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ORIGINAL ARTICLES

Shedding of Phosphatidylserine from Developing Erythroid Cells Involves Microtubule Depolymerization and Affects Membrane Lipid Composition

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Abstract Phosphatidylserine (PS), which is normally localized in the cytoplasmic leaflet of the membrane, flip-flops to the external leaflet during aging of, or trauma to, cells. A fraction of this PS undergoes shedding into the extracellular milieu. PS externalization and shedding change during maturation of erythroid cells and affect the functioning, senescence and elimination of mature RBCs. Several lines of evidence suggest dependence of PS shedding on intracellular Ca concentration as well as on interaction between plasma membrane phospholipids and microtubules (MTs), the key components of the cytoskeleton. We investigated the effect of Ca flux and MT assembly on the distribution of PS across, and shedding from, the membranes of erythroid precursors. Cultured human and murine erythroid precursors were treated with the Ca ionophore A23187, the MT assembly enhancer paclitaxel (Taxol) or the inhibitor colchicine. PS externalization and shedding were measured by flow cytometry and the cholesterol/phospholipids in RBC membranes and supernatants, by ^{1}H-NMR. We found that treatment with Taxol or colchicine resulted in a marked increase in PS externalization, while shedding was increased by colchicine but inhibited by Taxol. These results indicate that PS externalization is mediated by Ca flux, and PS shedding by both Ca flux and MT assembly. The cholesterol/phospholipid ratio in the membrane is modified by PS shedding; we now show that it was increased by colchicine and A23187, while taxol had no effect. In summary, the results indicate that the Ca flux and MT depolymerization of erythroid precursors mediate their PS externalization and shedding, which in turn changes their membrane composition.

Keywords Erythroid maturation · Membrane phospholipid · Phosphatidylserine · Shedding · Cholesterol/phospholipid ratio

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Introduction

The phospholipids (PLs) in the plasma membranes of all cells, including erythroid cells, are distributed asymmetrically (Zwaal et al. 2005). Lipids with the choline headgroup such as phosphatidylcholine are mainly present in the outer leaflet of the membrane, while aminophospholipids such as phosphatidylserine (PS) are mainly present in the cytoplasmic leaflet (Op den Kamp 1979). The PS distribution across the cell membrane is in a dynamic equilibrium; while the aminophospholipid translocase inserts it inward, the scramblase causes its externalization. Under oxidative stress the translocase activity is reduced; as a result, the equilibrium leans toward externalization. In erythroid cells, alterations in PL distribution, such as flip-flop of PS from the inner to the external membrane leaflet, lead to apoptosis of developing precursors in the bone marrow and removal of senescent RBCs from the circulation (Zwaal et al. 2005). A fraction of the external PS undergoes shedding into the extracellular milieu (Op den Kamp 1979). Such shedding is associated with the development of erythroid precursors and aging of mature RBCs (Freikman et al. 2008, 2009; Gifford et al. 2006; Greenwalt 2006; Pattanapanyasat et al. 2004).

PS externalization in nucleated cells as well as in RBCs is mediated by the intracellular concentration of calcium (Ca) through activation of the scramblase (Basse et al. 1996; Zhou et al. 1997). PS shedding was also reported to involve changes in Ca flux (Freikman et al. 2008, 2011). Thus, in nucleated cells Ca flux–induced membrane vesiculation is associated with apoptosis (Enjeti et al. 2008). In RBCs, shedding of PS-enriched microvesicles was shown to be stimulated by sustained elevation of intracellular Ca (Smith et al. 2001). Other events associated with changes in Ca influx include opening of Ca-dependent potassium channels leading to cytoskeletal alterations (Liu et al. 2005; Sato and Ohnishi 1983; Sikorski et al. 2000; Takakuwa and Mohandas 1988) and an alteration in the membrane lipid organization (Best et al. 2002; Smith et al. 2001).

Several lines of evidence suggest interactions between plasma membrane PLs and cytoskeletal components (Chichili and Rodgers 2009; Meiri 2004), including microtubules (MTs) (Caron and Berlin 1979; Reaven and Azhar 1981), the key components of the cytoskeleton (van der Vaart et al. 2009). MTs are made up of $\alpha\beta$-tubulin heterodimers (van der Vaart et al. 2009), and they readily polymerize and depolymerize in cells. MTs are involved in a variety of cellular processes such as cell division, maintenance of cell shape, cell signaling and migration and cellular transport (Bhattacharyya et al. 2008) as well as maturation and stress (Nogales 2001). During erythroid maturation, MTs undergo dramatic changes in distribution, and they completely disappeared in mature mammalian RBCs (Koury et al. 1989). It had been shown that at early stages of maturation of murine erythroid precursors MTs are radially arranged just under the plasma membrane. Addition of the MT depolymerization promoter colchicine or vinblastine caused MTs to disappear completely. This, however, did not affect enucleation (Koury et al. 1989). Addition of paclitaxel (Taxol), which enhances MT polymerization and stabilization, caused the resulting reticulocytes to contain abnormally high numbers of polymerized MTs (Koury et al. 1989). Treatment of patients with Taxol caused PS externalization and short survival of their RBCs (Lang et al. 2006).

