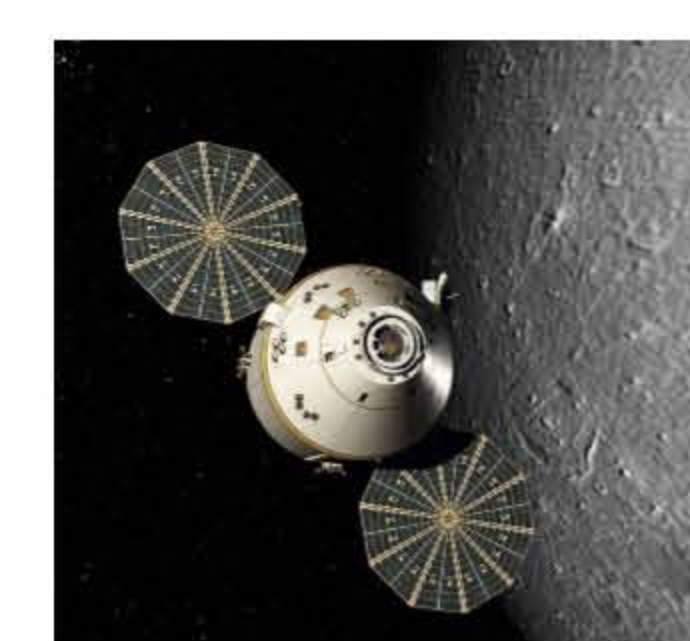


# Immune Alterations in Rats Exposed to Airborne Lunar Dust

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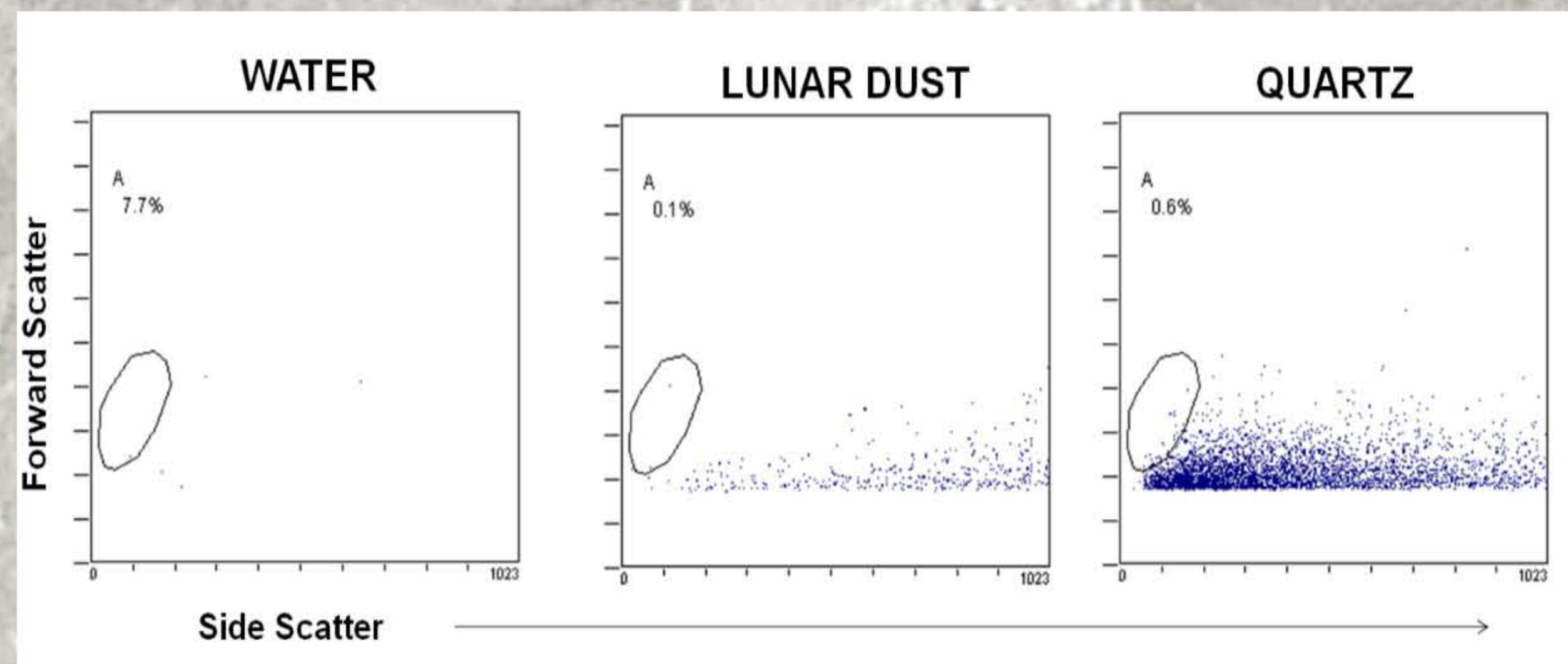
**Background** The lunar surface is covered by a layer of fine, reactive dust. Very little is known regarding the toxicity of lunar dust on human physiology. This study assessed the toxicity of airborne lunar dust exposure in rats on pulmonary and systemic immune parameters.

