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Evidence that endoplasmic reticulum (ER) stress and caspase-4 activation occur in human neutrophils

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ABSTRACT

Apoptosis can result from activation of three major pathways: the extrinsic, the intrinsic, and the most recently identified endoplasmic reticulum (ER) stress-mediated pathway. While the two former pathways are known to be operational in human polymorphonuclear neutrophils (PMNs), the existence of the ER stress-mediated pathway, generally involving caspase-4, has never been reported in these cells. Recently, we have documented that arsenic trioxide (ATO) induced apoptosis in human PMNs by a mechanism that needs to be further investigated. In this study, using immunofluorescence and electron microscopy, we present evidence of ER alterations in PMNs activated by the ER stress inducer arsenic trioxide (ATO). Several key players of the unfolded protein response, including GRP78, GADD153, ATF6, XBP1 and eIF2α are expressed and activated in PMNs treated with ATO or other ER stress inducers. Although caspase-4 is expressed and activated in neutrophils, treatment with a caspase-4 inhibitor did not attenuate the pro-apoptotic effect of ATO at a concentration that reverses caspase-4 processing and activation. Our results demonstrate for the first time that the ER stress-mediated apoptotic pathway operates in human neutrophils.

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Introduction

Although there is no doubt that both the extrinsic and intrinsic pathways of cell apoptosis are operational in polymorphonuclear cells (PMNs), there is no evidence that the recently identified endoplasmic reticulum (ER) stress-mediated cell apoptotic pathway exists in these cells. Caspase-4, an ER-resident caspase known to be implicated in ER stress-induced apoptosis [1], has, thus far, only been studied at the mRNA level in human PMNs; therefore, its protein expression and activity in these cells has never been reported.

Because of the existence of high concentrations of proteins, large amount of chaperone molecules are present in the ER in order to maintain proteins in a folding-competent state, and to prevent protein folding intermediates from aggregation. However, a number of cellular stress or cytotoxic conditions lead to accumulation and aggregation of unfolded and/or misfolded proteins in the ER lumen and cause so-called ER stress [2]. ER stress has been implicated in diverse diseases, including cancer, diabetes, cerebral ischemia, neurodegenerative and cardiovascular diseases [3].

ER stress leads cells to initiate self-protective mechanisms via activation of a range of stress-response signaling pathways termed the unfolded protein response (UPR). The UPR emanates from the ER, but may require both the nucleus and the Golgi apparatus for signal transduction [4]. The main goal for initiating UPR is to reduce unfolded protein load in the ER by attenuating translation (to limit further accumulation of misfolded proteins), promoting protein folding, secretion and degradation. In brief, the UPR is fundamentally a cytoprotective response. However, excessive or prolonged UPR (if ER homeostasis cannot be restored) may result in apoptosis via mitochondria-dependent or -independent mechanisms. Although the ER is now being recognized as an important organelle that can regulate programmed cell death, the signal-transducing events required for connecting ER stress to the apoptotic machinery are still under intensive investigation. At least three different classes of ER stress transducers have been identified, each defining a distinct arm of the UPR: inositol-requiring protein-1 (IRE1), activating transcription factor-6 (ATF6) and protein kinase RNA (PKR)-like ER kinase (PERK) [4,5], which serve as proximal sensors of protein folding status in the ER. PERK, ATF6 and IRE1 transmit this information across the ER membrane to the cytosol. Initially, during ER stress, the PERK pathway is activated, resulting in phosphorylation and inactivation of the translation initiation factor eIF2α. The cleaved cytoplasmic domain of ATF6 can function alone as a transcription factor for the regulation of genes encoding other transcription factors and molecular chaperones. Finally, activation of IRE1 results in unconventional splicing of mRNA of the transcription factor, XBP1. XBP1 mRNA encodes a

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potent transcriptional activator (XBP1s), whereas the unspliced XBP1 mRNA encodes XBP1u, an inhibitor of the UPR. Of note, both ATF6 and XBP1 can activate transcription of genes encoding molecular chaperones and proteins involved in ER-associated degradation. IRE1 can also act by an alternative route which, upon phosphorylation, leads to recruitment of TRAF2 (tumor necrosis factor receptor (TNFR)-associated factor-2), resulting in activation of c-Jun-NH_2-terminal kinase (JNK) [4,5]. In absence of ER stress, the UPR is not activated because the three ER luminal stress 'sensors' PERK, ATF6 and IRE1 proteins are sequestered as inactive complexes with Bip (immunoglobulin-heavy-chain-binding protein), also known as GRP78.

We have recently reported that arsenic trioxide (ATO) induced human PMN apoptosis by a caspase-dependent mechanism and via de novo protein synthesis, including some chaperones [6,7]. In addition, inhibition of H_2O_2 production by catalase was found to reverse both de novo protein synthesis and neutrophil apoptosis induced by ATO [6,7]. We have also demonstrated that ATO can activate both caspase-3 and caspase-8, indicating that the intrinsic and extrinsic pathways of cell apoptosis are activated by ATO in human PMNs [6]. In the present study, we found that ATO induced ER stress in human PMNs and this provide the first evidences that activation of the ER stress-mediated cell apoptotic pathway is part of the PMN apoptotic machinery. Furthermore, we demonstrate that although PMNs express caspase-4 which can be processed and activated in these cells, these events are not essential for ATO-induced apoptosis.

