Microparticle Detection and Phospatidlyserine Exposure in Airway Surface Liquid in Response to Nitrogen Mustard Inhalation in Rats

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Inhalation of mustard alkylating agents injures the respiratory tract, resulting in airway inflammation and procoagulant effects. Evidence suggests that microparticles (MP) released following exposure to toxicants play a role initiating inflammation and coagulopathy. In these studies we determined if MPs are involved in the adverse effects of nitrogen mustard (NM). Sprague-Dawley rats were exposed by intratracheal instillation to vehicle (PBS) or NM (0.15 mg/kg). Twenty-four hours later, rats were euthanized and MPs were analyzed in bronchoalveolar lavage fluid (BALF). To identify MPs, BALF was fractionated into three distinct populations by serial centrifugation (Fraction 1: 1,200 *g*/10 min; F2: 10,000 *g*/30 min; F3: 108,000 *g*/60 min). Western blot analysis revealed high level mitochondrial aconitase expression in protein extracts recovered from the initial two fractions, whereas the vesicular sorting protein ALIX, an exosomal marker, was detected almost exclusively within the third fraction. Flow cytometric analysis revealed a three-fold increase in MPs (0.15-1.0 μm) in all three fractions following NM exposure. Use of a lactadherin-FITC probe to detect phosphatidylserine+ MPs also demonstrated increased percentages of positive events in initial and intermediate MP fractions from NM-exposed subjects. Despite increases in MPs and phosphatidylserine positivity, no change in the level of ALIX was observed between NM and control groups. Our findings indicate MPs are increased in BALF from rats exposed to NM. The increasing proportion of phosphatidylserine+ events in the absence of any change in ALIX suggests the rise in MPs originates from apoptotic events rather than increased exosome release by airway cells.