Ultraviolet irradiation accelerates apoptosis in human polymorphonuclear leukocytes: protection by LPS and GM-CSF

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Abstract: Polymorphonuclear leukocytes (PMN) play a central role in host response to injury and infection. Understanding factors that regulate PMN survival may therefore have a major influence on the development of novel treatment strategies for controlling life-threatening infections, as well as local and systemic inflammatory responses. Unfortunately, the presently utilized in vitro culture model of PMN apoptosis makes the examination of early biochemical events surrounding PMN apoptosis very difficult. This study demonstrates that a short course of UV irradiation (15 min) can be used to induce rapid progression of PMN through the apoptotic process with 70–90% of PMN displaying features of apoptosis by 4 h after UV exposure. Bacterial lipopolysaccharide and granulocyte-macrophage colony-stimulating factor, which are known to prolong PMN survival during in vitro culture, also protected PMN from UV-accelerated apoptosis. The UV-accelerated model of PMN apoptosis provides another valuable tool for the investigation of early signaling pathways associated with inducing or delaying PMN apoptosis. *J. Leukoc. Biol.* 62: 517–523; 1997.

Key Words: *inflammation · necrosis · priming*

INTRODUCTION

Neutrophils (PMN) are the primary effector cells in the host response to injury and infection [1]. Normally, these terminally differentiated phagocytes have a circulating life span in vivo of 6–10 h, after which they undergo programmed cell death, or apoptosis [2]. The spontaneous entry of PMN into the apoptotic pathway minimizes the potential leakage of lytic enzymes and proinflammatory substances from PMN. Safe removal of apoptotic PMN by the reticuloendothelial system is a mechanism to limit the toxic potential of these cells [2–4]. Recent studies have shown that bacterial lipopolysaccharide (LPS) and several proinflammatory cytokines delay apoptosis of cultured PMN [5–10]. These agents are also known to prime many PMN antimicrobial functions. Extrapolation of these findings to the in vivo milieu suggests an increased probability for primed PMN to release lytic enzymes and proinflammatory substances, which may then cause damage to surrounding tissues and initiate a pathological inflammatory response. Understanding signaling pathways that modulate PMN apoptosis may therefore have a major influence on the development of treatment strategies for controlling local and systemic inflammatory responses.

Although there is considerable interest regarding the events leading to PMN apoptosis, the present model available for the study of the early biochemical phenomena associated with PMN apoptosis is not optimal for this purpose. PMN are cultured and observed for the morphological features of apoptosis as they age in vitro over a 24- to 48-h period [5–11]. During this time course PMN asynchronously proceed from viable to apoptotic to secondary necrotic. The lack of synchronicity in the in vitro culture model makes it difficult to study early biochemical events associated with PMN apoptosis. This study was undertaken to determine whether a short course of ultraviolet (UV) radiation could induce the characteristic features of PMN apoptosis and produce a rapid progression of PMN into the apoptotic pathway. This method of inducing apoptosis has been previously described for the human leukemia HL-60 cell line [12, 13]. We hypothesize that the morphological features of UV-induced PMN apoptosis are similar to those of PMN that have been aged in vitro, but that they occur in a more rapid and homogenous fashion. We further hypothesize that bacterial LPS and granulocyte-macrophage colony-stimulating factor (GM-CSF), which are known to delay PMN apoptosis and prolong PMN survival during in vitro culture, will protect PMN from UV-induced apoptosis.

Abbreviations: PMN, polymorphonuclear leukocytes; LPS, lipopolysaccharide; GM-CSF, granulocyte-macrophage colony-stimulating factor; UV, ultraviolet.

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MATERIALS AND METHODS

Reagents

All reagents were purchased from Sigma Chemical Co., St. Louis, MO, except where otherwise indicated.

Growth medium

RPMI 1640 growth medium (GIBCO, Grand Island, NY) containing 10% heat-inactivated fetal bovine serum, 2 mM L-glutamine, 100 units/mL penicillin, 100 μg/mL streptomycin, and 5 mM *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid buffer was utilized as growth medium (medium).