In the current study, we investigated the effect of MT depolymerization in developing erythroid cells on their membrane PS distribution and shedding. For this purpose, we treated cultured human normal erythroid (HNE) and murine erythroid leukemia (MEL) cells with an MT depolymerization enhancer, colchicine, and an inhibitor, Taxol. The effects of these modulators were studied on constitutive shedding as well as on shedding induced by a Ca ionophore, A23187 (Dedkova et al. 2000; Freikman et al. 2011). The cellular (inner and external) PS and the shed PS were measured by a novel quantitative flow cytometric methodology (Freikman et al. 2009). We found that treatment with colchicine and Taxol markedly increased both the constitutive and the induced PS externalization. PS shedding, however, was increased by colchicine but inhibited by Taxol.

Shedding is one of the mechanisms of membrane remodeling (Greenwalt 2006), including changes in the membrane cholesterol/PL ratio (Freikman et al. 2008, 2009). In the present study, using ^{1}H-NMR, we showed that colchicine, by enhancing shedding, increased the cholesterol/PL ratio, whereas Taxol, by inhibiting shedding, decreased the ratio.

We have previously shown that PS externalization and shedding, and the subsequently evolved membrane changes, are intrinsically involved in the process of erythroid cell development (Freikman and Fibach 2011). The present results suggest a contribution of the MT polymerization state to this process.

Materials and Methods

Cell Cultures

MEL cells and human myeloid leukemia (HL-60) cells were maintained by subculturing twice weekly at about 5×10^4/ml in alpha-minimal essential medium (α-MEM) supplemented with 10 % fetal bovine serum (both from Biological Industries, Beit-HaEmek, Israel). Cells were incubated at 37 °C in a humidified atmosphere of 5 % CO_2 in air. For experiments, MEL cells were used on day 3, while HL-60 cells were used after 6 days, without change of medium.

Primary cultures of HNE cells were established as previously described (Fibach and Rachmilewitz 1993). Briefly, peripheral blood mononuclear cells were cultured in α-MEM in the presence of a conditioned medium derived from the 5637 human bladder carcinoma cell line. After 1-week incubation, nonadherent cells were harvested, washed and recultured in the presence of 1 U/ml erythropoietin. Erythroid cells proliferated and differentiated during the next 12 days into nucleated hemoglobin-containing orthochromatic normoblasts. For experimentation, HNE cells were used 6 days after the addition of erythropoietin.

Treatment of Cells

MEL or HNE cells were concentrated to 1×10^7 /ml in phosphate-buffered saline (PBS) containing 1 mM $CaCl_2$ (Ca-PBS) and incubated at 37 °C for 1 h with A23187 (2 μM), colchicine (100 μM) (both from Sigma-Aldrich, Rehovot, Israel) or paclitaxel (Taxol, 10 μM) (a gift from Bristol Myers Squibb, New York, NY). Cells were then centrifuged and analyzed. Supernatants were centrifuged at $1{,}700\times g$ and kept at –80 °C until analysis.

Extraction and Measurement of Free or Polymerized Tubulin

Free or polymerized tubulins were extracted from HNE or MEL cells by Triton X-100-containing buffer as described previously (Ren et al. 2009). Lysed cells were centrifuged; the supernatant contained free tubulin, while the pellet contained polymerized tubulin (i.e., as MTs) (Ren et al. 2009). Equal amounts of total proteins from each fraction were analyzed for tubulin by Western blotting using an anti-α-tubulin antibody and a horseradish peroxidase–conjugated secondary antibody. Reactive bands were visualized using an enhanced chemiluminescence system. The films were scanned and subsequently analyzed using Tina software (Raytest, Straubenhardt, Germany). The optical density of the bands was presented in relation to β-actin.

Measurement of Cellular and Shed PS

PS was measured using fluorescein isothiocyanate–conjugated human recombinant annexin-V (IQ Products, Groningen, the Netherlands). Indirect, quantitative measurement of PS was performed by the two-step flow cytometric inhibition assay as previously described (Freikman et al. 2009). In brief, the outer PS of intact cells, total cellular PS (lysed cells) or shed PS (supernatants) was first bound to fluorescent annexin-V (step I), then the residual, nonbound annexin-V was quantified by binding to PS exposed on apoptotic HL-60 cells, which served as an indicator reagent (step II). The indicator cell fluorescence in step II was reciprocally proportional to the amount of PS in step I (Freikman et al. 2009).

