Materials and Methods

Plg binding- Plg binding was measured as described previously (Das R., et al Blood, 2007). To implicate PS in Plg binding, THP-1 cells were differentiated in absence or presence of various concentrations of annexin V (Enzyme Research laboratories, IN) or protein S (BioVision). Cells were washed and incubated with 200 nM human Alexa-488-Glu-Plg, with or without 100 mM 6-aminohexanoic acid (EACA, Sigma), for 1 h at 4°C in HBSS-BSA buffer. Specific Plg binding was measured on viable cells by FACS and was defined as the component of the Alexa-488-Glu-Plg binding that was inhibited by EACA.

Apoptosis assay- THP-1 cells treated with IFNγ+VD3, camptothecin or left untreated. To detect the extent of apoptosis, the cells were washed two times with PBS and stained with FITC-annexin V and propidium iodide according to the manufacturer's protocol (BD Bioscience)

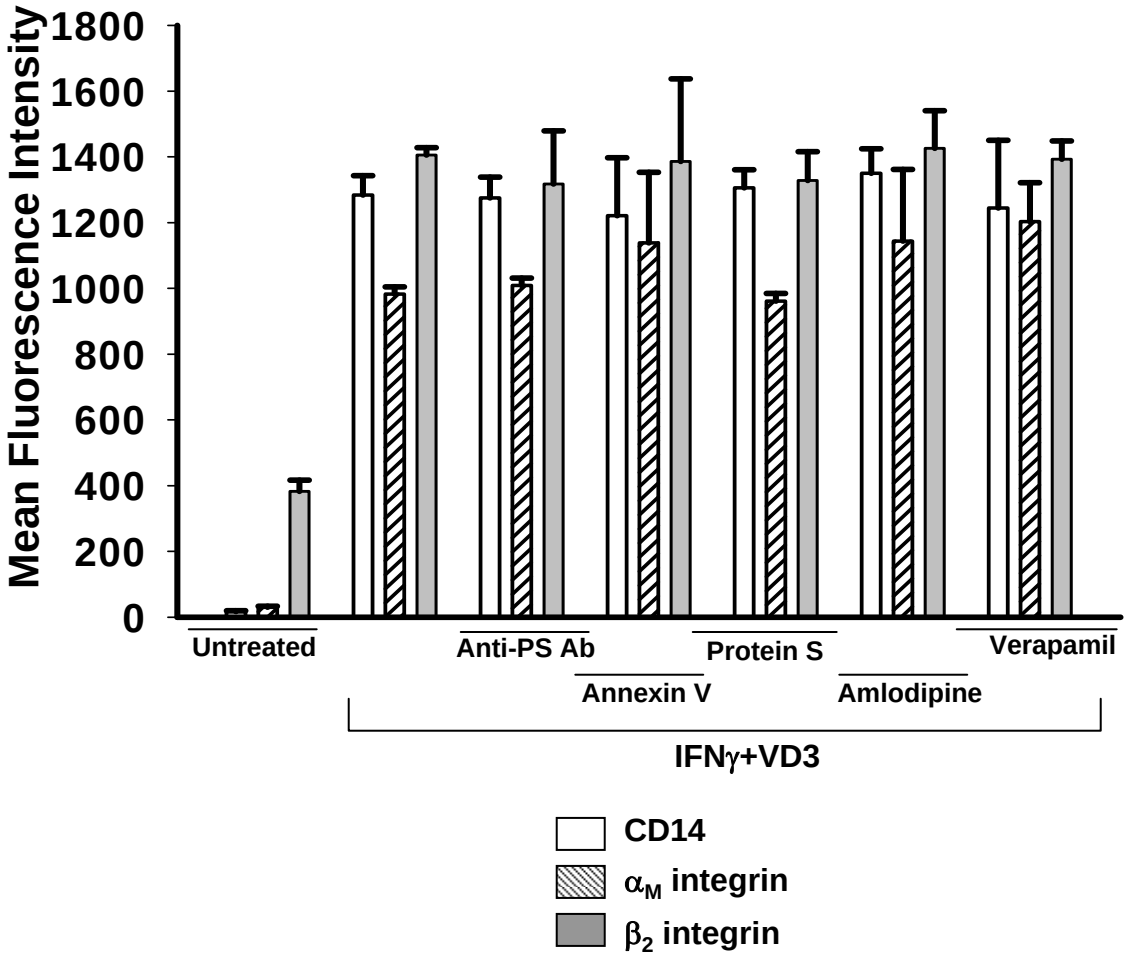
Intracellular Ca2+ measurements- THP-1 cells (2 X 10^6) were loaded with 2 μM Fluo-4AM (Invitrogen) in HBSS buffer containing 1%FBS and 2.5 mM probenecid (Invitrogen) for 30 min at 22°C. Cells were washed twice with HBSS buffer, and, after 30 min at 37°C, the cells were stained with annexin V-PE (eBioscience). Intracellular levels of Ca2+ and PS exposure were measured simultaneously by FACS using the FL-1 and FL-2 channels, respectively.

