**SUPPLEMENTAL FIGURES**

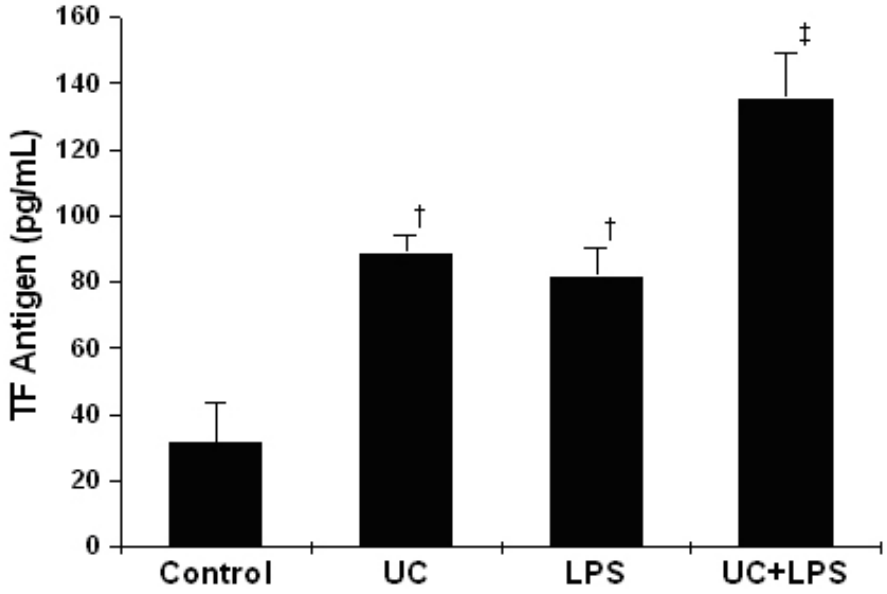
**for**

# Cholesterol enrichment of human monocyte/macrophages induces surface exposure of phosphatidylserine and the release of biologically active, tissue factor-positive microvesicles

*Ming-Lin Liu*, Michael P. Reilly†, Peter Casasanto†, Steven E. McKenzie†, and Kevin Jon Williams**

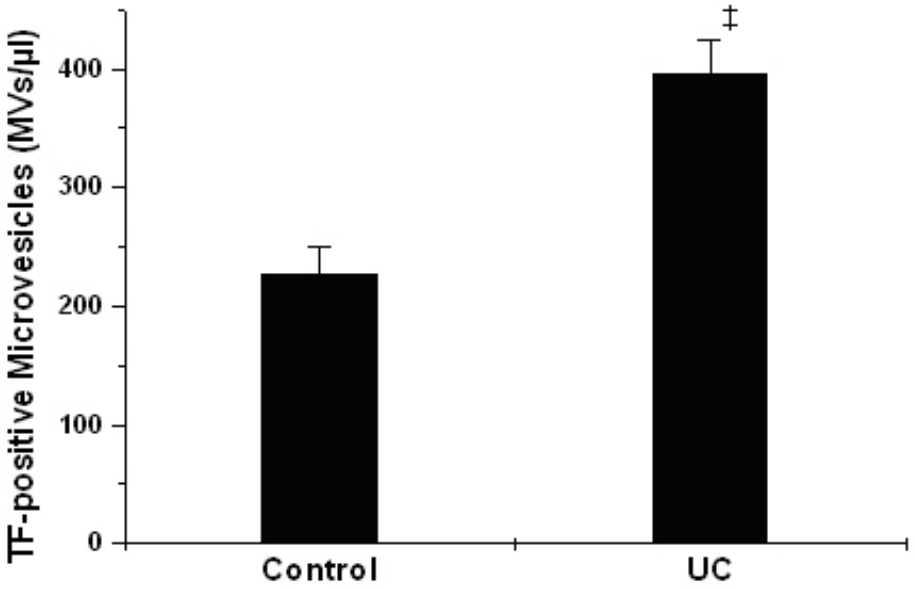
From the *Dorrance H. Hamilton Research Laboratories, Division of Endocrinology, Diabetes and Metabolic Diseases, and the †Cardeza Foundation for Hematologic Research, Division of Hematology, Department of Medicine, Jefferson Medical College of Thomas Jefferson University, Philadelphia, PA 19107, U.S.A.

