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Shyretha Brown
Tennessee State University

Margaret Whalen
Tennessee State University

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TRIBUTYLTIN ALTERS SECRETION OF INTERLEUKIN 1 BETA FROM HUMAN IMMUNE CELLS

Shyretha Brown[*] and **Margaret Whalen**

[*]Department of Biological Sciences, Tennessee State University, Nashville, TN, 37209

Department of Chemistry, Tennessee State University, Nashville, TN, 37209

Abstract

Tributyltin (TBT) has been used as a biocide in industrial applications such as wood preservation, antifouling paint, and antifungal agents. Due to its many uses, it contaminates the environment and has been found in human blood samples. Interleukin 1 beta (IL-1β) is a pro-inflammatory cytokine that promotes cell growth, tissue repair, and immune response regulation. Produced predominately by both monocytes and macrophages, IL-1β appears to increase the invasiveness of certain tumors. This study shows that TBT modifies the secretion of IL-1β from increasingly reconstituted preparations of human immune cells. IL-1β secretion was examined after 24h, 48h, or 6 day exposures to TBT in highly enriched human NK cells, monocyte-depleted (MD) peripheral blood mononuclear cells (MD-PBMCs), PBMCs, granulocytes, and a preparation combining both PBMCs and granulocytes (PBMCs+granulocytes). TBT altered IL-1β secretion from all of the cells preparations. The 200 nM concentration of TBT normally blocked the secretion of IL-1β, while lower concentrations (usually 5-50 nM) elevated secretion of IL-1β. Examination of the signaling pathway(s) responsible for the elevated secretion of IL-1β were carried out in MD-PBMCs. Pathways examined were IL-1β processing (Caspase-1), mitogen-activated protein kinases (MAPKs), and nuclear factor kappa B (NFκB). Results indicated that MAPK pathways (p44/42 and p38) appear to be the targets of TBT that lead to increased IL-1β secretion from immune cells. These results from human immune cells show IL-1β dysregulation by TBT is occurring *ex vivo*. Thus, potential for *in vivo* effects on pro-inflammatory cytokine levels may possibly be a consequence of TBT exposures.

Keywords

NK cells; PBMCs; Granulocytes; Tributyltin; Interleukin 1 beta

INTRODUCTION

Interleukin 1 beta (IL-1β) is a cytokine that regulates the inflammatory response and promotes cellular growth, and tissue repair (Apte *et al*., 2006; Arend, 2002; Dinarello, 1996; 2005; 2009). IL-1β is initially synthesized as a 31-kDa protein that is then processed by Caspase-1 to its active form (17-kDa) (Swaan *et al*., 2001; Dinarello, 1996). The enzyme

Correspondence: Margaret M. Whalen, Department of Chemistry, Tennessee State University, 3500 John A. Merritt Blvd., Nashville, TN 37209, mwhalen@tnstate.edu, Phone: 615-963-5247, Fax: 615-963-5326.

that proteolytizes IL-1β in monocytes is caspase-1 also referred to as IL-1β converting enzyme (ICE) (Kuida *et al*., 1995; Swaan *et al*., 2001). Cell types known for producing and secreting IL-1β include monocytes, macrophages, T cells, natural killer cells, keratinocytes, and fibroblasts (Burger and Dayer, 2002; Dinarello, 2005; Apte *et al*., 2006; Voronov *et al*., 2002). IL-1β appears to also be secreted by neutrophils; however, its processing in neutrophils may be independent of Caspase-1 (Guma *et al*., 2009). Additionally, it has been shown that IL-1β can act as a co-stimulator of the production of another pro-inflammatory cytokine, interferon gamma (IFNγ), in natural killer (NK) cells (Cooper *et al*., 2001). This pleiotropic cytokine can also contribute to chronic inflammation in some diseases such as rheumatoid arthritis and multiple sclerosis (Choy and Panayi, 2001; Lucas and Hohlfeld., 1995). Additionally, chronic inflammation plays a major role in the development of cancer. IL-1β has been detected in human cancers such as breast and pancreatic cancers and melanoma (Arlt *et al*., 2002; Elaraj *et al*., 2006; Jin *et al*., 1997; Lewis and Varghese, 2006; Muerkoster *et al*., 2006) and its levels of expression are linked to invasiveness of these tumors and are essential for angiogenesis and metastasis (Voronov *et al*., 2002). Expression of IL-1β at the site of tumor development boosts the ability of malignant cells to become adhesive and invade neighboring tissues (Chirivi *et al*., 1996; Vidal-Vanaclocha *et al*., 1996). IL-1β can stimulate tumor development as evidenced by studies showing increased IL-1β production in human and animal cancer cell lines. These cell lines include ovarian, sarcomas, and transitional cell carcinomas. Increased IL-1β production has been associated with poor prognoses in patients with breast, colon, and lung cancers as well as melanomas (Lewis and Varghese, 2006; Voronov *et al*., 2002).

Tributyltin (TBT) is an organotin compound that has been used as a biocide in a variety of industrial applications such as preservation of wood, controlling of slime in paper mills, antifungal agent in industrial cooling water systems and breweries, and antifouling paint (Antizar-Ladislao, 2008; Gipperth, 2009; Kannan *et al*., 1995; Kannan *et al*., 1997). It is also found in some household goods such as siliconized-paper baking parchments and shower curtains (Yamada *et al*., 1993). It is known to contaminate the marine and fresh water environment due to common use on boats and ships as an antifouling paint. TBT has been banned in the US since 1989 (Loganathan et al., 2001) and by the International Convention on the Control of Harmful Antifouling Systems on Ships in 2008 (Gipperth, 2009). However, it is still contaminating the environment due to continued application on ships and its chemical stability (Gipperth, 2009). TBT is found in fish and other foods (Kannan *et al*., 1995); as a result, TBT has been found in human blood samples (ranging as high as 261 nM) and other tissues (Antizar-Ladislao, 2008; Kannan *et al*., 1999; Whalen *et al*., 1999). Mammals with TBT exposure show increased incidences of tumors (Wester et al., 1990). TBT decreases the ability of human NK cells to destroy tumor cells with accompanying decreases in cytotoxic and cell surface protein expression (Dudimah *et al*., 2007; Thomas et al., 2004; Whalen et al., 2002). In addition, TBT both inhibits and stimulates the secretion of tumor necrosis factor alpha (TNFα) and interferon gamma (IFNγ) from human lymphocytes (depending on the exposure concentration) (Hurt *et al*., 2013; Lawrence *et al*., 2014). The importance of introducing TBT as a stress inducer to the cell environment is to understand whether exposure to TBT disrupts normal cellular functions such as immune cell secretion of the important regulatory cytokine IL-1β. Based on TBT's effect on TNFα and IFNγ

secretion (Hurt *et al*., 2013; Lawrence *et al*., 2014), we hypothesize that exposure to TBT will alter the ability of immune cells to secrete IL-1β.

In this study, an array of immune cell preparations were examined for the effects of TBT exposures on the secretion of IL-1β. The preparations studied included: human NK cells, human monocyte-depleted (MD) peripheral blood mononuclear cells (PBMCs) (MD-PBMCs), PBMCs, PBMCs combined with granulocytes (PBMCs+granulocytes), and granulocytes. The use of increasingly reconstituted immune cell preparation allows us to investigate the influence that various immune cell types may have on the ability of TBT to induce alteration in secretion of IL-1β, thus, better approximating the physiological situation. An additional goal of this study is to investigate the signaling pathways that may be involved in any TBT-induced alterations in IL-1β secretion.

MATERIALS AND METHODS

Preparation of PBMCs, MD- PBMCs, and Granulocytes

PBMCs were isolated from Leukocyte filters (PALL- RCPL or RC2D) obtained from the Red Cross Blood Bank Facility (Nashville, TN) as described in Meyer et al., 2005. Leukocytes were retrieved from the filters by back-flushing them with an elution medium (sterile PBS containing 5 mM disodium EDTA and 2.5% [w/v] sucrose) and collecting the eluent. The eluent was layered onto Ficoll-Hypaque (1.077g/mL) and centrifuged at 1200g for 30-50 min. Granulocytes and red cells pelleted at the bottom of the tube while the PBMCs floated on the Ficoll-Hypaque. Mononuclear cells were collected and washed with PBS (500g, 10min). Following washing, the cells were layered on bovine calf serum for platelet removal. The cells were then suspended in RPMI-1640 complete medium which consisted of RPMI-1640 supplemented with 10% heat-inactivated BCS, 2 m*M L*-glutamine and 50 U penicillin G with 50 μg streptomycin/mL. This preparation constituted PBMCs. Monocyte-depleted PBMCs (10-20% $CD16^+$, 10-20 % $CD56^+$, 70-80% $CD3^+$, 3-5% $CD19^+$, 2-20% $CD14^+$) were prepared by incubating the cells in glass Petri dishes (150 × 15 mm) at 37 °C and air/CO_2, 19:1 for 1 h. This cell preparation is referred to as MD-PBMCS cells. Granulocytes were isolated with the removal of mononuclear and red blood cells as described in Kuijpers *et al*., 2013. Granulocytes were collected and washed with PBS (2500 rpm, 15min). Red blood cells were lysed with NH_4CL isotonic solution (155mM NH_4Cl, 10mM $KHCO_3$, 0.1 mM EDTA) for 10 min. Cells were then washed with PBS and centrifuged at 800g for 10 min. The cells were then suspended in RPMI-1640 complete medium supplemented with 10% heat-inactivated BCS, 2 m*M L*-glutamine and 50 U penicillin G with 50 μg streptomycin/mL.