**Methods** Rats were exposed to 0, 2.1, 6.8, 20.8 or 60.6 mg/m<sup>3</sup> of respirable-size lunar dust for up to 4 weeks (6 h/day, 5 days/week). Intratracheal quartz (1 week) served as an experimental control. Subjects were then euthanized either 1 day, 1 week, 4 weeks or 13 weeks after the last exposure (Lam et al., *Inhal. Toxicol.* 25:661-678, 2013). Blood and lung lavage fluid samples were collected for analysis. Assays included leukocyte distribution by multicolor flow cytometry, electron/fluorescent microscopy, and lavage/plasma cytokine concentrations. Mitogen-stimulated cytokine production profiles were performed on whole blood samples only.



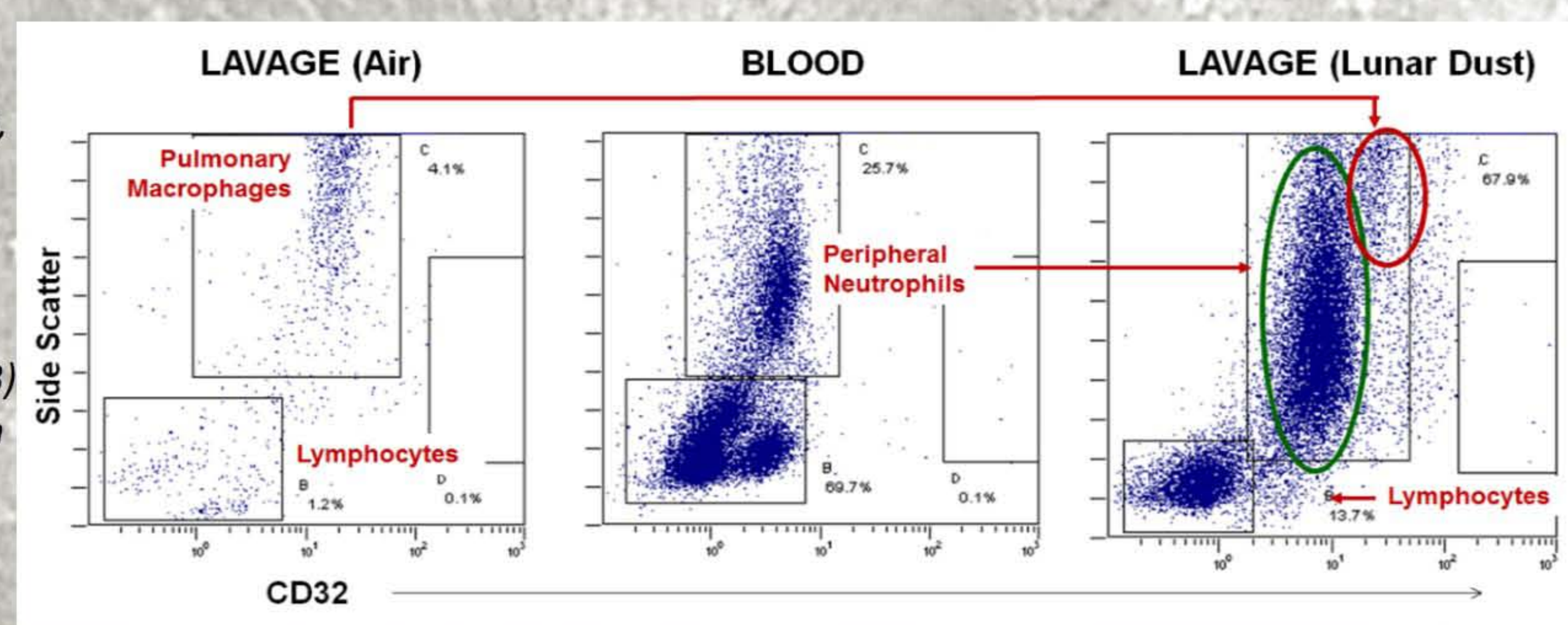
## Cytometry Controls

Representative flow cytometry dot plots demonstrate that the optical scatter properties for the lunar dust inhalation mixtures (and intratracheal quartz) did not overlap with the cellular analysis gate (typical lymphocyte size indicated by visible gate region).