Materials and methods

Reagents and antibodies. Arsenic trioxide (As_2O_3), tunicamycin (TM), thapsigargin (TP) and RPMI were purchased from Sigma–Aldrich (Saint-Louis, MI). The antibody against phosphospecific eIF2α antibody was purchased from Cell Signaling (Denvers, MA), the GRP78, GADD153 and caspase-4 antibodies were from Santa Cruz Biotechnology (Santa Cruz, CA) and the polyclonal Erk1/2 from Upstate Biotechnology (Lake Placid, NY). Annexin-V and PI were purchased from Biosource (Camarillo, CA). The caspase-4 substrate Ac-LEVD-AFC was purchased from Alexis Biochemicals (San Diego, CA). The caspase-4 inhibitor z-LEVD-FMK was purchased from BioVision (Moutain View, CA). Fluo-3-AM and ER-tracker were acquired from Molecular Probes (Eugene, OR, USA).

Neutrophil isolation. Neutrophils were isolated from venous blood of healthy volunteers as previously described [8–10]. Blood donations were obtained from consenting individuals according to institutionally approved procedures.

Immunofluorescence detection of ER. Cells were centrifuged for 2 min at 300 g on prewashed miscroscope slides and incubated with 1 μM of ER-tracker-Red (Molecular Probes) for 30 min at 37 °C. After two brief washes, cells were visualized (400×) with a Leica miscroscope equipped with a fluorescent lamp.

Electron microscopy. An aliquot of freshly isolated PMNs was reserved, and the rest was used for treatment with ATO or buffer (SA) for 20 h. Cells were fixed overnight with 2.5% glutaraldehyde in phosphate-buffered saline (PBS). After several washes in PBS with 3% sucrose, cells were fixed with 1.3% OsO_4 for 2 h and embedded in Spurr resin. Thin slices were prepared with an ultramicrotome and stained with 5% uranyl acetate and filtered lead citrate. Examinations of ultra thin sections were examined with a Hitachi H-7100 transmission electron microscope.

RT-PCR. Isolation of RNA was carried out using a commercially available kit (GenElute, Sigma). Synthesis of cDNA was done using 500 ng of RNA with Moloney Murine Leukaemia Virus Reverse Transcriptase (M-MLV). For detection of spliced XBP1, the method consisted of a first amplification at 94 °C, 5 min and 2 cycles at 95 °C, 30 s, 55 °C, 30 s, 72 °C, 30 s with the following primers: forward: 5′-CCTTGTAGTTGAGAACCAGG-3′; reverse: 5′-GGGCTT GGTATATATGTGG-3′. Then, PCR products were digested with 7.5 U of Pst1 enzyme and the PCR reaction was completed using the same primer for an additional 28 cycles. Amplification of other cDNA was done using a denaturation step of 5 min at 95 °C followed by 18 cycles (18S), 29 cycles (GADD153), 30 cycles (GRP78), or 36 cycles (ATF6) of 94 °C for 30 s, 56 °C (18S), 67 °C (GADD153), 61 °C (GRP78), or 55 °C (ATF6) for 30 s, 72 °C for 45 s and completed with a final step of 72 °C for 7 min. The following primers were used: 5′-CC GATAACGAACGAGACTC-3′ (sense) and 5′-CAGGGACTTAATCAACGC AA-3′ (antisense) for 18S; 5′-GAAACGGA-AACAGAGTGGTCATTCCCC -3′ (sense) and 5′-GTGGGATTGAGGGTCACATCATTG-GCA-3′ (antisense) for GADD153; 5′-GTTTGCTGAGGAAGACAAAAAGCTC-3′ (sense) and 5′-CACTTCCATAGAGTTTGCTGATAATTG-3′ (antisense) for GRP78; 5′-ATGAAGTTG-TGTCAGAGAAC-3′ (sense) and 5′-GGGT GCTATTGTAATGACTCA-3′ (antisense) for ATF6. The PCR products were separated using an ethidium bromide 2% agarose gel electrophoresis and visualized under ultraviolet light.

Western blot. PMNs (10×10^6 cells/mL RPMI-Hepes-P/S with 10% autologous serum) were stimulated with the respective agonists and for the indicated times. Cells were lysed in 2× Laemmli's sample buffer and aliquots corresponding to 1×10^6 cells were loaded onto 10% SDS–PAGE and transferred to nitrocellulose or polyvinylidene difluoride membranes. Membranes were blocked for 1 h at room temperature in Tris-buffered saline (TBS)-Tween containing nonfat dry milk (Carnation, Don Mills, Ontario, Canada) or BSA. After washing, the antibody directed against phospho-eIF2α (1:1000) or caspase-4 (1:1000), Erk1/2 (1:1000) was added in TBS-Tween. The membranes were kept overnight at 4 °C. Membranes were then washed with TBS-Tween and incubated for 1 h at room temperature with a goat anti-mouse or goat anti-rabbit horseradish (HRP) secondary antibody (Jackson ImmunoResearch Laboratories) at 1:20000 in TBS-Tween +5% nonfat dry milk followed by several washes. Protein expression was revealed using an enhanced chemiluminescence Western blotting detection system.