Preparation of NK cells

NK cells were prepared from buffy coats (from healthy adult donors) purchased from Key Biologics, LLC (Memphis, TN). Highly purified NK cells were prepared using a rosetting procedure. RosetteSep human NK cell enrichment antibody cocktail (0.6-0.8 mL) (StemCell Technologies, Vancouver, British Columbia, Canada) was added to 45 mL of buffy coat. The mixture was incubated for 20 min at room temperature (~ 25° C). Approximately 8 mL of the mixture was layered onto 4 mL of Ficoll-Hypaque (1.077 g/mL) (MP Biomedicals,

Irvine, CA) and centrifuged at 1200 g for 30-50 min. NK cells were collected and washed twice with phosphate buffered saline (PBS) pH 7.2 and stored in complete media (RPMI-1640 supplemented with 10% heat-inactivated bovine calf serum (BCS), 2 m*M L*-glutamine and 50 U penicillin G with 50 μg streptomycin/ml) at 1 million cells/mL at 37 °C and air/CO_2, 19:1.

Chemical Preparation

TBT was purchased from Sigma-Aldrich (96%) (St. Louis, MO). Desired concentrations of TBT were prepared by dilution of the stock into cell culture media. TBT was a neat standard, dissolved initially in deionized water to give a 1 mM solution.

Inhibitor Preparation

Enzyme inhibitors were purchased from Fischer Scientific (Pittsburgh, PA). The stock solution for each inhibitor was a 50 mM solution in dimethylsulfoxide (DMSO). Capase-1 inhibitor II, MEK 1/2 pathway inhibitor (PD98059), p38 inhibitor (SB202190), MEK 1/2 pathway inhibitor (U0126), and NFκB inhibitor (BAY11-7085) were prepared by dilution of the stock solution into cell culture media.

Cell Treatments

NK cells, MD-PBMCs, PBMCs, granulocytes (at a concentration of 1.5 million cells/ mL), or PBMCs+granulocytes (at a concentration of 0.75 million cells/mL each) were treated with TBT at concentrations of 2.5-200 nM TBT for 24h, 48h, or 6 days. Following the incubations, the cells were pelleted and supernatants were collected and stored at -70° C until assaying for IL-1β.

For pathway inhibitor experiments, MD-PBMCs (at a concentration of 1.5 million cells/ mL) were treated with pathway inhibitors 1h before adding TBT at concentrations of 5, 10, 25 nM TBT for 24h. Following the incubations, the cells were pelleted and supernatants were collected and stored at -70 C until assaying for IL-1β.

Cell Viability

Cell viability was assessed at the beginning and end of each exposure period. Viability was determined using the trypan blue exclusion method. Briefly, cells were mixed with trypan blue and counted using a hemocytometer. The total number of cells and the total number of live cells were determined for both control and treated cells to determine the percent viable cells.

IL-1 β Secretion Assay

IL-1 β levels were measured using the BD OptEIA™ Human IL-1 β enzyme-linked immunosorbent assay (ELISA) kit (BD-Pharmingen, San Diego, CA). Briefly, a 96-well micro well plate, designed for ELISA (Fisher, Pittsburgh, PA), was coated with a capture antibody for IL-1β diluted in coating buffer. The plate was incubated with the capture antibody overnight at 4 °C. After incubation, the capture antibody was removed by washing the plate three times with wash buffer (PBS and 0.05% Tween-20). Assay diluent (PBS and

bovine calf serum) was added to each well (blocking non-specific binding) and incubated at room temperature for 1h. The assay diluent was removed by washing the plate three times, and the cell supernatants and IL-1β standards were added to the coated plated and incubated for 2 h at room temperature. Following this incubation, the plate was thoroughly washed five times and then incubated for 1h with a detection antibody to IL-1β which was conjugated with horseradish peroxidase. The excess detection antibody was removed by washing seven times and a substrate solution was added for 30 min at room temperature to produce a colored product. The incubation with the substrate was ended by addition of acid and the absorbance was measured at 450 nm on a Thermo Labsystems Multiskan MCC/340 plate reader (Fisher Scientific).

Statistical Analysis

Statistical analysis of the data was performed by using ANOVA and Student's t test. Data were initially compared within a given experimental setup by ANOVA. A significant ANOVA was followed by pair wise analysis of control versus exposed data using Student's t test, a p value of less than 0.05 was considered significant.

RESULTS

Viability of NK cells, MD-PBMCs PBMCs, PBMCs+ Granulocytes, and Granulocytes exposed to TBT

Table 1 shows the effects of TBT exposures (2.5-200 nM) on the viability of highly purified NK cells, MD-PBMCs, PBMCs, PBMCs+granulocytes, and granulocytes. Exposure of NK cells to 2.5-100 nM TBT for 24 h had no effect on their viability as compared to the control. The highest concentration of TBT (200nM) caused a slight decrease in NK cell viability. Exposure to these same concentrations for 48h caused no decrease in viability compared to control cells except at the highest concentration of TBT. NK cells exposed to 2.5-50 nM TBT for 6 days showed no change in viability as compared to the control; but, 100nM and 200nM exposures did significantly reduce viability. MD-PBMCs showed a very slight decrease in viability with exposure to 200 nM TBT for 24 h. The viability of both MD-PBMCs and PBMCs was somewhat decreased by exposure to 200 nM TBT for 48 h. After 6 days both 100 nM and 200 nM TBT caused some decrease in viability of both MD-PBMCs and PBMCs. Exposure of PBMCs+granulocytes and granulocytes alone to 2.5-200 nM TBT for 24 h had no effect on their viability as compared to the control. Exposure to 200 nM TBT for 48h significantly diminished the viability of PBMCs+granulocytes. After 6 days of exposure to 200 nM TBT both, PBMCs+granulocytes and granulocytes showed some decrease in viability as compared to controls.

Viability of monocyte-depleted PBMCs with pathway inhibitors exposed to TBT

Table 2 shows the effects of TBT exposures (5, 10, 25nM) on the viability of MD-PBMCs that were treated with various signaling pathway inhibitors 1 hour prior to adding the appropriate concentrations of TBT. No significant changes in viability were seen with any of the inhibitors when compared to control cells.

Effects of TBT Exposure on Secretion of IL-1β by NK cells

Table 3 shows the effects of exposing highly purified NK cells to 0, 2.5, 5, 10, 25, 50, 100 and 200 nM TBT for 24 h, 48 h, and 6 days on IL-1β secretion from 4 donors tested (KB=Key Biologic buffy coat). All donors showed significant decreases in IL-1β when exposed to 200 nM TBT for all lengths of exposure. Cells from all 4 donors showed a significant increase in IL-1β secretion when exposed to 5, 10, and 25nM TBT. Concentrations that caused increases varied from one donor to the next. For instance, the cells from donor KB166 treated with 2.5, 5, 10, 25, 50, and 100 nM TBT showed significant increases of 1.1, 1.4, 1.7, 1.9, 2.1, and 1.4 fold respectively after 24 h while cells from donor KB169 showed significant increases of 1.7, 3.4, 6.7, 6.2, 7.7, and 6.3 fold at these same concentrations. When NK cells were exposed to TBT for 48 h, all 4 donors showed significant increases in IL-1β secretion at one or more concentration. After 6 d of exposure, all donors display significant decreases in IL-1β secretion at 200 nM TBT and increases at 10 and 25 nM TBT. Figure 1A shows the effects of TBT exposures on IL-1β secretion at each of the time points for an individual donor (KB166).

Effects of TBT Exposure on Secretion of IL-1β by MD-PBMCs

The effects of exposures to TBT on secretion of IL-1β from MD-PBMCs after 24 h, 48 h, and 6 d from 5 donors (F=filter obtained from the Red Cross) are shown in Table 4. This preparation is largely NK cells and T cells. When MD-PBMCs were exposed to TBT for 24 h there were statistically significant increases in IL-1β secretion induced by TBT for all donors at the 25 and 50 nM concentrations with variation in the fold increase among different donors (F142 showed 2.0 and 2.1 fold, respectively while F156 showed 4.1 and 5.4 fold, respectively). Significant decreases in IL-1β secretion were seen at 200 nM TBT for 3 of the 5 donors after a 24 h exposure. The results seen after a 48 h of exposure to TBT were similar to those seen after 24 h. Increases were seen in every donor after a 6 d exposure to TBT, however, the number of concentrations that showed significant increases were fewer. Figure 1B shows the effects of TBT exposures at each of the lengths of exposure for an individual donor (F142).

Effects of TBT Exposure on Secretion of IL-1β by PBMCs

Table 5 summarizes the effects of exposing PBMCs from 5 individual donors to TBT on IL-1β secretion (F=filter obtained from the Red Cross). As with NK cells and MD-PBMCs, there was rather wide variation in the baseline secretion of IL-1β from one donor to the next at each time point. A 24 h exposure to 200 nM TBT, caused a significant decrease ($P<0.05$) in IL-1β secretion in 4 of the 5 donors. Significant increases in IL-1β secretion were seen in all donors at 50 nM. Additional concentrations of TBT caused increases in IL-1β secretion depending on the donor. For instance, cells from donor F144 treated with 5, 10, 25, 50, and 100 nM TBT showed significant increases of 1.4, 1.4, 1.6, 2.6, and 2.2 fold, respectively while donor F147 treated with 2.5, 5, 10, 25, 50, and 100 nM TBT showed significant increases of 1.5, 1.6, 2.7, 2.6, 4.4, and 3.8 fold, respectively. 48 h exposures of PBMCs to 200 nM TBT caused significant decreases in IL-1β for all donors and significant increases at 50 nM. After 6 days of exposure, still 4 of the 5 donors showed a significant decrease in

IL-1β secretion compared to the control when exposed to 200 nM TBT. Figure 1C shows the effects of TBT exposures at each of the lengths of exposure for an individual donor (F144).