Measurement of Intracellular Calcium

Intracellular Ca^{2+} measurements were performed as described (Lang et al. 2006). Briefly, cells were loaded with Fluo-3/AM (Calbiochem, San Diego, CA) by addition of 4 µl of stock solution (2.0 mM in DMSO) to 1 ml of cell suspension (10^7 cells in PBS supplemented with 10 mM D-glucose) and incubation at 37 °C for 30 min under protection from light. Cells were then washed with PBS containing 0.5 % bovine serum albumin (Sigma) and with PBS without albumin. About 10^6 Fluo-3-loaded cells were then treated with colchicine, Taxol or A23187, as indicated in Results, and analyzed by flow cytometry.

Flow Cytometry

Cells were analyzed by a fluorescence-activated cell sorter (FACScalibur; Becton Dickinson, Immunofluorometry Systems, Mountain View, CA). Instrument calibration and settings were made using Cali-BRITE™-3 beads (Becton Dickinson). Cells were passed at a rate of ~1,000/s, with saline serving as the sheath fluid. A 488-nm argon laser beam was used for excitation. The sensitivity of the photomultiplying tubes was set by adjusting the voltage in each set of experiments. Arithmetic mean fluorescence channels were calculated by the FACS-equipped CellQuest® software (Becton Dickinson).

Measurement of Cholesterol and PLs

PLs and cholesterol were extracted by a modified Folch method (Folch et al. 1957) and measured by proton ^{1}H-NMR as previously described (Yoshioka et al. 2000). Spectra were obtained with an Inova500 spectrometer equipped with 11.74 T 51-mm Bore Oxford Magnet (Varian Medical Systems, Palo Alto, CA), operating at 500 MHz. The data were processed using VNMR software (Varian Medical Systems), including backward linear prediction. NMR peak assignments were determined by comparison with the spectra of commercial cholesterol and PL (all from Sigma-Aldrich) and by referring to the literature (Sparling et al. 1989; Yoshioka et al. 2000). The absolute concentrations of lipids were determined using the integral of the NMR signal of residual $CHCl_3$ in the solvent (chloroform-D1, 99.8 %) as a reference. Calibration curves for selected signal integrals relative to the $CHCl_3$ integral were prepared using commercial lipids.

Statistical Analysis

The data were expressed as mean ± SD from at least three independent experiments and further subjected to a two-tailed Student's t test. $P < 0.05$ was considered statistically significant.

Results

Effects of MT Depolymerization Modulators on PS Redistribution and Shedding

We first studied the effects of 1-h treatment with colchicine or Taxol on MT depolymerization in developing erythroid cells by Western blot analysis of polymerized and free tubulin (Fig. 1). The results show that treatment with colchicine (100 µM) increased the amount of free tubulin by 2- and 1.8-fold in MEL (Fig. 1a) and HNE (Fig. 1b) cells, respectively, while Taxol (10 µM) decreased it by 4- and 3.6-fold, respectively, compared with untreated controls. Reciprocal changes were observed in the amount of polymerized tubulin. These results indicated the promoting and inhibiting effects of colchicine and Taxol, respectively, on MT depolymerization in developing erythroid cells.

