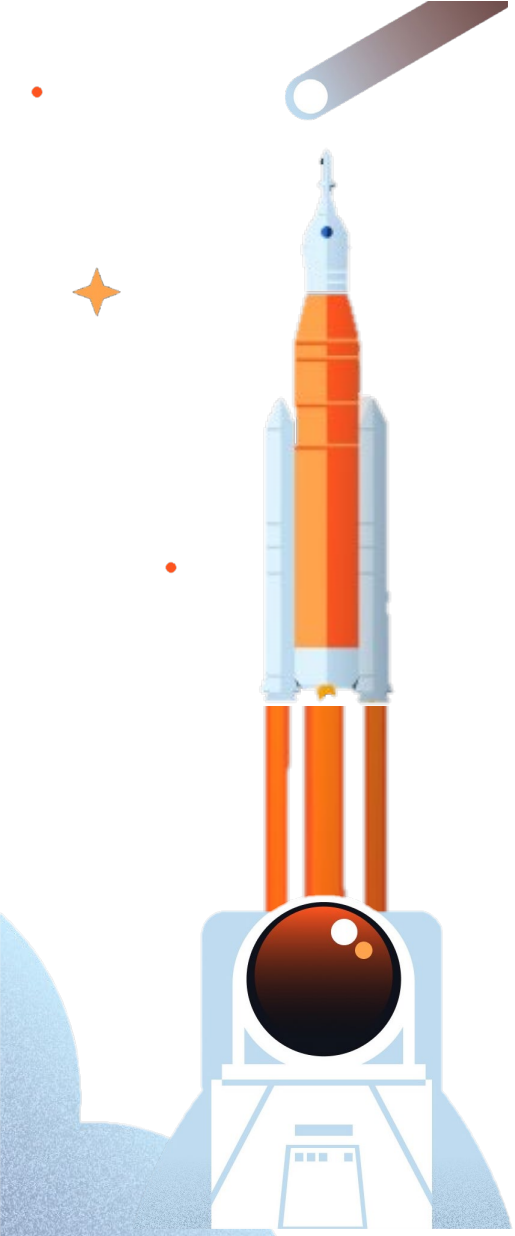

HAZARDS OF LUNAR SURFACE EXPLORATION: DETERMINING THE IMMUNOGENICITY/ALLERGENICITY OF LUNAR DUST

Audrie A. Colorado, PhD

2024 Human Research Program
Investigators' Workshop

Overview

- During the Apollo moon missions, there were consistent reports of lunar dust exposure leading to upper respiratory symptoms in both astronauts and ground support personnel
- Crew members and landing vehicles will inevitably be exposed to lunar dust in future lunar missions and the hazards associated with this are essentially unknown.
- The goal of this study is to determine, in a simple cell culture experiment, if lunar dust has the capacity to serve as an allergen, an adjuvant, or a cellular toxin.

Methods

- Co-cultures
  - PBMCs
  - Whole blood
  - Eosinophil cell line
  - Basophil cell line
- Stimulation
  - Staphylococcus enterotoxin B
  - Der p1 – common household dust mite
  - Fine ground silica quartz
  - Lunar dust
- Outcomes
  - Flow cytometry
  - Milliplex
  - ELISA
  - Cell proliferation
  - Microscopy

Flow Cytometry Data

Cellular identification and activation markers

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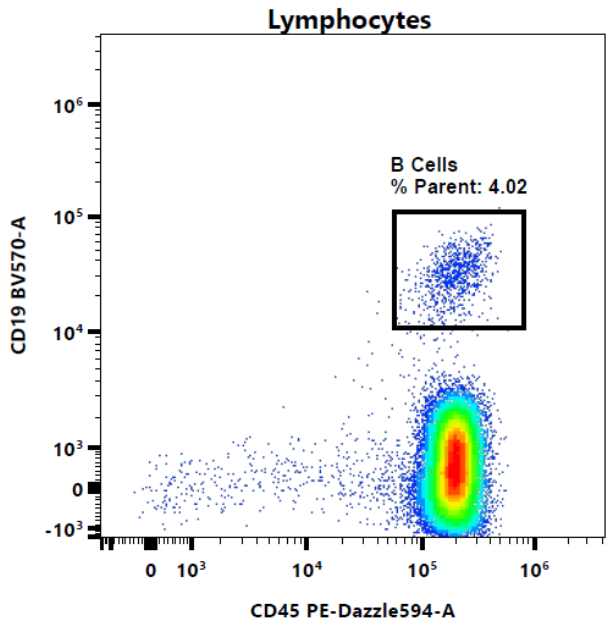
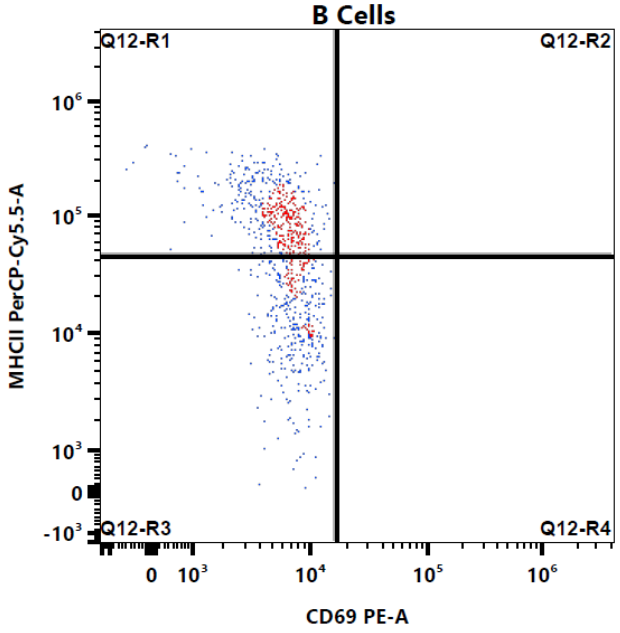
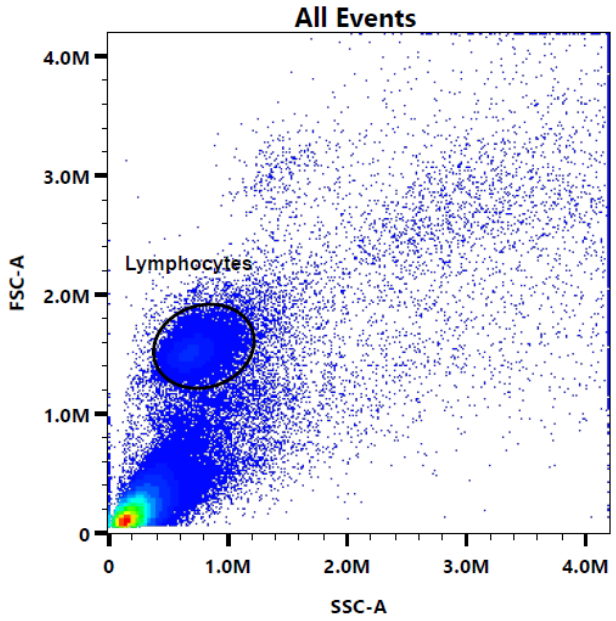
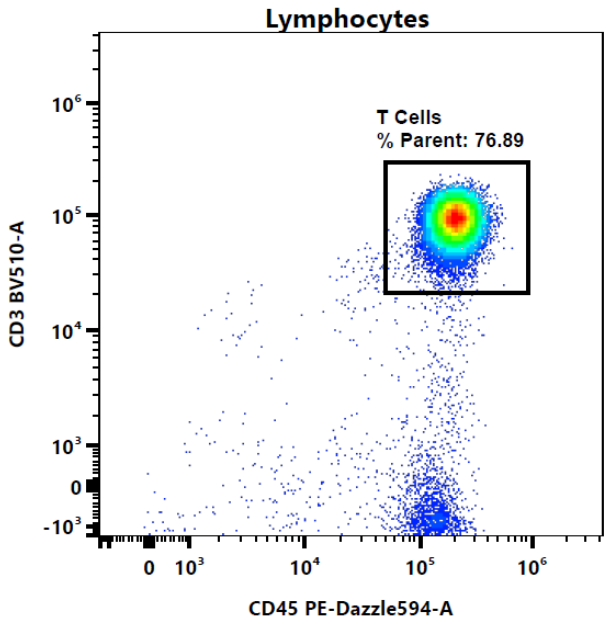
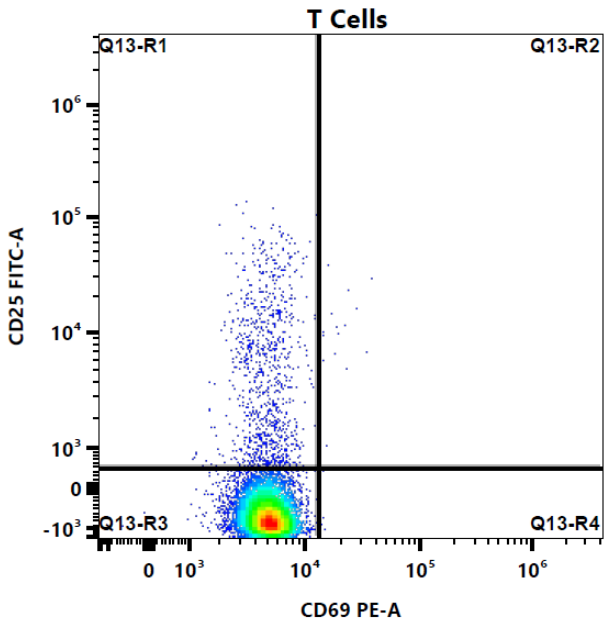
Gating Strategies