Effects of TBT Exposure on Secretion of IL-1β by PBMCs+Granulocytes

Effects of exposing PBMCs+granulocytes to TBT f on IL-1β secretion from the same 5 donors as for PBMCs alone are shown in Table 6 (F=filter obtained from the Red Cross). Like the previous cell types discussed, the concentrations that caused increases and decreases in IL-1β secretion varied from one donor to the next in all lengths of exposures as did baseline secretion. In contrast to the other cell types, including PBMCs alone, there were significant increases of IL-1β noted for 200 nM TBT for 4 of the 5 donors at 24h and 2 of the 5 donors at 48h. Cells from donor F144 treated with 10, 25, 50, 100, and 200 nM TBT showed fold increases at 1.5, 1.8, 1.5, 1.7, and 1.7, respectively at 24 h and 1.4, 1.5, 1.6, 1.6, and 1.2, respectively at 48 h. After 6 days of exposure, control secretion of IL-1β was diminished compared to 24 h and 48 h; and the changes in (increases or decreases) of IL-1β seen at each concentration of TBT were normally quite small. Figure 1D shows the effects of TBT exposures at each of the lengths of exposure for an individual donor (F144).

Effects of TBT Exposure on Secretion of IL-1β by Granulocytes

The effects of exposures to TBT on secretion of IL-1β from granulocytes after 24 h, 48 h, and 6 d from the same 5 donors as for PBMCs alone (F=filter obtained from the Red Cross) are shown in Table 7. Baseline secretion of IL-1β from granulocytes was quite low, being undetectable in 1 donor at 24 h and in all donors by 6 days. When granulocytes were exposed to TBT for 24 h there were statistically significant increases in IL-1β secretion induced by at least 1 concentration of TBT for 4 of 5 donors. Figure 1E shows the effects of TBT exposures at each of the lengths of exposure for an individual donor (F144). Although IL-1β secretion was lower for granulocytes than all other cell types tested, there were still significant decreases and increases in IL-1β secretion induced by 24 and 48 h exposures to TBT.

Effects of TBT Exposure on Secretion of IL-1β by MD-PBMCs treated with Selective Signaling Pathway Inhibitors

IL-1β Cleavage Inhibitor (Caspase-1 Inhibitor II)—The effects of exposures to 5, 10, and 25 nM TBT on secretion of IL-1β from MD-PBMCs where Caspase-1 had been inhibited with Caspase-1 inhibitor II (50 μM) are shown in Table 8. Caspase-1 inhibitor II inhibited baseline IL-1β production as this enzyme is required for IL-1β processing. However, cells exposed to TBT showed similar fold increases in IL-1β secretion in both the presence and absence of the inhibitor. Figure 2A shows the fold increases from a representative experiment (F182). There are 1.3, 1.6, and 1.7 fold increases when MD-PBMCs are exposed to 5, 10, and 25 nM in the absence of the Caspase-1 inhibitor. When the inhibitor is present those same TBT exposures are still able to cause 1.3, 1.6, and 2.0 fold increases in IL-1β secretion (albeit on a much lower baseline). This same trend was seen in all of the donors tested (Table 8). Thus, although the baseline secretion was greatly decreased by the presence of the inhibitor, TBT was still able to cause the same magnitude of increase in IL-1β secretion as was seen when no inhibitor was present. This indicates that

Caspase-1 (needed for IL-1β processing) is not being activated by TBT to increase IL-1β secretion, since the magnitude of the TBT-induced increase should have been diminished when caspase-1 was inhibited if TBT had been activating this enzyme in order to increase IL-1β secretion.

Nuclear Factor kappa B (NFκB) Inhibitor (BAY 11-7085)—The effects of exposures to 5, 10, and 25 nM TBT on secretion of IL-1β from MD-PBMCs where NFκB function had been inhibited with BAY 11-7085 (1.25 μM) are shown in Table 9. BAY 11-7085 inhibited baseline IL-1β production. However, cells exposed to TBT showed no diminution in fold increase in IL-1β secretion when the inhibitor was present. In Figure 2B (representative data from F218) there are 1.9, 3.0, and 4.8 fold increases when MD-PBMCs are exposed to 5, 10, and 25 nM in the absence of NFκB inhibitor. When the inhibitor is present those same TBT exposures cause 2.8, 4.9, and 5.3 fold increases in IL-1β secretion. Cells from each of the donors tested showed this same pattern (Table 9). Thus, like the Caspase-1, NFκB does not seem to be a cellular component that TBT is activating in order to increase the secretion of IL-1β from the cells, as the fold increase in secretion is not diminished by the inhibition NFκB function.

Mitogen activated protein kinase kinase (MAP2K), MEK, Inhibitor (U0126)—The effects of exposures to 5, 10, and 25nM TBT on secretion of IL-1β from MD-PBMCs where the mitogen activated protein kinase kinase (MAP2K) for p44/42, MEK, had been inhibited with U0126 (50 μM) are shown in Table 10. U0126 had varying effects on baseline IL-1β secretion; however, cells exposed to TBT showed a lower fold increase in IL-1β secretion in the presence of the inhibitor. In Figure 2C (representative data from F181) we see there are 2.1, 3.3, and 4.3 fold increases when MD-PBMCs are exposed to 5, 10, and 25 nM TBT in the absence of MEK inhibitor. When the inhibitor is present those same TBT exposures are able to cause 1.1, 1.3, and 1.2 fold increases in IL-1β secretion. In 3 of the 4 donors tested the ability of TBT to increase IL-1β secretion was blocked or significantly decreased when the p44/42 pathway was inhibited (Table 10) in the 4^{th} donor the ability of 5 nM TBT to increase IL-1β secretion was blocked. This indicates that the p44/42 MAPK pathway is being utilized by TBT to lead to the increase in IL-1β secretion.

p38 Inhibitor (SB202190)—The effects of exposures to 5, 10, and 25nM TBT on secretion of IL-1β from MD-PBMCs where p38 had been inhibited with SB202190 (25-50 μM) are shown in Table 11. p38 inhibitor inhibited baseline IL-1β secretion and also diminished the ability of TBT to stimulate IL-1β secretion from MD-PBMCs. In Figure 2D we see there are 2.5, 3.6, and 5.1 fold increases when MD-PBMCs are exposed to 5, 10, and 25 nM TBT in the absence of p38 inhibitor. When the inhibitor is present those same TBT exposures are able to cause 2.8, 1.5, and 2.6 fold increases in IL-1β secretion. Three of the 4 donors tested showed that inhibition of the p38 pathway diminished or completely blocked the ability of at least one concentration of TBT to simulate IL-1β secretion (Table 11). However, 1 donor showed no decrease in TBT-stimulation of IL-1β secretion when the p38 pathway was inhibited. These results suggest that the p38 pathway may be utilize by TBT to stimulate IL-1β secretion, with significant variation among donors.

DISCUSSION

IL-1β is an inflammatory cytokine that contributes to chronic inflammation which can increase tumor invasiveness (Voronov *et al*., 2003). TBT is known to contaminate the marine and fresh water environment due to common use on boats and ships as an antifouling paint (Gipperth, 2009) and levels as high as 261 nM have been found in human blood samples (Kannan *et al*., 1995; Whalen *et al*., 1999). TBT can decrease the ability of human NK cells to destroy tumor cells (Dudimah *et al*., 2007) and alter the secretion of TNFα and IFNγ from immune cells (Hurt *et al*., 2013; Lawrence et al. 2014). As alterations of levels of IL-1β can have profound effects on immune competency as well as tumor development and progression (Dinarello, 2009), it is important to determine whether TBT is able to alter the secretion of IL-β from immune cells in an ex vivo human model. In this study, increasingly reconstituted human immune cell preparations were used (NK cells, MD-PBMCs, PBMCs, PBMC+granulocytes, and granulocytes) to determine the effects of TBT on IL-β secretion. Additionally experiments to identify signaling pathways involved in TBT-induced alterations of IL-1β levels were also conducted.

Baseline secretion of IL-1β varied among the different cell preparations. NK cells and MD-PBMCs showed very similar baseline secretions. The presence of monocytes caused a greater baseline secretion for PBMCs. This is expected due to the fact that monocytes (along with macrophages) are the major IL-1β secreting cell type (Dinarello, 2009). Granulocytes alone showed very low baseline secretion of IL-1β. Granulocytes have been shown by others to be capable of secreting IL-1β (Guma et al., 2009). The concentration at which TBT caused the maximum fold increase in IL-β from each of the different cell types (after 24 h of exposure) varied among the donors. The average of the maximum fold increase (from each donor, 4 donors) for pure NK cells was 3.2 fold (this ranged from 1.3 fold to 7.8 fold over the range of 2.5-100 nM). For MD-PBMCs, the average of the greatest fold increase (5 donors) was 11.2 fold (ranging from 1.8-33 fold over 2.5-100 nM depending on the donor) and for PBMCs the value was 2.6 fold (again from 5 donors) increases ranged from1.4-4.4 fold. PBMCs plus granulocytes showed an average highest increase in TBT-stimulated IL-1β secretion of 4.7 fold (same 5 donors as the PBMCs alone) with at range of 1.3-16 fold. While the average of the maximum increase is higher from PBMCs plus granulocytes than from PBMCs alone this is entirely due to the highest value being much greater in one donor (16 fold) than was seen in the PBMCs. All other donors showed similar maximum secretion values in both the PBMCs alone and the PBMCs plus granulocytes. Granulocytes alone exhibited an average maximum increase of 3.8 fold (ranging from 2-12 fold). However, as mentioned above, the overall level of secretion was very small from this cell preparation. These results indicate that TBT has the greatest ability to increase IL-1β secretion from the MD-PBMCs (a preparation that is predominantly T and NK lymphocytes). This suggests that in the *in vivo* setting that those areas where there is an enrichment of T and NK lymphocytes may be more susceptible to potential dysregulation by exposures to TBT, such as the tumor microenvironment (Whiteside, 2008). The fact that there is greater average secretion stimulated in the MD-PBMCs suggests that there may be TBT-stimulated secretion of another factor when T cells are the dominant cell type that stimulates IL-1β secretion from the T cells and or other cell types present in this cell preparation. T cells are major

