Tobacco Smoke Induces the Generation of Procoagulant Microvesicles From Human Monocytes/Macrophages

Mingzhen Li, Demin Yu, Kevin Jon Williams, Ming-Lin Liu

Objective—To investigate whether exposure of human monocytes/macrophages to tobacco smoke induces their release of membrane microvesicles (MVs) that carry tissue factor (TF) released from cells, whether smoke-induced MVs are procoagulant, and what cellular processes might be responsible for their production.

Methods and Results—We found that exposure of human THP-1 monocytes and primary human monocyte–derived macrophages to 3.75% tobacco smoke extract (TSE) significantly increased their total and TF-positive MV generation. More importantly, MVs released from TSE-treated human monocytes/macrophages exhibited 3 to 4 times the procoagulant activity of control MVs, as assessed by TF-dependent generation of factor Xa. Exposure to TSE increased TF mRNA and protein expression and cell surface TF display by both THP-1 monocytes and primary human monocyte–derived macrophages. In addition, TSE exposure caused activation of C-Jun-N-terminal kinase (JNK), p38, extracellular signal regulated kinase (ERK) mitogen-activated protein kinases (MAPK), and apoptosis (a major mechanism for MV generation). Treatment of THP-1 cells with inhibitors of ERK, MAP kinase kinase (MEK), Ras, or caspase 3, but not p38 or JNK, significantly blunted TSE-induced apoptosis and MV generation. Surprisingly, neither ERK nor caspase 3 inhibition altered the induction of cell surface TF display by TSE, indicating an effect solely on MV release. Inhibition of ERK or caspase 3 essentially abolished TSE-induced generation of procoagulant MVs from THP-1 monocytes.

Conclusion—Tobacco smoke exposure of human monocytes/macrophages induces cell surface TF display, apoptosis, and ERK- and caspase 3–dependent generation of biologically active procoagulant MVs. These processes may be novel contributors to the pathological hypercoagulability of active and secondhand smokers. (Arterioscler Thromb Vasc Biol. 2010;30:1818-1824.)

Key Words: apoptosis ■ coagulation ■ smoking ■ microvesicles ■ monocytes

As of 2002, an estimated 1.3 billion people in the world actively smoked cigarettes, and even more individuals are exposed to secondhand tobacco smoke.1,2 Tobacco smoke substantially increases the risk of atherothrombotic disease in coronary, cerebral, and peripheral arteries1,3–8; and of venous thrombosis.9–11 Public smoking bans are associated with reductions in cigarette smoking.12,14,15 Much of the decline in cardiovascular events in America and Europe during the past 2 to 3 decades has been attributed to management of the major conventional cardiovascular risk factors, including reductions in cigarette smoking.12,14,15 Despite its wide importance, the underlying pathological mechanisms for cardiovascular harm from tobacco smoke remain poorly understood.3 Herein, we focused on microvesicles (MVs), also called microparticles, which are released from the plasma membrane during cell activation or apoptosis and have played an important role in thrombus formation.16–20 MVs transport factor (TF), a transmembrane molecule that initiates coagulation in vivo.16–20 Several studies have shown that cigarette smoking increases TF expression on peripheral monocytes,21 by cultured mouse alveolar macrophages,22 and in atherosclerotic lesions.23 Smokers have higher plasma concentrations of TF than do nonsmokers, and smoking just 2 cigarettes in a row increases their TF levels even further.24 Nevertheless, we are aware of no prior reports characterizing cellular mechanisms for increased plasma TF in smokers. In the current study, we sought to determine whether exposure of human monocytes/macrophages to tobacco smoke induces the release of MVs, whether these smoke-induced MVs are procoagulant, and what cellular processes might be responsible for their production.
Methods

The human THP-1 monocytic cell line (ATCC, Manassas, Va) was maintained in RPMI medium 1640 with 10% FBS. Primary human monocyte–derived macrophages (hMDMs) were prepared from fresh buffy coats by selecting monocytes by adherence followed by differentiation into macrophages. Tobacco smoke extract (TSE) was prepared as previously described. At the beginning of each experiment, THP-1 monocytes or hMDMs were transferred to serum-free RPMI medium 1640 plus BSA supplemented with different concentrations of TSE, ranging from 0% (control) to 3.75% (vol/vol), and then incubated at 37°C for 2 to 20 hours (0 hours denotes harvest immediately before adding TSE). In time-course studies of kinase activation, cells were placed into serum-free medium simultaneously. TSE was added at different times; and all cells were harvested simultaneously. In experiments using kinase or caspase 3 inhibitors, the compounds were added to cells 1 hour before the addition of TSE and remained until the end of the experiment, at concentrations as follows: SP600125, 10 μmol/L; SB202190, 10 μmol/L; U0126, 10 μmol/L; PD98059, 20 μmol/L; farnesyl transferase inhibitor (FTI), 20 μmol/L; and caspase 3 inhibitor Z-DEVD-FMK, 100 μmol/L. Flow cytometric characterization of MVs and cells was performed according to previously published protocols. Additional experimental details are provided in the supplemental materials (available online at http://atvb.ahajournals.org).

