reagent was obtained from Pierce (Rockford, IL). Thioglycollate broth without indicator was purchased from Difco Laboratories (Detroit, MI). Pf II was obtained as a stock solution containing 2.5 mg ml^{-1} in 0.9% NaCl (Photofrin Medical Inc., Raritan, NJ). The photosensitizer was diluted with phosphate-buffered saline (PBS) to give a final working solution of 1 mg ml^{-1}, from which the various dilutions in medium, cyt *c* or NBT were prepared. Acridine orange was obtained from Eastman Kodak (Rochester, NY).

2.2. Isolation of macrophages

Female Balb/c mice at 8–10 weeks of age were obtained from Simonsen (Gilroy, CA) and housed in the animal care facility at the University of California, Irvine. Prior to the collection of peritoneal cells (4–5 days before), the mice were injected intraperitoneally (i.p.) with 1 ml sterile thioglycollate broth as previously described [14, 15]. The mice were sacrificed by CO_2 inhalation. The abdominal skin was removed, and ice cold HBSS (10 ml) was injected using a 25G gauge butterfly catheter. The peritoneum was lavaged thoroughly. A 23G gauge needle was used to withdraw the injected solution. Usually, 9 ml of the 10 ml injected were recovered. The cells from five mice were pooled for each experiment.

The solution containing thioglycollate-elicited peritoneal Mϕs was centrifuged for 10 min at $200 \times g$ at 4 °C. The supernatant was removed and the cell pellet was resuspended in 1 ml ice cold ACK (ammonium chloride–potassium chloride) buffer to lyse red blood cells, followed by the addition of 9 ml ice cold plain RPMI 1640 medium. After centrifugation at 4 °C, the cells were resuspended carefully in RPMI 1640 medium supplemented with 10% FBS and plated into 96 well plates for the NBT and cyt *c* reduction assays.

To assess the number of Mϕs contained in the peritoneal cells, the cells (0.5 ml) adjusted to 1×10^6 ml^{-1} were incubated in a 24 well plate into each of which a sterile coverslip (12 mm in diameter) had been placed before the addition of the cells. After 1 h incubation at 37 °C, the cells were washed vigorously with HBSS and stained with Wright stain (Diff-Quick, American Scientific Products, McGaw Park, IL). The number of Mϕs was found to be 75 ± 5%, which was in good agreement with previous data [5].

2.3. Viability of macrophages

The viability of freshly isolated Mϕs was determined by mixing them with an equal amount of trypan blue and counting in a hemocytometer. To determine the viability of already adherent Mϕs following incubation with Pf II and/or PDT, the acridine orange–ethidium bromide (AO–EB) fluorescence assay was employed [16]. A 100× stock solution, containing 15 mg AO and 50 mg EB in a mixture of 1 ml 95% ethanol and 40 ml *dd*H_2O, was prepared. To obtain the working solution, the stock was diluted 100-fold in PBS and stored at 4 °C until use.

Mϕs were adjusted to 5×10^5 cells ml^{-1}, and 1 ml of the suspension was placed into each well of a 24 well plate into which sterile coverslips (12 mm in diameter) had been placed prior to the addition of the cells. A separate plate was prepared for each time point and for irradiated and unirradiated experiments. After adherence for 1 h, the cells were washed three times with HBSS, and the photosensitizer diluted in culture medium was added. The cells were shielded from ambient light by wrapping each plate in aluminum foil. To determine the effect of Pf II and light, the cells were irradiated immediately following the addition of the photosensitizer. During irradiation, the aluminum foil was removed from the plates. Following

incubation at 37 °C for different time periods, the medium containing the photosensitizer was removed, and 0.5 ml of the AO–EB mixture was added to each well at a time delay of 5–10 min. The fluorescence was observed using the filter set G436/FT510/LP515 (Zeiss, Oberkochen, F.R.G.). Pictures were taken at each data point. Two non-contiguous areas of approximately 100 cells were counted on each slide. The viability was calculated according to