Caspase-4 activity assay. PMNs were stimulated as specified in figure legends. Cells were washed in PBS and disrupted using a lysis buffer (Hepes 25 mM pH 7.5, EDTA 5 mM, 5 mM $MgCl_2$, DTT 10 mM, pepstatin 10 μg/mL, leupeptin 10 μg/mL, PMSF 2 mM, Triton X-100 0.5%). Protein concentration was determined using the Bradford assay. An amount of 10 μg of proteins was mixed in 200μL of reaction buffer containing Hepes 50 mM, sucrose 10%, CHAPS 0.1%, DTT 10 mM with 100 μM of the caspase-4 substrate Ac-LEVD-AFC for 1hr at 37 °C. Cleavage of the caspase substrate was monitored using a fluorometer at 405 nm (excitation) and 500 nm (emission).

Detection of apoptosis. Freshly isolated human PMNs (10×10^6 cells/mL in RPMI 1640-HEPES-P/S, supplemented with 10% heat-inactivated autologous serum) were incubated for 24 h in the presence or absence of an increasing concentration of ATO (0–100 μM). Cytocentrifuged samples of PMNs were prepared using a Cyto-tek® centrifuge (Miles Scientific, Naperville, IL), stained with the Hema 3 Stain staining kit according to the manufacturer's instructions and processed as documented previously [8]. Cells were examined by light microscopy at 400× final magnification, and apoptotic PMNs were defined as cells containing one or more characteristic, darkly stained pyknotic nuclei.

Statistics. Statistical analysis was performed with SigmaStat for Windows version 3.0 using Student's *t*-test. Statistical significance was established at $P < 0.05$.

Results

We first examined whether or not the ER network is perturbed in ATO-induced human PMNs, by monitoring the ER by immuno-