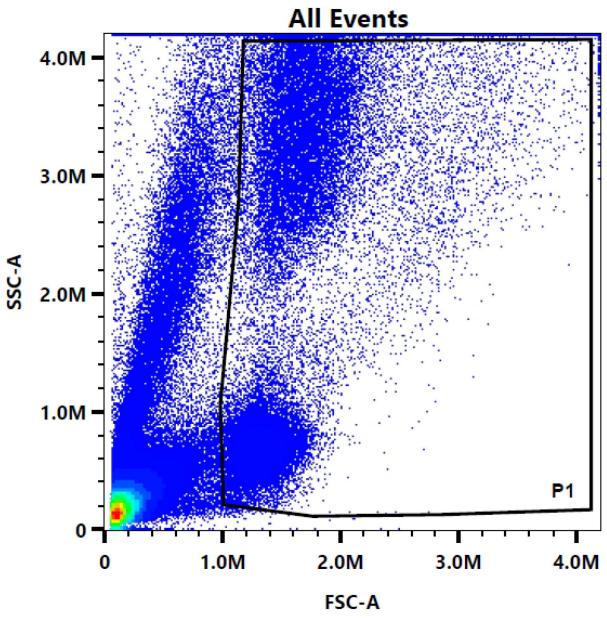


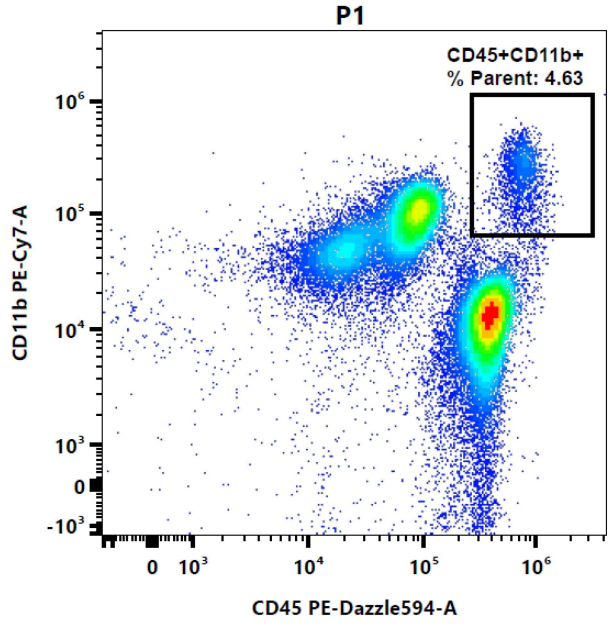


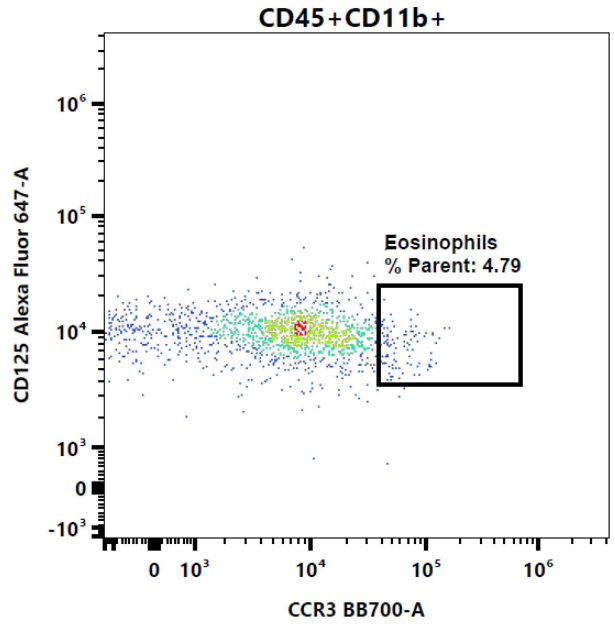


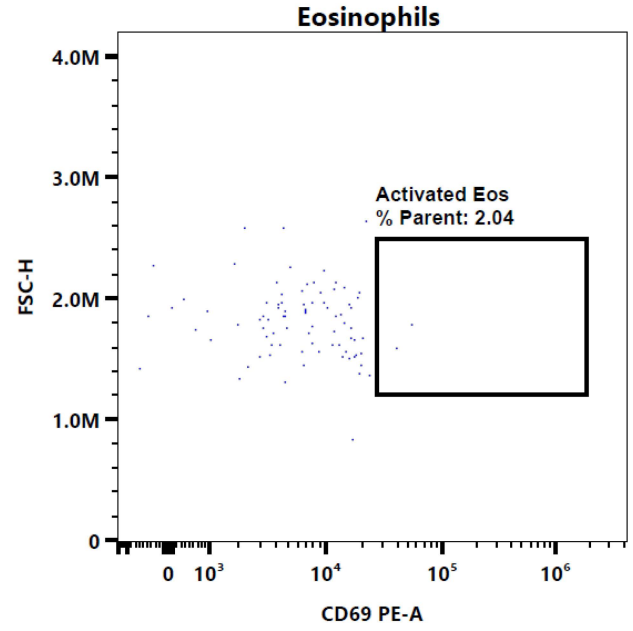


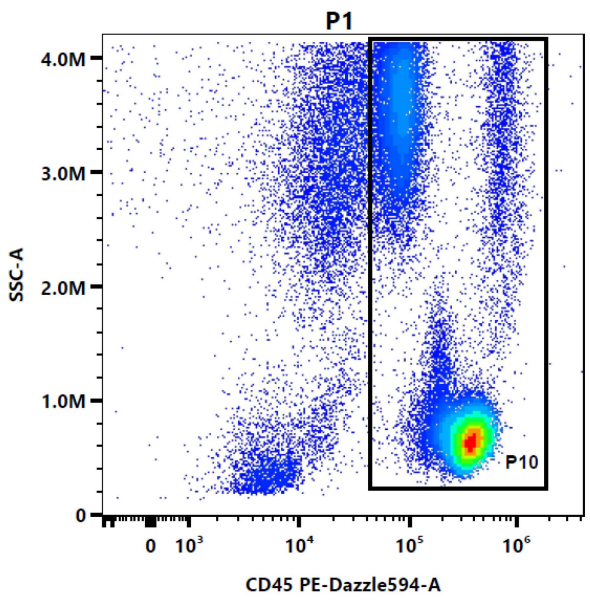


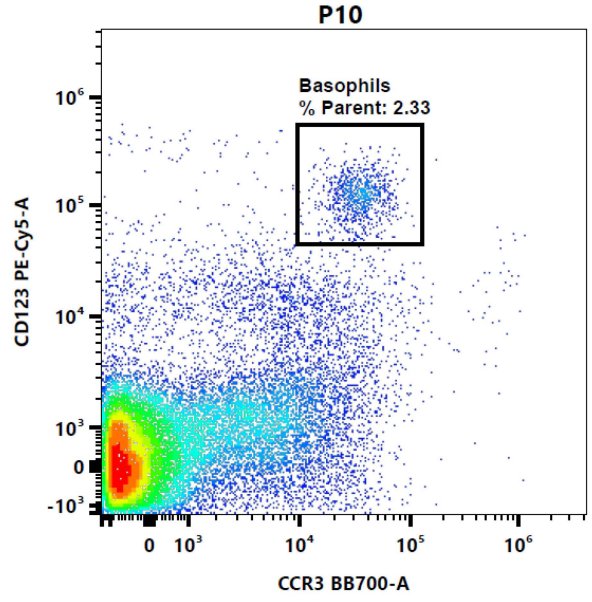

All Events
SSC-A
FSC-A
P1

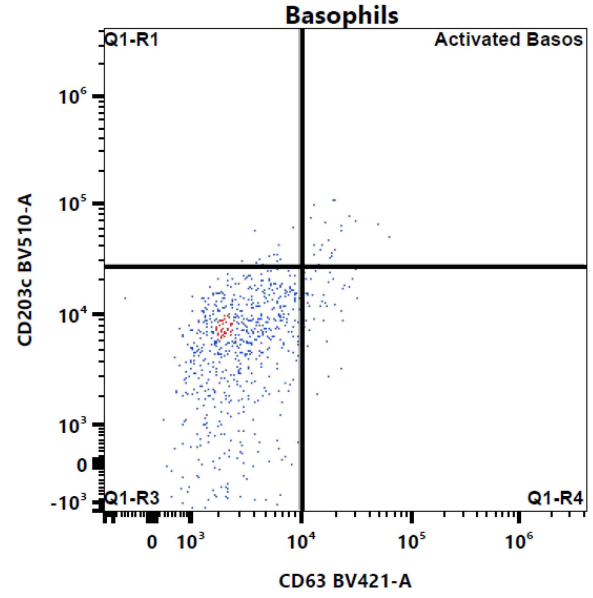

P1
CD45+CD11b+
% Parent: 4.63
CD11b PE-Cy7-A
CD45 PE-Dazzle594-A


CD45+CD11b+
Eosinophils
% Parent: 4.79
CD125 Alexa Fluor 647-A
CCR3 BB700-A


Eosinophils