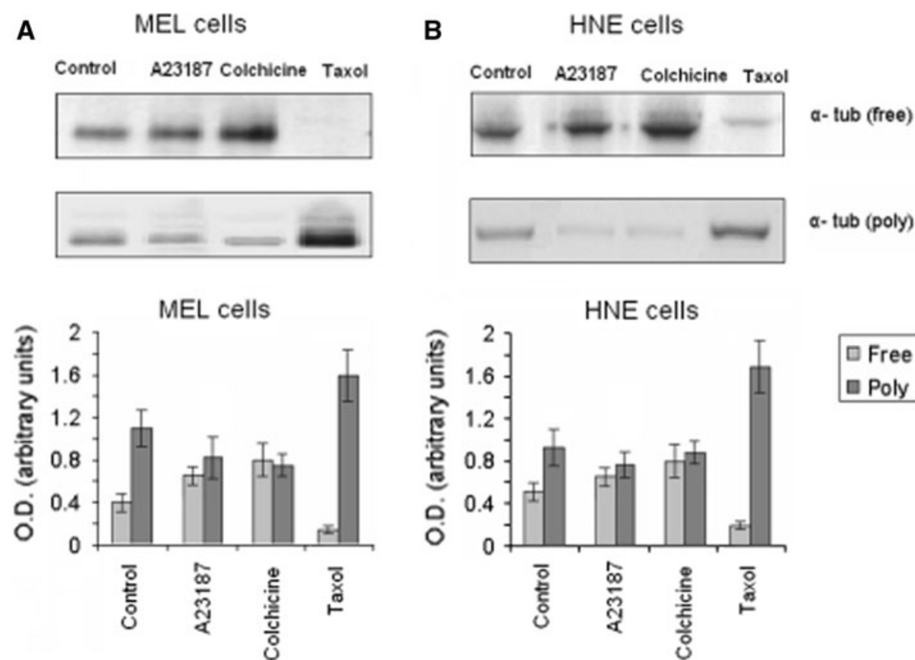

Fig. 1 Microtubule polymerization state in the membranes of erythroid precursors. Murine erythroleukemia (MEL) (**a**) and human normal erythroid (HNE) (**b**) cells were suspended in Ca-PBS and treated for 1 h with nothing (Control), A23187 (2 μM), colchicine (100 μM) or Taxol (10 μM). Free and polymerized tubulins were extracted as described in "Materials and methods" and analyzed by Western blotting. *Upper panels* show the location of the bands. *Bars in lower panels* depict the calculated optical density of the bands in relation to β-actin (mean ± SD, $n = 3$). The results show that treatment with colchicine significantly ($p < 0.05$) increased the amount of free tubulin, while Taxol decreased it ($p < 0.01$), compared with untreated controls. Reciprocal changes are observed in the amount of polymerized tubulin

Interestingly, like colchicine, the Ca ionophore A23187 (2 μM) had an inhibitory effect on tubulin polymerization and stabilization, probably due to elevation of intracellular Ca, which was reported to prevent MT polymerization (Dedman et al. 1979).

PS externalization and shedding were then determined by quantitative flow cytometry. The kinetics of the changes is presented in Figs. 2 and 3. The total PS content of untreated control MEL cells was 27.6 nmole/mg protein, only 10.6 % of which was found on the outer membrane leaflet. Following 1-h incubation in Ca-PBS, the total and inner PS slightly decreased and the outer PS—slightly increased (Fig. 2a). Treatment of these cells with A23187 (2 μM) had only a minor effect on the total PS but increased the outer PS in a time-dependent manner, which was accompanied by a decrease in the inner PS (Fig. 2b) compared to the untreated control (Fig. 2a). Similarly, treatment with Taxol (10 μM) or with colchicine (100 μM) alone or in combination with A23187 (2 μM) had only a slight effect on the total PS but caused (after 1 h) up to threefold increase in the outer PS and a 50 % decrease in the inner PS (Fig. 2c–f). As for the shed PS (Fig. 2g), while colchicine increased it after 1 h by 40 % compared to the untreated control, Taxol alone or in combination with A23187 decreased it about threefold.

Similar analysis in 6-day HNE cells (Fig. 3) indicated that untreated cells had only 5 nmole PS/mg protein on the outer membrane. Treatment with Taxol, alone or with A23187, increased the outer PS by 3.4-fold, while colchicine alone had no effect; but colchicine combined with A23187 increased it by 1.7-fold compared with untreated control cells (Fig. 3a). As for the shed PS (Fig. 3b), while colchicine alone or A23187 alone increased it by threefold, Taxol, alone or with A23187, decreased it by 70 % compared with untreated control cells. Taken together, although some differences were found in the extent of the response of MEL and HNE cells to these compounds, the results indicate that while both depolymerization modulators increase PS externalization, they have opposite effects on shedding—colchicine increases it, while Taxol inhibits. Interestingly, when A23187, which by itself increased externalization and shedding of PS (Fig. 2b), was added together with either colchicine or Taxol, it did not significantly change their effects.

Effect of MT Depolymerization Modulators on Intracellular Ca

Changes in intracellular Ca have been reported to be involved in PS externalization (Dedkova et al. 2000). The