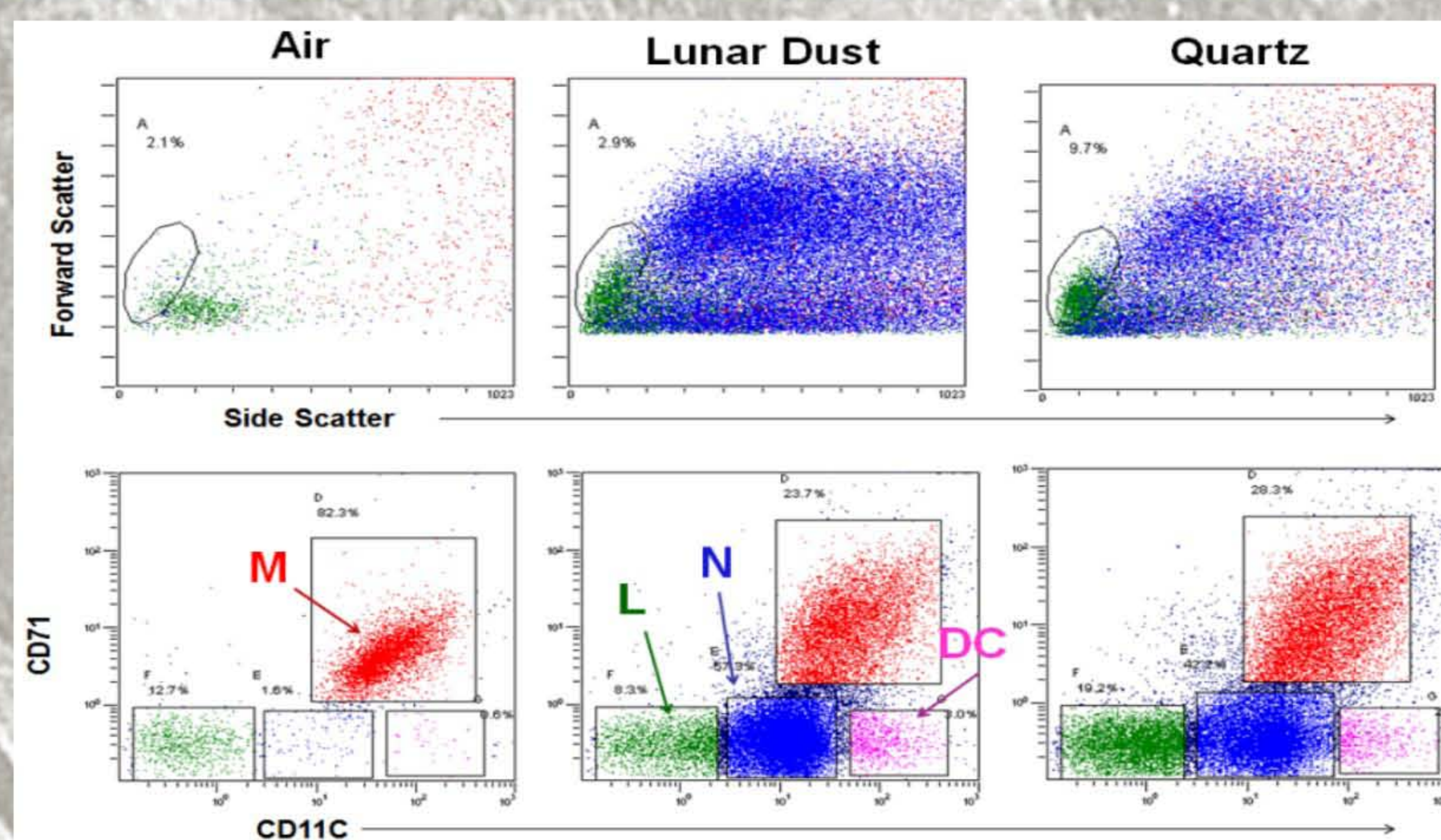
## Neutrophil Influx

Representative flow cytometry dot-plots, side-scatter vs. CD32 expression, illustrate the neutrophil influx into the peritoneal space following lunar dust inhalation. (A) control lavage consists primarily of pulmonary macrophages; (B) rat blood staining illustrates the location of peripheral granulocytes; (C) treated lavage fluid consists of both macrophages and neutrophils, with an increased population of lymphocytes.



## Lavage Cytometry

Flow cytometry cellular characterization of pulmonary lavage based on scatter properties and expression of CD71 and CD11c. Air treated normal lavage fluid consists primarily of CD11c+/CD71+ pulmonary macrophages (M). After lunar dust and quartz inhalation treatment, a pulmonary influx of neutrophils (N), dendritic cells (DC) and lymphocytes (L) is evident.



## Results

- Untreated lavage fluid was comprised primarily of pulmonary macrophages.
- High-dose lunar dust inhalation (>20.8 mg/m<sup>3</sup>) resulted in an influx of neutrophils and lymphocytes. The T cell CD4:CD8 ratio was unchanged.
- Lavage fluid showed increased levels of IL-1 $\beta$  and TNF $\alpha$ . These alterations generally persisted through the 13 week sampling.
- Blood analysis showed that by week 4 the peripheral granulocyte percentage was elevated in the treated rats, however plasma cytokine levels were unchanged in all treated rats.
- Blood culture indicated mitogen-stimulated production of IL-1 $\beta$  and IL-6, and decreased IL-2.
- Minimal adverse immune effects observed in either lung or peripheral blood, following low-dose exposure to  $\leq 6.8$  mg/m<sup>3</sup> lunar dust (data not shown).

