Docosahexaenoic acid and palmitic acid reciprocally modulate monocyte activation in part through endoplasmic reticulum stress

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Abstract

Palmitic acid (C16:0) and TLR2 ligand induce, but docosahexaenoic acid (DHA) inhibits monocyte activation. C16:0 and TLR2 or TLR4 ligand induce certain ER stress markers; thus, we determined whether ER stress induced by these agonists is sufficient to induce monocyte activation, and whether the ER stress is inhibited by DHA which is known to inhibit C16:0- or ligand-induced TLR activation. Monocyte activation and ER stress were assessed by TLR/inflammasome-induced IL-1 β production, and phosphorylation of IRE-1 and eIF2 and expression of CHOP, respectively in THP-1 cells. TLR2 ligand Pam3CSK4 induced phosphorylation of eIF2, but not phosphorylation of IRE-1 and CHOP expression. LPS also induced phosphorylation of both IRE-1 and eIF2 but not CHOP expression suggesting that TLR2 or TLR4 ligand, or C16:0 induces different ER stress responses. C16:0-, Pam3CSK4-, or LPS-induced IL-1 β production was inhibited by 4-phenylbutyric acid, an inhibitor of ER stress suggesting that IL-1 β production induced by these agonists is partly mediated through ER stress. Among two ER stress-inducing molecules, thapsigargin but not tunicamycin led to the expression of pro-IL-1 β and secretion of IL-1 β . Thus, not all types of ER stress are sufficient to induce inflammasome-mediated IL-1 β secretion in monocytes. Although both C16:0 and thapsigargin-induced IL-1 β secretion was inhibited by DHA, only C16:0-mediated ER stress was responsive to DHA. These findings suggest that the anti-inflammatory effects of DHA are at least in part mediated through modulating ER homeostasis and that the propensity of ER stress can be differentially modulated by the types of dietary fat we consume.

Keywords: monocyte; inflammation; inflammasome; fatty acid; ER stress; DHA

1. Introduction

Chronic inflammation is one of the key etiological conditions for the development and progression of many chronic diseases. Low-grade chronic inflammation (LGC inflammation) characterized by elevated circulating concentrations of pro-inflammatory cytokines, acute-phase proteins and adhesion molecules, is known to be associated with obesity and insulin resistance. What causes and mediates LGC inflammation and how it can be suppressed by

dietary means which can provide preventive efficacy for inflammatory chronic disease are challenging questions.

The Endoplasmic Reticulum (ER) is a major cellular anabolic organelle that plays a vital role in the folding, transport, and processing of newly synthesized proteins and the biosynthesis of cholesterol, steroids, and other lipids [1-3]. To maintain optimal function the ER has evolved a complex network of signaling pathways to respond and adapt to perturbations in cellular nutrient and energy status known as the ER stress response or

unfolded protein response (UPR). The primary objective of the UPR is to re-establish homeostasis and alleviate ER stress through increasing protein folding capacity and decreasing unfolded protein load. If a stress is prolonged or severe and these mechanisms of adaptation and survival are insufficient to recover ER homeostasis, the UPR initiates programmed cell death.

Blood monocytes are sentinel immune effector cells that detect and respond to invading pathogens and dietary components absorbed from the gut. Activated blood monocytes can extravasate into peripheral tissues and become resident macrophages or dendritic cells (DCs) that can trigger inflammatory signals in response to exogenous and endogenous stimuli [4, 5]. Recent animal studies have shown that a high fat diet increases infiltration of macrophages and dendritic cells, that originate from circulating blood monocytes, in adipose tissue where they play a central role in the development of chronic inflammation and insulin resistance [6]. Therefore, activation of pro-inflammatory pathways in circulating blood monocytes is the gateway toward enhanced inflammation in various tissues. A recent study showed elevated expression of ER stress markers in circulating blood monocytes of patients with type 2 diabetes [7].