Isolation of PMN

All materials and solutions used in the preparation of PMN were deemed non-pyrogenic, in that they contained undetectable (0.1 ng/mL) endotoxin according to the amoebocyte lysate assay (Sigma). Briefly, venous blood was collected from healthy volunteers into 1/6 volume acid citrate dextrose anticoagulant solution. PMN were then partially purified from red blood cells by dextran-70 sedimentation, and further purified by Percoll gradient separation as previously described by Haslett et al. [14]. This method of PMN isolation yields an unprimed PMN population (determined by measurement of oxidant generation in response to fMLP using the PHPA method developed by Hyslop and Sklar [15] of ≥98% purity. After isolation PMN were resuspended at a final concentration of 5×10^6/mL in medium for all experiments.

In vitro model of PMN apoptosis

PMN, at a final concentration of 5×10^6/mL, were combined with or without LPS (*Escherichia coli* serotype O55:B5, Sigma) in the wells of a 24-well polystyrene tissue culture plate. At varying time points PMN were harvested and assessed for the morphological features of apoptosis, using fluorescence microscopy (see below). Assessment of DNA fragmentation, using agarose gel electrophoresis, was also undertaken with DNA isolated from PMN (see below).

UV-accelerated PMN apoptosis

Induction of PMN apoptosis was achieved by using UV-irradiation as previously described [12, 13]. PMN (5×10^6/mL), incubated with or without LPS or GM-CSF (R & D Systems, Minneapolis, MN), were seeded in the wells of a 24-well polystyrene tissue culture plate (Costar). After cells had settled into an even monolayer, they were exposed from below to a 312-nm wavelength transilluminator source (Fotodyne Inc., model 3-3000) at a distance 2.5 cm from the transilluminator surface for varying time periods at room temperature. The UV intensity at this distance, as calculated per the manufacturer's instructions with a UVX Digital Radiometer (UVP, Inc., San Gabriel, CA), was 32 mW/cm^2. Exposure of cells to UV irradiation at a distance of less than 2.5 cm results in significant necrosis. After UV irradiation, cells were incubated at 37°C/5% CO_2 and features of apoptosis were determined at varying time points by use of the methods described below.

Determination of apoptotic index, cell viability, and cell morphology

At each experimental time point, the viability and apoptotic index of the cultured PMN was determined using a dye mixture of 10 μM acridine orange/10 μM ethidium bromide (Sigma) prepared in phosphate-buffered saline [16]. Acridine orange (fluorescent DNA-binding dye) intercalates into DNA, making it appear green, and binds to RNA making it appear red-orange. Ethidium bromide is only taken up by nonviable cells, overwhelming the fluorescence of acridine orange, making the chromatin of necrotic cells appear orange.

At each time point, 25 μL of the PMN monolayer was aspirated and mixed with 1 μL of the dye mixture. At least 200 cells were then examined from each sample, and the percentage of apoptotic, necrotic, and viable cells determined using the following criteria: viable cells with normal nuclei, fine reticular pattern of green stain in the nucleus and red-orange granules in the cytoplasm; apoptotic cells, green chromatin that is highly condensed or fragmented and uniformly stained by the acridine orange; necrotic cells, bright orange chromatin stained by ethidium bromide; % apoptotic cells, number of apoptotic cells/total number of cells counted; % necrotic cells, number of necrotic cells/total number of cells counted; % viable cells, 100 − (% apoptotic cells + % necrotic cells).

DNA fragmentation analysis

The characteristic laddering of DNA into multiples of ≈180 base pairs was demonstrated using a procedure described by Laird et al. with slight modifications [17]. At each time point PMN samples (5×10^6 cells) were lysed with 500 μL of buffer (100 mM Tris, 5 mM ethylenediaminetetraacetate, 200 mM NaCl, and 0.2% sodium dodecyl sulfate at pH 8.5) containing Proteinase K (Sigma) at a final concentration of 400 μg/mL. Samples were then incubated in a shaking water bath for 1 h at 50°C. DNA was then precipitated with an equal volume of cold isopropanol with gentle mixing. The precipitate was pelleted by centrifugation at 14,000 *g* for 30 min, air dried (after decanting the supernatant) under a fume hood for 20 min, and then resuspended in 100 μL of TE buffer (10 mM Tris, 1 mM ethylenediaminetetraacetate, pH 7.6) containing 80 μg/mL of RNase. After an overnight incubation at 25°C, 3 μL of a 10× loading buffer (0.25% bromophenol blue, 0.25% xylene cyanol FF, 30% glycerol) was added to 27 μL of the RNase-treated sample and 10 μL was transferred to each well of a 1% agarose containing 7 μg/gel of ethidium bromide. Horizontal agarose gel electrophoresis was performed at 125 mV for 1 h. The gel was photographed under UV light.