producers of IFNγ and IFNγ has been shown to increase secretion of IL-1β from human immune cells (Masters et al., 2010). Recently, we have shown that TBT greatly increases the secretion of IFNγ from MD-PBMCs over the range of 2.5-100 nM after 24 h (Lawrence et al., 2014) and this IFNγ may then lead to the increase in IL-1β seen in this particular cell preparation. 200 nM TBT normally blocked or decreased IL-1β secretion from NK cells, MD-PBMCs and PBMCs, while increases in IL-1β secretion were seen with TBT concentrations ranging from 2.5 to 100 nM (depending on the donor). These data suggest that TBT is affecting similar pathways in NK cells, T cells, and monocytes. However, when granulocytes are present in the reconstituted cell preparation (PBMCs + granulocytes) exposure to 200 nM TBT caused a significant increase in IL-1β production in 4 of the 5 donors. This is in stark contrast to the effects of 200 nM TBT on PBMCs, which caused a large decrease in IL-1β secretion in these same donors. The effects of 200 nM TBT exposures on granulocytes (alone) from these donors indicate that granulocytes were not responsible for the increased IL-1β secretion seen at 200 nM TBT in the PBMC+ granulocyte preparation. Interestingly, the effects of lower concentrations of TBT on granulocytes showed a similar pattern as seen with the other cell preparations (increased IL-1β secretion) albeit on a much lower baseline. This suggests that granulocytes are able to reverse the negative effects of 200 nM on IL-1β secretion from PBMCs, but do not appreciably alter the effects of TBT at lower concentrations. One explanation for this effect may be that, due to TBT binding to the granulocytes, a lower effective concentration of TBT is seen by the PBMCs. As the data shows, a small shift in the effective concentration of TBT could take it from a level that is inhibitory to one that is stimulatory of IL-1β secretion. It might be expected that TBT will bind to components within the cell with varying affinity. At higher concentrations of TBT such as 200 nM you would expect that the largest number of components capable of interacting with TBT would do so and that this would result in multiple (and possibly competing) effects including toxic effects that lead to an increased level of cell death over time (as is seen in the viability data). As the concentrations decreases (such as at 10 or 25 nM) TBT will interact with far fewer components and may show a completely opposite effect (from that seen at higher concentrations) depending on the nature of the component(s) that it influences. This appears to be the case with respect to IL-1β secretion. At 200 nM TBT is inhibitory to this process, while at 10 nM it is stimulatory. Overall, the data indicates that TBT-induced alterations of IL-1β secretion from human immune cells may be a significant consequence of TBT exposures and that TBT may potentially affect immune competence and cancer invasiveness.

Several signaling pathways are involved in the production/secretion of IL-1β from immune cells. They include caspase 1, NFκB, and MAPKs (p44/42 and p38). We found that inhibition of caspase-1 did not affect TBT-induced secretion, indicating that while this enzyme is critical for secretion of IL-1β from immune cells it does not seem to be necessary for TBT-induced increases in IL-1β secretion. Thus, it is apparently not one of the cellular components that is bound by TBT at concentrations of 5-25 nM. NFκB is a signaling component that is required for the transcription of several cytokine genes including the IL-1β gene (Cogswell *et al*., 1994; Perez *et al*., 1997; Scheibel *et al*., 2010). The results of this study indicate that, like the caspase-1 pathway, the NFκB pathway is also not a target of TBT at those concentrations where TBT causes increases in IL-1β secretion. p44/42 MAP

kinase (ERK 1 and ERK 2) plays an important role in cell growth regulation (Cowley *et al*., 1994; Hunter *et al*., 1995; Marshall, 1995). Activation of p44/42 leads to activation of the AP-1 transcription activator (composed of fos and jun genes) leading to an increase of IL-1β transcription (Glauser *et al*., 2007). When the p44/42 pathway was inhibited by a MEK inhibitor, TBT-induced increases in IL-1β secretion were blocked. These results suggest that TBT causes p44/42 pathway activation which then results in increased IL-1β synthesis and secretion. Previous studies have shown that TBT is capable of activating p44/42 in highly purified NK cells (Abraha *et al*., 2008). The current results indicate a need to examine p44/42 activation by TBT in MD-PBMCs. The MAPK, p38, has also been shown to regulate the production of cytokines (Davis *et al*., 1995; Young *et al*., 1993) including IL-1β by activation of C/EBPβ/NFIL-6 transcription activator (Baldassare *et al*., 1999). Inhibition of the p38 pathway diminished TBT-induced IL-1β secretion in 3 of the 4 donors tested. This suggests that a component(s) of the p38 may also be a target of lower concentrations of TBT leading to increased IL-1β secretion. Figure 3 summarizes the pathways that may be involved in regulating IL-1β synthesis and secretion.

Sterile inflammation is a term used to describe inflammation occurring in the absence of a microorganism (Chen and Nunez, 2010). Chemical exposure could result in sterile inflammation by its ability to induce the production of pro-inflammatory cytokines such as IL-1β. TBT may have this potential. Our *ex-vivo* results indicate that at low exposure concentrations TBT increases levels of IFNγ, TNFα (Lawrence et al., 2014; Hurt et al., 2013), and IL-1β. Concentrations of TBT as low as 2.5 nM were able to increase IL-1β secretion and we know from past studies a majority of donors tested had blood levels that exceeded 2.5 nM (Kannan et al., 1999; Whalen et al., 1999). If TBT were to have similar effects i*n vivo*, then exposure may have the potential to cause chronic inflammation, which has a host of negative consequences including, exacerbation of symptoms in multiple sclerosis and rheumatoid arthritis (Lucas and Hohlfeld, 1995; Choy and Panayi, 2001) as well as enhanced tumor development and progression (Lewis and Varghese, 2006).

The current study indicates that TBT alters IL-1β secretion from increasingly reconstituted preparations of human immune cells. The highest concentration of TBT tested blocked secretion of IL-1β from highly purified NK cells, MD-PBMCs, and PBMCs; however, when granulocytes were added to PBMCs, 200 nM TBT now increased IL-1β secretion. Lower concentrations of TBT elevated secretion of IL-1β from NK cells, MD-PBMCs, PBMCs, and granulocytes. This study also indicates that components of the p44/42 and possibly p38 MAPK pathways are targets of TBT. The fact that these pathways are both known to regulate IL-1β production (Baldassare *et al*., 1999; Glauser *et al*., 2007), and are needed for the TBT-induced secretion of IL-1β suggests that increased IL-1β transcription and translation are a requirement for this effect.

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References

Abraha AB, Whalen MM. The role of p44/42 activation in tributyltin-induced inhibition of human natural killer cells: effects of MEK inhibitors. Journal of Applied Toxicology. 2009; 29:165–73. [PubMed: 18989867]

Antizar-Ladislao B. Environmental levels, toxicity and human exposure to tributyltin (TBT)-contaminated marine environment. Environment International. 2008; 34:292–308. [PubMed: 17959247]

Apte RN, Dotan S, Elkabets M, White MR, Reich E, Carmi Y, Song X, Dvozkin T, Krelin Y, Voronov E. The involvement of IL-1 in tumorigenesis, tumor invasiveness, metastasis and tumor-host interactions. Cancer and Metastasis Reviews. 2006; 25:387–408. [PubMed: 17043764]

Arend WP. The balance between IL-1 and IL-1Ra in disease. Cytokine and Growth Factor Reviews. 2002; 13:323–340. [PubMed: 12220547]

Arlt A, Vorndamm J, Müerköster S, Yu H, Schmidt WE, Fölsch UR, Schäfer H. Autocrine Production of Interleukin 1β Confers Constitutive Nuclear Factor κB Activity and Chemoresistance in Pancreatic Carcinoma Cell Lines. Cancer Research. 2002; 62:910–916. [PubMed: 11830551]

Baldassare JJ, Bi Y, Bellone CJ. The role of p38 mitogen-activated protein kinase in IL-1 beta transcription. The Journal of Immunology. 1999; 162:5367–73. [PubMed: 10228013]

Burger D, Dayer J. Cytokines, Acute-Phase Proteins, and Hormones. Annals of the New York Academy of Science. 2002; 473:464–473.

Chen GY, Nunez G. Sterile inflammation: sensing and reacting to damage. Nature Reviews/ Immunology. 2010; 10:826–837.

Chirivi RG, Chiodoni C, Musiani P, Garofalo A, Bernasconi S, Colombo MP, Giavazzi R. IL-1α gene-transfected human melanoma cells increase tumor-cell adhesion to endothelial cells and their retention in the lung of nude mice. International Journal of Cancer. 1996; 67:856–863.

Choy EHS, Panayi GS. Cytokine Pathways and Joint Inflammation in Rheumatoid Arthritis. New England Journal of Medicine. 2001; 344:907–916. [PubMed: 11259725]

Cogswell JP, Codlevski MM, Wisely CB, Leesnitzer LM, Ways JP, Gray JG, Clay C. NF-kB Regulates IL-1B Transcription Through a Consensus NF-kB Binding Site and a Nonconsensus CRE-Like Site. The Journal of Immunology. 1994; 153:712–723. [PubMed: 8021507]

Cooper MA, Fehniger TA, Ponnappan A, Mehta V, Wewers MD, Caligiun MA. Interleukin-1β costimulates interferon-γ production by human natural killer cells. European Journal of Immunology. 2001; 31:792–801. [PubMed: 11241284]

Cowley S, Paterson H, Kemp P, Marshall CJ. Activation of MAP kinase kinase is necessary and sufficient for PC12 differentiation and for transformation of NIH 3T3 cells. Cell. 2014; 77:841–852. [PubMed: 7911739]

Davis RJ. Transcriptional regulation by MAP kinases. Molecular Reproduction and Development. 1995; 42:459–467. [PubMed: 8607977]

Dinarello CA. Biologic Basis for IL-1 in Disease. The Journal of The American Society of Hematology. 1996; 87:2095–2147.

