

Kinetic effect of collagen on intracellular doxorubicin accumulation. Jurkat cells were activated or not with collagen (Col) for 4h and then treated with doxorubicin (250ng/ml) (Dox) for an additional 4 or 24h. The cells were washed with PBS and the intracellular doxorubicin content was analyzed by flow cytometry using the FL-2 settings. A representative flow cytometric profile of intracellular doxorubicin content is shown.



Sustained expression of ABCC1 by collagen. HSB2 cells were activated or not with collagen (100µg/ml) (Col) for 4 or 10 h before adding doxorubicin (250ng/ml) (Dox) for an additional 2 h. The cells were washed with PBS and ABCC1 expression was assessed by flow cytometry using FITC-coupled anti-ABCC1 antibody as described in "Materials and Methods". The results represent the mean values \pm SD of positive cells (%) x the mean fluorescence intensity (MFI) from three independent experiments. * *P*<0.05 where indicated.



ABCC1 siRNA silencing in leukemic T cells. (A) Jurkat cells were transfected with ABCC1 or with control (Ctrl) siRNAs as described in Material and Methods. Cell lysates were prepared and analyzed by immunoblot using the anti-ABCC1 mAb (clone QCRL-1). The blots were stripped and reprobed with control anti- β -actin Ab to ensure equal loading. **(B)** ABCC1 levels in collagen-treated cells. Flow cytometry analysis of ABCC1 expression levels in collagen-treated cells transfected with control or with ABCC1 siRNA. Lower panels represent quantifications of ABBC1 levels. The results are representative of three independent experiments. * *P*<0.05 where indicated.



ABCC1 silencing abrogated the collagen-mediated escape from apoptosis induced by a 48 h exposure to doxorubucin. Jurkat cells were transfected with control or ABCC1 siRNAs. 24 h after transfection, the cells were left untreated (NT) or activated or not with collagen (100µg/ml) (Col) for 4 h and incubated with doxorubicin (250ng/ml) (Dox) for 48 h. The cells were washed and apoptosis was detrmined by annexin V staining and flow cytometry analysis. Data represent mean values \pm SD from three independent experiments.* *P*<0.05 where indicated.



DN-MEK-1 expression in transfected cells. Jurkat cells were transfected with DN-MEK1 or pcDNA (control) plasmids as described in "Materials and Methods" section. The cells were then washed in cold PBS and lysed in RIPA buffer containing protease and phosphatase inhibitors. Cell lysates were subjected to SDS–PAGE and analyzed by immunoblot using the anti-Flag mAb. The blots were stripped and reprobed with control anti- β -actin Ab to ensure equal loading. Immunoblots were visualized using an HRP-conjugated secondary antibody followed by enhanced chemiluminescence's detection.