Data presentation and statistical analysis

Data are expressed as mean ± SEM and were analyzed by two-tailed paired Student's *t*-test. Differences were taken to be significant for $P < 0.05$.

RESULTS

PMN undergo spontaneous apoptosis during in vitro culture

PMN suspended in 10% FCS at a concentration of 5×10^6/mL, were incubated at 37°C in 5% CO_2, and observed for the morphological changes of apoptosis at 12-, 24-, and 36-h time points using fluorescent microscopy with acridine orange/ethidium bromide staining. There was an initial increase in the percentage of PMN demonstrating apoptotic features over the first 12 h. This became maximal at 24 h and plateaued thereafter (**Fig. 1**). The percentage of PMN displaying necrotic features increased steadily throughout the same time course (Fig. 1). The net result was a decrease in the number of viable PMN with time (Fig. 1).

LPS delays PMN apoptosis and prolongs PMN survival during in vitro culture

PMN were cultured with *Escherichia coli* LPS at a final concentration of 10 ng/mL. PMN incubated in medium alone served as control. The cells were then placed in culture as described above and observed for the morphological features of apoptosis at 12 and 24 h using fluorescent microscopy with acridine orange/ethidium bromide staining. At

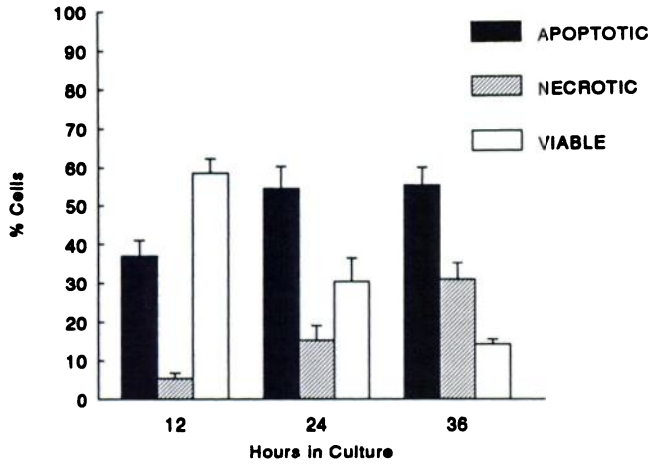

Fig. 1. In vitro aging model of PMN apoptosis. Results represent the mean of six experiments. Error bars represent standard error of the mean (SEM).

the 12-h time point, PMN treated with LPS demonstrated significantly fewer apoptotic features when compared with control (**Fig. 2A**). There was minimal evidence of necrosis in either treatment group. Similar findings were present at the 24-h time point. LPS-treated PMN demonstrated significantly less apoptotic and necrotic features when compared with control PMN (Fig. 2B). LPS treatment resulted in a significant overall increase in the percentage of viable PMN.

UV irradiation induces morphological features of apoptosis

To test the hypothesis that UV irradiation can synchronize and accelerate the apoptotic process in PMN we exposed PMN to a UV light source for 15, 30, and 60 min at room temperature. This method has been previously described for the human leukemia HL-60 cell line [12, 13]. Control PMN did not receive any UV exposure. After UV irradiation, cells were incubated at 37°C/5% CO_2 and features of apoptosis were determined at 0, 2, and 4 h post UV exposure by use of fluorescence microscopy with acridine orange/ethidium bromide staining. **Figure 3** illustrates the dose response of PMN to UV irradiation. It is clear from these results that the length of UV exposure determined the mode of PMN death. PMN irradiated for 15 or 30 min demonstrated minimal features of apoptosis immediately after UV exposure when compared with control PMN (Fig. 3B). At the 2- and 4-h time points PMN exposed to UV radiation for 15 and 30 min demonstrated a significant increase in the percentage of apoptotic PMN (Fig. 3A). UV irradiation of PMN for 60 min resulted in a completely different type of cell death. Although a significant percentage of PMN demonstrated features of apoptosis immediately after completion of the 60-min UV exposure, a significant percentage of PMN also demonstrated morphological features of necrosis (Fig. 3, A and B). This trend was present throughout the 4-h time course. UV irradiation of PMN for 30 min also significantly increased the percentage of necrotic cells at each time point when compared with control PMN (Fig. 3B). For PMN exposed to 15 min of UV irradiation, the percentage of cells demonstrating necrotic features was between 5 and 10% at all time points and this was not significantly different from control PMN (Fig. 3B). These results demonstrate that a short course of UV irradiation (15 min) accelerates the apoptotic process in PMN without inducing a significant amount of necrosis.