## Apollo Missions – Visible Adherence of Lunar Dust



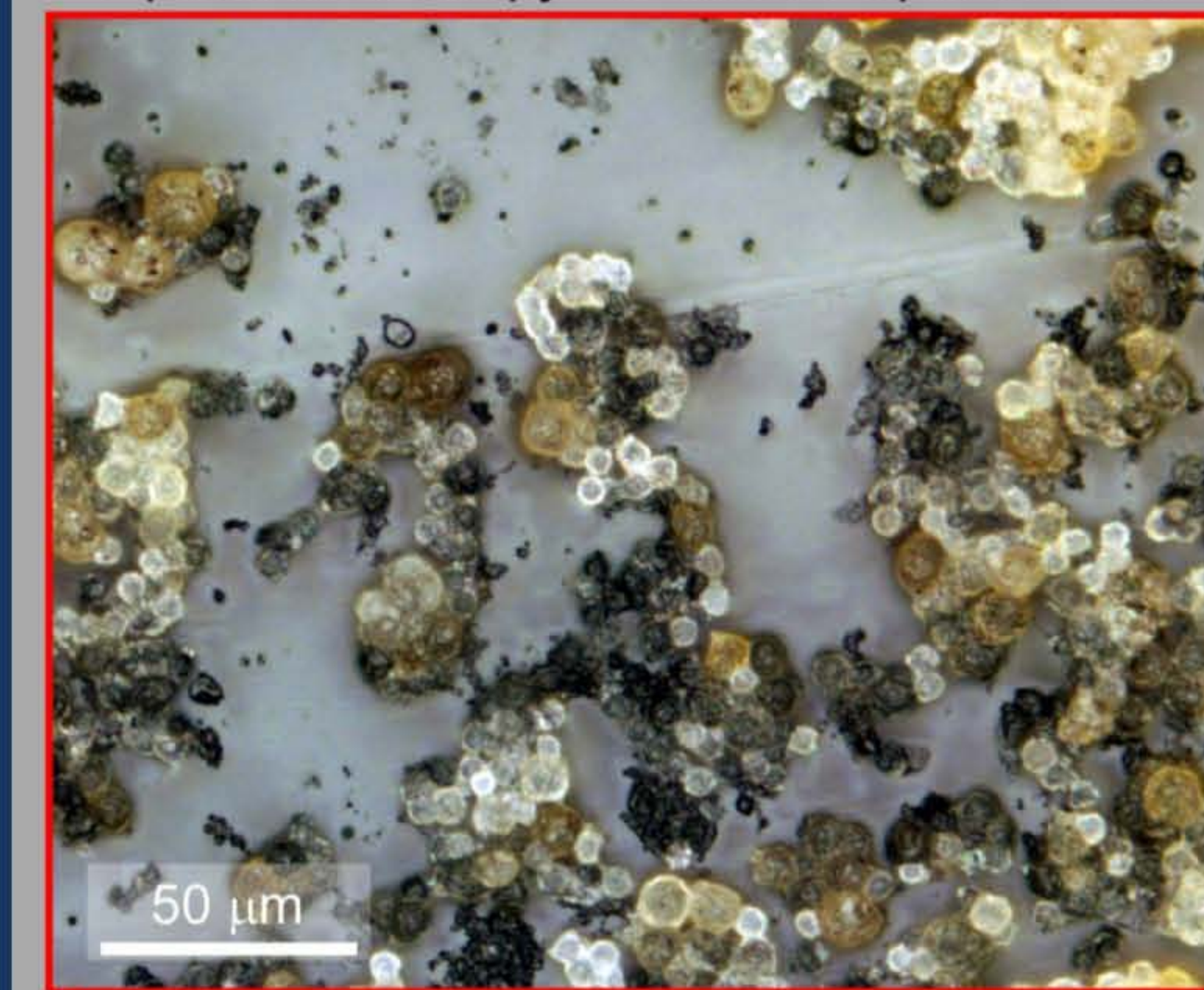
Gene Cernan – Apollo 17



Harrison Schmitt – Apollo 17

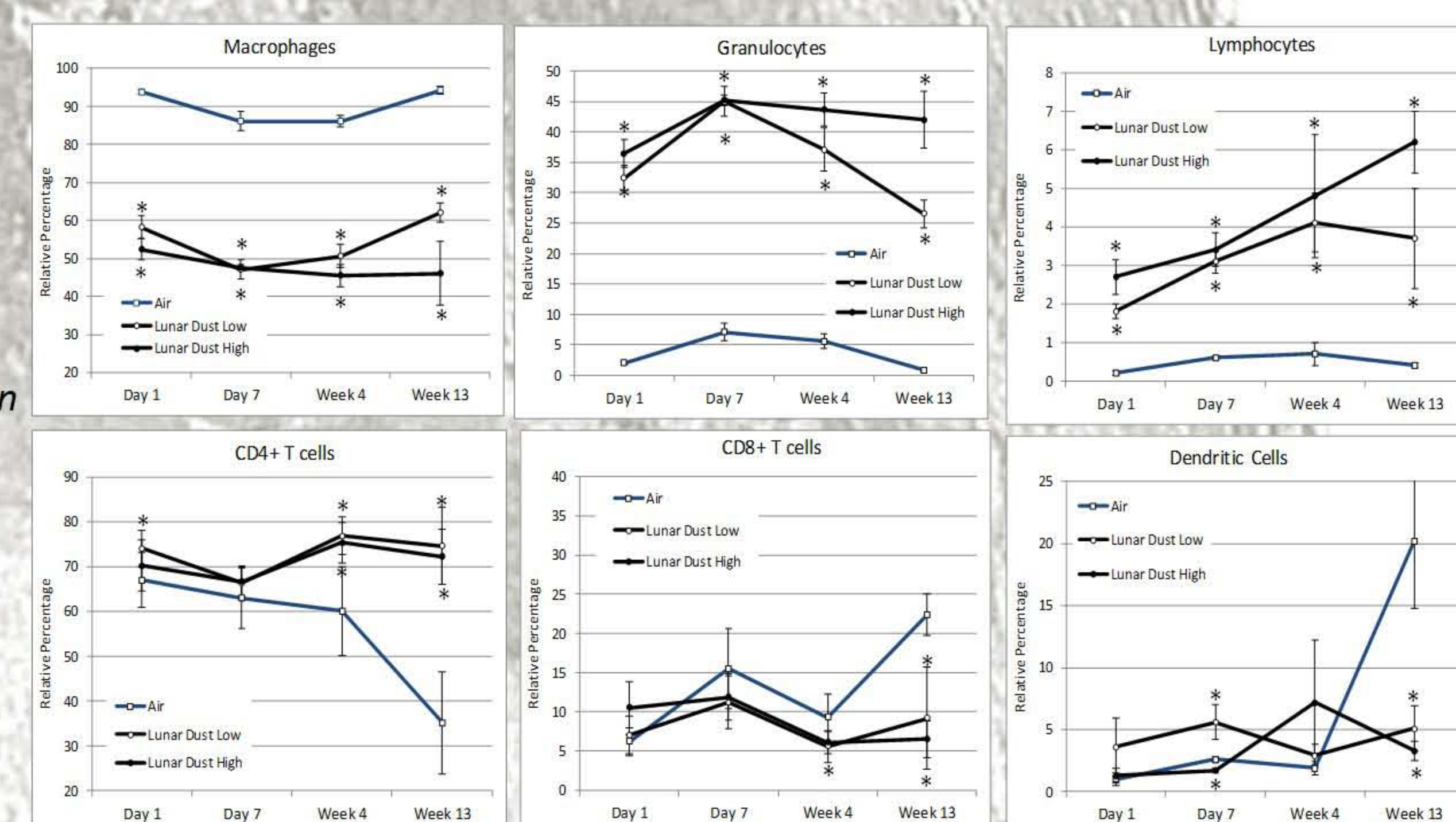