Activated Eos
% Parent: 2.04
FSC-H
CD69 PE-A

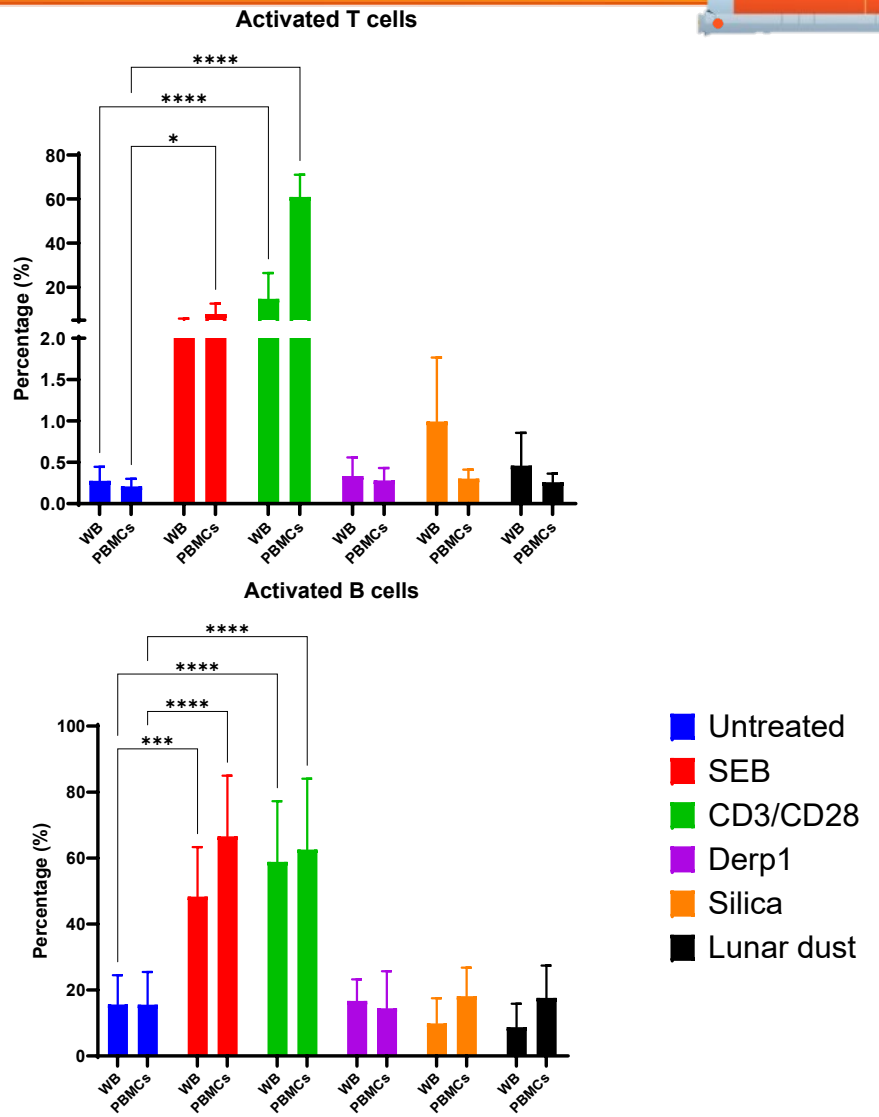

P1
P10
SSC-A
CD45 PE-Dazzle594-A


P10
Basophils
% Parent: 2.33
CD123 PE-Cy5-A
CCR3 BB700-A


Basophils
Q1-R1
Activated Basos
Q1-R3
Q1-R4
CD203c BV510-A
CD63 BV421-A

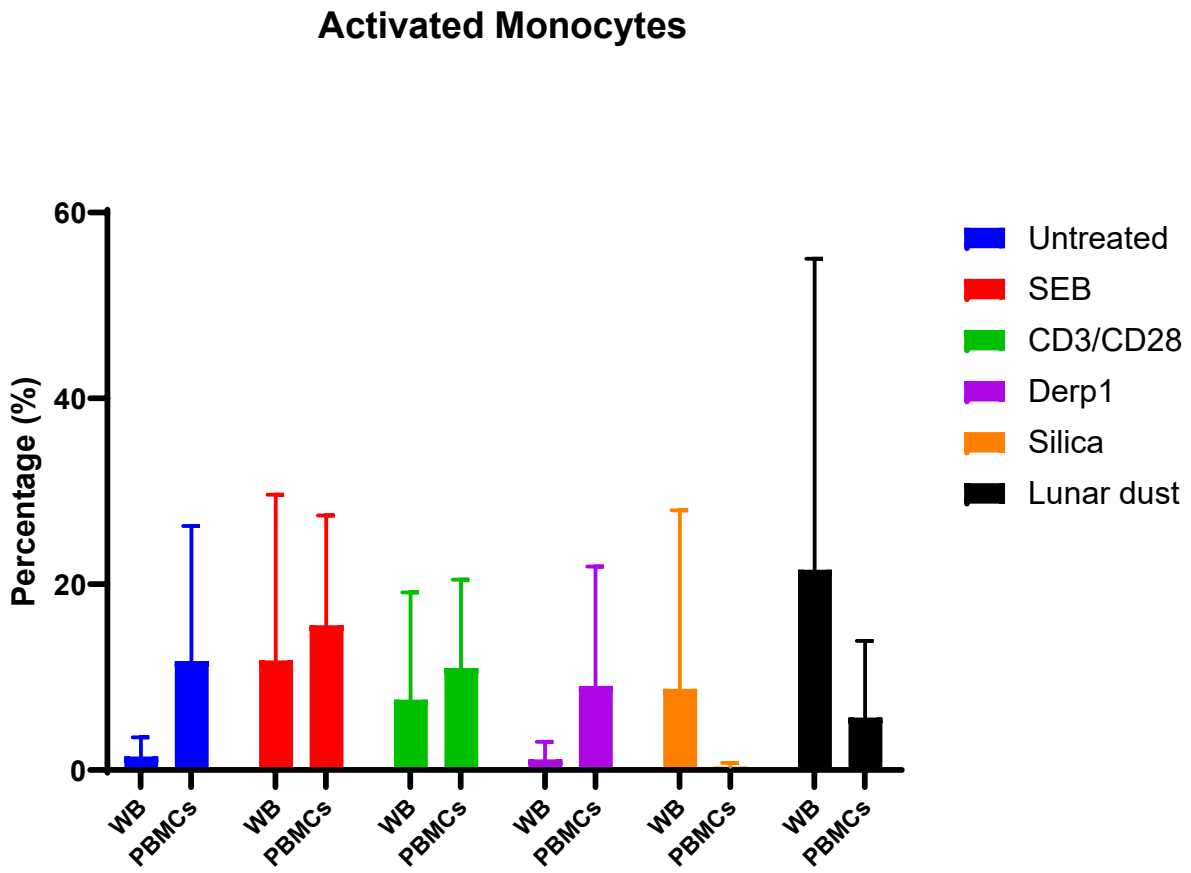
T and B Cells

- SEB and CD3/CD28 significantly activate T and B cells compared to untreated in WB and PBMC cultures



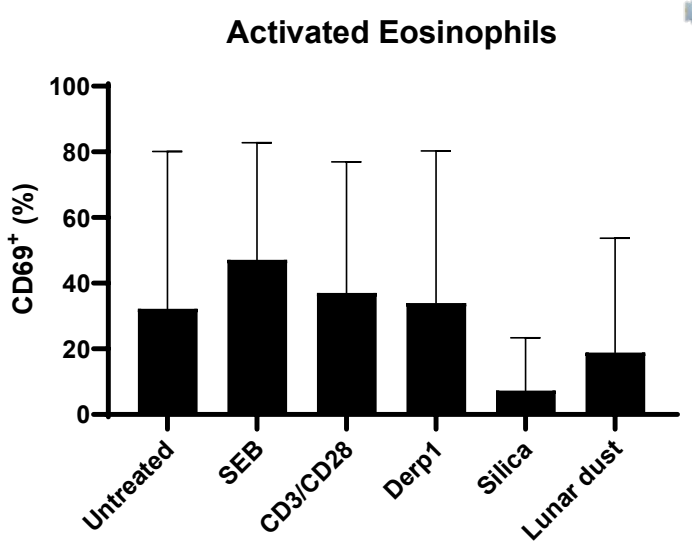
Monocytes