viability (%) = (number of live cells/total cell count) × 100

2.4. Irradiation procedure

A model 90-K krypton ion laser (Coherent, Palo Alto, CA) emitting at 405 nm was employed for the irradiations. A mask of cardboard was prepared containing an aperture with a diameter of one well of the 96 well plate (for reduction assays) or 24 well plate (for viability assays). This mask prevented irradiation of adjacent wells. The diameter of the dye laser beam was adjusted to the appropriate spot size using a handpiece optical fiber with a 2 mm lens. The final energy density was 5 J cm^{-2}. For the spot size of the well of a 96 well plate, the power density was 150 mW cm^{-2} and the irradiation time was 33 s; for the well of a 24 well plate, the power density was 125 mW cm^{-2} and the irradiation time was 40 s. The energy density was measured using a Coherent 210 powermeter.

2.5. Measurement of superoxide anion generation

2.5.1. NBT reduction

Table 1 illustrates the procedure for the NBT and cyt *c* assays. To inhibit NBT reduction, and to obtain a blank adjusting for Mϕs and photosensitizer absorbances, Mϕs containing NBT + stimulant were compared with Mϕs incubated with NBT + stimulant + iodoacetamide [17]. The stimulant is either PMA or Pf II at one of the indicated concentrations. Those wells used for the blanks were preincubated with 0.2 ml of a 10 mM solution of iodoacetamide for 10 min at 37 °C. The solutions were removed and 0.1 ml NBT

TABLE 1

Experimental set-up for the NBT reduction assay

Stimulant[a]	Blank	Irradiation parameters	Absorbance[b]
Pf II 10.0 μg ml^{-1}	Pf II 10.0 μg ml^{-1} + IAc	Wavelength, 405 nm	Measured at 550 nm
1.0 μg ml^{-1}	1.0 μg ml^{-1} + IAc	Energy density, 5 J cm^{-2}	Bandwidth, ± 1 nm
0.1 μg ml^{-1}	0.1 μg ml^{-1} + IAc		
PMA 160 ng ml^{-1}	PMA 160 ng ml^{-1} + IAc		
NBT (negative control)	NBT + IAc		

[a]The dilutions of the stimulants were prepared in NBT solution (1 mg ml^{-1} in HBSS) and then pipetted into the wells containing the Mϕs.

[b]Measured every 20 min up to four times in the NBT assay, but only once 90 min after addition of the stimulant or irradiation in the cyt *c* assay.

(1 mg ml^{-1}) containing stimulant was added to untreated wells, whereas NBT containing stimulant and iodoacetamide (10 mM) was added to wells preincubated with iodoacetamide. Two plates were set up separately. One plate was irradiated using the parameters described in Section 2.4. The absorbance of the irradiated plate was measured at 550 nm before and immediately after the irradiation, and then in time intervals of 20 min. Usually, four readings were taken.

2.5.2. Cytochrome c reduction

This assay was performed in a similar manner to the NBT measurements. The suspension containing the Mϕs was adjusted to 1×10^6 ml^{-1}. From this suspension, 0.2 ml was pipetted into each well of a 96 well plate to give a final concentration of 2×10^5 cells $well^{-1}$. Since 75% of the cells were shown to be Mϕs (Section 2.2), the final concentration of Mϕs was 1.5×10^5 Mϕs $well^{-1}$. After allowing the cells to adhere for 1–2 h, they were washed three times with HBSS to remove non-adherent cells. A stock solution of ferricytochrome *c* (160 μM) was prepared, and aliquots containing PMA (160 ng ml^{-1}), Pf II (concentrations of 10.0, 1.0 or 0.1 μg ml^{-1}, PMA+SOD (300 units (U) ml^{-1}) and Pf II (at the three concentrations)+SOD were mixed in the dark. The various solutions thus prepared were added to the wells in triplicate. Wells containing Pf II at one concentration were blanked against the wells containing Pf II and SOD to adjust for the absorbance of Pf II at the wavelength used to determine the reduction of cyt *c*. The absorbance was determined at 550 nm (the bandwidth of the filter was ±1 nm). The absorbances were converted to nanomoles of oxygen based on the extinction coefficient of (reduced minus oxidized) cyt *c*: $\Delta E=21\times10^3$ M^{-1} cm^{-1} [17]. The height of the solution in the well of a 96 well plate was 6 mm (0.2 ml $well^{-1}$). A second type of cyt *c* reduction was performed as described above, but in the presence of 2 mM NaN_3. As has been described [5], NaN_3 inhibits cyt *c* oxidase and prevents reoxidation of cyt *c*.