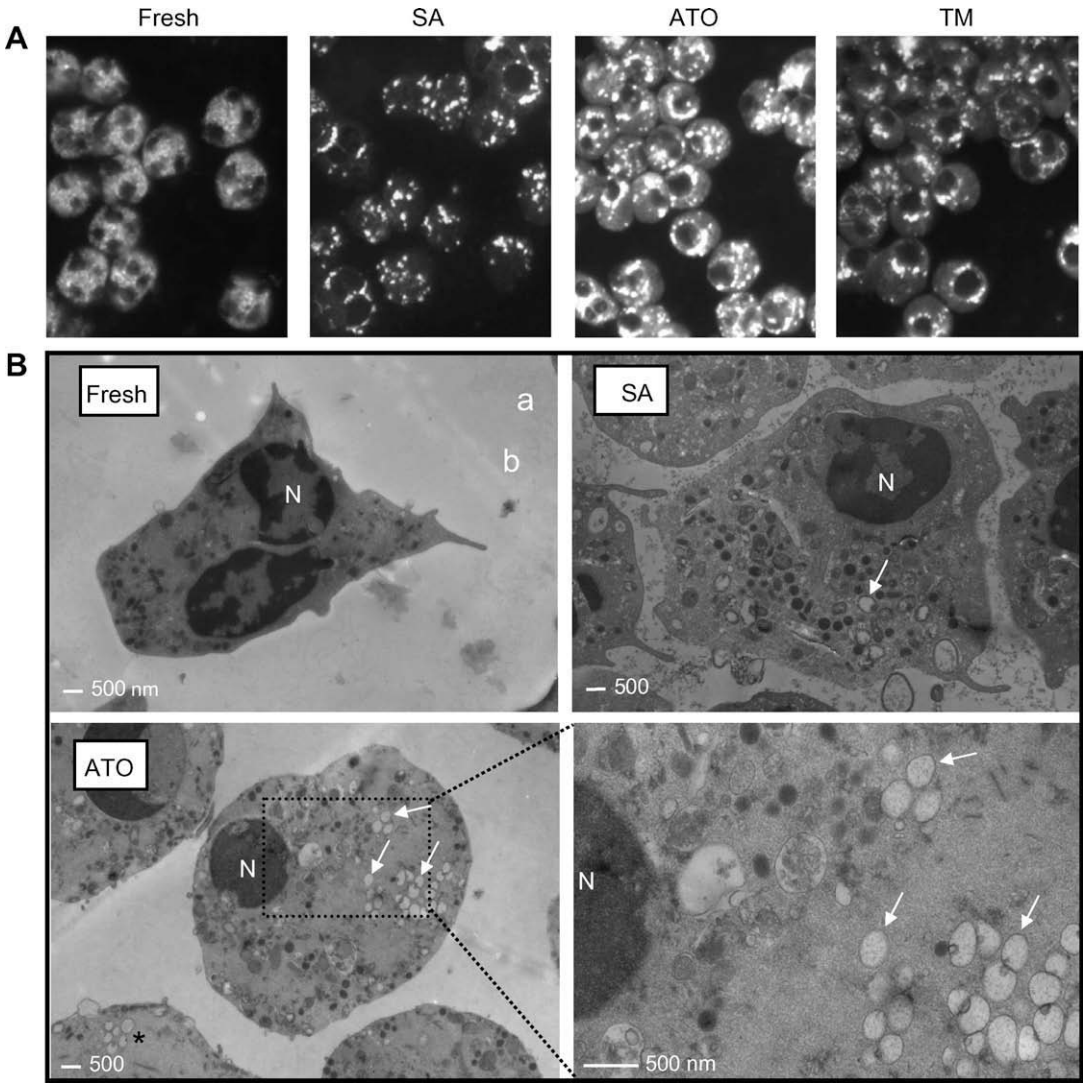

Fig. 1. Alteration of the endoplasmic reticulum in apoptotic neutrophils. (A) Freshly isolated cells (Fresh) were incubated the presence of buffer (spontaneous apoptosis or SA), ATO or TM for 20 h and then processed for immunofluorescence (A) or electron microscopy (B) for the observation of morphological changes. (A) The ER was stained with the ER-tracker (400× magnification). Results are representative of at least four different experiments. (B) Dilatation of the ER (arrows) is not observed in freshly isolated cells but is a characteristic of apoptotic cells seen during SA or ER stress-induced neutrophil apoptosis (5000×).

fluorescence using an ER-tracker dye. As illustrated in Fig. 1A, only a weak diffuse signal is detected in freshly isolated PMNs (Fresh) but an intense signal is observed in apoptotic PMNs during spontaneous apoptosis or after treatment with the ER stress inducers ATO or TM. Of note, the signal was reproducibly more intense with ATO than TM. To support further ER alterations in apoptotic PMNs, we examined cells by electron microscopy in order to confirm the presence of some vacuoles recently reported to correspond to dilatation of the ER [11–13]. As illustrated in Fig. 1B such vacuoles were observed in SA and in ATO-induced PMNs but not in fresh cells.

Expression and regulation of important key players involved during the unfolded protein response (UPR) in human PMNs

The above results indicate that an ER stress occurs in apoptotic PMNs, allowing the possibility that an UPR-like event may be induced in these cells. To support this, we investigated the possibility that one or more of the three arms of the UPR, namely PERK, ATF6 and IRE1 is/are activated in PMNs. Because UPR induction is initially characterized by up-regulation of several ER-resident chaperones, including the well-known GRP78/BiP and GADD153, we investigated whether or not these two key players are modulated in PMNs. As illustrated in Fig. 2A and B, the expression of GRP78 mRNA was increased after 1 h of treatment with ATO and TM, whereas up-regulation of GADD153 mRNA occurred later, after 3 h in response to ATO and TM. These results indicated that the modulation of GRP78 and GADD153 can be observed and is variable in PMNs, depending upon which ER stress inducers are tested. Knowing that these two ER chaperones (especially GRP78 known to be associated with the three ER sensors) are modulated in PMNs, we then studied the possibility that the PERK signaling pathway operates in these cells. As illustrated in Fig. 2C, eIF2α, the prototype downstream marker of PERK activation is activated in response to ATO. In order to determine if the two other cell signaling pathways occurring in response to ER stress are operational in PMNs, we monitored gene expression of ATF6 and XBP1. As illustrated in Fig. 2D, PMNs express ATF6 and its expression was markedly increased by ATO after 5 h. After 7 h of treatment, the expression of ATF6 was further increased by ATO to a level that was also observed in response to TP but not TM, indicating that ATF6 expression is differentially modulated according to different