## Lavage Microscopy

### Optical Microscopy Extended Depth-of-Field



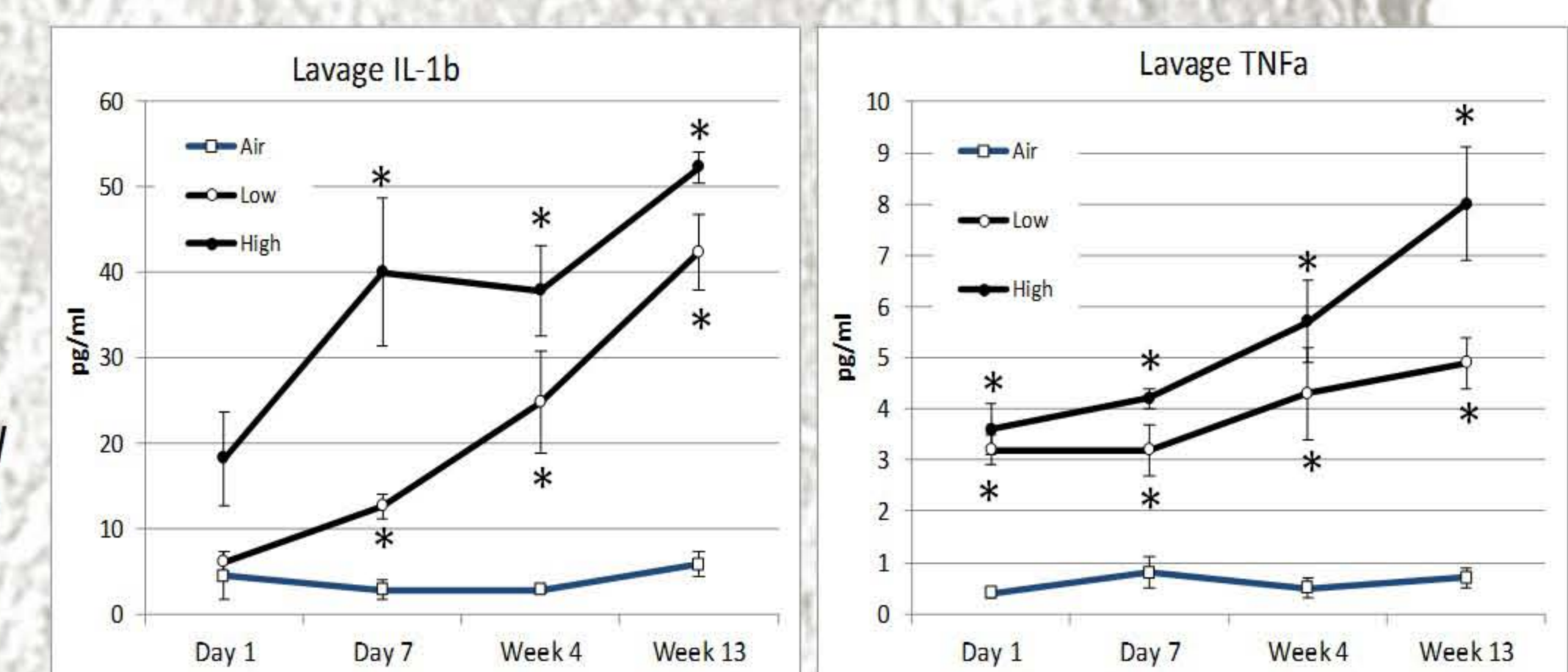
## Lavage: Leukocyte Distribution

Mean alterations in lavage leukocyte distribution by inhalation treatment and timecourse. Data are expressed as relative percentage mean  $\pm$  SEM for the indicated parameters. Significant differences are indicated (\*).