2.6. Spectra

In order to evaluate the effect of Pf II on the spectral characteristics of cyt *c* and NBT (to indicate a possible oxidation due to singlet oxygen formation), the spectra were recorded on a Beckman DU-7 spectrophotometer.

The readings of all the assays using 96 well plates were taken on an ELISA plate reader from Molecular Devices (THERMOmax™, model 0200-0600, Molecular Devices Corp., Menlo Park, CA).

2.7. Statistical evaluation

The statistical significance was determined using the two-tailed Student's *t*-test. Values of $p<0.05$ were considered to be statistically significant.

3. Results

In order to determine the dark and light toxicity of the photosensitizer on the viability of murine peritoneal Mϕs, 2×10^5 Mϕs per well were incubated

with various concentration of Pf II (0.1, 1.0 and 10.0 $\mu g\ ml^{-1}$) for increasing time intervals. As indicated in Fig. 1(a), a dark toxicity of approximately 25% was observed for the highest concentration of 10.0 $\mu g\ ml^{-1}$ Pf II at

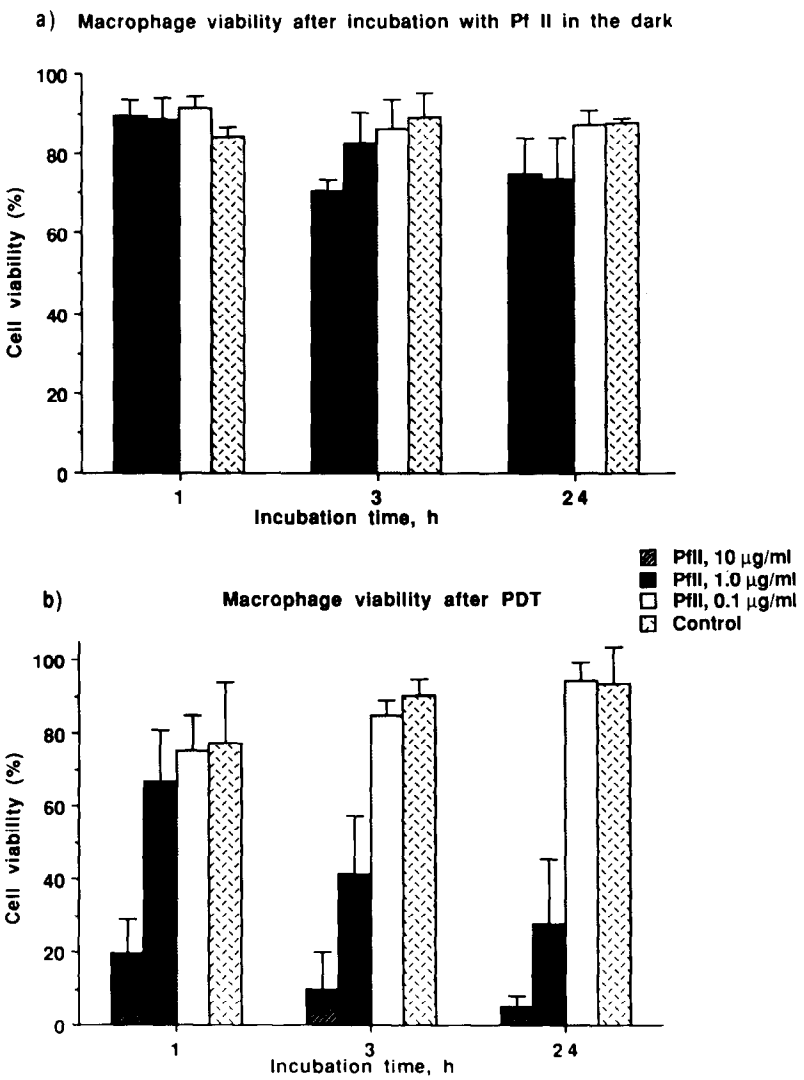

Fig. 1. Effect of Pf II on the viability of mouse peritoneal Mϕs. Peritoneal Mϕs (0.2 ml) ($1 \times 10^6\ ml^{-1}$), collected as described in Section 2.2, were placed in each well of a 24 well plate. Each well contained a coverslip (12 mm in diameter). After adherence of the macrophages to the coverslips (about 1 h at 37 °C), the cells were washed three times with HBSS. Pf II at 10.0, 1.0 and 0.1 $\mu g\ ml^{-1}$ was added. One plate was irradiated, while another set was shielded from ambient light. After incubation for the indicated time periods, the viability of the Mϕs was assessed using acridine orange–ethidium bromide. The data are the mean ± standard deviation (SD) of two independent experiments. (a) Without irradiation. (b) Irradiated at 405 nm ($5\ J\ cm^{-2}$).

the incubation times of 3 and 24 h. At this concentration and for an incubation time of 1 h, cell viability was in the region of the control. At 1 μg ml^{-1} and 24 h, cell viability decreased to 73.3%$\pm$10.4%, but at incubation times of 3 and 1 h, the cell viability was the same as for the control.

After irradiation at 405 nm, which followed immediately after the addition of the photosensitizer, the cells were incubated for the indicated time periods. The viability decreased dramatically for 10.0 and 1.0 μg ml^{-1} of Pf II (Fig. 1(b)). After 1 h incubation following irradiation, viability had decreased to 66.7%$\pm$14.2% in the case of 1.0 μg ml^{-1}, and to 19.5%$\pm$9.5% in the case of 10.0 μg ml^{-1}. Only the lowest concentration of Pf II (0.1 μg ml^{-1}) showed viabilities in the range of the control.