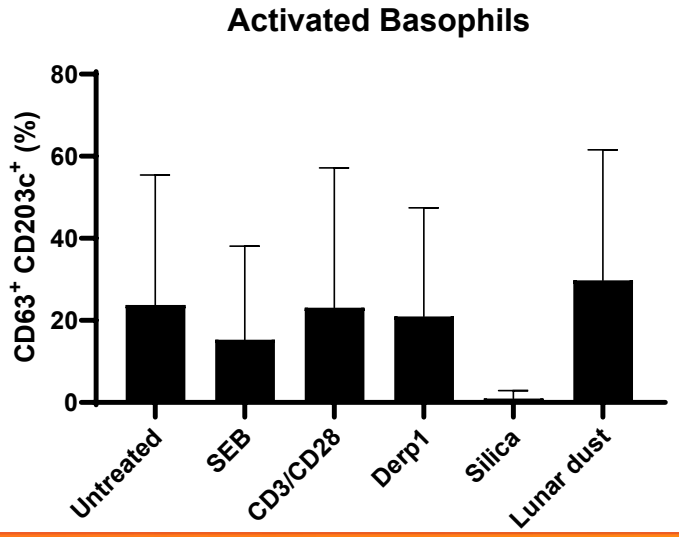
- No significant findings in monocyte activation in response to stimuli



Eosinophils and Basophils

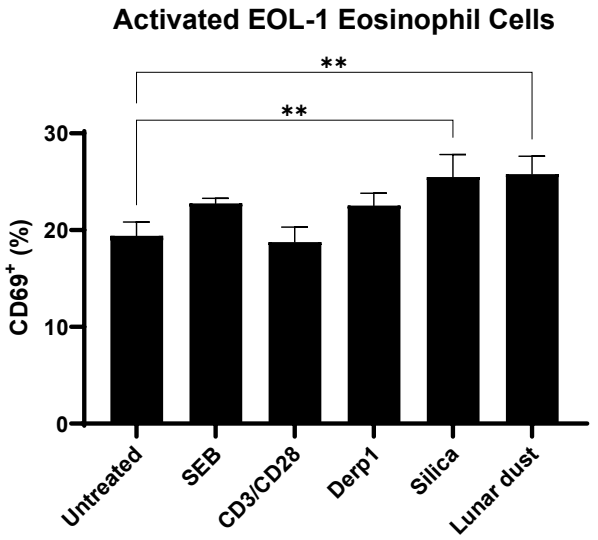
- No significant findings in activation of eosinophils or basophils

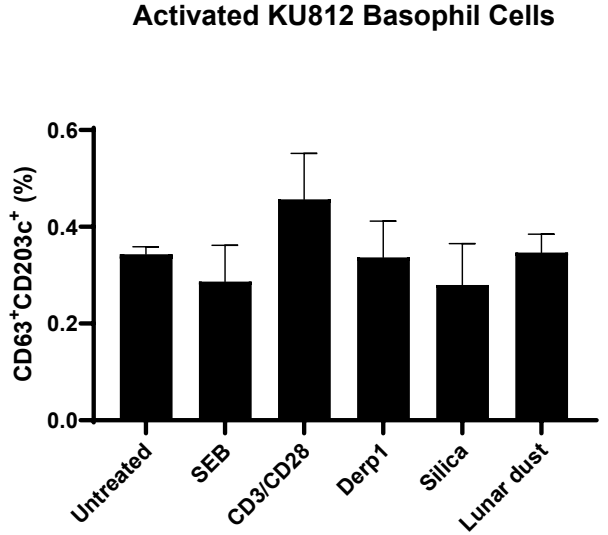




Cell Lines

- Silica and lunar dust treatment significantly induce eosinophil activation in the single cell line cultures
- No significant findings in basophil cell line cultures