**Supplemental Figure I**



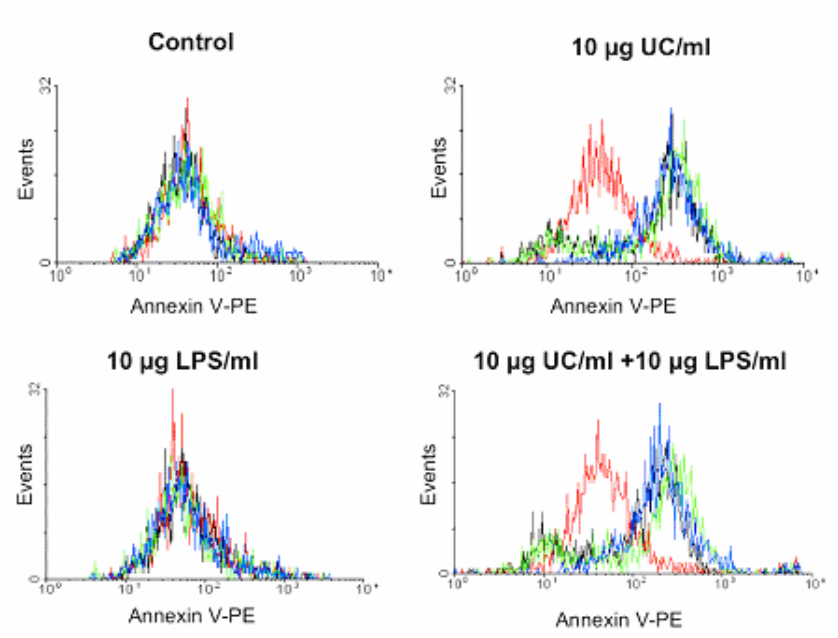

**Supplemental Figure I.** Cholesterol enrichment of monocytic cells increases TF antigen release. Suspensions of human THP-1 monocytes were treated for 4h at 37ºC with UC, LPS, both, or neither (*Control*), as indicated. Displayed are TF ELISA data from culture supernatants prepared from the same samples as in Figure 2A. $P<0.01$ by ANOVA. †$P<0.01$ and ‡$P<0.001$ *vs* the control group by the Student-Newman-Keuls test.