Results

We found that exposure of human THP-1 monocytes to TSE significantly increased total MV release, in a dose- (Figure 1A) and a time-dependent manner (supplemental Figure I). Smoke-induced MV release was confirmed by 2 independent assay methods (Figure 1A and C). Likewise, TSE significantly stimulated total MV generation from primary hMDMs (Figure 1B and D). In addition, the numbers of TF-positive MVs released from TSE-treated human THP-1 cells (Figure 1E) and hMDMs (Figure 1F) were significantly higher than those from control cells incubated without TSE. More importantly, we found that TSE treatment of THP-1 cells (Figure 1G) or hMDMs (Figure 1H) for 20 hours tripled the procoagulant activity of their MVs.

Because MVs are released from the plasma membrane, we examined whether tobacco smoke affects expression and cell surface display of TF on human THP-1 monocytes and hMDMs. We found that exposure to 3.75% TSE for 2 hours increased TF mRNA levels in both THP-1 cells (Figure 2A) and primary hMDMs (Figure 2B), measured by real-time quantitative PCR. Exposure of THP-1 cells and hMDMs to
TSE for 6 hours substantially increased their content of TF protein (Figure 2C and D) and stimulated cell surface TF display (Figure 2E and F). In a time course, display of TF on the surface of THP-1 cells peaked at 6 hours after the addition of TSE and was still elevated at 20 hours, compared with baseline (supplemental Figure II). The decline in cell-surface TF from 6 to 20 hours may reflect, in part, the release of cellular TF into the medium on MVs.

Tobacco smoke has been reported to activate a number of intracellular signaling pathways,27–33 but their roles in smoke-induced MV generation are unknown. Consistent with prior literature in other cell types,27,34,35 we found that exposure of THP-1 monocytes to TSE increased the phosphorylation of 3 major mitogen-activated protein (MAP) kinases (ie, c-Jun-N-terminal kinase [JNK], p38, and extracellular signal regulated kinase [ERK]) (Figure 3). The time courses differed considerably. Phosphorylated JNK was increased at 0.5 hours but returned to nearly its baseline level after 1 to 6 hours of TSE exposure (Figure 3A). In contrast, phosphorylated p38 and phosphorylated ERK were partially induced at 0.5 hours and peaked at 2 to 4 hours; the increase lasted throughout the entire 6-hour period of TSE exposure (Figure 3B and C). Then, we sought to determine which, if any, of these activated MAP kinases might be involved in TSE-induced generation of biologically active MVs. Inhibitors of ERK or its upstream molecules (ie, MEK and Ras) significantly decreased total MV generation from TSE-exposed THP-1 cells, in some cases to levels statistically indistinguishable from control, as measured by flow cytometry (supplemental Figure III) or ELISA (Figure 3).
However, inhibitors of JNK or p38 did not affect TSE-induced MV generation, and none of the previously mentioned inhibitors significantly affected MV generation in the absence of TSE (Figure 4A [ERK inhibitor] and data not shown [remainder of the inhibitors]). Surprisingly, treatment of THP-1 cells with the ERK inhibitor did not significantly affect TSE-induced display of TF on the cell surface (supplemental Figure IV); however, ERK inhibition decreased TSE-induced generation of TF-positive MVs to a level indistinguishable from control, implying an effect solely on MV release (Figure 4B). Most important, ERK inhibition essentially blocked the ability of TSE to stimulate the release of procoagulant MVs from THP-1 monocytes (Figure 4C and D). Thus, TSE activates the ERK MAP kinase pathway, and this activation is crucial for the generation of procoagulant MVs.