Cytokine Data

13 plex array

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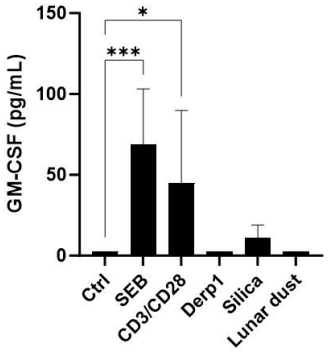
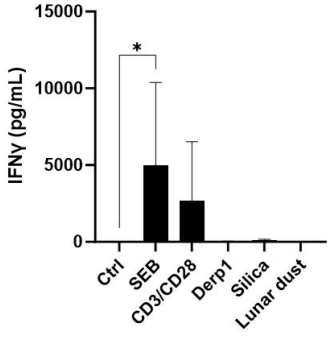
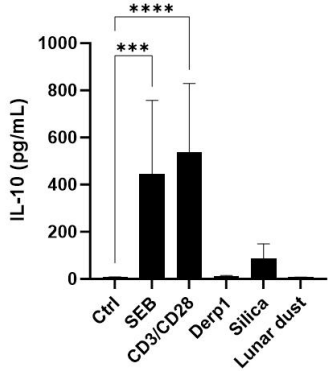
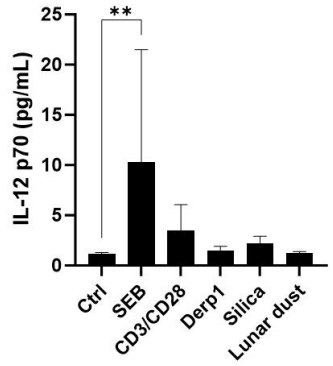
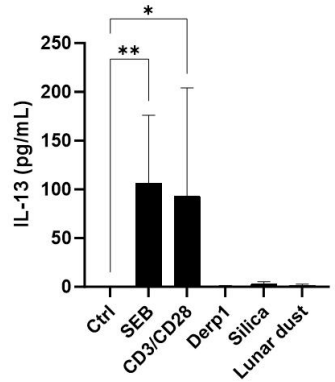
Human whole blood cultures