Dinarello CA. Interleukin-1, Interleukin-1 Receptors and Interleukin-1 Receptor Antagonist. International Reviews of Immunology. 1998; 16:457–499. [PubMed: 9646173]

Dinarello CA. Blocking IL-1 in systemic inflammation. The Journal of Experimental Medicine. 2005; 201:1355–1359. [PubMed: 15867089]

Dinarello CA. Immunological and Inflammatory Functions of the Interleukin-1 Family. Annual Review of Immunology. 2009; 27:519–550.

Dudimah FD, Odman-Ghazi SO, Hatcher F, Whalen MM. Effect of tributyltin (TBT) on ATP levels in human natural killer (NK) cells: Relationship to TBT-induced decreases in NK function. Journal of Applied Toxicology. 2007; 27:86–94. [PubMed: 17149696]

Elaraj DM, Weinreich DM, Varghese S, Puhlmann M, Hewitt SM, Carroll NM, Feldman ED, Turner EM, Alexander HR. The role of interleukin 1 in growth and metastasis of human cancer xenografts. Clinical Cancer Research. 2006; 12:1088–1096. [PubMed: 16489061]

Gipperth L. The legal design of the international and European Union ban on tributyltin antifouling paint: Direct and indirect effects. Journal of Environmental Management. 2009; 90:S86–S95. [PubMed: 18976851]

Glauser DA, Schlegel W. Sequential actions of ERK1/2 on the AP-1 transcription factor allow temporal integration of metabolic signals in pancreatic beta cells. FASEB Journal: Official Publication of the Federation of American Societies for Experimental Biology. 2007; 21:3240–3249. [PubMed: 17504975]

Guma M, Ronacher L, Liu-Bryan R, Takai S, Karin M. Caspase 1- Independent Activation of Interleukin-1 beta in Neutrophil-Predominant Inflammation. Arthritis and Rheumatism. 2009; 60:3642–3650. [PubMed: 19950258]

Hunter T. Protein kinases and phosphatases: The Yin and Yang of protein phosphorylation and signaling. Cell. 2014; 80:225–236. [PubMed: 7834742]

Hurt K, Hurd-Brown T, Whalen M. Tributyltin and dibutyltin alter secretion of tumor necrosis factor alpha from human natural killer cells and a mixture of T cells and natural killer cells. Journal of Applied Toxicology. 2013; 33:503–510. [PubMed: 23047847]

Jin L, Yuan RQ, Fuchs A, Yao Y, Joseph A, Schwall R, Schnitt SJ, Guidae A, Hastings HM, Andres J, Turkel G, Polverini PJ, Goldberg ID, Rosen EM. Expression of interleukin-1β in human breast carcinoma. Cancer. 1997; 80:421–434. [PubMed: 9241076]

Kannan K, Tanabe S, Tatsukawa R. Occurrence of butyltin residues in certain foodstuffs. Bulletin of Environmental Contamination and Toxicology. 1995; 55:510–516. [PubMed: 8555674]

Kannan K, Senthilkumar K, Loganathan BG, Takahashi S, Odell DK, Tanabe S. Elevated accumulation of tri-butyltin and its breakdown products in bottle nose dolphins (Tursiops truncates) found stranded along the U.S. Atlantic and Gulf coasts. Enviorn Sci Technol. 1997; 31:296–301.

Kannan K, Senthilkumar K, Giesy JP. Occurrence of butyltin compounds in human blood. Environmental Science and Technology. 1999; 33:1776–1779.

Kuida K, Lippke JA, Ku G, Harding MW, Livingston DJ, Su MS, Flavell RA. Altered cytokine export and apoptosis in mice deficient in interleukin-1 beta converting enzyme. Science. 1995; 267:2000–2003. [PubMed: 7535475]

Kuijpers TW, Tool ATJ, van der Schoot CE, Ginsel LA, Onderwater JJM, Roos D, Verhoeven AJ. Membrane surface antigen expression on neutrophils: a reappraisal of the use of surface markers for neutrophil activation. Blood. 2013; 78:1105–1111. [PubMed: 1907873]

Lawrence S, Reid J, Whalen MM. Secretion of interferon gamma from human immune cells is altered by exposure to tributyltin and dibutyltin. Environmental Toxicology. 2014 in press.

Lewis A, Varghese S. Interleukin-1 and cancer progression: the emerging role of interleukin-1 receptor antagonist as a novel therapeutic agent in cancer treatment. Journal of Translational Medicine. 2006; 12:1–12.

Loganathan, BG.; Kannan, K.; Sajwan, KS.; Owen, DA. Butyltin compounds in freshwater ecosystems. In: Lipnick, RL.; Hermens, J.; Jones, K.; Muir, D., editors. Persistent, Bioaccumulative and Toxic Chemicals I: Fate and Exposure. Vol. 772. American Chemical Society; Washington, DC: 2001. p. 134-149.ACS Monograph Series

Lucas K, Hohlfeld R. Different aspects of cytokines in the immunopathology of multiple sclerosis. Neurology. 1995; 45:S4–S5. [PubMed: 7783911]

Marshall CJ. Specificity of receptor tyrosine kinase signaling: Transient versus sustained extracellular signal-regulated kinase activation. Cell. 2014; 80:179–185. [PubMed: 7834738]

Masters SL, Mielke LA, Cornish AL, Sutton CE, O'Donnell J, Cengia LH, Roberts AW, Wicks IP, Mills KHG, Croker BA. Regulation of interleukin-1β by interferon-γ is species specific, limited by suppressor cytokine signalling 1 and influences interleukin-17 production. EMBO reports. 2010; 11:640–646. [PubMed: 20596075]

Meyer TP, Zehnter I, Hofmann B, Zaisserer J, Burkhart J, Rapp S, Weinauer F, Schmitz J, Illert WE. Filter Buffy Coats (FBC): A source of peripheral blood leukocytes recovered from leukocyte depletion filters. Journal of Immunological Methods. 2005; 307:150–166. [PubMed: 16325197]

Muerkoster SS, Lust J, Arlt A, Hasler R, Witt M, Sebens T, Schreiber S, Folsch UR, Schafer H. Acquired chemoresistance in pancreatic carcinoma cells: induced secretion of IL-1beta and NO lead to inactivation of caspases. Oncogene. 2006; 25:3973–3981. [PubMed: 16474845]

Perez R, Ritzenthaler J, Roman J. Transcriptional regulation of the interleukin-1 β promoter via fibrinogen engagement of the CD18 integrin receptor. American Journal of Respiratory Cell and Molecular Biology. 1999; 20:1059–1066. [PubMed: 10226077]

Scheibel M, Klein B, Merkle H, Schulz M, Fritsch R, Greten FR, Arkan MC, Schneider G, Schmid RM. IkappaBbeta is an essential co-activator for LPS-induced IL-1beta transcription in vivo. The Journal of Experimental Medicine. 2010; 207:2621–30. [PubMed: 20975042]

Swaan P, Knoell D, Helsper F, Wewers M. Sequential Processing of Human ProIL-1β by Caspase-1 and Subsequent Folding Determined by a Combined In Vitro and In Silico Approach. Pharmaceutical Research. 2001; 18:1083–1090. [PubMed: 11587477]

Thomas LD, Shah H, Green SA, Bankhurst AD, Whalen MM. Tributyltin exposure causes decreased granzyme B and perforin levels in human natural killer cells. Toxicology. 2004; 200:221–233. [PubMed: 15212818]

Vidal-Vanaclocha F, Alvarez A, Asumendi A, Urcelay B, Tonino P, Dinarello CA. Interleukin 1 (IL-1)-Dependent Melanoma Hepatic Metastasis In Vivo; Increased Endothelial Adherence by IL-1-Induced Mannose Receptors and Growth Factor Production In Vitro. Journal of the National Cancer Institute. 1996; 88:198–205. [PubMed: 8632494]

Voronov E, Shouval DS, Krelin Y, Cagnano E, Benharroch D, Iwakura Y, Dinarello CA, Apte RN. IL-1 is required for tumor invasiveness and angiogenesis. Proceedings of the National Academy of Sciences of the United States of America. 2002; 100:2645–2650. [PubMed: 12598651]

Wester PW, Krajnc EI, van Leeuwen FXR, Loeber JG, van der Heijden CA, Vaessen HA, Helleman PW. Chronic toxicity and carcinogenicity of bis(tri-n-butyltin)oxide (TBTO) in the rat. Food and Chemical Toxicology. 1990; 28:179–196. [PubMed: 2344992]

Whalen MM, Loganathan BG, Kannan K. Immunotoxicity of Environmentally Relevant Concentrations of Butyltins on Human Natural Killer Cells in Vitro. Environmental Research. 1999; 81:108–116. [PubMed: 10433842]

Whalen MM, Ghazi S, Loganathan BG, Hatcher F. Expression of CD16, CD18 and CD56 in Tributyltin-exposed human natural killer cells. Chemico-Biological Interactions. 2002; 139:159–176. [PubMed: 11823004]

Whiteside TL. The tumor microenvironment and its role in promoting tumor growth. Oncogene. 2008; 27:5904–5912. [PubMed: 18836471]

Yamada S, Fuji Y, Mikami E, Kawamura N, Hayakawa J. Small-scale survey of organotin compounds in household commodities. Journal of AOAC International. 1993; 76:436–441.