Next, we examined the role of programmed cell death in the production of procoagulant MVs after TSE exposure. Consistent with prior literature in other cell types, TSE exposure of THP-1 monocytes caused cell surface exposure of phosphatidylserine (supplemental Figure VA and B) and dose-dependent DNA fragmentation detected by TUNEL staining (Figure 5A and B). Likewise, exposure of human primary hMDMs to 3.75% TSE also substantially increased apoptosis, as determined by TUNEL staining (supplemental Figure VC and D). The inhibition of ERK, MEK, or Ras, but not JNK or p38, partially decreased TSE-induced apoptosis, as assessed by TUNEL staining (Figure 5C and D); none of these inhibitors affected TUNEL staining of THP-1 cells in the absence of TSE (data not shown). Finally, the caspase 3 inhibitor, Z-DEVD-FMK, substantially inhibited TSE-induced apoptosis (Figure 6A and B) and decreased TSE-induced generation of total MVs, measured by flow cytometry (Figure 6C) and MV-ELISA (Figure 6D). Z-DEVD-FMK, like the ERK inhibitor, did not affect TSE-induced TF display on the cell surface (data not shown) but decreased TSE-induced procoagulant activity of the purified MVs released from THP-1 cells essentially to baseline (Figure 6E).

**Discussion**

Our results demonstrate that exposure of human monocytes and primary human macrophages to tobacco smoke provokes the generation of highly procoagulant membrane MVs, in a process requiring ERK activation and apoptosis. These cellular mechanisms may contribute to the link, in clinical and population studies, between tobacco smoke exposure (both active and secondhand) and a high risk of thrombotic events. Additional studies reported that exposure of experimental animals to tobacco smoke induces apoptosis of several different cell types, which we infer should increase their production of MVs. Thus, our findings are likely to be relevant in vivo.

Regarding intracellular signaling pathways, we found that TSE exposure caused transient activation of JNK and sustained activation of p38 and ERK (Figure 3), but only ERK appeared to be involved in TSE-induced apoptosis and MV generation. ERK MAP kinases are known for their involvement in cell survival, but recent evidence suggests that ERK activation can also contribute to cell death under certain conditions.
44,45 For example, the Ras/MEK/ERK pathway was reported to play a key role in apoptosis induced by UV-A irradiation46 or a calcium ionophore.44 Based on our results, TSE induces TF expression and cell surface display (Figure 2) through a process independent of ERK or caspase 3. In other circumstances, TF expression is either regulated47,48 or not regulated49 through ERK activation. Then, our TSE-exposed cells underwent apoptosis and extensive apoptotic

Figure 5. Tobacco smoke exposure induces apoptosis and MV generation in part through the Ras/MEK/ERK pathway. A, representative histograms from flow cytometry of TUNEL staining of THP-1 monocytes after exposure to control medium (0% TSE, filled gray curve) or medium supplemented with 1.25% (dotted line), 2.5% (thin black line), or 3.75% (heavy black line) TSE for 20 hours. FITC indicates fluorescein isothiocyanate. B, Quantification of TUNEL-positive THP-1 cells by flow cytometry after exposure to the indicated concentrations of TSE. C, Representative histograms of TUNEL staining of THP-1 monocytes after exposure to control medium (0% TSE, filled gray curve) or medium supplemented with 3.75% TSE without (thin black line) or with (heavy black line) indicated inhibitors for 20 hours; the x-axis scale is logarithmic. The horizontal lines indicate intensities considered to be TUNEL positive for quantification in D. B and D, Quantifications of TUNEL-positive cells from 3 to 4 independent experiments. \( P < 0.001 \) by ANOVA for B and D. Individual pairwise comparisons by SNK are displayed.

Figure 6. Apoptosis is required for TSE-induced MV generation by human THP-1 monocytes. A, Representative histograms of TUNEL staining of THP-1 cells after exposure to 0% TSE (control, filled gray curve) or 3.75% TSE without (heavy black line) or with (dashed line) caspase-3 inhibition by Z-DEVD-FMK (caspase3i). As an additional control, the thin black line shows TUNEL-stained cells after treatment with caspase3i without TSE. The horizontal line (M1) indicates intensities considered to be TUNEL positive for quantification in B. FITC indicates fluorescein isothiocyanate. B, Quantifications of TUNEL-positive THP-1 cells after exposure to the indicated agents. C and D, Total MV generation from THP-1 cells, measured by flow cytometry (C) or ELISA (D), after exposure to control medium (Control), 3.75% TSE, and/or the Caspase3i, as indicated. E, The procoagulant activity of isolated MVs released by THP-1 cells after exposure to control medium (Control), 3.75% TSE, and/or the Caspase3i, as indicated. In B through E, results from 3 to 6 independent experiments are shown. \( P < 0.01 \) by ANOVA for each of those panels; and the individual pairwise comparisons were performed by SNK. In all panels, TSE exposure lasted 20 hours and treatment with Caspase3i began 1 hour before the addition of TSE and remained until the end of the study.
vesication, which depends on ERK and caspase 3 and leads to the release of biologically active TF on highly procoagulant MVs (Figures 1, 4, and 6). In addition, the presence of phosphatidylserine on the MV surface is a key identifying characteristic during flow cytometry and ELISA analyses, and this unique surface phospholipid promotes the catalytic efficiency of complexes in the blood coagulation cascade.\(^{19,20,50}\) especially for apoptotic MVs.\(^{20,51–53}\)