LPS protects PMN from UV-accelerated apoptosis

Several investigators have shown that LPS prolongs PMN survival during in vitro culture [5, 6]. We therefore felt it a logical step to investigate whether LPS could delay UV-accelerated apoptosis in PMN. PMN were preincubated for 60 min with 10-fold serial dilutions of LPS ranging from

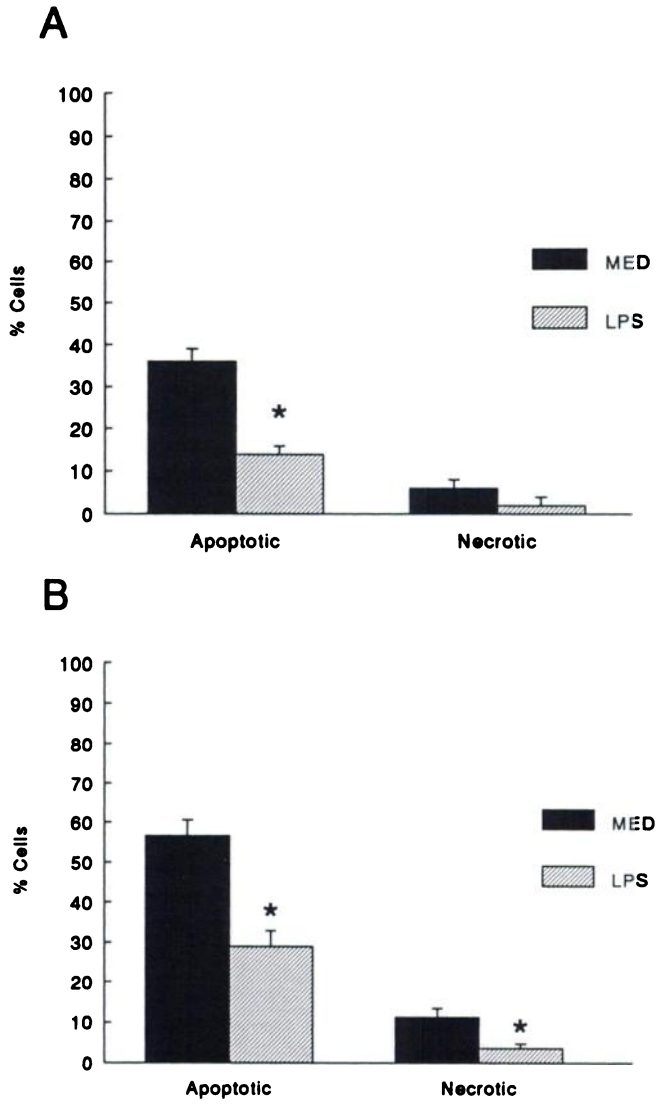

Fig. 2. LPS delays PMN apoptosis. PMN were incubated in medium (solid bars) or with 10 ng/mL LPS for 12 (A) and 24 (B) h at 37°C. Results represent the mean of seven experiments. Error bars represent SEM. $*P < 0.01$ paired Student's *t*-test.