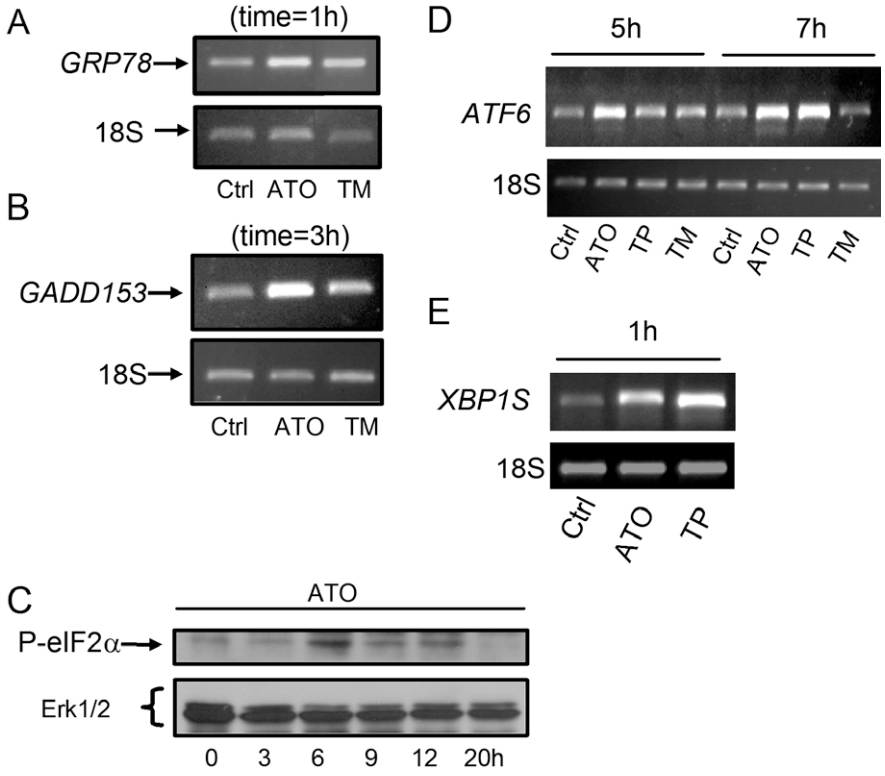

Fig. 2. Activation of ER stress markers in human PMNs. Cells were treated with the indicated agonist for different period of time and mRNA expression of GRP78 (A), GADD153 (B), ATF6 (D) and XBP1S (e) were assessed by RT-PCR as described in Materials and methods. Phosphorylation of eIF2α protein (C) was monitored by immunoblotting. Results are representatives of at least three independent experiments. 18S and eIF2α are presented here as loading controls.

ER stress inducers. Fig. 2E reveals that PMNs also express *XBP1s* (the spliced form) and that its expression increases in response to ATO and TP. Collectively, the above results indicate that the three signaling pathways of the UPR known to be triggered during ER stress exist, are operational in PMNs and are further activated by ER stress inducers.

Expression and activation of caspase-4 in human PMNs

ATO is known to induce apoptosis in PMNs [6,7] and to induce ER stress in other cells [14]. Because of the importance of caspase-4 during ER stress [1,5], we examined whether or not PMNs express this caspase at the protein level and, if so, if it is processed and activated in ATO-induced cells. As illustrated in Fig. 3A, PMNs express procaspase-4 and its cleavage occurs in ATO-induced PMNs, as judged by the appearance of the active form of caspase-4, a cleaved polypeptide of ~29 kDa, as reported in other cells [1]. Since caspase cleavage is not always indicative of caspase activity, we further demonstrated that caspase-4 was effectively activated in PMNs by measuring the release of a fluorophore from a synthetic peptide target sequence. As illustrated in Fig. 3B, caspase-4 activity was detected during PMN apoptosis. Using the pharmacological

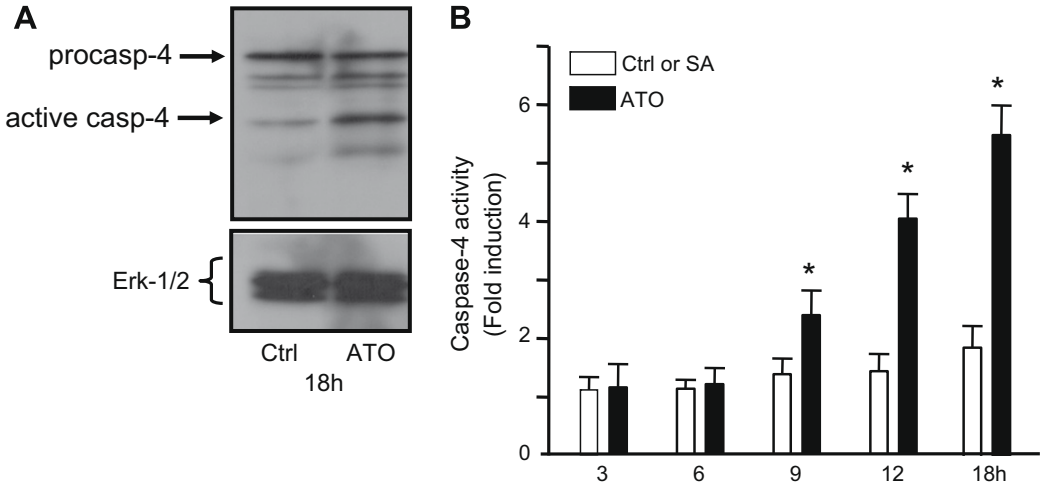

Fig. 3. Expression, processing, and activity of caspase-4 in human PMNs. (A) Expression and processing (or cleavage) of procaspase-4 was assessed by immunoblotting. Neutrophils were incubated with 5 μM of ATO for 18 h. Results are representatives of three other experiments. (B) The caspase-4 activity was determined by measuring the generation of the fluorescent cleavage product from Ac-LEVD-AFC as described in Materials and methods for the indicated periods of time during SA or in response to ATO. Activity is expressed as fold increase over untreated cells (time = 0 h). Results are means ± SEM ($n = 3$). $^*P < 0.05$ vs. SA.