Unlike other pro-inflammatory cytokines, IL-1 β production is tightly regulated by a unique two-signal mechanism. The primary signals induce the expression of pro-IL-1 β mediated in part through the activation of Toll-like receptors (TLRs). The secondary signals activate the nucleotide-binding oligomerization domain-like receptor (NLR) pyrin domain-containing (NALP3) inflammasome, an intracellular signaling complex composed of NALP3, pro-caspase-1, and the adaptor protein apoptosis-associated speck-like protein containing a caspase recruitment domain (ASC). Formation of the inflammasome complex results in proteolytic cleavage of pro-caspase-1 to yield active caspase-1 which in turn cleaves pro-IL-1 β producing mature and active IL-1 β . Mature IL-1 β released extracellularly binds its cognate receptor in a paracrine manner resulting in amplification of pro-inflammatory responses [8, 9, 10, 11]. Blood monocytes are a unique immune cell in that they have constitutively active NALP3 inflammasome; therefore, any signal which induces the expression of pro-IL-1 β alone is

sufficient to induce inflammasome-mediated IL-1 β production which is one of hall marks for monocyte activation [12-14].

The predominant dietary saturated fatty acid palmitic acid is a potent inducer of ER stress in many cell types including pancreatic β -cells, hepatocytes, and muscle cells [15-17]. Ligands for TLR4 and TLR2 can also induce ER stress [18, 19]. Our previous studies revealed that saturated fatty acids can activate but docosahexaenoic acid (DHA) inhibits pattern recognition receptors (PRRs) including TLR4, TLR2 [20-23]. Furthermore, we have also shown that palmitic acid induces but docosahexaenoic acid (DHA) inhibits TLR2/inflammasome-mediated IL-1 β release in human monocytes [24]. Therefore, in this study, we determined whether palmitic acid-induced ER stress is directly linked to monocyte activation as assessed by inflammasome-mediated IL-1 β , and whether this process is inhibited by n-3 polyunsaturated fatty acids DHA.

2. Materials and methods

2.1 Reagents

LPS (Cat #: 421) was purchased from List Biological Laboratories, Inc. (Campbell, CA). Pam₃CSK₄ (Cat #: tlr1-pms) and TAK-242 (Cat #: tlr1-cli95) and nigericin (cat #: tlr1-nig) were purchased from Invivogen (San Diego, CA). Bovine serum albumin (BSA, Cat #: 30-AB79, Lot #: A10072001) was purchased from Fitzgerald Industries International (Acton, MA). Sodium palmitate (Cat #: P9767), sodium salt docosahexaenoic acid (DHA) (Cat #: D8768), endotoxin free water, Thapsigargin (Cat #: T9033), 4-Phenylbutyric acid (Cat #: P21005) and antibody for β -actin were purchased from Sigma (Saint Louis, MO). Apocynin (Cat #: 178385) and Tunicamycin (Cat #: 654380) were purchased from EMD Chemicals Inc. (Darmstadt, Germany).

2.2 Cell cultures

THP-1 cells (human monocytic cell line, ATCC TIB-202) were cultured in RPMI-1640 medium (ATCC, cat #: 30-2001) containing 10% (v/v) Fetal Bovine Serum (FBS, Premium Select, cat #: S11550, Lot#: K0109 and K11050, Atlanta Biologicals, Lawrenceville, GA), 0.05 mM 2-mercaptoethanol, 100 units/ml penicillin, and 100 mg/ml streptomycin. Cells were maintained at 37°C in a 5% CO₂/air environment. Cells were seeded at 1 x 10⁶/ml and incubated in serum-poor medium (0.25% and 1.0% FBS) prior to treatment. In order to differentiate THP-1 cells into macrophages the monocytic THP-1 cells were treated with 5 ng/ml phorbol-12-myristate-13-acetate (PMA) for two days in 6-well plates at 1.6-2 x 10⁶/well. The differentiated THP-1 cells were cultured in Opti-MEM medium (Cat #: 31985, Life technologies, Grand Island, NY) containing 0.25% FBS for

the treatments with ER stress inducers and the inflammasome activator nigericin.

2.3 Fatty acid preparations

A stock solution of 100 mM sodium palmitate (C16:0) was prepared in 70% ethanol in a glass vial and heated to 60°C. Sodium palmitate was reheated to 60°C and vortexed before use to give appropriate final concentration indicated in figures. A stock solution of 10 mM DHA sodium salt was prepared in endotoxin free water, flushed with nitrogen, then sealed in air tight tube and stored at -20°C until use.