Young PR, McLaughlin MM, Kumar S, Kassis S, Doyle ML, McNulty D, Gallagher TF, Fisher S, McDonnell PC, Carr SA, Huddleston MJ, Seibel G, Porter TG, Livi GP, Adams JL, Lee JC. Pyridinyl Imidazole Inhibitors of p38 Mitogen-activated Protein Kinase Bind in the ATP Site. Journal of Biological Chemistry. 1997; 272:12116–12121. [PubMed: 9115281]

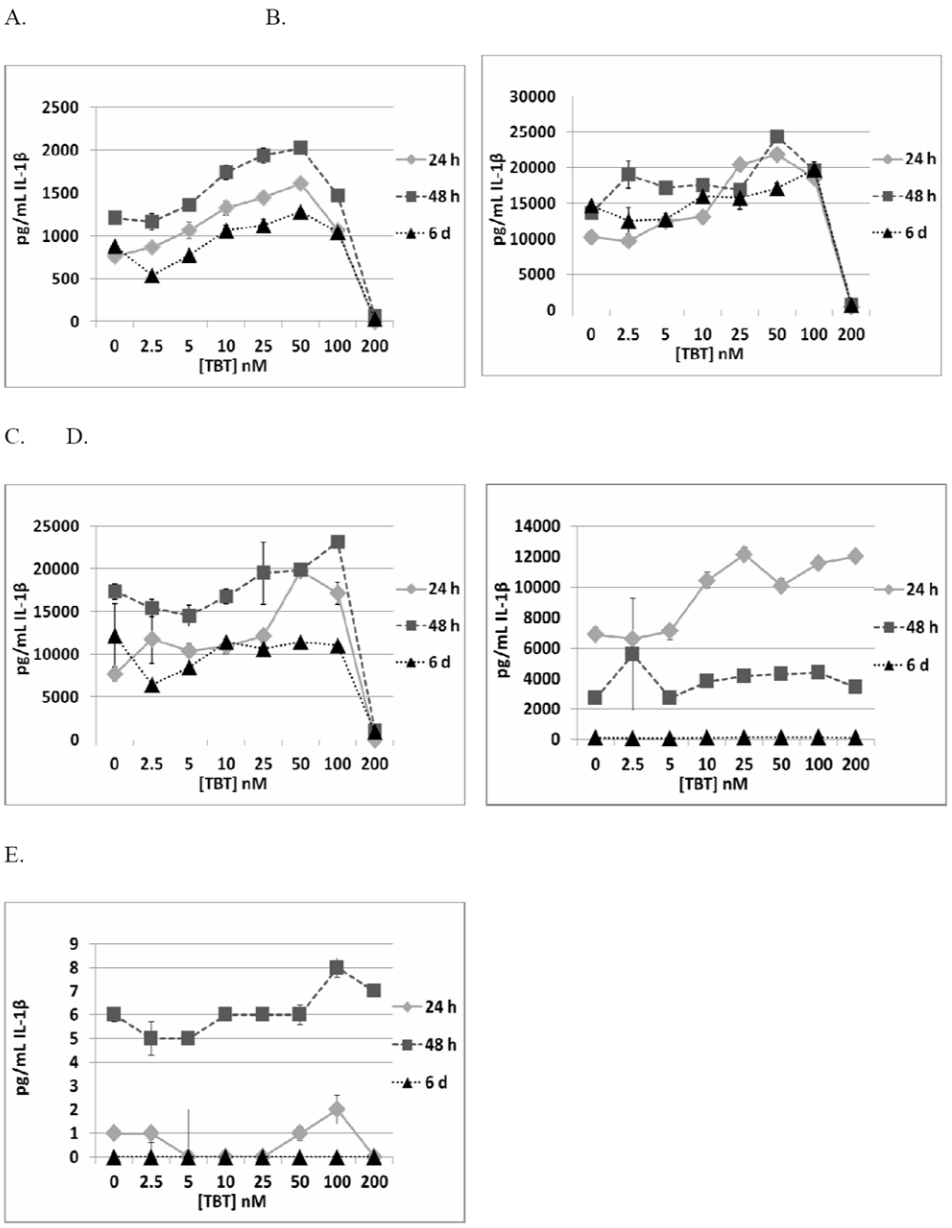

Figure 1.

Effects of 24 h, 48 h and 6 day exposures to TBT on IL-1β secretion from highly purified human NK cells, monocyte-depleted PBMCs, PBMCs, PBMCs plus granulocytes, and granulocytes in individual donors. A) NK cells exposed to 0-200 nM TBT (donor KB166). B) Monocyte-depleted PBMCs exposed to 0-200 nM TBT (donor F142). C) PBMCs exposed to 0-200 nM TBT (donor F144). D) PBMCs plus granulocytes exposed to 0-200 nM TBT (donor F144). E) Granulocytes exposed to 0-200 nM TBT (donor F144).

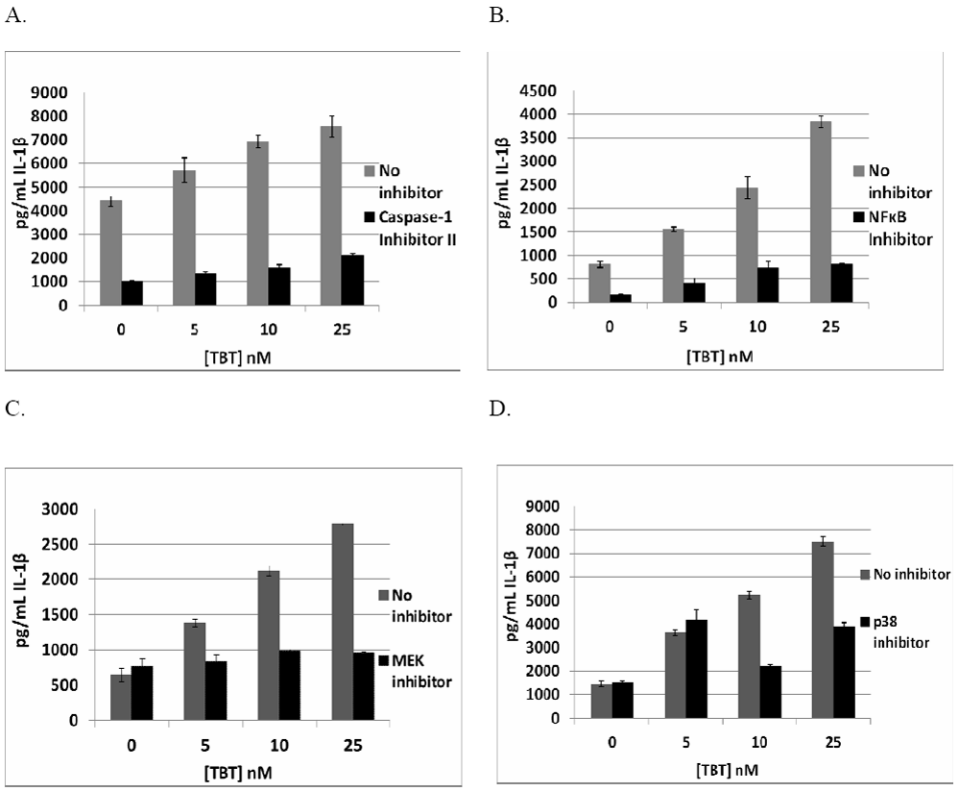

Figure 2.

Effects of 24 h exposure to 5, 10, and 25 nM TBT on IL-1β secretion from monocyte-depleted PBMCs treated with selective pathway component inhibitors in individual donors. A) IL-1β Cleavage Inhibitor (Caspase-1 Inhibitor II) (donor F182). B) NFκB Inhibitor (BAY 11-7085) (donor F218). C) MEK ½(p44/42) Inhibitor (U0126) (donor F181). D) P38 Inhibitor (SB202190) (donor F183).

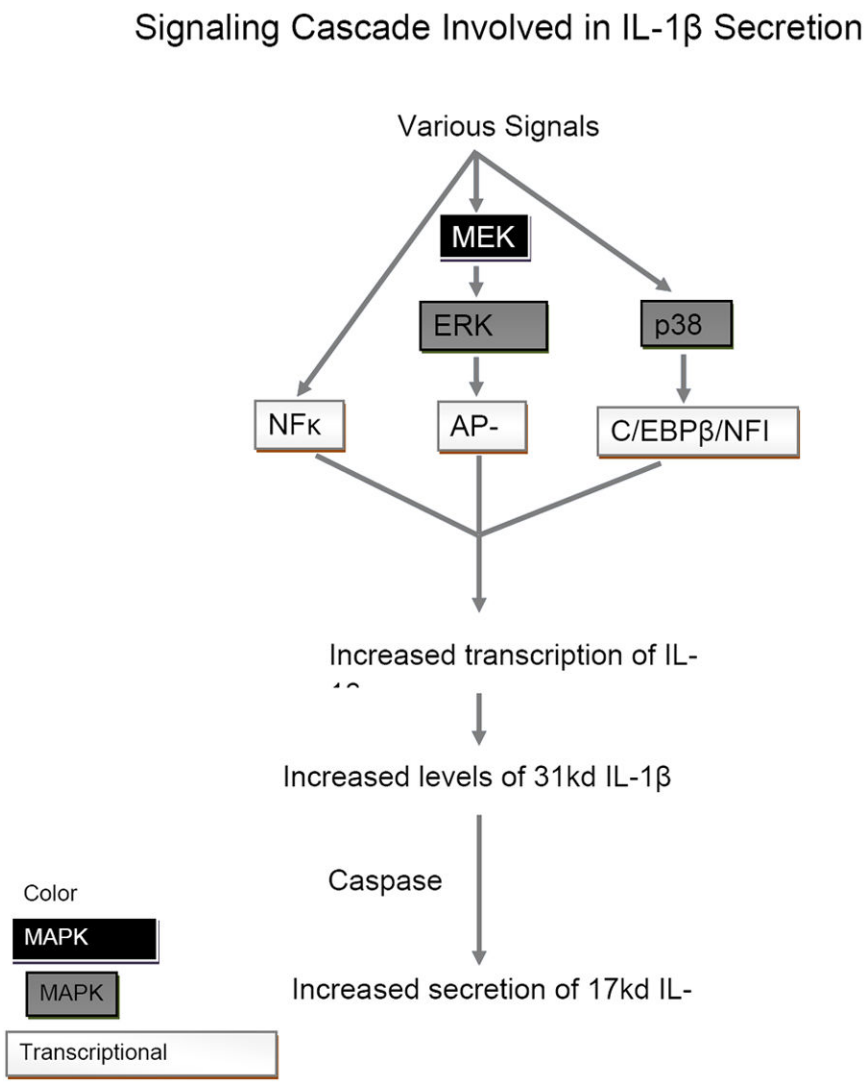

Figure 3.
Signaling pathways regulating IL-1β secretion.

