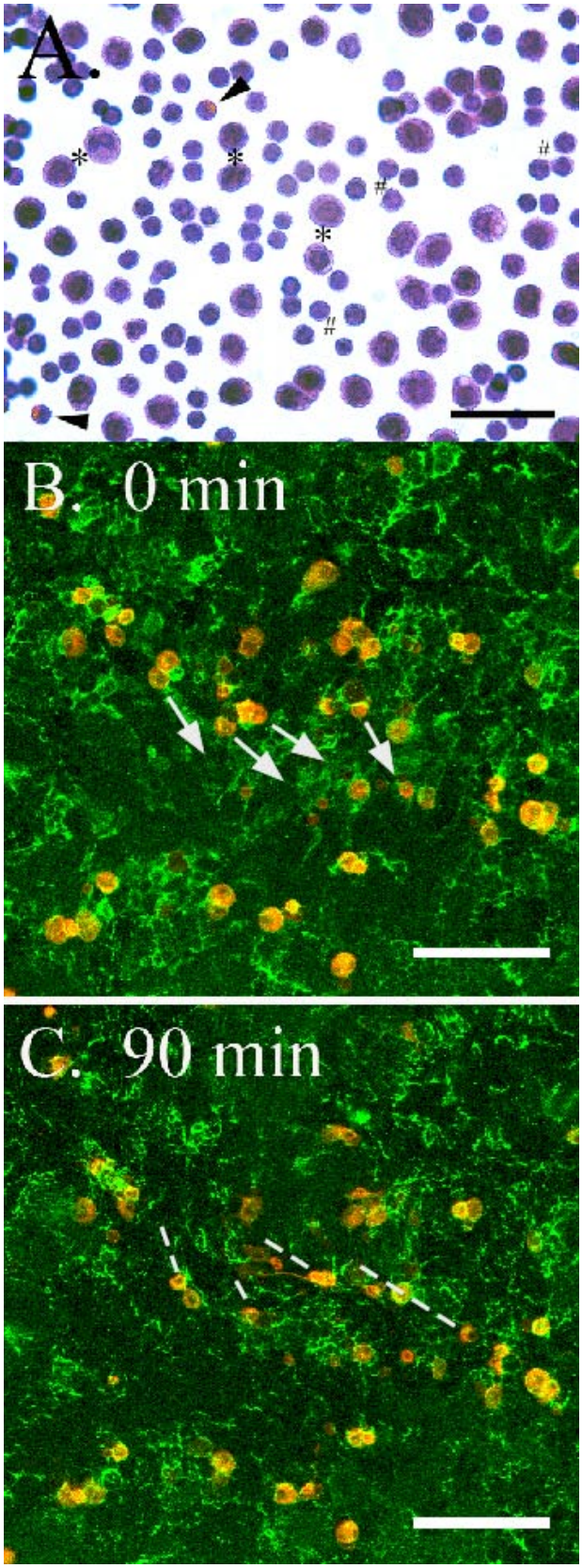

Murine peritoneal lavage leukocytes bound to hyaluronan on the apical surface of differentiated murine airway epithelial cells, treated with tunicamycin, migrate following hyaluronan degradation. BALB/c mice were euthanized by CO_2 asphixiation. The peritoneal cavity was opened with surgical scissors and rinsed twice with 2 ml (each) PBS containing 0.5 mM EDTA. The lavage cells were counted on a hemacytometer followed by centrifugation at 300 g for 10 minutes, resuspending the pellet in PBS-EDTA at 1 million/ml. The cells were labeled with CM-DiI and applied to the airway epithelial cells in the same manner as the U937 cells (no EDTA). Panel A portrays the cell population we obtained from the lavage. Lymphocytes (pound sign) and macrophages (asterisk) predominated, with eosinophils (arrowhead) in minor quantities (mag. 63x, mag. bar at 25 µm). Panel B shows the peritoneal lavage cells (red) bound to hyaluronan (green) on the apical surface of the epithelial cells (black) while the cultures were at 4°C (original image at 20x mag. zoomed to approximately 40x, mag. bar at 100 µm). Both large (i.e. macrophages with bi-lobed nuclei) and small diameter cells (lymphocytes and eosinophils) were found bound to hyaluronan (panel B). Confocal z-step images were taken every 5 min. until the end point at 90 min. (panel C) after exposure to room temperature. A movie was generated from these images (supplement). Over the time-course, hyaluronan appeared to be removed/degraded followed by the migration of, primarily, the smaller diameter leukocytes through the regions where hyaluronan was removed. Arrows (panel B) mark the trajectory of this migration and dashed lines (panel C) portray the path taken by the leukocytes. In this experiment, it remains to be determined the extent to which the hyaluronan removal/degradation was accomplished by the leukocytes or the epithelial cells.