SUPPLEMENTARY METHOD

Quantification of EMPs by flow cytometry

Human umbilical vein endothelial cells were incubated with indoxyl sulfate (250 μg/mL) for 24 hours. The supernatants were harvested from each well and assayed immediately. Culture supernatants were centrifuged for 10 minutes at 5,000 g at 4°C and then ultracentrifuged for 1.5 hours at 100,000 g at 4°C. In order to define the endothelial microparticle (EMP) phenotypes prior to flow cytometry analysis, the pellets were resuspended with phosphate buffered saline (PBS) and stained with following antibodies: phycoerythrin (PE)-conjugated anti-human CD31 antibody (clone WM59, mouse IgG1, BD Biosciences, San Jose, CA, USA) and FITC-conjugated anti-human CD42b antibody (clone HIP1, mouse IgG1, BD Biosciences). PE- and FITC-conjugated, isotype-matched monoclonal antibodies (clone MOPC21, mouse IgG1, BD Biosciences) were used to exclude irrelevant specificity. Each 50 μL sample was incubated with 5 μL PE-conjugated anti-human CD31 plus 5 μL FITC-conjugated anti-human CD42 for 30 minutes with gentle regular shaking at room temperature. Then, 500 μL of PBS was added, and the EMPs were analyzed by flow cytometry. The light scatter and fluorescence channels were set at a logarithmic gain. The region of particles smaller than 1 μm was defined and gated by comparison with calibration beads (Sigma, St. Louis, MO, USA). Fluorescence-positive particles were further separated on another histogram based on this size range. EMPs were defined as the particles that appeared in the CD31 + CD42− region. Sample analysis concluded after 10,000 events. The level of nonspecific staining was determined using PE- and FITC isotype controls. Samples were acquired on a FACSCalibur system (BD Biosciences) and were analyzed using CellQuest software (BD Biosciences). The absolute EMP count per tube was measured via a Trucount tube (BD Biosciences).