2.4 Immunoblot and ELISA assays

Immunoblotting was performed as previously described (17). Briefly, THP-1 cells were lysed by sonication in cell lysis buffer (Cell Signaling Technology, Danvers, MA) containing 20 mM Tris-HCl (pH 7.5), 150 mM NaCl, 1 mM Na₂EDTA, 1 mM EGTA, 1% Triton, 2.5 mM sodium pyrophosphate, 1 mM β -glycerophosphate, 1 mM sodium orthovanadate, and 1 μ g/ml leupeptin plus 1 mM phenylmethylsulfonyl fluoride. Lysate supernatants were collected by centrifugation and subjected to 10% or 10-20% SDS-PAGE followed by protein transfer to polyvinylidene difluoride membrane (Bio-Rad, Hercules, CA). The membrane was blocked in TBST buffer [20 mM Tris-HCl (pH 7.4), 150 mM NaCl, and 0.05% (v/v) Tween 20] containing 5% nonfat milk or BSA. The membrane was probed with primary antibody for 1 hour at room temperature or overnight at 4°C followed by incubation with horseradish peroxidase-conjugated secondary antibody (Amersham Biosciences, Piscataway, NJ) for 1 hour at room temperature. Proteins were detected by ECL Western blot detection reagents (Amersham Biosciences) followed by exposure to autoradiography film (BioExpress, Kaysville, UT). Anti-IL-1 β (3ZD) antibody was obtained from the National Cancer Institute Preclinical Repository and anti-phospho-IRE1 (cat # 3881) antibody was purchased from Epitomics (Burlingame, CA). Anti-eIF2 α (cat #: 2103), anti-phospho-eIF2 α (cat #: 3597), anti-IRE1 α (cat #: 3294), and anti-CHOP (cat #: 2895) antibodies were purchased from Cell Signaling Technology (Danvers, MA). Cell culture supernatants were analyzed for IL-1 β using BD OptEIA Human IL-1 β Enzyme-linked immunosorbent assay (ELISA) using a Synergy 2 plate reader (BioTek, Winooski, VT) according to the manufacturer's instructions.

2.5 Statistical analyses

One-way ANOVA was used to determine significance of IL-1 β concentration differences in cell culture supernatants. Tukey's multiple comparison test was used as a post test if any differences were noted with ANOVA (GraphPad Software, La Jolla, CA). A *P* value of < 0.05 was considered statistically significant.

3. Results

3.1 Palmitic acid, Pam₃CSK₄, and LPS induce markers of ER stress in THP-1 monocytes.

TLR4 and TLR2 activation induced markers of ER stress in murine macrophages [18]. Since palmitic acid has been previously shown to activate TLR2 in THP-1 monocytes [24] we determined whether palmitic acid, which is known to induce ER stress in several cell types [15-17, 25-28], induces markers of ER stress in THP-1 monocytes. Treatment of cells with sodium palmitate, TLR2 ligand Pam₃CSK₄ or TLR4 ligand LPS led to activation of the UPR as determined by phosphorylation and expression levels of ER stress related proteins in a time-dependent manner (Fig 1). Sodium palmitate induced phosphorylation of IRE-1 and eIF2 and the expression of CHOP. Pam₃CSK₄ induced phosphorylation of eIF2 but did not induce phosphorylation of IRE-1 or the expression of CHOP. LPS also failed to induce CHOP but induced the phosphorylation of both IRE-1 and eIF2. Activation of TLR2 and TLR4 induced the ER stress responses in THP-1 monocytes, but failed to induce CHOP. These results also suggest that activation of TLR2 or TLR4 induces different ER stress responses as assessed by IRE1, eIF2, and CHOP.

3.2 Palmitic acid-, Pam₃CSK₄-, or LPS-induced IL-1 β production in THP-1 monocytes is inhibited by chemical chaperone 4-phenylbutyric acid (4PBA).

Since TLR2 and TLR4 ligands induced ER stress, we attempted to determine whether Pam₃CSK₄, LPS, or palmitic acid-induced secretion of IL-1 β in THP-1 monocytes is mediated by ER stress. Therefore, we pretreated cells with an ER stress inhibitor, 4-phenylbutyric acid (4PBA). 4PBA is a small molecule chemical chaperone shown to stabilize proteins and assist in their folding and translocation across membranes [29-31] and thus, decreases UPR signaling [32, 33]. Treatment of THP-1 monocytes with sodium palmitate, Pam₃CSK₄, and LPS led to the expression of pro-IL-1 β in a time-dependent manner (Fig. 2A). Pre-treating with 4PBA dose-dependently inhibited palmitic acid-induced expression of pro-IL-1 β (Fig. 2C) and the secretion of IL-1 β after 24 hour treatment with sodium palmitate, Pam₃CSK₄, or LPS (Fig. 2B). These results imply that Pam₃CSK₄-, LPS-, and palmitic acid-induced expression of pro-IL-1 β and

subsequent release of IL-1 β in THP-1 monocytes are at least in part mediated through ER stress.