Figure Legends

Figure S1. Effect of anti-PS antibody, annexin V, protein S, amlodipine, verapamil on IFNγ+VD3 mediated THP-1 cell differentiation. THP-1 cells were pretreated with anti-PS (50 μg/ml), annexin V (2 μM), protein S (2 μM), amlodipine (10 μM) or verapamil (10 μM) for 1 h and then treated with IFNγ+VD3 for 48 h. To determine the extent of differentiation, the cells were stained with anti-CD14, anti-α_M Ab (clone 904) or anti-β2 (clone IB4) and analyzed by FACS. Specific mean fluorescence intensity (MFI) values were quantified by subtracting the binding obtained with secondary antibody alone. Data are means ± SD from triplicate experiments. IFNγ+VD3 induced CD14, α_M and β_2 expression, and the extent of expression of these surface markers were not significantly changed by anti-PS antibody, annexin V, protein S, amlodipine or verapamil.

Figure S2. Effect of IFNγ+VD3 on camptothecin-induced apoptosis and of camptothecin on differentiation of THP-1 cells. IFNγ+VD3 (48 h) and camptothecin-treated (24 h) cells were stained for FACS using either FITC annexin V and PI (panel A) to detect apoptosis or anti-CD14 (black open), anti-α_M (blue open), and anti-β_2 (red open) antibodies (panel B) to detect differentiation. Data for panel A are representative of triplicate experiments. Specific mean fluorescence intensity (MFI) values for Panel B histograms are tabularized below the figure. Data are means ± SD from triplicate histograms.

Figure S3. Camptothecin treatment of THP-1 cells enhances intracellular Ca2+ levels and PS surface expression. THP-1 cells were either pretreated with amlodipine or verapamil for 1 h or were untreated. The cells were then induced to undergo apoptosis with camptothecin (5 μM) for 24 h. Cells were then loaded with Fluo-4AM and subsequently stained with Annexin V-PE. Intracellular Ca2+ ($[Ca2+]_{in,}$ striated bars) and PS exposure (black bars) were measured by FACS. Data represent the mean fluorescence intensities of triplicate samples. Camptothecin treated cells show an increased $[Ca2+]_{in}$ and PS exposure compared to untreated control cells. The level of Ca2+ is lowered in cells pretreated with amlodipine and verapamil. *$p<0.001$ vs. untreated cells, #$p\leq 0.001$ vs. camptothecin striated bar, †$p\leq 0.001$ camptothecin black bar as analyzed by a one way ANOVA test.


Mean Fluorescence Intensity
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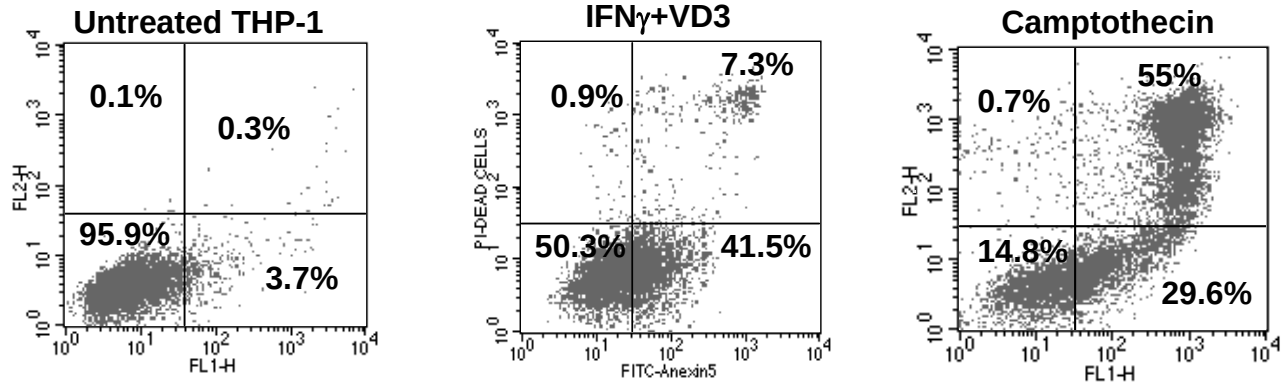
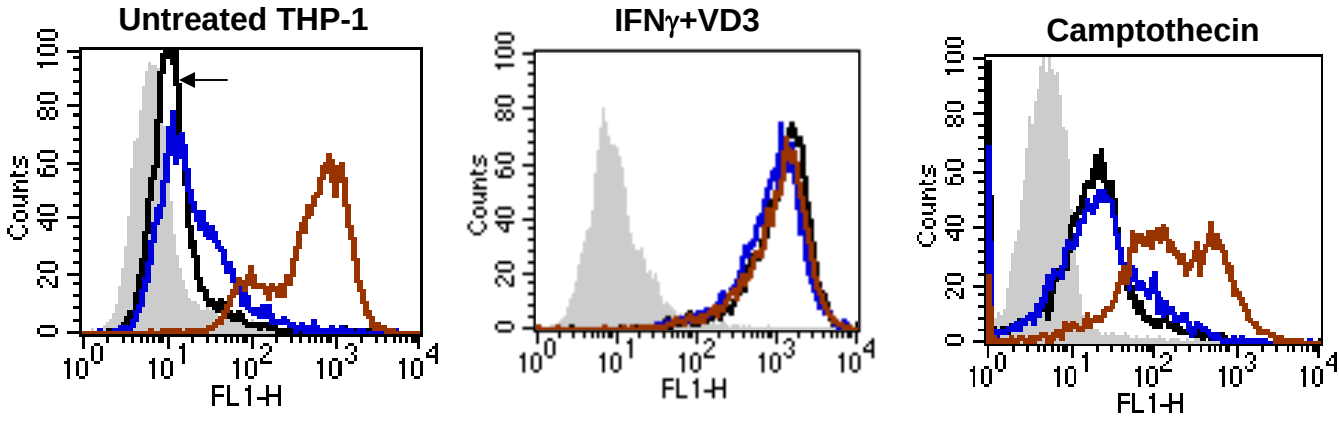
1600
1400
1200
1000
800
600
400
200
0
Untreated
Anti-PS Ab
Protein S
Verapamil
Annexin V
Amlodipine
IFNγ+VD3
CD14
αM integrin
β2 integrin