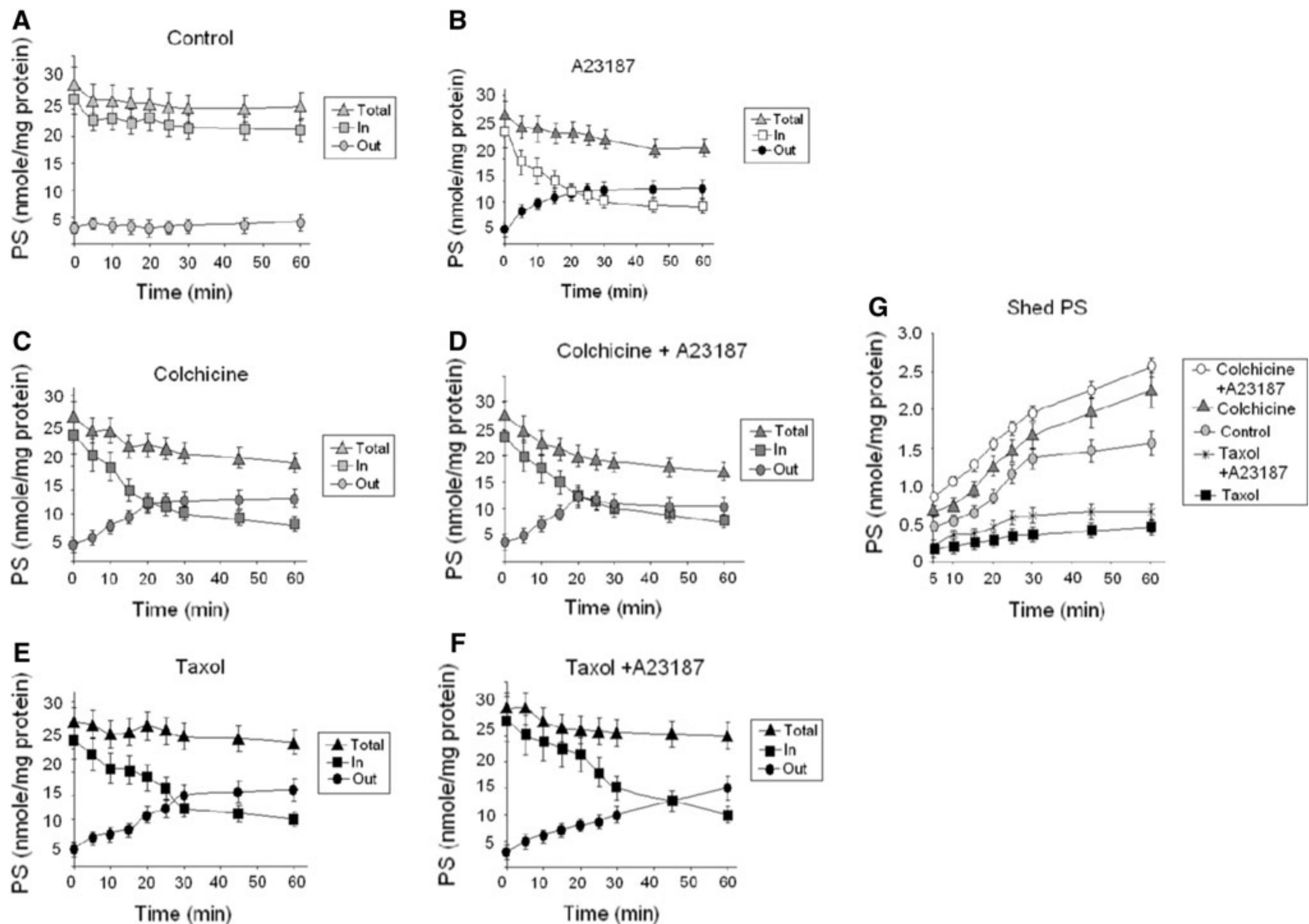

Fig. 2 Effect of microtubule (MT) modulators on phosphatidylserine (PS) distribution and shedding in MEL cells. Cells were suspended in Ca-PBS and incubated for the indicated durations at 37 °C with nothing (Control) (**a**), A23187 (2 μM) (**b**), colchicine (100 μM) (**c**), colchicine with 2 μM A23187 (**d**), Taxol (10 μM) (**e**) and Taxol with 2 μM A23187 (**f**). The inner (in), outer (out) and total PS (**a**–**e**) as well as shed PS (**g**) were quantitatively analyzed using indirect fluorescent assay as described in "Materials and methods". Data are expressed as nanomoles per milligram protein (mean ± SD, $n = 5$). The results indicate that Taxol and colchicine, alone or in combination with A23187, only slightly affected total PS but markedly increased (compared to untreated control) outer PS while decreasing inner PS ($p < 0.01$). Shed PS was increased by colchicine or colchicine + A23187 but decreased by Taxol or Taxol + A23187 ($p < 0.01$). Although A23187 alone increased externalization and shedding (**b**) when added together with either colchicine or Taxol, it did not significantly change their effects

similar effect of colchicine and Taxol on PS externalization could be attributed to their effect on Ca. To study this point, we measured intracellular Ca by flow cytometry using Fluo-3. In MEL cells, Fluo-3 fluorescence was significantly increased by treatment with Taxol or with colchicine alone ($p < 0.01$, $n = 5$) or in combination with A23187 ($p < 0.001$, $n = 5$) compared to untreated cells, indicating their ability to increase intracellular Ca (Fig. 4a). The Ca ionophore A23187, which served as a positive control, caused an up to 8.6-fold increase in fluorescence, but no significant additional increase was observed in combination with Taxol or colchicine. Similar results were obtained in HNE cells (Fig. 4b).