3.3 Thapsigargin but not tunicamycin induces the expression of pro-IL-1 β and secretion of mature IL-1 β in THP-1 monocytes.

A recent study showed that palmitic acid triggers ER stress in β -cells through changes in Ca^{2+} handling [34]. To determine if changes in calcium handling could in part explain how palmitic acid-induced ER stress leads to IL-1 β production in THP-1 monocytes, we induced ER stress through both calcium-dependent and calcium-independent mechanisms and analyzed IL-1 β secretion. To induce ER stress by disrupting calcium homeostasis we treated cells with thapsigargin. Thapsigargin is a sesquiterpene lactone extracted from the Mediterranean plant *Thapsia garganica* used experimentally as a potent and selective inhibitor of sarcoplasmic and endoplasmic reticulum Ca^{2+} ATPase (SERCA) which functions to maintain a 10,000-times higher concentration of calcium in the ER relative to cytosol [35]. Inhibition of SERCA by thapsigargin leads to a rapid depletion of ER Ca^{2+} ($\text{Ca}^{2+}_{\text{ER}}$) and a subsequent elevation of cytosolic Ca^{2+} ($\text{Ca}^{2+}_{\text{cyt}}$) [35, 36]. To induce ER stress by a non-intracellular calcium disrupting mechanism we used tunicamycin. Tunicamycin is a pharmacologic ER stress inducer produced by *Streptomyces lysosuperificus* and consists of a mixture of homologous antibiotics which contain uracil, N-acetyl glycosamine, an 11-carboxyaminodialdose called tunicamine, and a fatty acid linked to the amino group which collectively inhibit protein glycosylation and palmitoylation in the ER [2, 37]. Tunicamycin-induced ER stress has not been shown to involve intracellular calcium disruption. Treatment of THP-1 cells with thapsigargin and tunicamycin dose-dependently induced ER stress as assessed by the phosphorylation of IRE-1 and the expression of CHOP (Fig. 3A). Although both pharmacologic agents induced ER stress, only thapsigargin induced the expression of pro-IL-1 β (Fig. 3B) and secretion of mature IL-1 β in THP-1 monocytes (Fig. 3C). These results suggest that only ER stress mediated by perturbations in calcium homeostasis can induce IL-1 β , and that ER stress-induced IL-1 β release mediated by palmitic acid, Pam₃CSK

or LPS may involve disruption of calcium homeostasis.

3.4 Thapsigargin and tunicamycin also induce ER stress but fail to induce IL-1 β release in differentiated THP-1 cells.

Next, we determined whether ER stress can activate inflammasome leading to the release of mature IL-1 β in the differentiated THP-1 macrophages. Thapsigargin or tunicamycin dose-dependently induced ER stress as assessed by the phosphorylation of IRE-1 and eIF2 and the expression of CHOP, similar to undifferentiated THP-1 monocytes (Fig. 4); however, they fail to induce the release of mature IL-1 β in the differentiated THP-1 cells (Fig. 5). The release of mature IL-1 β was potentiated only after the treatment with Nigericin (Fig. 5). This result suggests that ER stress can provide the primary signal to induce pro-IL-1 β expression but not the secondary signal to release mature IL-1 β .

3.5 Docosahexaenoic acid (DHA) inhibits palmitic acid- or thapsigargin-induced IL-1 β secretion in THP-1 cells.

DHA is a well characterized inhibitor of inflammatory signaling pathways and a pan-inhibitor of TLR activation [20, 23, 38-40]. DHA has also been shown to modulate intracellular Ca^{2+} concentrations ($[\text{Ca}^{2+}]_i$) by inhibiting ER Ca^{2+} release [41-43]. Therefore we determined whether DHA inhibits palmitic acid- and thapsigargin-induced markers of ER stress and the secretion of IL-1 β . Pre-treating THP-1 cells with DHA dose-dependently inhibited palmitic acid-induced phosphorylation of IRE-1 and CHOP expression (Fig. 6A). In contrast, DHA failed to inhibit thapsigargin-induced phosphorylation of IRE-1 and CHOP protein expression (Fig. 6B) as well as thapsigargin-induced CHOP and GRP78 mRNA (data not shown). Furthermore, DHA dose-dependently inhibited palmitic acid-induced IL-1 β secretion but only modestly suppressed thapsigargin-induced IL-1 β secretion (Fig. 6C). These results suggest DHA does not inhibit ER stress through $[\text{Ca}^{2+}]_i$ modulation in monocytes but likely through additional mechanisms.