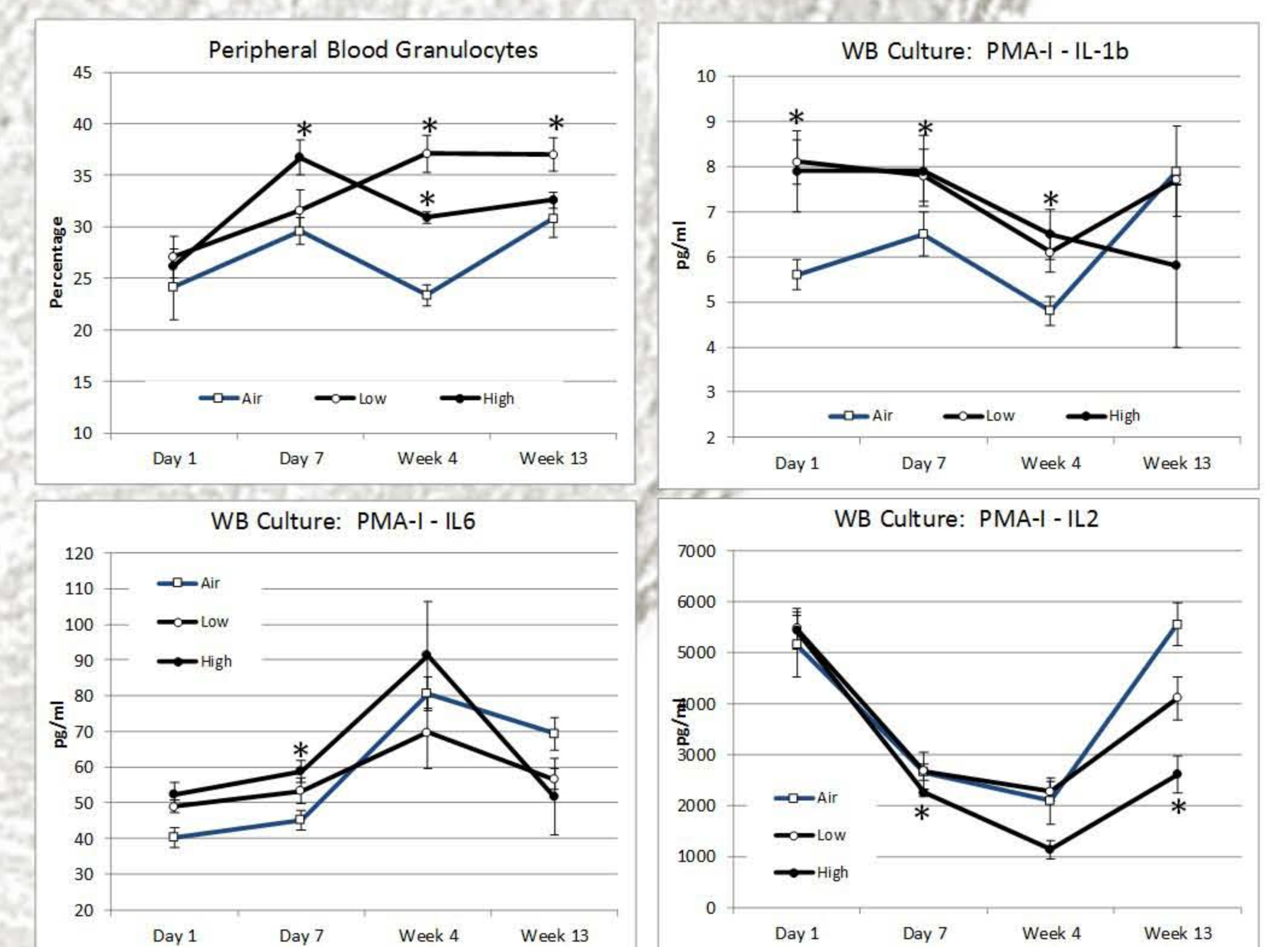
## Lavage: Cytokine Concentration

Mean alterations in lavage cytokine concentration by inhalation treatment and timecourse. Data are expressed as mean pg/ml cytokine concentration  $\pm$  SEM for the indicated analytes. Significant differences are indicated (\*).



## Blood Alterations

Mean alterations in peripheral blood leukocyte distribution and mitogen-stimulated cytokine response by inhalation treatment and timecourse. Data are expressed as mean (relative percentage of pg/ml concentration as indicated)  $\pm$  SEM for the indicated parameters. Significant differences are indicated (\*).



**Conclusion** Exposures to  $\geq 20.8$  mg/m<sup>3</sup> lunar dust resulted in lung inflammation; dust uptake by pulmonary immunocytes, and some systemic immune dysregulation that did not subside even 13 weeks after the dust exposure. This information is beneficial in deriving an exposure limit of airborne lunar dust, and for spacecraft engineers considering dust mitigation systems in lunar landers or habitats.