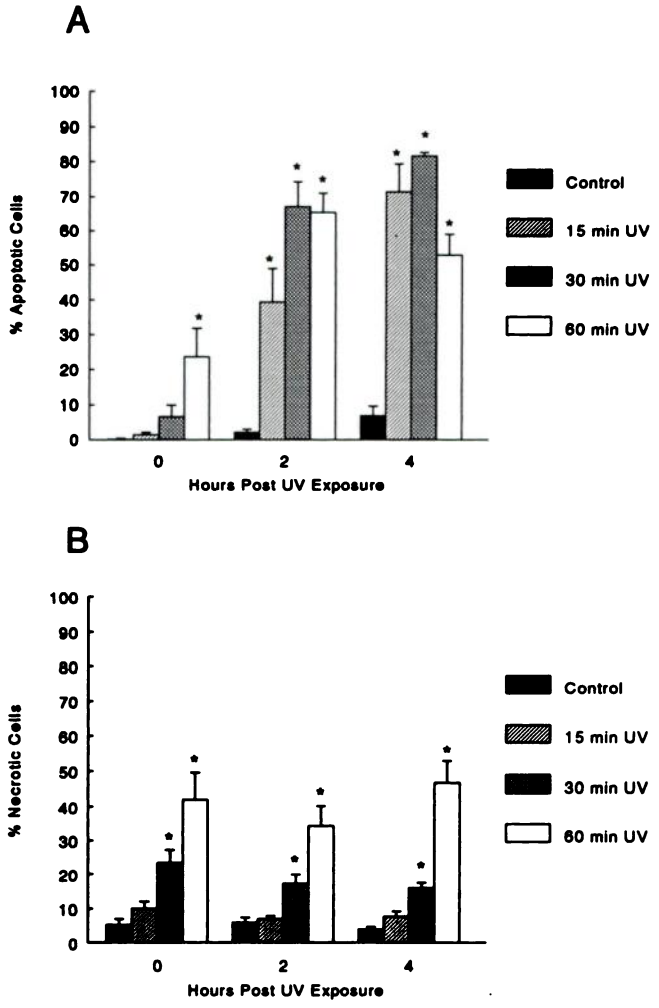

Fig. 3. UV irradiation induces apoptosis. PMN were UV-irradiated for 0, 15, 30, and 60 min at a dose of 32 mW/cm^2 at room temperature and then incubated at 37°C. Morphological features of apoptosis (A) and necrosis (B) were determined with fluorescence microscopy utilizing acridine orange/ethidium bromide staining. Results represent the mean of four experiments. Error bars represent SEM. $*P < 0.05$ paired Student's *t*-test.

1 ng/mL to 10 μg/mL. After LPS preincubation, PMN were UV-irradiated for 15 min, and then followed for the morphological features of apoptosis at 2 and 4 h. Approximately 90% of control PMN demonstrated morphological features of apoptosis 4 h after UV exposure (**Fig. 4**). LPS preincubation conveyed a significant protective effect on UV-accelerated PMN apoptosis at concentrations between 10 ng/mL and 10 μg/mL. LPS doses of 1 ng/mL or less did not protect PMN from UV-induced apoptosis. Similar results were obtained when evaluating PMN morphology 2 h after UV exposure (data not shown).

To determine the optimal timing of LPS pretreatment on UV-accelerated PMN apoptosis, PMN were preincubated with LPS (20 ng/mL) for 15, 30, and 60 min, before being exposed to UV irradiation for 15 min. PMN incubated in medium alone served as control. PMN morphology was then evaluated 2 and 4 h after UV exposure. These experiments demonstrate the time-dependent nature of LPS preincubation on protection of PMN from UV-accelerated apoptosis (**Fig. 5**). As the length of LPS preincubation increased there was a concomitant decrease in the percentage of apoptotic PMN. Protection from apoptosis was greatest in these experiments when PMN were pretreated with LPS for 60 min.

LPS prevents DNA fragmentation

To confirm the morphological evidence of apoptosis in the previous experiments, agarose gel electrophoresis was undertaken with DNA extracted from PMN 2 and 4 h after UV exposure and after 24 h of in vitro culture (**Fig. 6**). At 2 h after UV treatment, minimal evidence of DNA fragmentation was noted in any of the treatment groups. This corresponded with the morphological data at 2 h for this particular experiment and suggests that a threshold level of apoptotic cells greater than 20% is required before laddering is seen. At 4 h after UV treatment, DNA extracted from control PMN demonstrated a pattern of DNA laddering that is much clearer than DNA isolated from PMN after 24 h of in vitro culture (Fig. 6; lane 6 vs. lane 10). DNA extracted from PMN that were preincubated with LPS for 15, 30, or 60 min (Fig. 6; lanes 7, 8, and 9, respectively), demonstrated a decrease in DNA laddering that correlated well with the morphological features of apoptosis at the four time points. DNA extracted from LPS-treated PMN after 24 h of in vitro culture demonstrated marked inhibition of chromatin fragmentation on gel electrophoresis compared with control PMN at 24 h (Fig. 6; lane 11). These results demonstrate that LPS decreases DNA fragmentation in both models of PMN apoptosis and reinforces the concept that protection of PMN from UV-accelerated apoptosis by LPS depends on the period of LPS exposure before acceleration of apoptosis with UV irradiation.