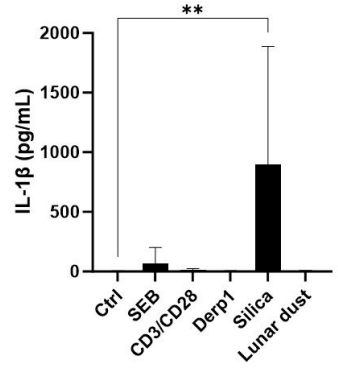


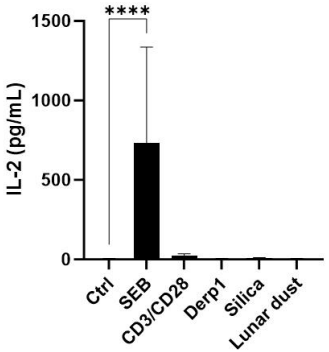


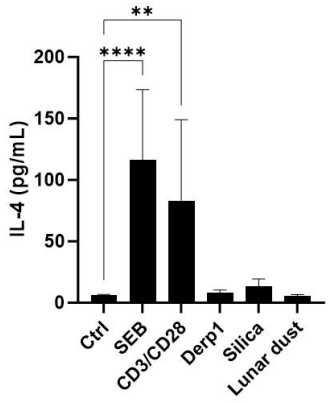


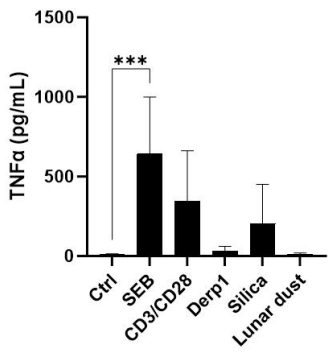


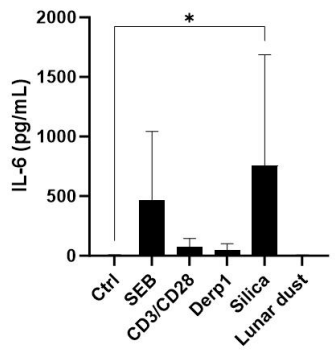


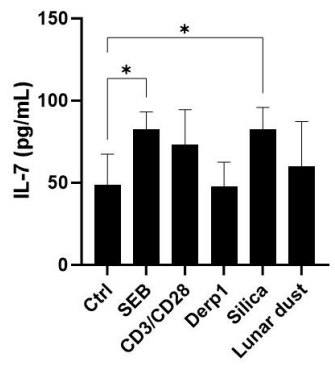


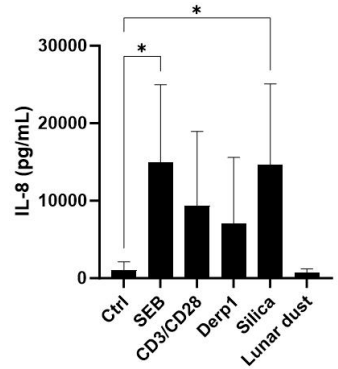




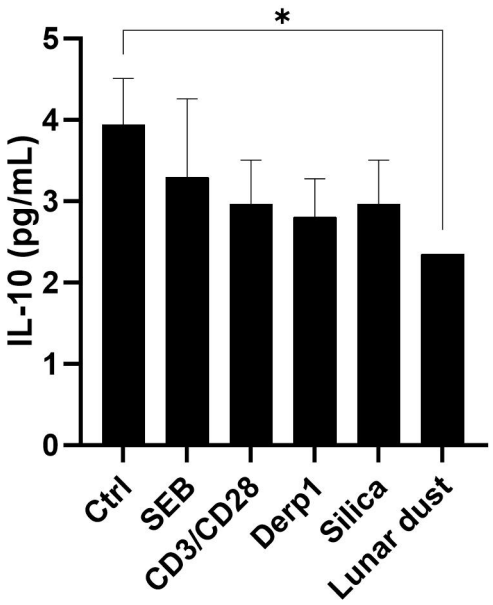


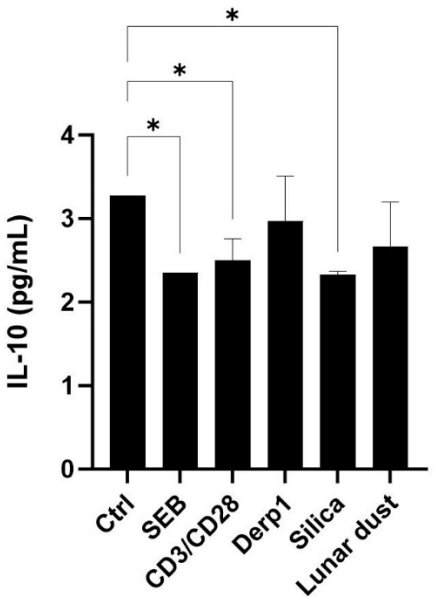




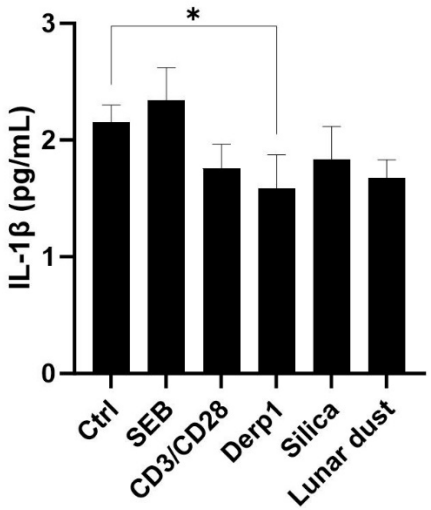


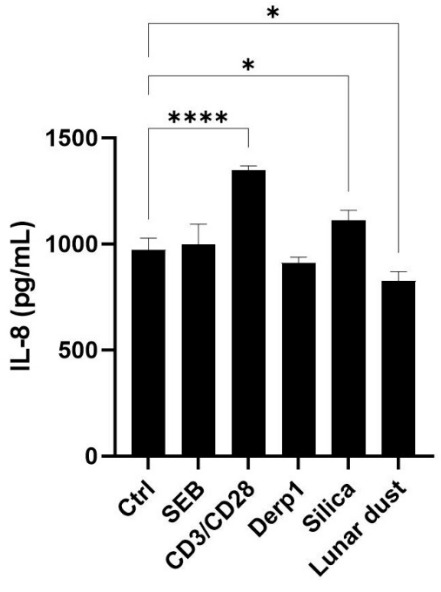
Eosinophil Cell Line





Basophil Cell Line





ELISA Data

Histamine, IgE, Leukotriene

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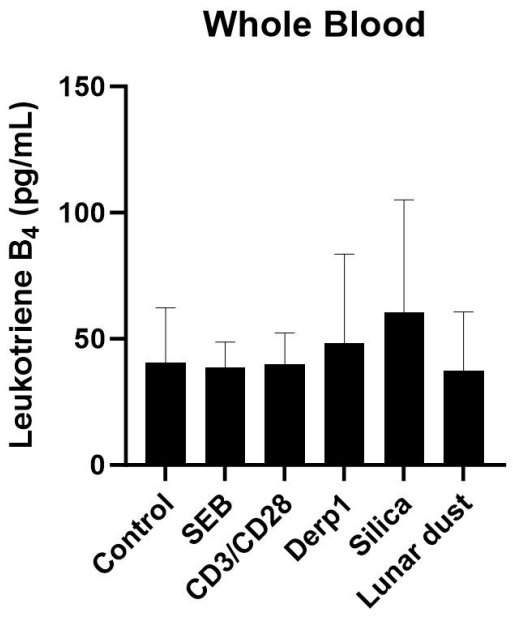
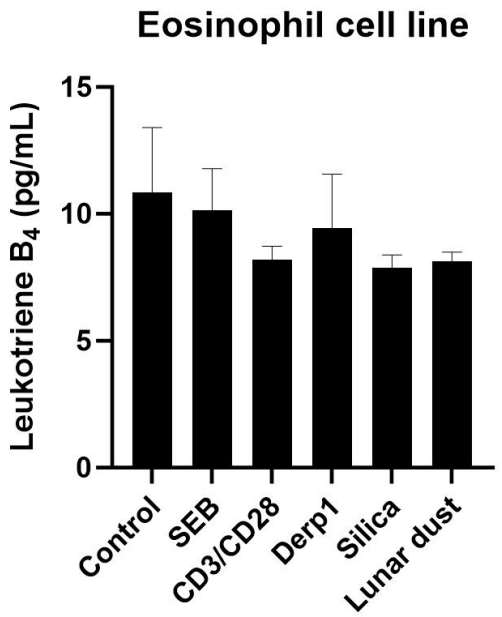
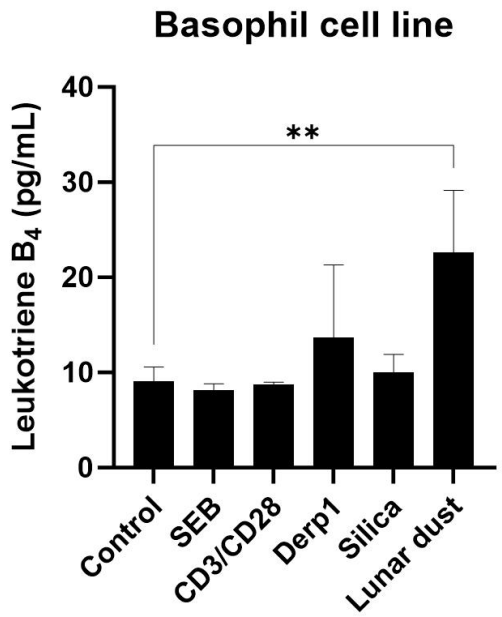
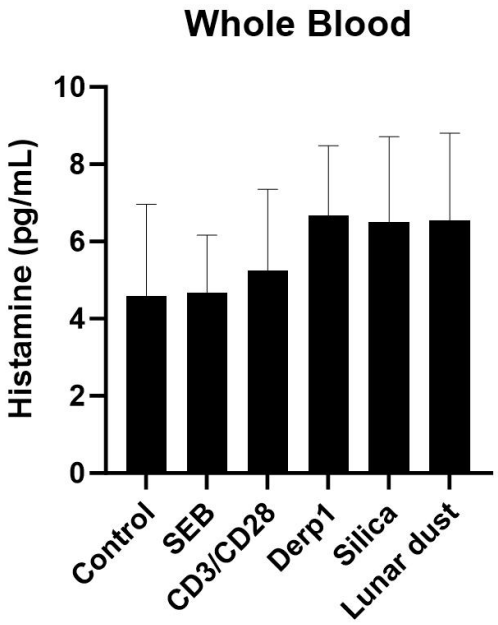
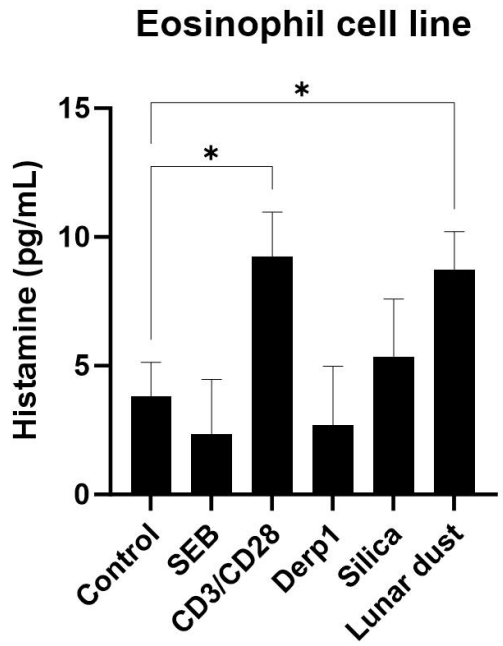
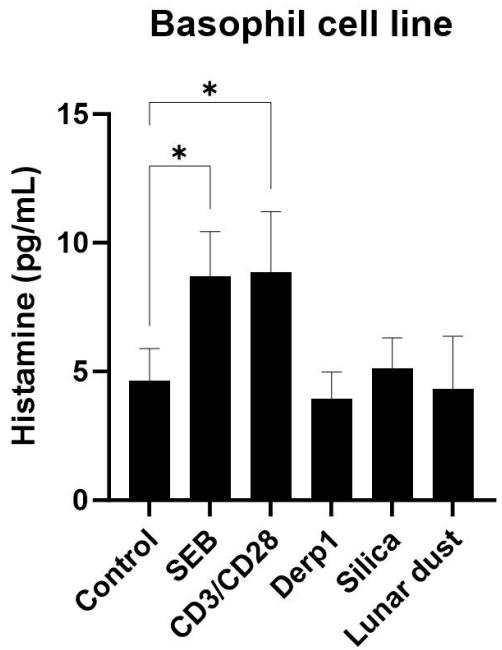

Whole Blood
Leukotriene B4 (pg/mL)
150
100
50
0
Control
SEB
CD3/CD28
Derp1
Silica
Lunar dust