Effects of Shedding on the Membrane Cholesterol/PL Ratio

PLs and cholesterol are the major components of the membrane lipids, and both interact with the MTs (Chichili and Rodgers 2009; Garg et al. 2009; Kwik et al. 2003; Liu and Fletcher 2006; Sheetz et al. 2006). We studied the effect of shedding on the kinetics of the cholesterol/PL ratio by high-resolution ^{1}H-NMR. Treatment with colchicine slightly decreased the cellular cholesterol but significantly decreased (by 2.7-fold) the PL content of MEL membranes, thereby increasing, in a time-dependent manner, their ratio up to 2.5-fold after 1 h compared to

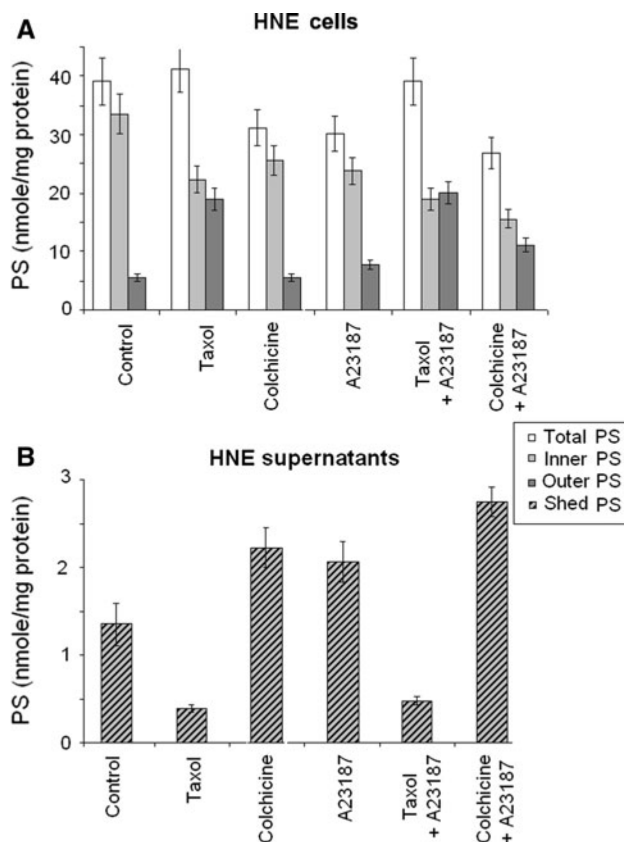

Fig. 3 Effect of MT modulators on PS distribution and shedding in HNE cells. HNE cells were harvested on day 6 of phase II (after addition of erythropoietin), diluted and treated and analyzed as in Fig. 2

untreated cells (Fig. 5a). Similar results were obtained following treatment with A23187 alone. A combined treatment with colchicine and A23187 increased the ratio by 20 % compared to colchicine alone (Fig. 5a). In contrast, treatment with either Taxol or a combined treatment with Taxol and A23187 did not significantly change the cholesterol/PL ratio compared to untreated control.

We also measured the kinetics of the cholesterol/PL ratio in MEL supernatants following 1-h incubation. Treatment with colchicine, alone or with A23187, decreased the ratio in MEL supernatants by up to 1.7- and 2.5-fold, respectively, compared to the supernatants of untreated control cells (Fig. 5b). In contrast, Taxol, either alone or in combination with A23187, had a significant enhancing effect. Similar results were obtained in HNE cells (Fig. 5c, d). Taken together, these results indicate that increase in MT depolymerization by colchicine caused the cells to shed membrane components selectively, relatively enriching the membrane with cholesterol. On the other hand, a decrease in MT depolymerization by Taxol, which inhibited shedding of PLs, prevented changes in membrane composition.

Discussion

PS shedding undergoes biphasic modulation during development and aging of RBCs (Freikman and Fibach 2011). It decreases during maturation in the bone marrow, where it mediates a decrease in cell size and changes in membrane composition and functionality, and increases again as the peripheral mature RBCs age (or undergo accelerated senescence in pathological cases). We have previously demonstrated that these changes in PS shedding are mediated by oxidative stress–induced modifications in Ca flux (Freikman et al. 2011). Several lines of evidence suggest an interaction between plasma membrane PLs and cytoskeletal components (Chichili and Rodgers 2009; Meiri 2004), including the MTs (Caron and Berlin 1979; Reaven and Azhar 1981). The latter undergo changes in