From these data we can conclude that Mϕs incubated with Pf II, but not irradiated, showed some cell death at 10.0 and 1.0 μg ml^{-1} for the longer incubation times of 24 and 3 h, but not for 1 h. However, irradiation at 5 J cm^{-2} and 405 nm induced considerable cell death even at a low Pf II concentration.

The effect of Pf II on the generation of the superoxide anion is illustrated in Figs. 2 and 3. In the NBT assay, an increase in absorbance at 550 nm caused by the reduction of NBT was measured. This reduction occurs due to the generation of a reducing agent such as superoxide anion. The increase in absorbance was rapid when 1.0 μg ml^{-1} Pf II was used without irradiation (Fig. 2(a)). The reduction must have started immediately after the addition of the photosensitizer and persisted for the time of observation. This is in contrast with the stimulation via PMA, where a continuous increase in absorbance was observed. On irradiation of Mϕs stimulated with 1.0 μg ml^{-1} Pf II (Fig. 2(b)), the absorbance showed no change relative to the absorbance without irradiation, but was as prominent as for the positive control PMA. However, the reduction of NBT by Mϕs stimulated with PMA occurred faster after irradiation than without irradiation, reaching the saturation point earlier.

Figure 3 shows the final absorbances 60 min after irradiation. The statistical significance for Pf II at 10 μg ml^{-1} and 1.0 μg ml^{-1} *vs.* the negative control (Mϕ incubated with NBT alone) was $p < 0.05$ for unirradiated samples and $p < 0.01$ for irradiated samples. However, there was no statistically significant difference between irradiated and unirradiated Mϕs. Furthermore, at the lowest concentration of 0.1 μg ml^{-1}, no increase in absorbance was observed. Here, the values were in the region of the absorbance of the negative control.

The results of the cyt *c* assay are depicted in Fig. 4. None of the concentrations of Pf II employed stimulated the Mϕs to release an increased amount of superoxide anion. A dose-dependent decrease in absorbance, *i.e.* decrease in the release of superoxide anion, in relation to spontaneous oxygen release (control), was observed. Irradiation of cells in the presence of PMA slightly increased the generation. For our control, which consisted of Mϕ incubated with cyt *c* alone, oxygen generation doubled. The negative value for superoxide anion at 10.0 μg ml^{-1} Pf II corresponds to a smaller absorbance of Mϕ+Pf II *vs.* Mϕ+Pf II+SOD, indicating oxidation of cyt *c* at 550 nm.