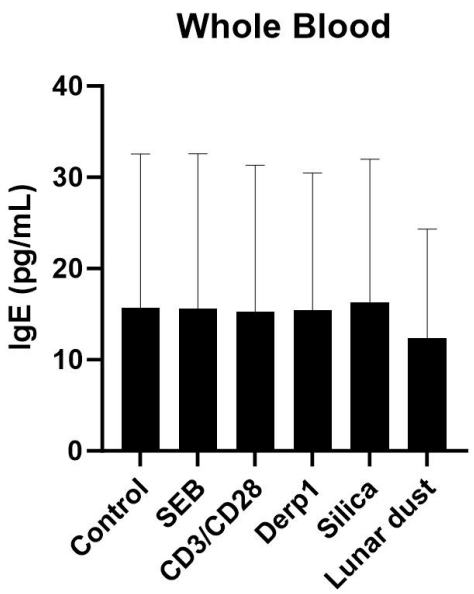

Eosinophil cell line
Leukotriene B4 (pg/mL)
15
10
5
0
Control
SEB
CD3/CD28
Derp1
Silica
Lunar dust

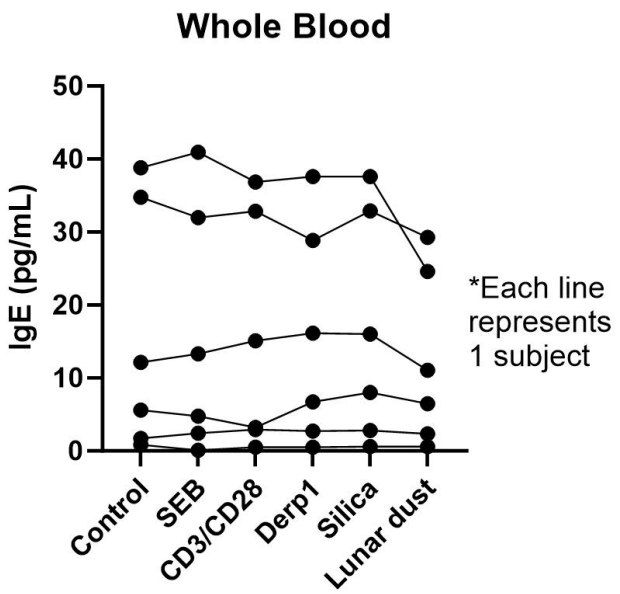

Basophil cell line
Leukotriene B4 (pg/mL)
**
40
30
20
10
0
Control
SEB
CD3/CD28
Derp1
Silica
Lunar dust


Whole Blood
Histamine (pg/mL)
0
2
4
6
8
10
Control
SEB
CD3/CD28
Derp1
Silica
Lunar dust


Eosinophil cell line
Histamine (pg/mL)
0
5
10
15
*
*
Control
SEB
CD3/CD28
Derp1
Silica
Lunar dust


Basophil cell line
Histamine (pg/mL)
0
5
10
15
*
*
Control
SEB
CD3/CD28
Derp1
Silica
Lunar dust


Whole Blood
IgE (pg/mL)
40
30
20
10
0
Control
SEB
CD3/CD28
Derp1
Silica
Lunar dust


Whole Blood
IgE (pg/mL)
50
40
30
20
10
0
Control
SEB
CD3/CD28
Derp1
Silica
Lunar dust
*Each line represents 1 subject

Microscopy Images

ESEM analysis of cell-lunar dust interactions

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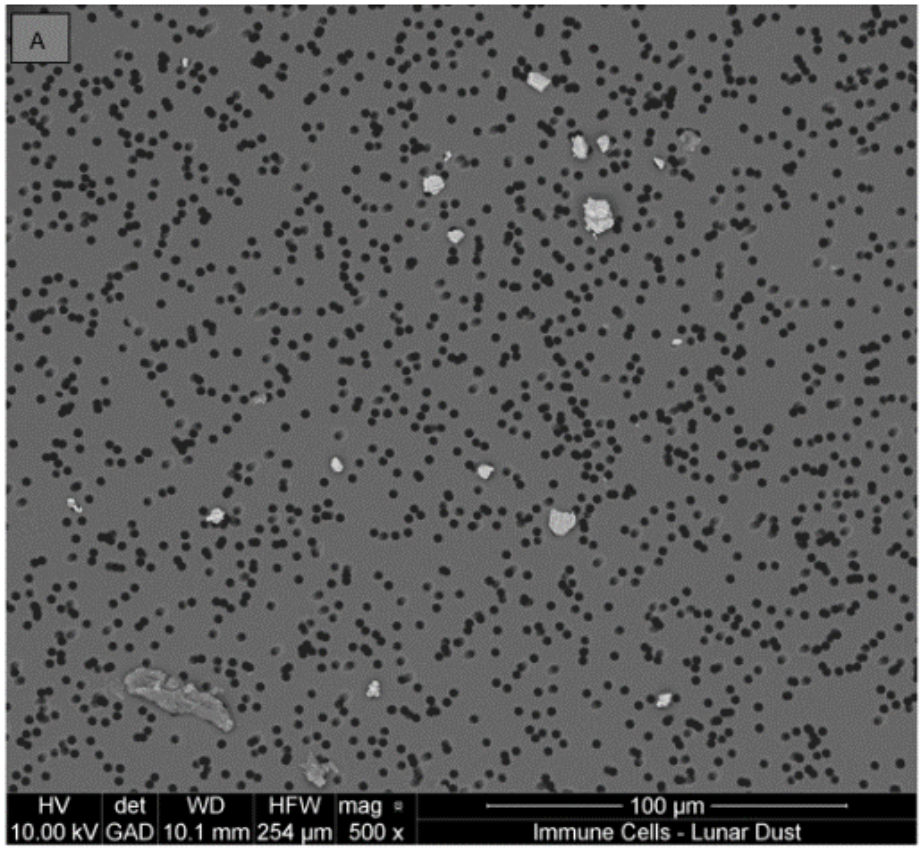
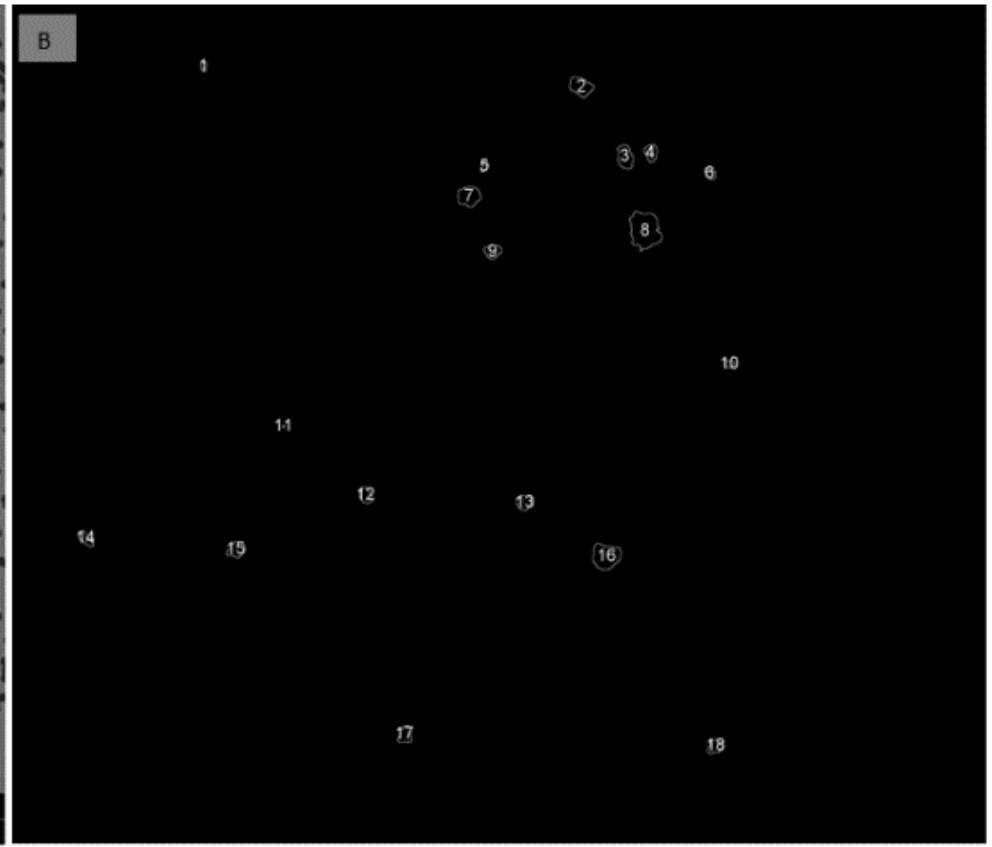
Lunar Dust – Particle Size Distribution