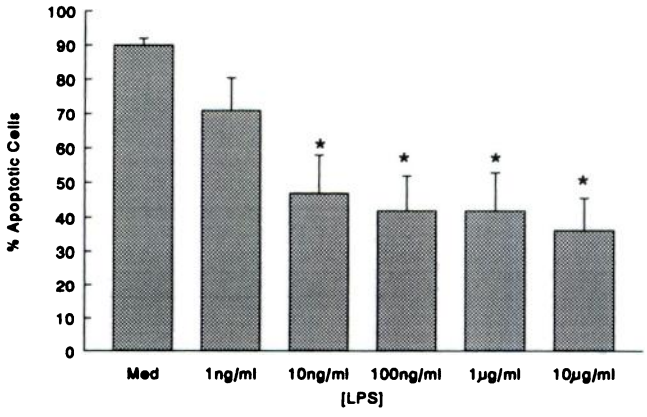

Fig. 4. LPS dose response for protection of UV-accelerated PMN apoptosis. PMN were preincubated with 10-fold serial dilutions of LPS for 60 min at 37°C, UV irradiated for 15 min at room temperature at a dose of 32 mW/cm^2, and then reincubated at 37°C. Morphological features of apoptosis were determined 4 h after UV irradiation with fluorescence microscopy utilizing acridine orange/ethidium bromide staining. Results represent the mean of four experiments. Error bars represent SEM. $*P < 0.05$, paired Student's *t*-test.

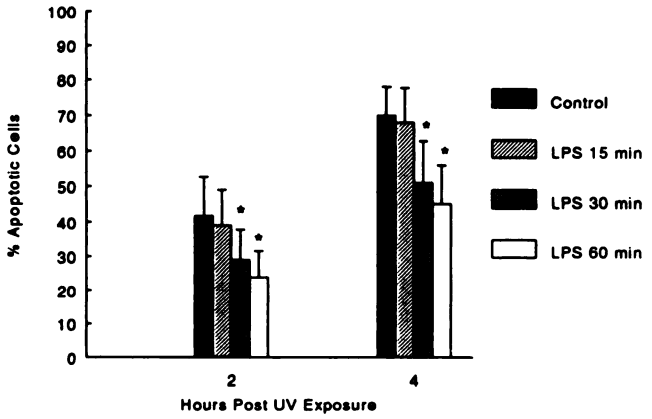

Fig. 5. LPS time response for protection of UV-accelerated PMN apoptosis. PMN were preincubated with 20 ng/mL of LPS for 0, 15, 30, and 60 min at 37°C, UV-irradiated for 15 min at a dose of 32 mW/cm^2 at room temperature, and then reincubated at 37°C. Morphological features of apoptosis were determined 2 and 4 h after UV irradiation with fluorescence microscopy utilizing acridine orange/ethidium bromide staining. Results represent the mean of seven experiments. Error bars represent SEM. $*P < 0.05$ paired Student's t-test.

GM-CSF protects PMN from UV-accelerated apoptosis

The next set of experiments with GM-CSF were undertaken to determine whether a PMN specific cytokine, which is known to prolong PMN survival during in vitro culture, can also protect PMN from UV-accelerated apoptosis. PMN preincubated with GM-CSF for 60 min were significantly more resistant to UV irradiation than were control PMN (**Fig. 7**). There was no significant difference between the 100-unit/mL and the 1000-unit/mL dose of GM-CSF.

DISCUSSION

PMN are terminally differentiated phagocytes that play a sentinel role in host defense against microbial invasion [1]. There is also considerable evidence that PMN contribute to the tissue injury associated with the initiation and propagation of systemic inflammatory responses and multisystem organ failure in critically ill patients. Understanding factors that modulate PMN apoptosis may therefore have a major influence on the development of treatment strategies for controlling local and systemic inflammatory responses. Unfortunately, the accepted in vitro culture model of PMN apoptosis makes the study of early biochemical events associated with PMN apoptosis very difficult if not impossible. In this study we have demonstrated that UV irradiation can accelerate and synchronize PMN apoptosis. This form of PMN apoptosis was also found to be sensitive to the survival prolonging effect of LPS and GM-CSF.