4. Discussion

Our results demonstrate that palmitic acid, a predominant dietary saturated fatty acid, as well as PAMPs Pam₃CSK₄ and LPS induce markers of ER stress in THP-1 monocytes (Fig. 1). These results support the findings that palmitic acid as well as TLR2 and TLR4 ligands induce the UPR [15-19]. However, differential induction of ER stress markers by Pam₃CSK₄ and LPS in THP-1 monocytes suggests that TLR2 activation induces a different UPR than TLR4 activation. TLR2 signaling consists of two main pathways; the MyD88-dependent which is utilized by both TLR2 and TLR4 and the MyD88-independent which is utilized by TLR4 but not TLR2. Therefore the different UPRs in THP-1 monocytes mediated by Pam₃CSK₄ and LPS may reflect ER stress stemming from different TLR signaling pathways. Although palmitic acid is well known to induce markers of ER stress [15-17, 25-27, 44, 45], our results revealed that ER stress markers induced by palmitate is not in the same pattern as TLR2 or TLR4 ligands; namely, palmitate induced CHOP expression, whereas TLR2 or TLR4 ligand did not. These results further suggest that palmitate-induced ER stress is mediated through both TLR2 dependent and independent pathways.

Next, we determined to what extent TLR-induced ER stress is responsible for pro-inflammatory cytokine production in THP-1 monocytes. Pro-IL-1 β is one of the TLR target gene products. Since monocytes contain active caspase-1 due to presence of constitutively active NALP3 inflammasome, signals inducing the expression of pro-IL-1 β are sufficient for inflammasome-mediated IL-1 β production. Pretreating cells with the chemical chaperone phenylbutyric acid (4PBA) inhibited palmitic acid-, Pam₃CSK₄-, and LPS-induced IL-1 β production (Fig. 2B). These results suggest that ER stress mediates TLR-induced production of IL-1 β in THP-1 monocyte. How chemical chaperone inhibits TLR/inflammasome-mediated IL-1 β production in THP-1 monocytes is an intriguing question. A recent study showed that activation of TLR2 and TLR4 by Pam₃CSK₄ and LPS respectively induced phosphorylation of IRE-1 and its downstream target, transcription factor X-box binding protein-1 (XBP1) in bone marrow macrophages which was required for sustained production of innate immune mediators [18].

XBP1 expression enhances productive protein folding by chaperone gene induction, suggesting an accumulation of unfolded proteins following TLR activation. The possibility that TLR activation increases the unfolded protein load as a means to activate IRE-1/XBP1 and sustain production of innate immune mediators would be an interesting hypothesis.

To better understand the mechanism by which ER stress mediates IL-1 β secretion in THP-1 cells, we sought to examine the effects of two ER stress-inducing molecules thapsigargin and tunicamycin. We found that although both pharmacologic ER stress inducing molecules robustly induced markers of ER stress (Fig. 3A), only thapsigargin treatment led to the expression of pro-IL-1 β and secretion of mature IL-1 β in THP-1 monocytes (Fig. 3B, 3C). These results suggest that not all ER stressors are sufficient to induce IL-1 β secretion in THP-1 cells. How thapsigargin but not tunicamycin induces the expression of pro-IL-1 β and secretion of mature IL-1 β is an intriguing question. Both chemicals induce ER stress but through different mechanisms. Tunicamycin suppresses glycosylation and posttranslational modification of proteins whereas thapsigargin disrupts ER calcium homeostasis. These differential mechanisms of action suggest that ER stress stemming from homeostatic calcium disruption may underlie the mechanisms by which ER stress induces IL-1 β secretion. Such an interpretation is supported by a recent animal study showing that mice fed a high fat diet had increased calcium transport dysfunction due to altered lipid composition in the ER membrane which resulted in chronic ER stress [46]. Collectively these results suggest that palmitic acid- as well as Pam₃CSK₄- and LPS-induced IL-1 β may be in part mediated through the disruption of calcium homeostasis.