The relative importance of TSE-induced TF released on MVs or remaining on cells may depend on the context. For example, biologically active TF transported by monocyte-derived MVs can become targeted into developing thrombi via the interaction of P-selectin glycoprotein ligand-1 (PSGL1) on the MV surface, with P-selectin displayed by newly activated platelets.\(^{17}\) In contrast, the direct recruitment of intact monocytes or macrophages into early experimental atherosclerotic plaques contain MV-associated TF\(^{56}\) and cell-associated TF,\(^{23,57}\) both of which we presume would increase in smokers.\(^{23,24}\) and thereby contribute to thrombus formation and acute vessel occlusion on plaque rupture.

Overall, our findings indicate that exposure of human monocytes/macrophages to tobacco smoke induces cell surface TF display, apoptosis, and ERK- and caspase 3-dependent generation of biologically active procoagulant MVs. These processes may be novel contributors to pathological hypercoagulability caused by active and secondhand exposure to tobacco smoke.

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Disclosures

None.

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SUPPLEMENTARY MATERIALS

For

Tobacco Smoke Induces the Generation of Procoagulant Microvesicles from Human Monocyte/Macrophages

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SUPPLEMENTAL METHODS

Reagents and antibodies
PhosphoPlus antibody kits against three major mitogen-activated protein kinases (MAPK), namely, Jun N-terminal kinase (JNK, catalog #9250), p38 MAPK (cat#9210), and extracellular signal-regulated kinase (ERK, cat#9100), were purchased from Cell Signaling Technology (Beverly, MA, USA). Monoclonal antibodies (mAb) against human TF, with or without FITC labeling, were from American Diagnostica (Stamford, CT). Phycoerythrin (PE)-labeled annexin-V was from BD Pharmingen (San Jose, CA). Inhibitors of JNK (SP600125, Cat#5567), p38 (SB202190, Cat#7067), ERK (U0126, Cat#120), MAP kinase/ERK kinase (MEK, PD98059), Ras (farnesyl transferase inhibitor, FTI) were obtained from Sigma Chemical Company (St. Louis, MO). The caspase 3 inhibitor, Z-DEVD-FMK, was from R&D Systems (Minneapolis, MN).

Preparation of tobacco smoke extract
Research-grade cigarettes were obtained from the Reference Cigarette Program at the University of Kentucky. Tobacco smoke extract (TSE, 100%) was prepared by using a device to bubble mainstream smoke from five cigarettes through 10 ml of serum-free RPMI medium containing 0.2% BSA (RPMI/BSA), at one cigarette per 7-8 min, simulating the burning rate of typical smoking. The TSE was adjusted to a pH of 7.4 and then sterilized by passage through a filter with a 0.22-µm size cut-off. Our initial studies indicated that a single freeze-thaw cycle did not alter the biologic effects of TSE on cultured cells, and so TSE was aliquoted and stored at -80°C. To ensure consistency among preparations, each batch of TSE was standardized according to its absorbance at 320 nm by comparison with aliquots of a standard preparation that was made at the beginning of the study.

Cell culture
The human THP-1 monocytic cell line (ATCC, Manassas, VA) was maintained in suspension culture in RPMI-1640 supplemented with 10% FBS and 1% penicillin/streptomycin, as recommended by the ATCC. Primary human monocyte-derived macrophages (hMDMs) were prepared from fresh Buffy coats by selecting monocytes by adherence followed by differentiation into macrophages as described. At the beginning of each experiment, THP-1 monocytic cells or hMDMs were transferred to serum-free RPMI/BSA supplemented with different concentrations of TSE, ranging from 0% (control) to 3.75% (v:v), and then incubated at 37°C for 2-20 h (0 h denotes harvest immediately before adding TSE). In time-course studies of kinase activation, cells were placed into serum-free medium simultaneously and then harvested simultaneously; TSE was added at different times before harvest. In experiments using kinase or caspase 3 inhibitors, the compounds were added to cells 1 h before the addition of TSE and remained until the end of the study, at concentrations of 100 µM Z-DEVD-FMK, 10 µM SP600125, 10 µM SB202190, 10 µM U0126, 20 µM PD98059, and 20 µM FTI.