Table 1

Percent viability of NK cells, Monocyte-depleted PBMCs, PBMCs, PBMCs combined with Granulocytes, and Granulocytes exposed to TBT for 24 h, 48 h, and 6 days.

		Percent Viable Cells				
24 h exposure	**[TBT] nM**	**NK**	**monocyte-depleted PBMCs**	**PBMCs**	**PBMCs with Granulocytes**	**Granulocytes**
	0	95±3	96±2	94±4	93±2	96±2
	2.5	99±0.7	97±1	94±6	92±5	97±1
	5	98±1	96±1	95±5	92±2	97±2
	10	97±0.4	97±1	94±6	94±5	97±2
	25	98±1	95±3	95±4	90±6	98±1
	50	96±2	95±4	95±2	94±5	98±2
	100	96±3	96±1	94±6	92±4	97±2
	200	89±2*	90±3*	87±9	90±3	93±3
48 h exposure	0	93±3	93±4	93±3	89±5	92±4
	2.5	96±2	95±1	92±6	92±3	94±1
	5	96±4	96±2	94±3	93±1	95±2
	10	96±1	96±2	94±5	90±4	96±1
	25	95±1	95±2	92±5	92±3	96±2
	50	94±4	94±6	93±7	85±5	96±3
	100	94±5	91±9	89±10	81±13	89±6
	200	72±18	80±5*	74±11*	79±8*	72±24
6 day exposure	0	68±15	82±9	89±6	85±9	82±10
	2.5	75±3	89±7	87±10	84±11	86±12
	5	75±6	88±8	86±10	83±21	81±12
	10	70±15	85±8	88±7	87±10	82±6
	25	68±5	86±11	88±9	88±9	80±11
	50	64±9	78±15	83±9	85±7	80±9
	100	47±10	63±15*	70±16*	71±11	67±14
	200	34±6*	41±18*	56±19*	52±5*	57±18*

Values are mean±S.D. of triplicate determinations.

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* Indicates a significant decrease in viability compared to control cells (cells treated with vehicle alone), $p<0.05$

Table 2

Percent viability of Monocyte-depleted PBMCs exposed to TBT for 24 h with selective enzyme inhibitors

		Percent Viable Cells				
24 h exposure	**[TBT] nM**	**Caspase1 Inhibitor**	**NFκB Inhibitor (BAY11-7085,1.25uM)**	**MEK1/2 Inhibitor (U0126, 50uM)**	**MEK 1/2 Inhibitor (PD98059, 50uM)**	**p38 Inhibitor (SB202190, 25-50uM)**
	0	97±2	97±1	94±4	96±3	97±4
	5	95±3	97±3	96±3	94±4	96±3
	10	95±4	96±2	94±6	94±7	95±3
	25	96±3	97±2	95±4	92±8	97±0
	0+inhibitor	96±5	98±2	88±8	95±5	92±5
	5+inhibitor	97±2	96±0	85±7	94±3	90±4
	10+inhibitor	97±1	99±1	95±5	92±8	90±6
	25+inhibitor	97±1	96±2	96±17	93±4	93±3

Values are mean±S.D. of triplicate determinations. Control cell (0) are cells treated with vehicle alone

Table 3

Effects of 24 h, 48 h, 6 day exposures to TBT on IL-1β secretion from highly purified human NKs.

24 h	**Interleukin 1 beta secreted in pg/mL (mean±S.D.)**			
[TBT] nM	**KB166**	**KB167**	**KB168**	**KB169**
0	761±14	294±5	1623±46	71±9
2.5	869±44*	455±82	2034±84*	125±13*
5	1063±97*	424±10*	1943±59*	245±11*
10	1326±82*	463±4*	1730±11*	474±21*
25	1444±47*	439±78	2135±49*	443±24*
50	1609±20*	358±15*	1621±59	551±10*
100	1065±22*	99±101	362±6*	446±9*
200	0±10*	0±26*	0±2*	0±10*
48 h	**Interleukin 1 beta secreted in pg/mL (mean±S.D.)**			
[TBT] nM	KB166	KB167	KB168	KB169
0	1206±8	402±10	2101±322	281±10
2.5	1161±96	383±16	2791±125*	327±39
5	1358±67	379±31	2624±36	909±50*
10	1740±82*	430±24	2647±78	1302±47*
25	1937±91*	444±6*	2427±156	1578±75*
50	2025±27*	435±28	2039±31	1726±48*
100	1475±32*	18±6*	510±10*	801±51*
200	61±52*	0±11*	0±11*	0±5*
6day	**Interleukin 1 beta secreted in pg/mL (mean±S.D.)**			
[TBT] nM	KB166	KB167	KB168	KB169
0	883±30	101±11	1748±24	490±18
2.5	535±42*	221.±33*	1821±106	389±29*
5	773±13*	225±49*	2065±32*	438±16*
10	1062±62*	180±33*	1968±41*	1051±23*
25	1124±69*	213±7*	1960±67*	954±24*
50	1281±40*	129±9*	1623±64	1026±31*
100	1042±18*	0±2*	250±11*	390±8*
200	32±3*	0±14*	0±4*	0±16*

Values are mean±S.D. of triplicate determinations.

* Indicates a significant change in secretion compared to control cells (cells treated with vehicle alone), $p<0.05$

Table 4

Effects of 24 h, 48 h, 6 day exposures to TBT on IL-1β secretion from monocyte-depleted PBMCs.

24 h	**Interleukin 1 beta secreted in pg/mL (mean±S.D.)**				
[TBT] nM	**F137**	**F142**	**F145**	**F152**	**F156**
0	130±8	10227±249	1816±79	261±40	1230±42
2.5	1073±40*	9693±719	2402±373	801±64*	3115±313*
5	709±227*	12460±712*	1997±131	616±28*	3067±91*
10	469±153	13127±663*	2050±145	986±33*	6144±187*
25	467±127*	20420±502*	2226±117*	1558±9*	5082±282*
50	262±46*	21827±160*	3335±340*	2907±108*	6637±194*
100	180±34	18533±722*	2707±136*	8695±244*	13500±339*
200	0±10*	487±31*	0±79*	2119±64*	1222±78
48 h	**Interleukin 1 beta secreted in pg/mL (mean±S.D.)**				
[TBT] nM	F137	F142	F145	F152	F156
0	70±57	13622±269	1802±40	459±10	2953±193
2.5	1151±134*	19037±1933*	3374±216*	495±34	2779±77
5	1141±341*	17178±791*	2446±347	495±17*	3393±144*
10	289±180	17615±450*	2798±276*	749±43*	4099±275*
25	190±41*	16882±817*	2771±206*	942±23*	5330±240*
50	182±26	24393±258*	2184±102*	1627±46*	9792±137*
100	232±22*	19615±777*	2214±155*	5826±58*	10480±402*
200	0±16*	696±207*	0±17*	1993±33*	4345±140*
6day	**Interleukin 1 beta secreted in pg/mL (mean±S.D.)**				
[TBT] nM	F137	F142	F145	F152	F156
0	0±33	14652±537	809±47	147±4	1572±13
2.5	175±47*	12533±1883	887±31	158±12	1813±115

24 h	Interleukin 1 beta secreted in pg/mL (mean±S.D.)				
[TBT] nM	**F137**	**F142**	**F145**	**F152**	**F156**
5	90±98	12793±752*	833±12	178±4*	2353±149*
10	7±50	16000±922	1045±7*	268±13*	3320±57*
25	0±15	15770±1576	1047±43*	300±2*	4006±312*
50	0±6	17141±711*	624±16*	265±5*	4572±238*
100	0±0	12630±12401	772±25	152±5	7934±194*
200	0±5	719±256*	0±10*	75±11*	1858±111*

Values are mean±S.D. of triplicate determinations.

* Indicates a significant change in secretion compared to control cells (cells treated with vehicle alone), $p<0.05$

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Table 5

Effects of 24 h, 48 h, 6 day exposures to TBT on IL-1β secretion from PBMCs.

24 h	**Interleukin 1 beta secreted in pg/mL (mean±S.D.)**				
[TBT] nM	**F131**	**F135**	**F143**	**F144**	**F147**
0	20610±1130	15028±315	12431±276	7693±869	4566±115
2.5	26640±485*	15963±737	13743±929	11786±2845	6983±284*
5	24781±1143*	16291±3257	17331±929*	10386±858*	7233±682*
10	32589±1235*	21484±3026	12366±548	10893±855*	12133±678*
25	49519±2975*	17589±677*	14545±967	12133±748*	11750±86*
50	37377±1948*	19414±669*	18393±344*	19693±641*	20016.7±340*
100	61125±30274	12279±294*	19954±162*	17133±1300*	17433±737*
200	2297±504*	993±126*	13949±1844	0±83*	0±160*
48 h	**Interleukin 1 beta secreted in ng/mL (mean±S.D.)**				
[TBT] nM	F131	F135	F143	F144	F147
0	30556±933	26778±1190	18909±227	17333±907	3276±411
2.5	35475±1156*	21101±408*	19927±875	15384±1020	11805±2594*
5	32456±913	17896±750*	20157±1087	14512±1261*	14600±1734*
10	34812±3254	22176±710*	22375±233*	16779±862	16707±960*
25	43629±1256*	23488±683	22266±1029*	19507±3656	11688±12813
50	38403±1126*	31165±49*	21236±838*	19866±350*	19933±1869*
100	38418±1462*	25606±542	28375±1126*	23148±661*	17237±68*
200	3721±161*	0±149*	12630±539*	1128±138*	0±94*
6day	**Interleukin 1 beta secreted in ng/mL (mean±S.D.)**				
[TBT] nM	F131	F135	F143	F144	F147
0	31802±777	17139±637	14142±305	12153±3740	4815±208
2.5	36017±6409	15558±1971	14333±1464	6441±467	6924±884*

24 h	Interleukin 1 beta secreted in pg/mL (mean±S.D.)				
[TBT] nM	**F131**	**F135**	**F143**	**F144**	**F147**
5	45723±3592*	15443±1594	16202±742*	8441±277	10474±1271*
10	46827±645*	16139±527	14512±1660	11436±248	11090±330*
25	45450±460*	15005±756*	15964±1271	10615±751	16774±1005*
50	59830±2003*	18491±3148	16785±1482	11446±423	6999±8505
100	48748±933*	9920±5989	18928±1180*	11046±273	13932±300*
200	4533±310*	586±333*	8440±1526*	923±30*	690±2386

Values are mean±S.D. of triplicate determinations.