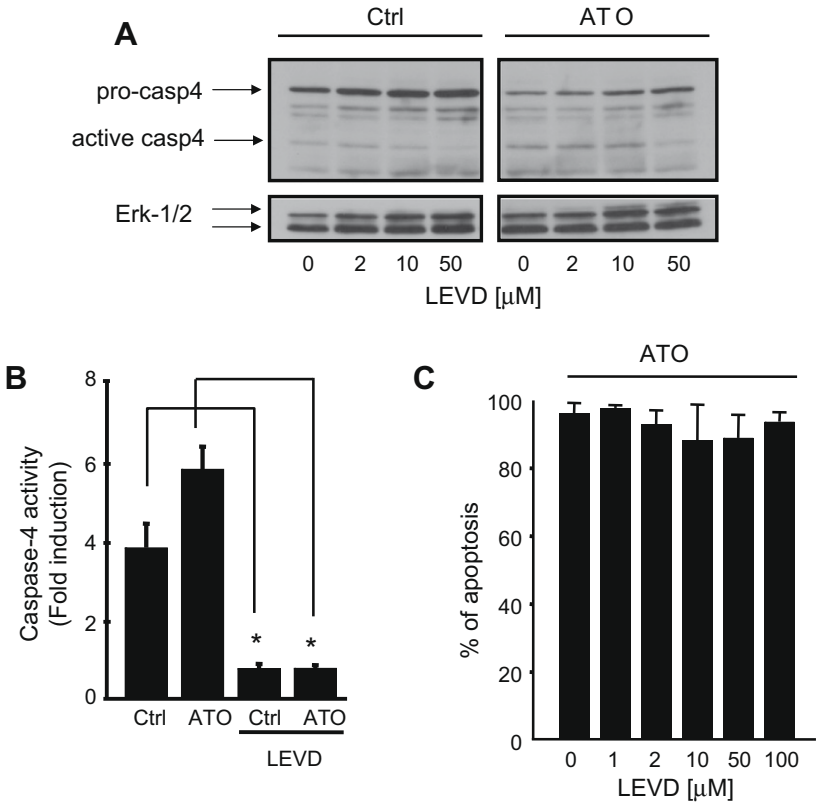

Fig. 4. The caspase-4 inhibitor reverses caspase-4 processing and activity but not ATO-induced human neutrophil apoptosis. (A) Cells were exposed to diluent (SA) or ATO (5 μM) for 18 h in the presence of increasing concentrations of the caspase-4 inhibitor LEVD-fmk (μM) and caspase-4 processing was assessed by immunoblotting. Erk1/2, a MAPK that is not activated by ATO in PMNs [27], was used as a loading control. Results are representatives of three other experiments. (B) LEVD-fmk (50 μM) abolishes caspase-4 activity during spontaneous apoptosis (SA) and in response to ATO after 18 h. Results are means ± SEM ($n = 3$). $^{*}P < 0.05$ vs. SA without caspase-4 inhibitor; $^{**}P < 0.05$ vs. ATO without caspase-4 inhibitor. (C) Apoptosis was assessed by cytology after 18 h. $^{*}P < 0.05$. Results are means ± SEM ($n = 3$).

inhibitor LEVD-fmk, a caspase-4 inhibitor, we found that the cleavage of caspase-4 during apoptosis was reversible in a concentration-dependent manner (Fig. 4A). Pre-treatment with the caspase-4 inhibitor abolishes the caspase-4 activity completely (Fig. 4B). These results corroborate those obtained by immunoblotting and confirm that caspase-4 is expressed and activated in PMNs. Because of these results, we next investigated the role of caspase-4 during neutrophil apoptosis. Cells were pre-treated with increasing concentrations of the caspase-4 inhibitor and then incubated in the presence of ATO. As expected, the number of apoptotic PMNs was always above 85% after 24 h of treatment (Fig. 4C). However, the apoptotic rate was unaffected by the caspase-4 inhibitor treatment even at a concentration of 100 μM. These results were confirmed by another method based on CD16 shedding in apoptotic cells as monitored by flow cytometry (*data not shown*).

Discussion

In this study, we provide the first evidence that the recently identified ER stress-mediated pathway of cell apoptosis is operational in PMNs. First, we have observed alteration of the ER by immunofluorescence and electron microscopy. Second, we demonstrated that genes involved in the three arms of the UPR are expressed and upregulated in apoptotic PMNs. Third, we observed activation of eIF2α as judged by its phosphorylation. ER stress and intracellular signaling pathways in response to the UPR have been relatively well-defined in cells undergoing massive protein secretion, such as plasma B cells or hepatocytes [15,16]. However, until now, no study has revealed a link between PMN apoptosis and the ER stress pathway. Some studies have proposed that this pathway operates in promyelocytes, which are progenitors of PMNs [17]. In addition, the expression of GADD153, which is involved in a pro-apoptotic branch in UPR signaling, has been shown to be upregulated during retinoic acid differentiation of promyelocytic cells [18]. This suggests that GADD153 may be implicated in activation of PMN apoptosis.

We have recently demonstrated that ATO induced de novo protein synthesis in PMNs [6] and this supports an ER stress response, since the UPR is initially engaged to circumvent a cytotoxic signal by activating protein synthesis, particularly those involved in protein folding, including chaperones [4]. This is in agreement with the recent observation that among the proteins that are newly synthesized in response to ATO, some are chaperones [7]. However, activation of the UPR in PMNs is probably prolonged, leading to an irreversible cell condition causing death by initiating apoptosis. We propose that this occurs not only in ATO (or other agents)-induced apoptosis, but also during SA, where H_2O_2 can accumulate in the medium over time [19]. This is supported by our previous study where treatment of human PMNs with catalase markedly inhibited both de novo protein synthesis and PMN apoptosis [6].