Flow cytometric characterization of microvesicles and cells, Total MV quantification by ELISA
Quantifications of MV generation, TF display by MVs and cells, and markers of early and late apoptosis were performed according to our published protocols. In brief, at the end of each incubation, THP-1 cells, which grow in suspension, were fixed in their conditioned medium by the addition of paraformaldehyde to a final concentration of 1%. Conditioned medium from hMDMs was also fixed and then supplemented with latex beads as a reference for MV counts. The number of MVs
in each sample was quantified by flow cytometry (FACSCalibur, Becton Dickinson), using gating
criteria based on particle size, as detected by forward scatter, and surface exposure of PS, as detected
by staining with PE-labeled annexin-V (BD Pharmingen).\textsuperscript{9, 10} For THP-1 cell suspensions, results are
reported as MVs per 1000 cells. Because hMDMs are adherent, we added 25000 15-µm latex beads
(Molecular Probes) to each 100 µL of their conditioned medium as a reference, and our results are
reported as absolute numbers of MVs per ml of conditioned culture medium. The portion of these PS-
positive MVs that were also TF-positive was quantified by simultaneous staining with a FITC-
labeled anti-human TF mAb (Cat# 4508CJ, American Diagnostica)\textsuperscript{9}.

As a second, independent method, the Zymuphen MP-Activity ELISA kit (Anira, Mason, OH) was
used to quantify total PS-positive MVs in culture supernatants that we prepared by low-speed
centrifugation to remove cells. The MV ELISA assessment was performed according to the
manufacture’s instruction by using 20-fold dilutions of THP-1-conditioned media and 5-fold
dilutions of hMDM-conditioned media. Results are expressed as PS equivalents (nM PS eq).

Cell-surface display of TF was analyzed by flow cytometry using the same FITC-labeled anti-TF
mAb.\textsuperscript{9} Cell-surface exposure of PS, an early marker of apoptosis, was detected by staining of cells
with PE-labeled annexin-V following by flow cytometric analysis. Late-stage apoptosis was assessed
by terminal deoxynucleotidyl transferase FITC-dUTP nick end labeling of the cells (TUNEL; APO-
DIRECT kit, BD Pharmingen), also quantified by flow cytometry.

Assessments of tissue factor mRNA and protein expression and tissue factor procoagulant activity
Total RNA from 2 x 10\textsuperscript{6} cells was isolated with 1 ml Trizol\textsuperscript{®} reagent (GibCo BRL, Carlsbad, CA)
according to the manufacturer’s instructions. Tissue factor mRNA expression was assessed by real-
time quantitative reverse-transcriptase PCR (qPCR). Primers and probes for qPCR were synthesized
by the Gene Expression Facility at the University of North Carolina (Dr. Hyung Suk Kim, Director;
Chapel Hill, NC), using the following sequences:

Human tissue factor mRNA:

\[
5'- gat aaa gga gaa aac tac tgt ttc ag -3' (sense primer) \\
5'- tac cgg gct gtc tgt act ct -3' (antisense primer) \\
F-5'- tca agc agt gat tcc ctc ccg aac ag -3'-Q (probe, where F and Q denote the positions of the 
fluorophore and quencher)
\]

Human ß-actin mRNA:

\[
5'- ggt cat cac cat tgg caa tg -3' (sense primer) \\
5'- tag ttt cgt gga tgc cac ag -3' (antisense primer) \\
F-5'- cag cct tcc ttc ctg ggc atg ga -3'-Q (probe)
\]

Tissue factor procoagulant activity (PCA) was measured with fresh (unfixed, unfrozen) samples
using a chromogenic assay for the activation of clotting factor X (active factor Xa; Actichrome TF
activity kit, American Diagnostica). In brief, cell-culture supernatants were obtained by low-speed
centrifugation of conditioned medium from THP-1 cells or hMDMs after treatment without or with
TSE. MVs from 4ml of each supernatant were purified by high-speed centrifugation (100,000 x g at
4°C for 1h), then washed, re-isolated, and re-suspended in 0.2ml of 50 mM Tris-HCl and 100 mM
NaCl. The PCA of these purified MVs was analyzed according to the manufacturer’s instructions,
except that to avoid saturation of the assay, we measured the absorbance at 405 nm of the reaction solutions in a 96-well plate every minute for 60 min with a BioTek Powerwave XS spectrophotometer. The linear portion of the curve was used to calculate the PCA.