**Supplemental Figure II**



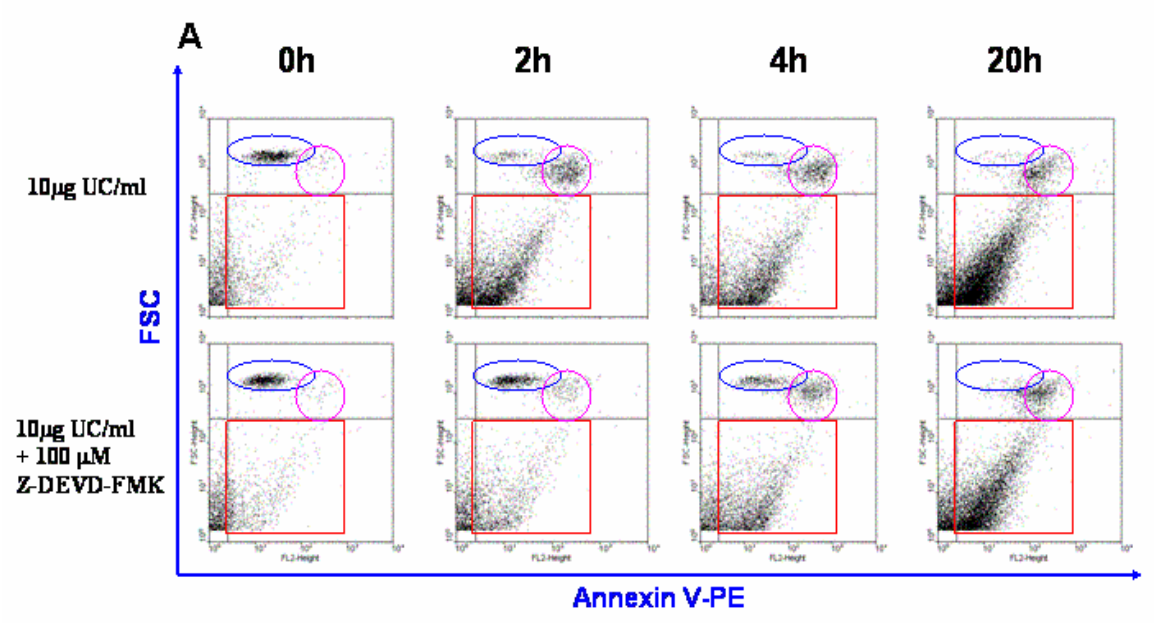

**Supplemental Figure II.** Cholesterol enrichment of primary human monocyte-derived macrophages increases the generation of TF-positive microvesicles. Adherent primary human MDMs in 6-well plates were treated without (*Control*) or with UC for 20h. Displayed are quantitative counts of PS-positive MVs that were also TF-positive, from the same samples as in Figure 3. ‡P<0.001 *vs* the control group by Student's unpaired t-test.

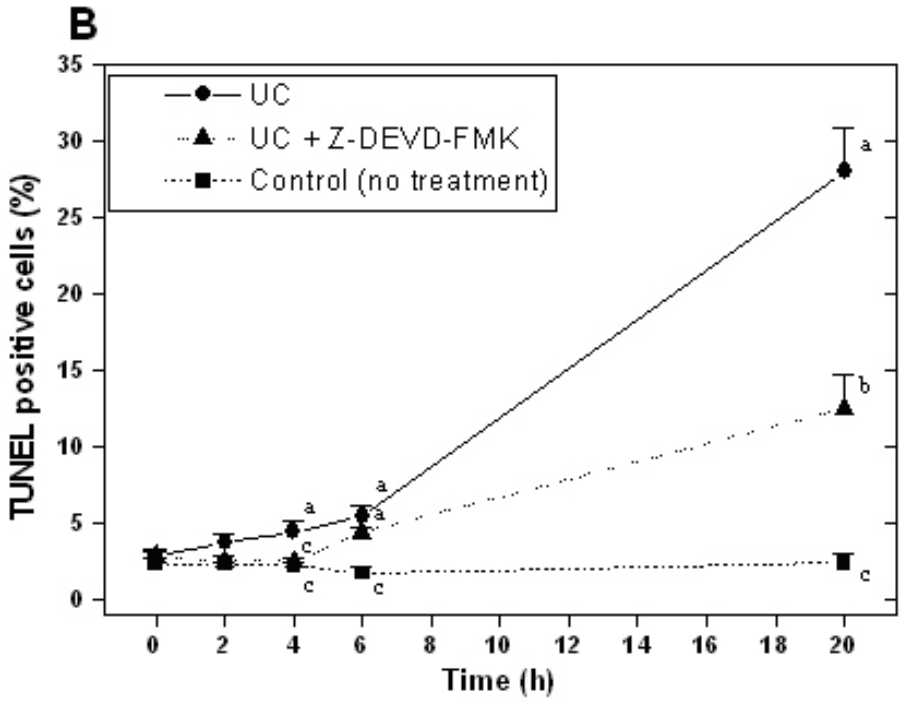
**Supplemental Figure III**




**Supplemental Figure III.** Cholesterol enrichment of monocytes increases cell-surface display of PS. THP-1 monocytes were incubated at 37ºC with or without UC, LPS, or combinations, as indicated, then analyzed by flow cytometry. Displayed are representative histograms of annexin-V staining, gated to include only cells, after 0h (*red*), 2h (*black*), 4h (*green*), and 20h (*blue*), from the same samples as in Figure 4. Each histogram includes approximately 1000 cells.

**Supplemental Figure IV**



**Supplemental Figure IV.** Role for apoptosis in cholesterol-induced microvesicle generation. THP-1 monocytic cells were incubated at 37ºC without or with UC, Z-DEVD-FMK (a caspase-3 inhibitor), or combinations, as indicated, then analyzed by flow cytometry. Panel *A* displays representative dot-plots for UC-treated THP-1 cells without or with Z-DEVD-FMK. Populations of MVs and cells are indicated as in Figure 1A. Panel *B* shows quantitative counts of TUNEL-positive cells after the indicated treatments, to demonstrate a significant inhibition of UC-induced apoptosis by the caspase-3 inhibitor. In panel B, $P<0.01$ by ANOVA. Symbols labeled with different lowercase letters are statistically different by the Student-Newman-Keuls test ($P<0.05$). Results are displayed from the same samples as in Figure 5.