DHA is known to inhibit many inflammatory signaling pathways [23, 39] and is a pan inhibitor of TLR activation [20, 22, 23, 38, 40, 47]. Our results showed that DHA inhibits palmitic acid-induced ER stress in THP-1 monocytes (Fig. 6A). DHA also inhibited thapsigargin- and palmitic acid-induced IL-1 β secretion in THP-1 cells (Fig. 6C). Although DHA has been reported to modulate [Ca²⁺]_i in cell types including astrocytes, T-cells, and endothelial cells [41-43, 48], our results suggest that DHA does not inhibit ER

stress through $[Ca^{2+}]_i$ modulation in THP-1 monocytes. Recently DHA was reported to inhibit NALP3 inflammasome activation in LPS-primed bone-marrow-derived macrophages [49]. This mechanism likely supports our observation that although DHA dose-dependently inhibited both palmitic acid-induced ER stress and IL-1 β secretion, it failed to suppress thapsigargin-induced ER stress while still modestly reducing IL-1 β secretion.

5. Conclusion

In summary, the predominant dietary saturated fatty acid palmitic acid induces ER stress and the secretion of IL-1 β which are inhibited by the n-3 polyunsaturated fatty acid DHA. Palmitic acid-induced IL-1 β is at least partially mediated through ER stress since it was inhibited by the chemical chaperone, 4PBA. In contrast, ER stress mediated by the intracellular calcium disrupting molecule thapsigargin was not inhibited although

IL-1 β secretion was modestly reduced by DHA. These results suggest a possible mechanism by which palmitic acid and DHA reciprocally modulate IL-1 β secretion in THP-1 monocytes involves modulation of ER homeostasis. The detail signaling pathways through which the cross-talk among fatty acids and TLR ligands leading to induction of different types of ER stress markers remain to be elucidated. However, such a reciprocal induction of ER stress by palmitate and DHA implies that the propensity of ER stress and its consequences can be differentially modulated by the types of dietary fat we consume.

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Fig. 1. Palmitic acid, Pam₃CSK₄, and LPS induce ER stress in THP-1 monocytes. THP-1 cells were serum starved in 0.25% FBS-RPMI-1640 for 12 hours. Cells were treated with palmitic acid (C16:0) (150 μM), Pam₃CSK₄ (Pam3) (100 ng/ml), and LPS (1 μg/ml) for the indicated times and cell lysates were immunoblotted for phosphorylated IRE-1, total IRE-1, phosphorylated eIF2, total eIF2, and CHOP. Data shown are representative of three independent experiments.

Fig. 2. Inhibitor of ER stress inhibits Palmitic acid-, Pam₃CSK₄-, and LPS-induced IL-1β secretion in THP-1 monocytes. THP-1 cells were serum starved in 0.25% FBS-RPMI-1640 for 12 hours then treated with LPS (1 μg/ml), Pam₃CSK₄ (Pam3) (100 ng/ml), and palmitic acid (C16:0) (150 μM) for the indicated times. Cell lysates were immunoblotted for pro-IL-1β (A). THP-1 cells were serum starved in 0.25% FBS-RPMI-1640 for 12 hours. Cells were pretreated with 4PBA (0.5 and 1.0 mM) for 1 hour then coincubated with palmitic acid (C16:0) (150 μM), Pam₃CSK₄ (Pam3) (10 ng/ml), and LPS (100 ng/ml). After 24 hours cell culture supernatants were analyzed for IL-1β by ELISA (B). THP-1 cells were serum starved in 0.25% FBS-RPMI-1640 for 12 hours. Cells were pretreated with 4PBA (0.5 and 1.0 mM) for 1 hour then coincubated with C16:0 (150 μM) for 9 hours. Cell lysates were immunoblotted for pro-IL-1β (C). ELISA data are expressed as mean ± SEM of three independent experiments. Significance was determined by ANOVA (# P < 0.001 significantly different from untreated, **P < 0.01, ***P < 0.001 significantly different from agonist only). Immunoblots shown are representative of three independent experiments.