Figure S1

A



B



Reagent	Color coding	MFI values		
		Untreated	IFNγ+VD3	Camptothecin
Secondary antibody	Grey filled	11.5 ± 5	13.9 ± 2	10.2 ± 4
Anti-CD14	Black open	19.2 ± 5	1230.5 ± 25	33.7 ± 6
904 (anti–α_M integrin)	Blue Open	32.5 ± 10	1030 ± 33	40.7 ± 8
IB4 (anti-β_2 integrin)	Red Open	358.3 ± 21	1340 ± 43	282.2 ± 16

Figure S2

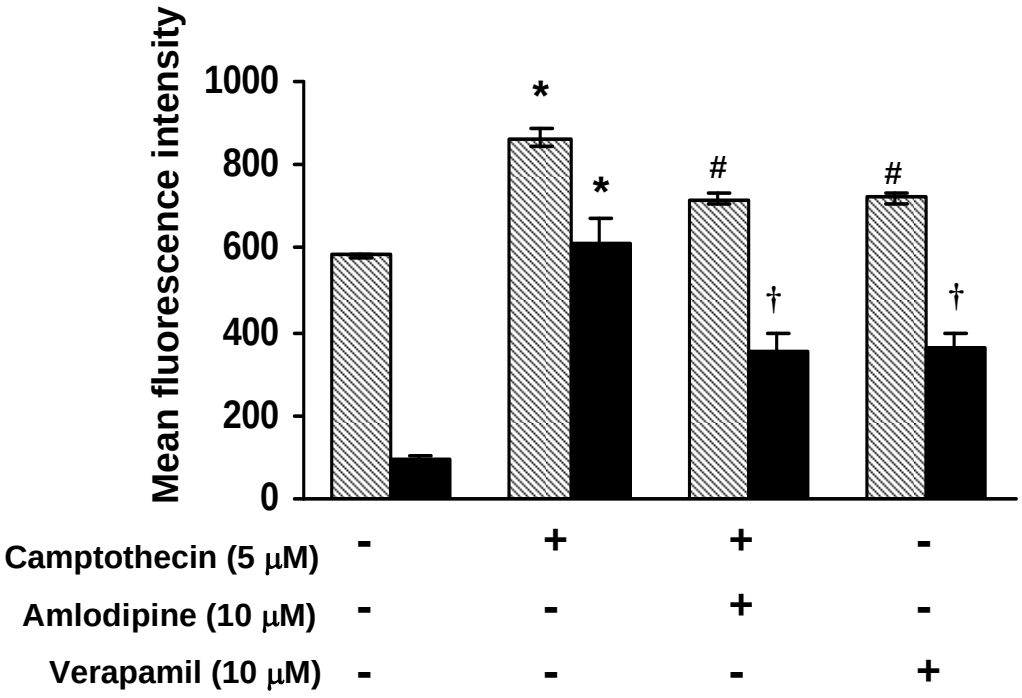

Mean fluorescence intensity
1000
800
600
400
200
0
*
*
#
#
†
†
Camptothecin (5 μM) - + + -
Amlodipine (10 μM) - - + -
Verapamil (10 μM) - - - +

Figure S3