*Indicates a significant change in secretion compared to control cells (cells treated with vehicle alone), $p<0.05$

Table 6

Effects of 24 h, 48 h, 6 day exposures to TBT on IL-1β secretion from PBMCs plus Granulocytes.

24 h	**Interleukin 1 beta secreted in pg/mL (mean±S.D.)**				
[TBT] nM	**F131**	**F135**	**F143**	**F144**	**F147**
0	4525±250	3729±400	14367±580	6893±482	600±254
2.5	7746±2480	6189±1486	14183±2273	6613±311	2658±941*
5	5026±218	3039±366	10767±690*	7120±302	1316.7±146.5*
10	6104±688*	3706±287	12917±729	10427±561*	2092±293*
25	5560±526	4510±329	16533±525*	12133±474*	2083±267*
50	10509±736*	7062±464*	12567±501*	10080±500*	3133±63*
100	9912±435*	947±20*	19367±1020*	11560±174*	9675±43*
200	11992±423*	0±121*	16400±937*	12000±280*	3325±152*
48 h	**Interleukin 1 beta secreted in pg/mL (mean±S.D.)**				
[TBT] nM	F131	F135	F143	F144	F147
0	7877±1285	5000±249	8872±512	2735±171	634±37
2.5	3848±480*	5152±286	9569±2439	5571±3710	584±319
5	2443±188*	3552±433*	9262±308	2735±52	499±89
10	2601±286*	5143±187	8082±108	3808±288*	882±33*
25	3246±221*	3457±174*	12821±256*	4170±352*	1166±74*
50	3819±662*	4848±400	11139±708*	4294±393*	1748±151*
100	2715±151*	1133±100*	8072±418	4384±391*	4315±119*
200	7705±329	0±1048*	7323±267*	3412±204*	1549±77*
6day	**Interleukin 1 beta secreted in pg/mL (mean±S.D.)**				
[TBT] nM	F131	F135	F143	F144	F147
0	22±1	11±1	54±1	100±5	4±0
2.5	9±7	6±2*	60±25	58±8*	21±31

24 h	Interleukin 1 beta secreted in pg/mL (mean±S.D.)				
[TBT] nM	**F131**	**F135**	**F143**	**F144**	**F147**
5	15±0*	7±0*	44±13	69±1*	4±0
10	20±1*	8±0*	44±3*	96±5	5±0*
25	22±1	9±0*	48±2*	116±3*	6±0*
50	25±1*	8±0*	44±2*	119±2*	7±0*
100	32±1*	6±0*	45±3*	139±5*	12±1*
200	24±1	2±1*	49±26	99±7	4±0

Values are mean±S.D. of triplicate determinations.

* Indicates a significant change in secretion compared to control cells (cells treated with vehicle alone), $p<0.05$

Table 7

Effects of 24 h, 48 h, 6 day exposures to TBT on IL-1β secretion from Granulocytes.

24 h	**Interleukin 1 beta secreted in pg/mL (mean±S.D.)**				
[TBT] nM	**F131**	**F135**	**F143**	**F144**	**F147**
0	124±4	33±2	32±5	1±0	1±0
2.5	130± 19	33±2	75±5*	1±0	1±0
5	112±8	35±1	56±13	0±0	2±1
10	167±5*	44±1	17±3*	0±0	1±0
25	155±8*	49±3*	23±15	0±0	3±0
50	198±3*	66±3*	14±11	1±0	3±1
100	340±12*	32±1*	20±1*	2±1	12±1*
200	123±5	0±0*	0±1*	0±0	0±0
48 h	**Interleukin 1 beta secreted in pg/mL (mean±S.D.)**				
[TBT] nM	F131	F135	F143	F144	F147
0	58±5	3±1	99±27	6±0	4±0
2.5	59±15	11±12	21±8*	5±1	4±1
5	48±4*	5±2	11±2*	5±0*	3±1
10	55±2	6±0*	14±0*	6±0	4±1
25	68±2	7±0*	22±4*	6±0	5±0
50	91±3*	6±1	34±8*	6±0	6±0*
100	131±3*	2±0	80±22	8±0*	10±1*
200	79±3*	0±1*	139±61	7±0*	5±1
6day	**Interleukin 1 beta secreted in pg/mL (mean±S.D.)**				
[TBT] nM	F131	F135	F143	F144	F147
0	0±0	0±0	0±0	0±8	0±1
2.5	0±0	0±1	0±2	0±1	0±1
5	0±1	0±1	0±2	0±2	0±1

24 h	Interleukin 1 beta secreted in pg/mL (mean±S.D.)				
[TBT] nM	**F131**	**F135**	**F143**	**F144**	**F147**
10	0±0	0±1	0±2	0±0	0±2
25	0±0	0±1	0±2	0±0	0±1
50	0±0	0±0	0±2	0±0	0±2
100	0±0	0±1	0±0	0±0	0±2
200	0±0	0±0	0±0	0±0	0±2

Values are mean±S.D. of triplicate determinations.

* Indicates a significant change in secretion compared to control cells (cells treated with vehicle alone), $p<0.05$

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Table 8

Effects of IL-1β Cleavage Inhibition (Caspase-1 Inhibitor II (CI)) on TBT-induced IL-1β secretion from MD-PBMCs

24 h	Interleukin 1 beta secreted in pg/mL (mean±S.D.)			
[TBT] nM	**F181**	**F182**	**F183**	**F185**
0	853±23	4401±220	1416±147	7634±426
0 + CI	44±7	1029±38	215±8	2544±174
5	1376±72*	5725±513*	2484±147*	8148±394
5 + CI	152±65	1324±110**	333±35**	2434±190
10	1770±68*	6928±268*	3712±156*	7838±308
10 + CI	227±56**	1599±121**	575±82**	2846±36
25	2347±202*	7575±447*	5041±154*	10049±183*
25 + CI	246±12**	2087±105**	1018±35**	3998±194**

Values are mean±S.D. of triplicate determinations.

* indicates a significant increase compared to no TBT (0), $p<0.05$;

** indicates a significant increase compared to no TBT+inhibitor (0+CI), $p<0.05$

Table 9

Effects of NFκB Inhibition (BAY 11-7085 (BAY)) on TBT-induced IL-1β secretion from MD-PBMCs.

24 h	Interleukin 1 beta secreted in pg/mL (mean±S.D.)		
[TBT] nM	**F218**	**F219**	**F225**
0	805±64	754±54	520±151
0 + BAY	152±37	234±28	190±21
5	1551±57*	1749±141*	753±42
5 + BAY	420±92**	923±254**	1042±212**
10	2434±239*	3185±96*	1921±473*
10 + BAY	739±149**	1523±266**	1476±331**
25	3838±120*	2647±132*	1616±121*
25 + BAY	805±29**	1278±213**	1476±25**

Values are mean±S.D. of triplicate determinations.

* indicates a significant increase compared to no TBT (0), $p<0.05$;

** indicates a significant increase compared to no TBT+inhibitor (0+BAY), $p<0.05$

Table 10

Effects of p44/42 Pathway Inhibition (U0126 (U)) on TBT-induced IL-1β secretion from MD-PBMCs.

24 h	Interleukin 1 beta secreted in pg/mL (mean±S.D.)			
[TBT] nM	**F181**	**F182**	**F183**	**F185**
0	643±96	2778±121	1519±68	6310±100
0 + U	768±102	4969±96	1437±116	2836±106
5	1377±50*	4544±233*	2836±134*	7608±342*
5 + U	830±104	5280±309	3044±540**	3042±384
10	2117±74*	6100±237*	4279±205*	8560±166*
10 + U	981±27	5406±344	2246±314**	5187±228**
25	2783±9*	7000±149*	5771±185*	8836±186*
25 + U	955±18	3755±392	2574±130**	6691±122**

Values are mean±S.D. of triplicate determinations.

* indicates a significant increase compared to no TBT (0, $p<0.05$);

** indicates a significant increase compared to no TBT+inhibitor (0+U), $p<0.05$

Table 11

Effects of p38 Inhibition (SB202190 (SB)) on TBT-induced IL-1β secretion from MD-PBMCs.

24 h	Interleukin 1 beta secreted in pg/mL (mean±S.D.)			
[TBT] nM	**F183**	**F185**	**F236**	**F237**
0	1459±119	6153±322	2537±180	968±113
0 + SB	1493±96	1249±73	483±62	541±29
5	3640±110*	6711±201	4365±369*	1280±95*
5 + SB	4176±442**	1454±54**	1618±238**	1510±53**
10	5222±158*	9791±250*	4356±513*	2289±103*
10 + SB	2199±87**	872±50	1477±90**	1479±19**
25	7504±204*	7679±30*	6807±629*	4348±71*
25 + SB	3871±200**	1048±12	1701±157**	1065±22**

Values are mean±S.D. of triplicate determinations.

* indicates a significant increase compared to no TBT (0), $p<0.05$;

** indicates a significant increase compared to no TBT+inhibitor (0+SB), $p<0.05$