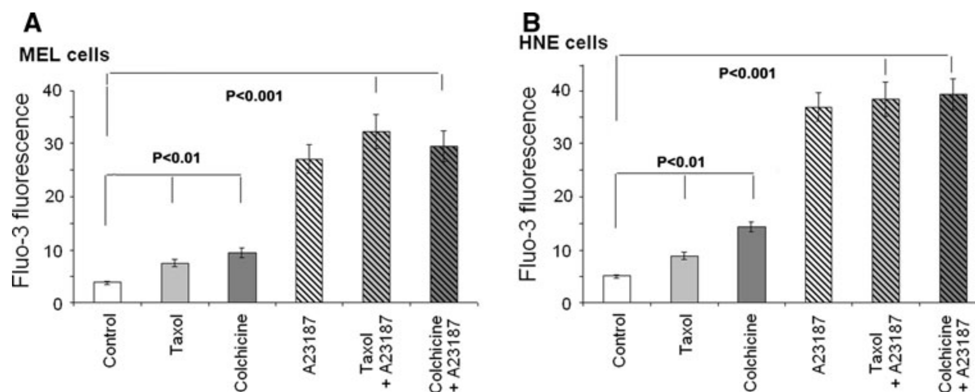

Fig. 4 Effect of MT modulators on intracellular Ca. MEL (**a**) and HNE (**b**) cells were loaded with Fluo-3, washed and treated with MT modulators as described in Fig. 2, then analyzed by flow cytometry (as described in "Materials and methods"). Results are expressed as the average of the mean fluorescence channel (±SD, $n = 5$). In both cell types, treatment with the Ca ionophore A23187 (a positive control) as well as with Taxol or colchicine alone ($p < 0.01$) or in combination with A23187 ($p < 0.001$) significantly increased fluorescence, indicating their ability to increase intracellular Ca

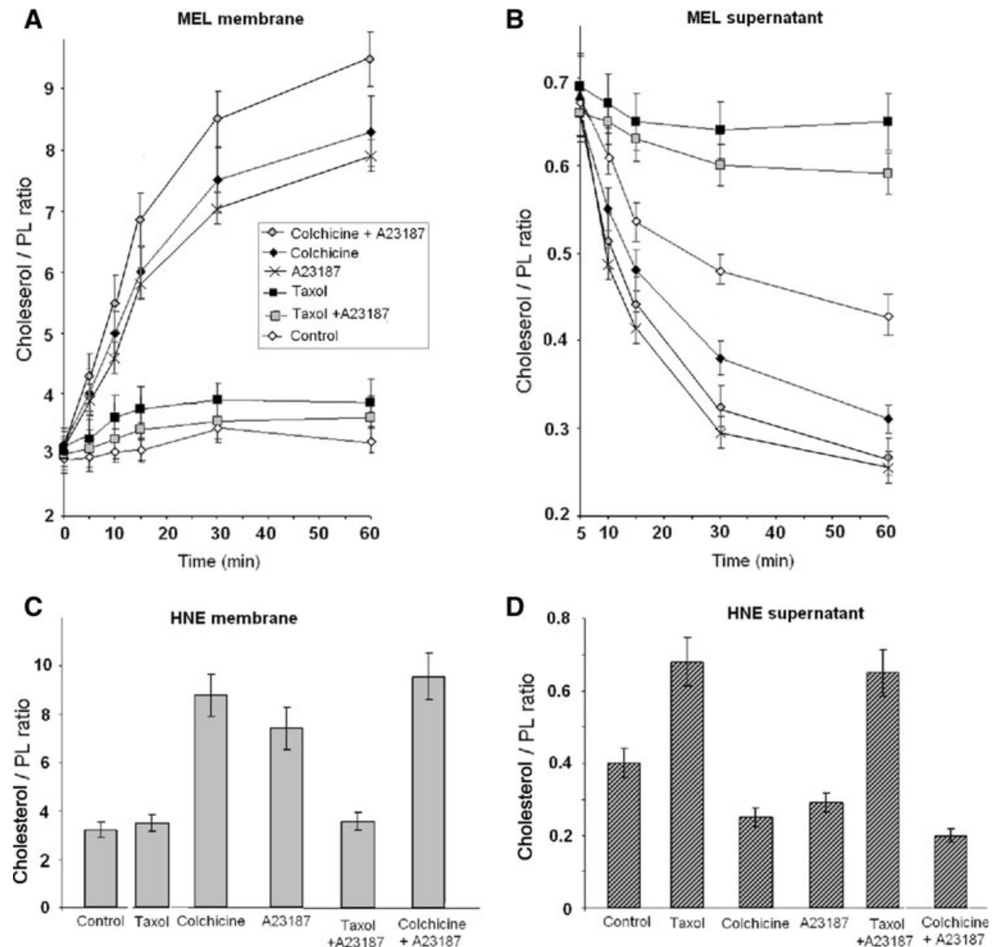

Fig. 5 Effects of shedding on the RBC membrane cholesterol/phospholipid (PL) ratio. MEL (**a**, **b**) and HNE (**c**, **d**) cells were treated with MT modulators as described in Fig. 2. At the indicated time points, cell aliquots containing about 1×10^7 cells and supernatants were collected and their lipids extracted and analyzed by ^{1}H-NMR. The cholesterol/PL molar ratios of the MEL and HNE membranes (**a** and **c**, respectively) and supernatants (**b** and **d**, respectively) were calculated as described in "Materials and methods" (mean ± SD, $n = 3$). The results indicate a significant ($p < 0.01$) increase in the cholesterol to PL ratio by A23187 and colchicine, while Taxol had no effect (**a**, **c**). Opposite results were found in the cell supernatants (**b**, **d**). Differences between the results of colchicine and colchicine + A23187 or Taxol and Taxol + A23187 did not reach statistical significance