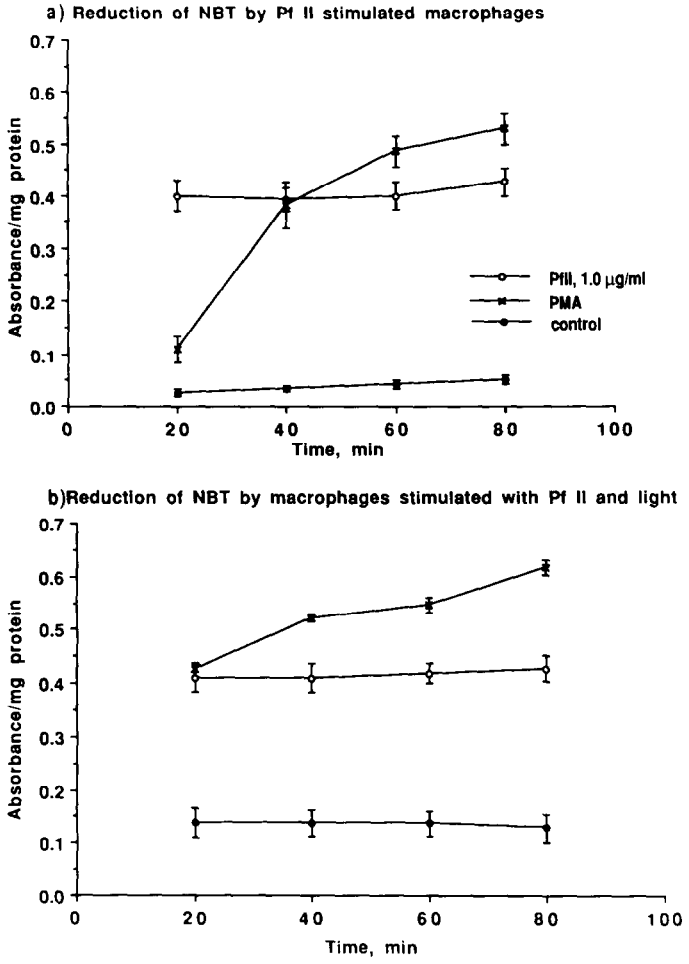

Fig. 2. Reduction of NBT by Mϕs in response to stimulation with Pf II. Macrophages (1.5×10^5 per well) were incubated with the stimulants (either PMA, which served as a positive control, or Pf II at 1 μg ml^{-1}). Immediately following the addition of the stimulants, one plate was irradiated at 5 J cm^{-2} and 405 nm, and the absorption was measured. Absorbance of the unirradiated plate was measured immediately after addition of Pf II. The data shown represent one experiment performed in triplicate (mean value $\pm$ SD) out of three independent experiments. (a) Unirradiated samples. (b) Irradiated samples. Control denotes the negative control consisting of Mϕs incubated with NBT alone, blanked *vs.* NBT plus iodoacetamide.

Both the NBT and cyt *c* assays were performed using Pf II at the same concentrations as indicated above in the absence of Mϕs in order to determine the reaction of the photosensitizer itself with the substrates. In both cases, no pronounced reduction or oxidation of either NBT or cyt *c* was observed (data not shown).

In a second experimental set-up, the Mϕs were incubated with Pf II for either 1 or 24 h prior to irradiation. The cells were washed to remove excess