Several studies have identified caspase-4 as a key player in the ER stress-mediated pathway of apoptosis in humans or caspase-12 in rodents [1,3–5,20]. Here, we report for the first time the protein expression of caspase-4 as well as its processing and activation during PMN apoptosis. However, the role of caspase-4 appears to be dispensable in ER stress-induced PMN apoptosis. This is surprising, considering that caspase-4 has been described as an initiator caspase, based on structural evidence [21]. Activation of caspase-4 was found to occur prior to activation of caspase-3 or -9 in melanoma cells subjected to TM or TP [22]. On the other hand,

caspase-4 was also described as an intermediate activator of Fas-mediated apoptosis, transmitting the death signal from caspase-8 to caspase-3 [20]. In addition, the ER stress inducer edelfosine was found to activate caspase-8 and -3 before the onset of apoptosis. In this case, caspase-4 was suggested to be activated alongside with ASK1/JNK and caspase-8/BAP31 signaling pathways [23]. One possible explanation for the different role of caspase-4 may be explained by the absence of caspase-2 in PMNs. Caspase-2 was identified as an ER-resident and proximal caspase in brefeldin A- and TM-induced apoptosis [24]. Therefore, it is plausible that caspase-4 may be confined to other roles in PMNs, such as amplifying mitochondrial or death-receptor associated apoptosis, due to lack of caspase-2 expression. Of note, caspase-4 deficient cell lines were found to be equally sensitive to ER stress inducers TM, TP and brefeldin A [25]. Caspase-12 was proposed to be part of the pro-apoptotic pathway of ER stress response involving TRAF2 and JNK [26]. Interestingly, we have previously demonstrated that ATO activates JNK in PMNs; however, addition of a JNK inhibitor did not rescue the cells from SA or ATO-induced apoptosis [27], suggesting that activation of the TRAF2-JNK pathway is not necessary for PMN apoptosis. A non-apoptotic role has been proposed recently for caspase-4 [25]. In that study, the authors demonstrated that LPS-induced IL-1β, CCL4 (MIP1β) and CXCL8 (IL-8) mRNA expression were downregulated in caspase-4-deficient THP1 cells. Thus, it is also possible that caspase-4 is involved in some way in the production of cytokines in PMNs, although this remains to be determined.

Conclusions

This study provides the first evidence that the ER stress-mediated pathway of cell apoptosis is operational in human PMNs. Caspase-4 is expressed, processed and activated in these cells, but could be involved in other functions than apoptosis.