distribution during erythroid maturation and completely disappear in RBCs (Koury et al. 1989). In the present study we investigated the involvement of the MT polymerization state on externalization and shedding of PS in developing erythroid precursor cells and its consequences on their membrane composition.

For this purpose, we utilized cultured MEL and HNE erythroid cells. MEL is an in vitro established cell line originally derived from the spleen of a virus-induced erythroleukemic mouse. These leukemic cells can be induced by various chemicals (e.g., DMSO) to undergo erythroid differentiation. In the present study we used them in their undifferentiating state, i.e., in the absence of a differentiation inducer. NHE cells are primary human erythroid precursors derived from progenitors present in the peripheral blood of normal donors (Fibach and Rachmilewitz 1993). They were harvested for experimentation on day 6 following addition of erythropoietin, when most of the cells had reached the state of basophilic erythroblasts. Both systems have served as models for developing erythroid cells in numerous studies (Tsiftsoglou et al. 2009). Cells were treated with an MT depolymerization promoter, colchicine (Risinger et al. 2009), and inhibitor, Taxol (Horwitz 1994). Using Western blot analysis of polymerized and free tubulin, we first confirmed that colchicine and Taxol have promoting and inhibitory effects, respectively, on MT depolymerization in developing erythroid cells (Fig. 1). Using a novel quantitative indirect flow cytometry (Freikman et al. 2009), we next studied their effects on cellular and shed PS. The results showed that although both modulators increased external PS (Fig. 2b, d), PS shedding was increased by colchicine (up to 45 %) while Taxol drastically decreased it (by 90 %) (Fig. 2e). Interestingly, when A23187, which by itself increased both PS externalization and shedding (Fig. 2b), was added together with either colchicine or Taxol, it did not significantly change their effects. These data point toward the importance of the state of MT polymerization, in addition to the changes in Ca flux, on PS externalization and shedding.

The interaction between MT polymerization and Ca flux and their involvement in PS externalization and shedding seems to be a complex phenomenon: PS externalization and shedding are enhanced by increased intracellular Ca. We have previously demonstrated this relationship by treating RBCs with A23187 or by depleting the Ca from the incubation medium (Freikman et al. 2011). We now

found out that colchicine, Taxol and A23187 increased intracellular Ca (Fig. 4) as well as PS exposure (Figs. 2, 3); but while colchicine and A23187 increased PS shedding, Taxol inhibited it. Therefore, PS externalization appears to depend on Ca flux rather than on MT depolymerization, while PS shedding appears to involve both processes.

We have previously shown that shedding induces modulation of lipids, specifically the cholesterol/PL ratio, in the membranes of mature and developing RBCs (Freikman et al. 2011). These changes in lipid membrane composition are associated with plasma membrane functionality (Gonzalez et al. 2009; Wilson-Ashworth et al. 2006). Since both PLs and cholesterol interact with the MTs (Caron and Berlin 1979; Reaven and Azhar 1981), we determined, by ^{1}H-NMR spectroscopy, the amounts and the ratio of these components in the membranes and the supernatants of developing erythroid cells treated with the MT modulators. The results (Fig. 5) indicated that MT depolymerization promoters (A23187, colchicine), which enhance PS shedding, increased the cholesterol/PL ratio in the cell membranes, while Taxol, an inhibitor of PS shedding, had no effect. Opposite results were found in the cell supernatants.

In summary, PS externalization and shedding, and the subsequently evolved membrane changes, are intrinsically involved in the process of erythroid maturation (Freikman and Fibach 2011). The present results suggest that PS shedding is affected by changes in the MT polymerization state of developing erythroid cells. Many compounds that alter the polymerization dynamics of MTs block mitosis and, consequently, induce cell death by apoptosis (Jordan 2002). One of these compounds, paclitaxel (Taxol), a promoter of MT polymerization and stabilization, is being used to treat patients with various malignancies such as breast cancer (Chang et al. 2005) or ovarian cancer (McGuire et al. 1996). A significant side effect of this treatment is severe anemia, which is related to its effect on PS externalization of mature RBCs (Lang et al. 2006). In line with the importance of PS shedding in erythroid development, the present finding that Taxol affects PS shedding may suggest that anemia in patients treated with Taxol might be due to its effect on erythropoiesis in the bone marrow.

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