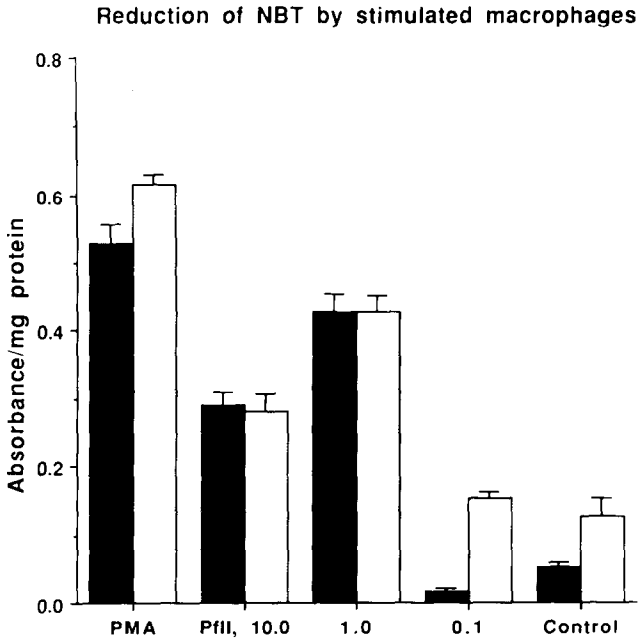

Fig. 3. Reduction of NBT by Mϕs in response to stimulation with varying concentrations (μg ml^{-1}) of Pf II. The absorbance per milligram of macrophage protein is given 60 min after the addition of the stimulant. Unirradiated samples (shaded) are compared with irradiated samples (no shading). The data shown represent one experiment performed in triplicate (mean value $\pm$ SD) out of three independent experiments.

photosensitizer, and the NBT substrate mixed with PMA (160 ng ml^{-1}) was added. One plate was irradiated, while the other plate was shielded from ambient light. Immediately after irradiation, the absorbances were measured in the microplate reader. None of the samples incubated with Pf II showed an increase in NBT reduction *vs.* the control wells which had not received the photosensitizer (data not shown).

4. Discussion

Recently, it has been reported that cells of the Mϕ lineage strongly respond to PDT [10, 11]. In this study, the viability of Mϕs, incubated with the photosensitizer Pf II, was strongly impaired on irradiation at 405 nm. A dark toxicity of about 25% was observed at the highest Pf II concentration of 10 μg ml^{-1}. However, irradiation of wells containing Pf II at a low concentration of 1 μg ml^{-1} induced a cell killing of more than 60% after 24 h incubation. These results on the viability of the Mϕs were unexpected because we had assumed that these cells were more resistant to PDT than tumor cells. According to reports on the cell viability of the human colon adenocarcinoma cell line WiDr [18, 19]. Pf II alone below 100 μg ml^{-1} was not toxic to the cells. Incubation with 10 μg ml^{-1} in a medium containing

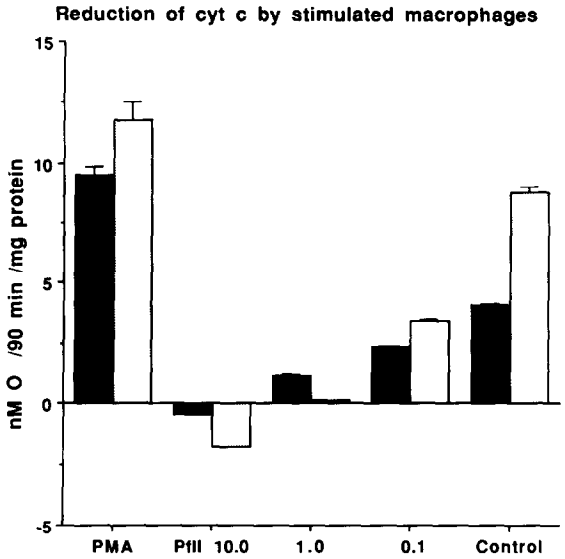

Fig. 4. Release of superoxide anion by Mϕs in response to Pf II measured by reduction of cyt *c*. Macrophages (1.5×10^5 per well) were incubated with cyt *c* mixed with either PMA or Pf II at 10.0, 1.0 and 0.1 μg ml^{-1}. Unirradiated samples (dark shading) are compared with irradiated samples (light shading). The data shown represent one experiment performed in triplicate (mean value $\pm$ SD) out of three independent experiments. Control denotes cyt *c* incubated with the cells without any additions.

FBS and irradiation with white light in the range 300–1100 nm at an energy density of 4 J cm^{-2} resulted in a sharp decline in cell viability. Using this concentration of 10 μg ml^{-1} Pf II in a medium with FBS, the Mϕs also responded with a strong decrease in viability following irradiation with 405 nm laser light. This toxic effect can be explained by the high phagocytic activity of Mϕs, resulting in a high uptake of Pf II, and by the activation of Pf II at the Soret wavelength.

