Online Supplement

Reagents

Platelet Activating Factor (1-*O*-hexadecyl-2-acetoyl-*sn*-glycero-3-phosphocholine, PAF), its enantiomer, 1-*O*-hexadecyl-*sn*-glycero-3-phosphocholine (C_{16} lyso-PAF), LPC and 1-palmitoyl-2-azelaoyl-*sn*-glycero-3-phosphocholine (Az-PC), d_4-PAF were purchased from Cayman Chemical (Ann Arbor, MI). [^{3}H-acetyl]PAF was purchased from Perkin Elmer (Waltham, MA). Fluorescent NBD-PAF (1-[12-[7-nitro-2-1, 3-benzoxadiazol-4-yl) amino]dodecanoyl]-2-acetoyl-*sn*-glycero-3-phosphocholine) and fluorescent AzPAF (1-[7-nitro-2-1, 3-benzoxadiazol-4-yl) amino]dodecanoyl]-2-azalaoyl-*sn*-glycero-3-phosphocholine) were custom synthesized by Avanti Polar Lipids (Alabaster, AL). Anti-CD31 and Alexa647-conjugated anti-mouse were from BioLegend (San Diego, CA), while endotoxin free Human Serum Albumin was a product of Baxter Healthcare (Deerfield, IL). TMEM30a siRNA and its scrambled control were from Thermo Scientific Dharmacon (Lafayette, CO). HUVEC nucleofector kits were from Lonza (Allendale, NJ). MCDB105 medium and endothelial cell growth supplement are from Sigma (St. Louis, MO). QuantiTect SYBR Green RT-PCR MASTER Mix is from Qiagen Inc (Valencia, CA), 3'TMEM30a primer cataaacacgttgccctc and 5'primer ccgcgagatcgagattga were from Invitrogen (Carlsbad, CA).

Animals

Wild-type and apoE$^{-/-}$ mice in a C57BL6 background were purchased from Jackson Laboratory. PAFR$^{-/-}$ mice were the very kind gift of Takao Shimizu (University of Tokyo) provided from a colony backcrossed for 10 generations and maintained by Jeffery Travers (Indiana University). PAF-AH$^{-/-}$ backcrossed eight times into C57BL6 were generously provided by Diana Stafforini (Huntsman Cancer Center, University of Utah).

Cells

HUVEC were cultured in MDCB105 medium, with 15% FBS, 15% endothelial cell growth supplement and heparin (90 μg/ ml) at 37°C in an atmosphere containing 5% CO_2. Trypsinized cells ($5x10^5$) were mixed with siRNA (10 pM in 100 μl master mix) and nuclearporated by program A-34 before 48 h of culture. Lipid uptake in transfected cells (2 x 10^6 cells/ml) was determined by physical suspension, washing in HBSS twice, and resuspending the cells in either 1 μM NDB-PC or NDB-PE at room temperature for 10 min. The labeled cells were washed twice with HBSS containing 1% (w/v) BSA before analysis by single color flow cytometry. TMEM30a was quantitated using the 3'TMEM30a primer cataaacacgttgccctc and the 5'primer ccgcgagatcgagattga.

In Vivo Phospholipid Metabolism

100 μCi of [^{3}H-acetyl]PAF was dried with nitrogen and dissolved in 1 ml PBS containing 0.5% human serum albumin by vortexing and ultrasonic dispersion for 2 min. 100 μl of the buffered [^{3}H-acetyl]PAF, with or without a 1,000-molar excess of enantiomeric PAF or a 2,000-fold excess of synthetic AzPC, was injected into mice via the retro-orbital plexus. Blood (~100 μl) was collected by cardiac puncture at 30 seconds, 1', 2' and 5 min post-injection, expressed into a microfuge tube, and a measured aliquot then immediately transferred to a glass tube containing methanol. Tissues were harvested 5 min post-injection by perfusing 10 ml PBS through the vascular system before lung, liver, spleen, kidney, heart and brain were recovered, in that sequence. Organs were immediately frozen with liquid nitrogen. Organs were then thawed, rapidly weighed and minced. [^{3}H]PAF was extracted from blood and organ homogenates by methanol/chloroform extraction[18] before radiation was quantified by liquid scintillation counting.

Identification and Quantification of Phospholipids by Mass Spectrometry After the addition of d_4-PAF to the monophase extract as an internal standard, the lipids were extracted and the organic phase washed thrice[18]. The chloroform extracts were dried under a stream of nitrogen, resuspended in 100 μl of methanol/water (85:15 v/v), absorbed to a HyperSep NH_2 column (Thermo Scientific) and the polar lipids isolated with a 100% methanol wash. The eluate was dried under nitrogen, resuspended in 200 μl methanol/water (85:15 v/v) and filtered. The phospholipid extract was injected onto a reverse phase C_{18} HPLC column (2 × 150 mm, 5 μm, ODS; Phenomenex, Rancho Palos Verdes, CA) at a flow rate of 0.2 ml/min generated by a Waters Alliance 2690 HPLC (Waters, Wilmington, DE). Phospholipids were resolved using a ternary gradient system comprised of mobile phase A (water containing 0.2% formic acid) and mobile phase B (methanol containing 0.2% formic acid). The column was equilibrated with 85% mobile phase B/mobile phase A mixture and held at this composition for 29 min after the injection. HPLC column effluent was introduced into a Quattro Ultima triple quadrupole mass

spectrometer (Micromass, Manchester, UK). The mass spectrometer was configured with capillary voltage at 3.0 kV, cone voltage at 40 V, collision energy at 20 V, source temperature at 120 °C, and a desolvation temperature at 250 °C. The flow rate for the nitrogen in the cone gas and desolvation gas was 80 and 600 l/h, respectively. Collision-induced dissociation was obtained using argon gas. Mass spectrometric analyses were performed on-line using electrospray ionization tandem mass spectrometry (ESI/MS/MS) in the positive ion mode with multiple reaction monitoring mode. The multiple reaction monitoring transitions used to identify and quantify individual PC molecular species were the m/z for the molecular cation [MH]+ and the daughter ion m/z 184 (the phosphocholine group). Calibration curves were constructed with a fixed amount of d_4-PAF internal standard and varying mol % of each authentic synthetic phospholipid prior to extraction and LC/MS/MS analysis.

Immunnohistochemistry

10 μg of fluorescent NBD-PAF or NBD-AzPAF was dried under a stream of nitrogen, dissolved in 100 μl PBS with 0.5% human serum albumin and dispersed with ultrasonic irradiation for 5 min and introduced to the circulation through retro-orbital injection as before. Organs were recovered 1 minute or 5 min post retro-orbital injection after flushing the vasculature with 10 ml PBS. Lung, liver, spleen, kidney, heart and brain were excised as before, immediately frozen in liquid nitrogen, and embedded in OTC media for sectioning.