During in vitro culture, PMN spontaneously undergo apoptosis. However, this is not a synchronous process and results in an inhomogenous population of PMN at any given time point. The number of apoptotic cells in our experiments increased at 12 and 24 h of culture, but plateaued by 36 h. During this same time course, there was a progressive increase in the percentage of PMN displaying features of necrosis. In vivo, apoptotic PMN are rapidly removed from the circulation and tissues by the reticuloendothelial system, minimizing the release of lytic enzymes and proinflammatory substances from PMN [2–4]. In the in vitro model, apoptotic PMN are not removed by the reticuloendothelial system and begin displaying features of necrosis as their cell membrane integrity is lost. The increase in the percentage of necrotic PMN with time therefore represents secondary necrosis. Regardless, the prolonged time course and inhomogenous population of apoptotic, necrotic, and viable cells make this model ineffective for the study of early events surrounding PMN apoptosis.

In an attempt to produce a rapid progression of PMN into the apoptotic pathway, we investigated the ability of UV irradiation to accelerate PMN apoptosis. Depending on the duration of exposure, we demonstrated that UV irradiation could induce two distinct forms of cell death. Sublethal injury with 15 min of UV irradiation triggered the cell suicide mechanism, ultimately resulting in PMN apoptosis. This short course of UV irradiation induced rapid synchronous progression of PMN through the apoptotic process,

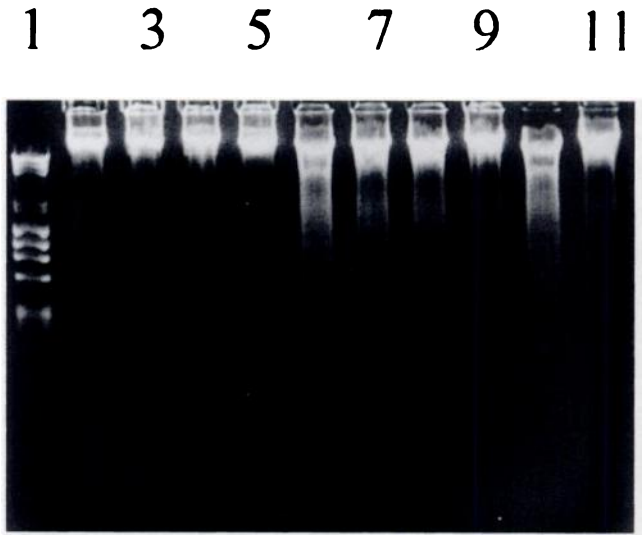

Fig. 6. Agarose gel electrophoresis of DNA extracted from PMN 2 and 4 h after UV irradiation and after 24 h of in vitro culture. UV-irradiated PMN were pretreated with LPS (20 ng/mL) for the respective times before being UV irradiated for 15 min at a dose of 32 mW/cm^2 as described in Materials and Methods. Lane 1, DNA markers (Hind III, HAE III digests; GIBCO); lanes 2–5, PMN 2 h after UV irradiation; lanes 6–9, PMN 4 h after UV irradiation; lane 10, control PMN after 24 h in vitro culture; lane 11, LPS-treated PMN after 24 h in vitro culture. Lanes 2 and 6, no LPS before UV irradiation; lanes 3 and 7, PMN pretreated with LPS for 15 min before UV irradiation; lanes 4 and 8, PMN pretreated with LPS for 30 min before UV irradiation; lanes 5 and 9, PMN pretreated with LPS for 60 min before UV irradiation. The percentage of cells with morphological features of apoptosis were as follows for each treatment group: lane 2, 22% apoptotic; lane 3, 18% apoptotic; lane 4, 18% apoptotic; lane 5, 8% apoptotic; lane 6, 68% apoptotic; lane 7, 72% apoptotic; lane 8, 46% apoptotic; lane 9, 20% apoptotic; lane 10, 62% apoptotic, 24% necrotic; lane 11, 19% apoptotic, 1% necrotic.

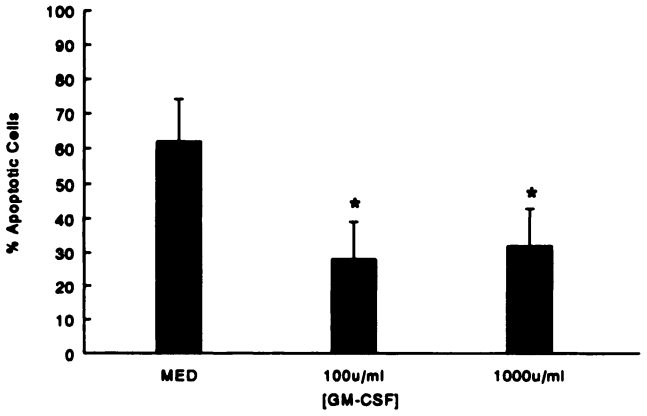

Fig. 7. GM-CSF protects PMN from UV-accelerated apoptosis. PMN were preincubated with GM-CSF for 60 min at 37°C, UV irradiated for 15 min at room temperature, and then re-incubated at 37°C. Morphological features of apoptosis were determined 4 h after UV irradiation with fluorescence microscopy utilizing acridine orange/ethidium bromide staining. Results represent the mean of three experiments. Error bars represent SEM. $*P < 0.05$, paired Student's *t*-test.