For the irradiation parameters employed (5 J cm^{-2}; 405 nm; 150 mW cm^{-2}), induction of hyperthermia is very unlikely, because our control cells showed a viability of better than 95%. We were interested in cell viability under the conditions employed in the NBT and cyt *c* assays. The photosensitizer was not removed prior to irradiation because it was observed that preincubation and removal of Pf II did not result in a higher generation of oxygen. An enhanced cell death due to the generation of $^1O_2$ by Pf II and light in the extracellular medium is unlikely due to the short lifetime of singlet oxygen [8].

Strong fluorescence of Pf II was observed inside the murine Mϕs after 15 min incubation time (data not shown). A high retention of the photosensitizer in tumor Mϕs has been described by Krosl *et al.* [20] and was suggested as a possible basis for tumor localization of Pf II. These workers have shown that tumor-associated Mϕs take up more Pf II than the tumor cells themselves.

Cells other than Mϕs rapidly take up HpD within an incubation time of 10 min [21]. The photosensitizer binds to the mitochondria and strongly affects them.

Incubation of Mϕs with Pf II resulted in the generation of a reducing agent intracellularly, most probably the reactive oxygen derivative superoxide anion. According to Pick [17], NBT reduction is an empirical method for superoxide anion generation. Iodoacetamide inhibitable reduction of NBT indicates that the mitochondria are involved in the activation process pointing to the generation of a reactive oxygen intermediate. This generation is rapid, immediately after the addition of the photosensitizer to the cells. Saturation of the amount of the reducing agent was accomplished within 20 min following the exposure of the Mϕ to Pf II. The observation that there is hardly any difference between the absorbances of unirradiated and irradiated wells for Pf II at 1.0 μg ml^{-1} may be attributed to the fact that irradiation of photosensitized cells results in a pronounced immediate cell death of more than 30% at this concentration. Most importantly, the intracellular generation occurred with as well as without irradiation, which implies a strong effect of the photosensitizer alone.

When the photosensitizer was mixed with the substrate, and the measured absorbance was calculated *vs.* its blank for each concentration, no reduction was observed. Thus, it is obvious that it is the Mϕs which are responsible for the reduction. These results support the observations by Gibson *et al.* [22] and Carraro and Pathak [23], who showed in *in vitro* studies that HpD does not reduce NBT or cyt *c*.

Release of superoxide anion into the extracellular medium was measured using the standard cyt *c* assay [24]. Obviously, such a release does not occur (Fig. 4). If superoxide anion had been released, it might have been dismutated immediately to H_2O_2 or $OH^{\cdot}$, therefore escaping measurement. Rapid intracellular conversion of superoxide anion into an oxidizing molecule may also be responsible for the relatively low concentration of this anion measured in the NBT assay. We did not attempt to measure the generation of H_2O_2 as, according to Cohn [25], thioglycollate-primed Mϕs do not produce measurable amounts of this oxidizing agent.

Our inability to measure extracellular superoxide anion is in agreement with the study by Marshall *et al.* [26]. In this *in vivo* study, peritoneal Mϕs isolated 24 h following Pf II injection, were neither activated nor impaired. Both findings contradict our preliminary hypothesis that tumor-associated Mϕs may also be involved in the killing of the tumor cells in addition to the cytotoxic effects of photosensitizer plus light. It is probable that these immunologically relevant cells contribute to the inflammatory reactions observed after PDT [27]. Experiments conducted in our laboratory [28] indicate that the complement system is involved in the PDT-induced acute inflammation. As Mϕs can produce some of the complement proteins and express complement receptors [29–31], a direct relationship may exist. The existence of such a relationship should be clarified in further investigations.

5. Conclusions

Pf II plus irradiation at 405 nm can strongly impair the viability of murine peritoneal Mϕs. Without irradiation and at a sublethal concentration of Pf II, a reducing agent, most probably superoxide anion, is generated intracellularly. Although this generation is not enhanced on irradiation of cells containing Pf II, the reaction rate of the stimulation with PMA (positive control) is increased. Release of superoxide anion into the extracellular medium does not occur. The intracellular generation of a reducing agent can be considered as a trigger for oxidative burst activation, which is a prerequisite for the PGE_2 release reported previously [11].

Acknowledgments

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