Fig. 3. Thapsigargin and tunicamycin induce ER stress but only thapsigargin induces the expression of pro-IL-1β and secretion of mature IL-1β in THP-1 monocytes. THP-1 cells were serum starved in 0.25% FBS-RPMI-1640 for 12 hours then treated with thapsigargin and tunicamycin for 9 hours. Cell lysates were immunoblotted for phosphorylated IRE-1, total IRE-1, and CHOP (A). THP-1 cells were serum starved in 0.25% FBS-RPMI-1640 for 12 hours then treated with thapsigargin and tunicamycin. After 24 hours cell culture supernatants were analyzed for IL-1β by ELISA (B). THP-1 cells were serum starved in 0.25% FBS-RPMI-1640 for 12 hours then treated with thapsigargin or tunicamycin. After 9 hours cell lysates were immunoblotted for pro-IL-1β (C). ELISA data are expressed as mean ± SEM of three independent experiments. Significance was determined by ANOVA (***P < 0.001 significantly different from untreated). Immunoblots shown are representative of three independent experiments.

Fig. 4. Thapsigargin and tunicamycin induce ER stress in differentiated THP-1 cells. THP-1 cells were differentiated with 5 ng/ml phorbol-12-myristate-13-acetate (PMA) in 10% FBS-RPMI-1640 for 2 days. The differentiated cells were serum starved in Opti-MEM medium containing 0.25% FBS for 1 hour and then treated with thapsigargin (A) or tunicamycin (B) at the indicated concentrations for 6 hours. Cell lysates were immunoblotted for phosphorylated IRE-1, total IRE-1, phosphorylated-eIF2, total eIF-2, CHOP, and β-actin. Immunoblots shown are representative of three independent experiments.

Fig. 5. Thapsigargin but not tunicamycin induces the expression of pro-IL-1β and secretion of mature IL-1β in differentiated THP-1 cells. THP-1 cells were differentiated with 5 ng/ml phorbol-12-myristate-13-acetate (PMA) in 10% FBS-RPMI-1640 for 2 days. The differentiated cells were serum starved in Opti-MEM medium containing 0.25% FBS for 1 hour then treated with thapsigargin or tunicamycin for 6 hours. Cell lysates were immunoblotted for pro-IL-1β and β-actin (A). The differentiated cells were serum starved as in (A) then treated with thapsigargin for 6 hours (open bars) or treated with thapsigargin for 6 hours, washed, and then treated with 5 μM nigericin for 1 hour (black bars). Cell culture supernatants were analyzed for IL-1β by ELISA (B). To verify that the THP-1 cells were successfully differentiated the differentiated cells were treated with Pam₃CSK₄ (Pam, the TLR2 agonist) for 3 hours, washed and then treated with or without 5 μM nigericin (Nig) for 1 hour (strike bars). *P<0.05, **P < 0.01 significantly different from untreated for the thapsigargin and tunicamycin experiments or Pam alone for the differentiation verification experiments. Immunoblots shown are representative of three independent experiments.

1 Fig. 6. Docosahexaenoic acid (DHA) inhibits palmitic acid- or thapsigargin-induced ER stress and IL-1 β
2 secretion in THP-1 cells. THP-1 cells were serum starved in 0.25% FBS-RPMI-1640 for 12 hours. Cells
3 were pretreated with DHA (5 and 10 μ M) for 1 hour then coincubated with palmitic acid (C16:0) (150
4 μ M). After 9 hours cell lysates were immunoblotted for phosphorylated IRE-1, total IRE-1, and CHOP
5 (A). THP-1 cells were serum starved in 0.25% FBS-RPMI-1640 for 12 hours. Cells were pretreated with
6 DHA (5 and 10 μ M) for 1 hour then coincubated with thapsigargin (100 nM). After 9 hours cell lysates
7 were immunoblotted for phosphorylated IRE-1, total IRE-1, and CHOP (B). THP-1 cells were serum
8 starved in 0.25% FBS-RPMI-1640 for 12 hours. Cells were pretreated with DHA (2.5, 5, 10 μ M) for 1
9 hour then coincubated with thapsigargin (100 nM) and C16:0 (150 μ M). After 24 hours cell culture
10 supernatants were analyzed for IL-1 β by ELISA (C). ELISA data are expressed as mean $\pm$ SEM of three
11 independent experiments. Significance was determined by ANOVA (**P < 0.01, ***P < 0.001
12 significantly different from thapsigargin or C16:0 alone). Immunoblots shown are representative of three
13 independent experiments.
14

Fig. 1

Fig. 2

Fig. 3

A

B

C

Fig. 4

Fig. 5

Fig. 6

A

B

C