with 70–90% of cells displaying features of apoptosis by 4 h. The percentage of apoptotic PMN at each time point in the in vitro model was never greater than 55–60% in our experiments, and this was also associated with a significant number of PMN expressing morphological features of necrosis. Higher doses of UV irradiation (30 and 60 min) also resulted in progressively larger numbers of cells displaying features of necrosis. This bimodal response to injurious stimuli is well characterized in several other models of cell death. At low doses, the calcium ionophore A23187 induces apoptosis in rodent thymocytes and HL-60 cells [18, 19]. At higher doses the same agent induces necrosis. Similar responses to heat shock and chemotherapeutic agents have also been reported for lymphocytes and HL-60 cells, respectively [20, 21].

A biochemical hallmark of apoptosis is the activation of endogenous endonucleases resulting in the fragmentation of DNA into multiples of ≈ 180 base pairs. To confirm that the morphological features of UV-treated PMN were truly representative of apoptosis, we undertook agarose gel electrophoresis of DNA extracted from UV-treated PMN. We clearly documented a distinct ladder pattern of DNA cleavage at 4 h after UV treatment, which was clearer when compared with DNA extracted from the same PMN after 24 h of in vitro culture. These observed differences are most likely related to the inhomogenous population of apoptotic and necrotic PMN after 24 h of in vitro culture. Loss of cell membrane integrity and rupture of lysosomal compartments during PMN necrosis releases a whole spectrum of proteases capable of degrading histones. Any DNA fragmentation that takes place during necrosis results in a smear, because the entire length of DNA is exposed to nuclease attack. The lack of DNA laddering at the 2 h time point is also an important observation. It implies that internucleosomal cleavage of DNA seen at 4 h after UV treatment is a result of endogenous endonuclease activation and not from direct effects of UV irradiation. By itself, UV irradiation is known to cause direct damage to DNA, which can interfere with DNA replication and translation [22]. However, in the presence of known DNA repair mechanisms, this is unlikely to kill the cell directly. More likely UV irradiation triggers constituitively expressed signaling pathways involved in PMN programmed cell death, which ultimately lead to endonuclease activation and DNA laddering, and the morphological features of apoptosis.

Several investigators have documented that PMN survival during in vitro culture can be significantly prolonged by bacterial LPS [5, 6]. We confirmed this finding and extended it to the UV-accelerated model of PMN apoptosis. LPS pretreatment of PMN provided significant protection from UV-accelerated apoptosis. LPS is known to have profound effects on PMN function both in vivo and in vitro. In physiologically relevant concentrations, LPS has been shown to increase the expression of PMN cell surface receptors [23], induce the production of cytokines [24], and prime PMN for an enhanced oxidative burst [25, 26]. It is interesting to note that the optimal time and dose response kinetics associated with protection of PMN from UV-accelerated apoptosis by LPS are very similar to those required for maximal priming of the oxidative burst in PMN [26].

LPS is a nonspecific stimulator of immune function. We were therefore interested to determine whether GM-CSF, which is a more specific activator of PMN functions, would also be able to protect PMN from UV-induced apoptosis. GM-CSF has been shown to prolong PMN survival during in vitro culture [8, 10]. Our data demonstrate that GM-CSF is also capable of protecting PMN from UV-accelerated apoptosis.

In summary, we have demonstrated that a short course of UV irradiation can be used to accelerate PMN apoptosis without inducing a significant amount of necrosis. LPS and GM-CSF, two agents that are known to prolong the survival of PMN during in vitro culture, also protected PMN from UV-accelerated apoptosis. Investigation of signaling pathways associated with initiating or delaying PMN apoptosis, utilizing the UV-accelerated model of PMN apoptosis, may provide significant insight into the regulation of PMN survival and lead to the development of novel treatment strategies for the control of local and systemic inflammatory responses.

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