Effect of Nitrogen Mustard Inhalation on Microparticle Formation in Rat Airways: Probing at the Limits of Detection

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Microparticles (MPs) are submicron-sized membrane vesicles released from activated or injured cells and are detectable by flow cytometry. In circulating blood, MPs have been used as biomarkers to evaluate cell injury or inflammation in patients with pathological conditions. Here, we hypothesize that MPs are increased in airway surface liquid of rats exposed to nitrogen mustard (NM). To determine this, we quantified overall MP numbers in bronchoalveolar lavage fluid (BALF) from control and injured subjects, defined MP burden in BALF by subclass (epithelial or hematopoietic), and tested MPs for nucleic acid content. Methods: BALF was obtained from rats 24 hr after airway instillation of PBS or 0.1 mg/kg of NM. Flow cytometry was used to enumerate MPs (0.3-1.0 µm) in BALF after labeling with specific antibodies for epithelial (E-Cadherin), hematopoietic (CD45) cell markers, and phosphatidylserine expression by lactadherin binding. A standardized protocol using counting beads was employed to determine absolute MP number. To assess for nucleic acid (DNA or RNA), MPs were isolated by ultracentrifugation and stained with propidium iodide. Results: Levels of phosphatidylserine+ MPs increased in BALF after NM exposure [27,555 MPs/µL] compared with control subjects exposed to PBS alone [9,903 MPs/µl]. The overall percentage of E-Cadherin+ MPs declined [93.1 to 35.4%] in response to injury, while CD45+ MPs increased [13.4 to 34.0%]. Additionally, NM exposure increased nucleic acid-containing MPs from 4.24% to 23.52%. Conclusions: MPs are elevated in BALF from rats exposed to NM, compared with control subjects. Reduction in the proportion of E-Cadherin positivity and concomitant increase in CD45+ MPs suggests enhanced microvesicle release, or apoptosis within macrophages/ neutrophil populations. Additionally, we identified a five-fold increase in MPs possessing nucleic acid after NM exposure. These latter findings suggest that MPs may harbor genetic information, specifically microRNAs, and could have an immunomodulatory role in the lung microenvironment. Supported by NIH R25ES020721, P30ES005022, and U54AR055073.