**Immunoblots**

Cells were extracted into a lysis buffer containing 20 mM Tris/HCl, Ph 7.4, 150 mM NaCl, 1mM EGTA, 1% Triton X-100, 1% sodium deoxycholate, 1 mM EDTA, 2.5mM sodium pyrophosphate, 1mM Na3VO4, and a commercial protease inhibitor cocktail tablet (Roche Diagnostics GmbH, Germany) for 30 min on ice. Samples containing the same amount of total protein were electrophoresed through a 10% SDS-polyacrylamide gel, followed by transfer to a nitrocellulose membrane (Bio-Rad). The membranes were then blocked with skim milk (5%, w:v) and probed with primary antibodies against human JNK, p38, ERK, and their phosphorylated (active) forms, or against TF or β-actin. Thereafter, detection was accomplished with horseradish peroxidase-conjugated secondary antibodies, to generate a chemiluminescent product (Amersham™ ECL™ Western blot analysis system, GE Healthcare, USA). Signals were quantified using Image J densitometry software and normalized in each independent experiment to values from control (0% TSE) cells.

**Statistical analyses**

Normally distributed data are shown as means ± SEM (n = 4-6). Comparisons amongst three or more groups were performed using one-way analysis of variance (ANOVA), followed by the Student-Newman-Keuls (SNK) test, with p<0.05 considered significant. Comparisons between two groups used the Student’s unpaired, two-tailed t-test. Comparisons between two groups of non-normally distributed data used the Mann-Whitney rank-sum test.
Supplemental Figure I. Exposure of human THP-1 monocytes to TSE increases total MV generation in a time-dependent manner. THP-1 cells were treated for 0 to 20h without (control) or with 3.75% TSE, as indicated, and then samples were analyzed by flow cytometry. *p < 0.05, ‡p < 0.01 compared by Student’s t-test to control values.

Supplemental Figure II. The figure shows flow cytometric histograms of cell-surface TF display by THP-1 cells after 0h (black curve), 6h (red curve) or 20h (orange curve) of incubation with 3.75% TSE. THP-1 cells treated with 3.75% TSE for 6h but stained with an isotype control antibody are shown by the blue curve.
Supplemental Figure III. Treatment of THP-1 monocytes with inhibitors of ERK (U0126, indicated as \textit{ERKi}) or the upstream molecules MEK (PD98059, indicated as \textit{MEKi}) or Ras (FTI, indicated as \textit{Rasi}) essentially blocked the ability of TSE (3.75%) to induce the generation of total MVs, as measured by flow cytometry. \textit{P} < 0.001 by ANOVA; individual pairwise comparisons were performed by SNK.

Supplemental Figure IV. No effect of ERK inhibition on TSE-induced display of TF on the surface of THP-1 monocytes. The \textbf{black curve} shows a representative histogram from flow cytometry of TF display on the surface of control THP-1 cells incubated for 6h without TSE. The \textbf{blue curve} shows the surface TF staining of THP-1 cells after 6h treatment with ERK inhibitor U0126. The \textbf{red curve} shows surface TF staining of THP-1 cells after a 6h treatment with 3.75% TSE. The \textbf{green curve} shows surface TF staining of THP-1 cells treated with both the ERK inhibitor U0126 and 3.75% TSE.
Supplemental Figure V. TSE treatment induces cell-surface PS exposure and nuclear TUNEL staining. Panel A (dose-response) shows representative histograms from flow cytometry of Annexin V staining after 20-h incubations of THP-1 cells with 0 (filled grey), 1.25% (dash line), 2.5% (light line), and 3.75% (heavy line) TSE. Panel B (time course) shows histograms of Annexin V staining after 0h (filled grey), 6h (dash line), or 20h (heavy line) incubations of THP-1 cells with 3.75% TSE. Panel C shows representative histograms of TUNEL staining of hMDMs after 20-h exposure to 0% TSE (filled grey curve) or 3.75% TSE (black line). The horizontal line (M1) indicates intensities considered to be TUNEL-positive for quantification in Panel D. Panel D indicates quantification of TUNEL-positive hMDMs after exposure to 0% (Control) or 3.75% TSE (TSE). The P-value was computed using Student’s t-test.
References