Acknowledgments

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References

[1] J. Hitomi, T. Katayama, Y. Eguchi, T. Kudo, M. Taniguchi, Y. Koyama, T. Manabe, S. Yamagishi, Y. Bando, K. Imaizumi, Y. Tsujimoto, M. Tohyama, Involvement of caspase-4 in endoplasmic reticulum stress-induced apoptosis and Abeta-induced cell death, J. Cell Biol. 165 (2004) 347–356.
[2] L.H. Chen, C.C. Jiang, R. Watts, R.F. Thorne, K.A. Kiejda, X.D. Zhang, P. Hersey, Inhibition of endoplasmic reticulum stress-induced apoptosis of melanoma cells by the ARC protein, Cancer Res. 68 (2008) 834–842.
[3] L. Zhao, S.L. Ackerman, Endoplasmic reticulum stress in health and disease, Curr. Opin. Cell Biol. 18 (2006) 444–452.
[4] D. Ron, P. Walter, Signal integration in the endoplasmic reticulum unfolded protein response, Nat. Rev. Mol. Cell. Biol. 8 (2007) 519–529.
[5] D.J. Todd, A.H. Lee, L.H. Glimcher, The endoplasmic reticulum stress response in immunity and autoimmunity, Nat. Rev. Immunol. 8 (2008) 663–674.
[6] F. Binet, H. Cavalli, E. Moisan, D. Girard, Arsenic trioxide (AT) is a novel human neutrophil pro-apoptotic agent: effects of catalase on AT-induced apoptosis, degradation of cytoskeletal proteins and de novo protein synthesis, Br. J. Haematol. 132 (2006) 349–358.
[7] F. Binet, S. Chiasson, D. Girard, Arsenic trioxide induces de novo protein synthesis of annexin-1 in neutrophils: association with a heat shock-like response and not apoptosis, Br. J. Haematol. 140 (2008) 454–463.
[8] D. Girard, M.E. Paquet, R. Paquin, A.D. Beaulieu, Differential effects of interleukin-15 (IL-15) and IL-2 on human neutrophils: modulation of phagocytosis, cytoskeleton rearrangement, gene expression, and apoptosis by IL-15, Blood 88 (1996) 3176–3184.
[9] M. Pelletier, D. Girard, Biological functions of interleukin-21 and its role in inflammation, ScientificWorldJournal 7 (2007) 1715–1735.
[10] M. Pelletier, A. Bouchard, D. Girard, In vivo and in vitro roles of IL-21 in inflammation, J. Immunol. 173 (2004) 7521–7530.
[11] H. Kadara, L. Lacroix, D. Lotan, R. Lotan, Induction of endoplasmic reticulum stress by the pro-apoptotic retinoid *N*-(4-hydroxyphenyl)retinamide via a reactive oxygen species-dependent mechanism in human head and neck cancer cells, Cancer Biol. Ther. 6 (2007) 705–711.
[12] X. Xue, J.H. Piao, A. Nakajima, S. Sakon-Komazawa, Y. Kojima, K. Mori, H. Yagita, K. Okumura, H. Harding, H. Nakano, Tumor necrosis factor alpha (TNFalpha) induces the unfolded protein response (UPR) in a reactive oxygen species (ROS)-dependent fashion, and the UPR counteracts ROS accumulation by TNFalpha, J. Biol. Chem. 280 (2005) 33917–33925.
[13] G. Faria, C.R. Cardoso, R.E. Larson, J.S. Silva, M.A. Rossi, Chlorhexidine-induced apoptosis or necrosis in L929 fibroblasts: a role for endoplasmic reticulum stress, Toxicol. Appl. Pharmacol. 234 (2009) 256–265.
[14] H. Zhang, G. Duncan, L. Wang, P. Liu, H. Cui, J.R. Reddan, B.F. Yang, I.M. Wormstone, Arsenic trioxide initiates ER stress responses, perturbs calcium signalling and promotes apoptosis in human lens epithelial cells, Exp. Eye Res. 85 (2007) 825–835.
[15] J.N. Gass, N.M. Gifford, J.W. Brewer, Activation of an unfolded protein response during differentiation of antibody-secreting B cells, J. Biol. Chem. 277 (2002) 49047–49054.
[16] J.N. Gass, H.Y. Jiang, R.C. Wek, J.W. Brewer, The unfolded protein response of B-lymphocytes: PERK-independent development of antibody-secreting cells, Mol. Immunol. 45 (2008) 1035–1043.
[17] D.S. Grenda, M. Murakami, J. Ghatak, J. Xia, L.A. Boxer, D. Dale, M.C. Dinauer, D.C. Link, Mutations of the ELA2 gene found in patients with severe congenital neutropenia induce the unfolded protein response and cellular apoptosis, Blood 110 (2007) 4179–4187.
[18] S. Gery, D.J. Park, P.T. Vuong, D.Y. Chih, N. Lemp, H.P. Koeffler, Retinoic acid regulates C/EBP homologous protein expression (CHOP), which negatively regulates myeloid target genes, Blood 104 (2004) 3911–3917.
[19] Y. Kasahara, K. Iwai, A. Yachie, K. Ohta, A. Konno, H. Seki, T. Miyawaki, N. Taniguchi, Involvement of reactive oxygen intermediates in spontaneous and CD95 (Fas/APO-1)-mediated apoptosis of neutrophils, Blood 89 (1997) 1748–1753.
[20] S. Kamada, M. Washida, J. Hasegawa, H. Kusano, Y. Funahashi, Y. Tsujimoto, Involvement of caspase-4(-like) protease in Fas-mediated apoptotic pathway, Oncogene 15 (1997) 285–290.
[21] E.A. Obeng, L.H. Boise, Caspase-12 and caspase-4 are not required for caspase-dependent endoplasmic reticulum stress-induced apoptosis, J. Biol. Chem. 280 (2005) 29578–29587.
[22] C.C. Jiang, L.H. Chen, S. Gillespie, Y.F. Wang, K.A. Kiejda, X.D. Zhang, P. Hersey, Inhibition of MEK sensitizes human melanoma cells to endoplasmic reticulum stress-induced apoptosis, Cancer Res. 67 (2007) 9750–9761.
[23] T. Nieto-Miguel, R.I. Fonteriz, L. Vay, C. Gajate, S. Lopez-Hernandez, F. Mollinedo, Endoplasmic reticulum stress in the proapoptotic action of edelfosine in solid tumor cells, Cancer Res. 67 (2007) 10368–10378.
[24] H.H. Cheung, N. Lynn Kelly, P. Liston, R.G. Korneluk, Involvement of caspase-2 and caspase-9 in endoplasmic reticulum stress-induced apoptosis: a role for the IAPs, Exp. Cell Res. 312 (2006) 2347–2357.
[25] U. Lakshmanan, A.G. Porter, Caspase-4 interacts with TNF receptor-associated factor 6 and mediates lipopolysaccharide-induced NF-kappaB-dependent production of IL-8 and CC chemokine ligand 4 (macrophage-inflammatory protein-1), J. Immunol. 179 (2007) 8480–8490.
[26] T. Yoneda, K. Imaizumi, K. Oono, D. Yui, F. Gomi, T. Katayama, M. Tohyama, Activation of caspase-12, an endoplastic reticulum (ER) resident caspase, through tumor necrosis factor receptor-associated factor 2-dependent mechanism in response to the ER stress, J. Biol. Chem. 276 (2001) 13935–13940.
[27] F. Binet, D. Girard, Novel human neutrophil agonistic properties of arsenic trioxide: involvement of p38 mitogen-activated protein kinase and/or c-jun NH_2-terminal MAPK but not extracellular signal-regulated kinases-1/2, J. Leukoc. Biol. 84 (2008) 1613–1622.