ESEM Imaging of LD



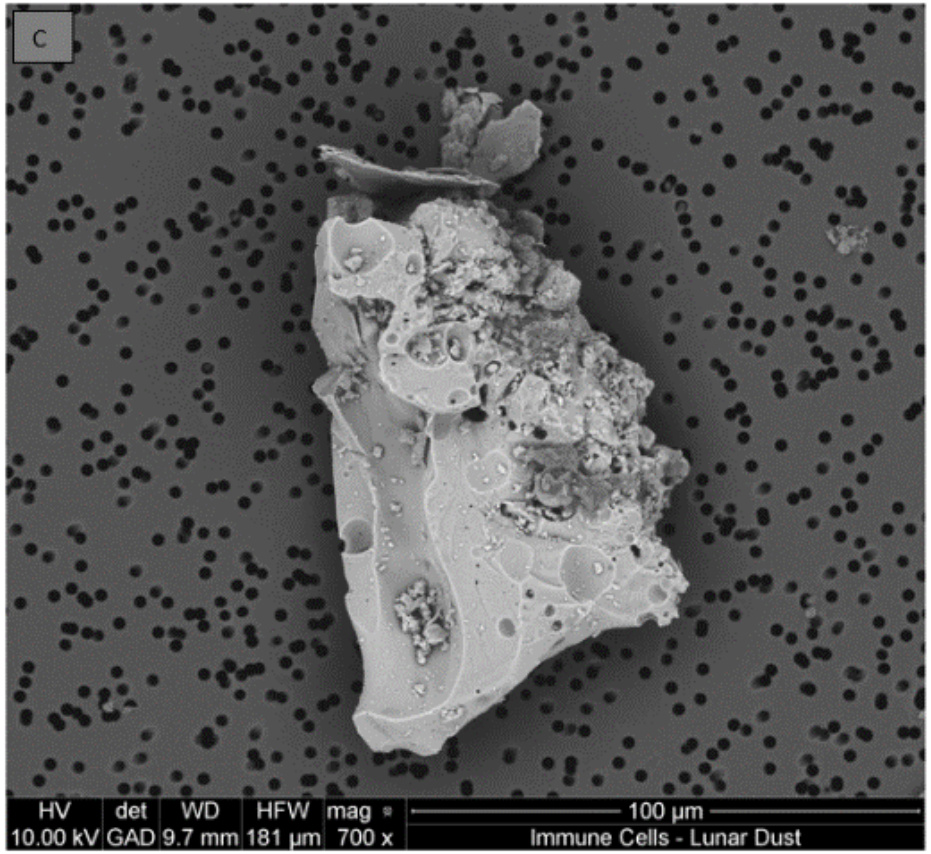
Particle Id For Size Distribution



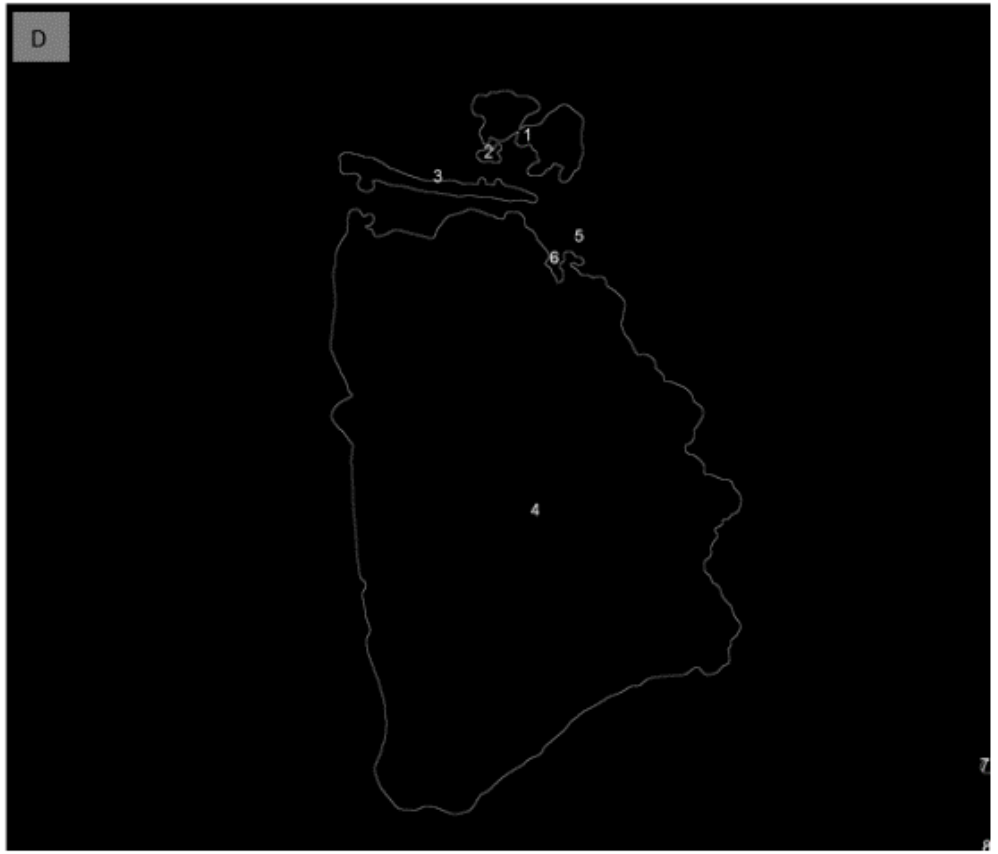
Section	Area (µm2)	Width (µm)	Height (µm)
1	3.19E+00	1.61E+00	2.48E+00
2	2.16E+01	6.33E+00	5.46E+00
3	1.96E+01	4.09E+00	6.45E+00
4	1.13E+01	3.47E+00	4.71E+00
5	3.26E+00	1.99E+00	2.73E+00
6	5.40E+00	2.85E+00	2.85E+00
7	2.40E+01	6.08E+00	5.33E+00
8	6.03E+01	8.56E+00	1.04E+01
9	1.20E+01	4.59E+00	3.72E+00
10	3.96E+00	2.73E+00	1.86E+00
11	3.23E-01	7.44E-01	4.96E-01
12	1.00E+01	3.35E+00	4.22E+00
13	1.24E+01	4.47E+00	3.97E+00
14	7.80E+00	4.22E+00	4.09E+00
15	1.35E+01	4.96E+00	4.34E+00
16	3.79E+01	7.32E+00	6.95E+00
17	1.16E+01	3.72E+00	4.47E+00
18	1.15E+01	4.22E+00	3.85E+00

Size distribution of lunar dust sample used in co-culture of Immune cells. Size distribution range from surface area 0.323 μm^2 with a width of 0.744 µm and height of 0.709 µm, up to a surface area of 60.3 μm^2 with a width of 8.56 µm and height of 10.4 µm. This depicts the large variability in the size distribution of the Lunar dust samples used for this study.

ESEM Imaging of LD In Cell Co-culture Sample



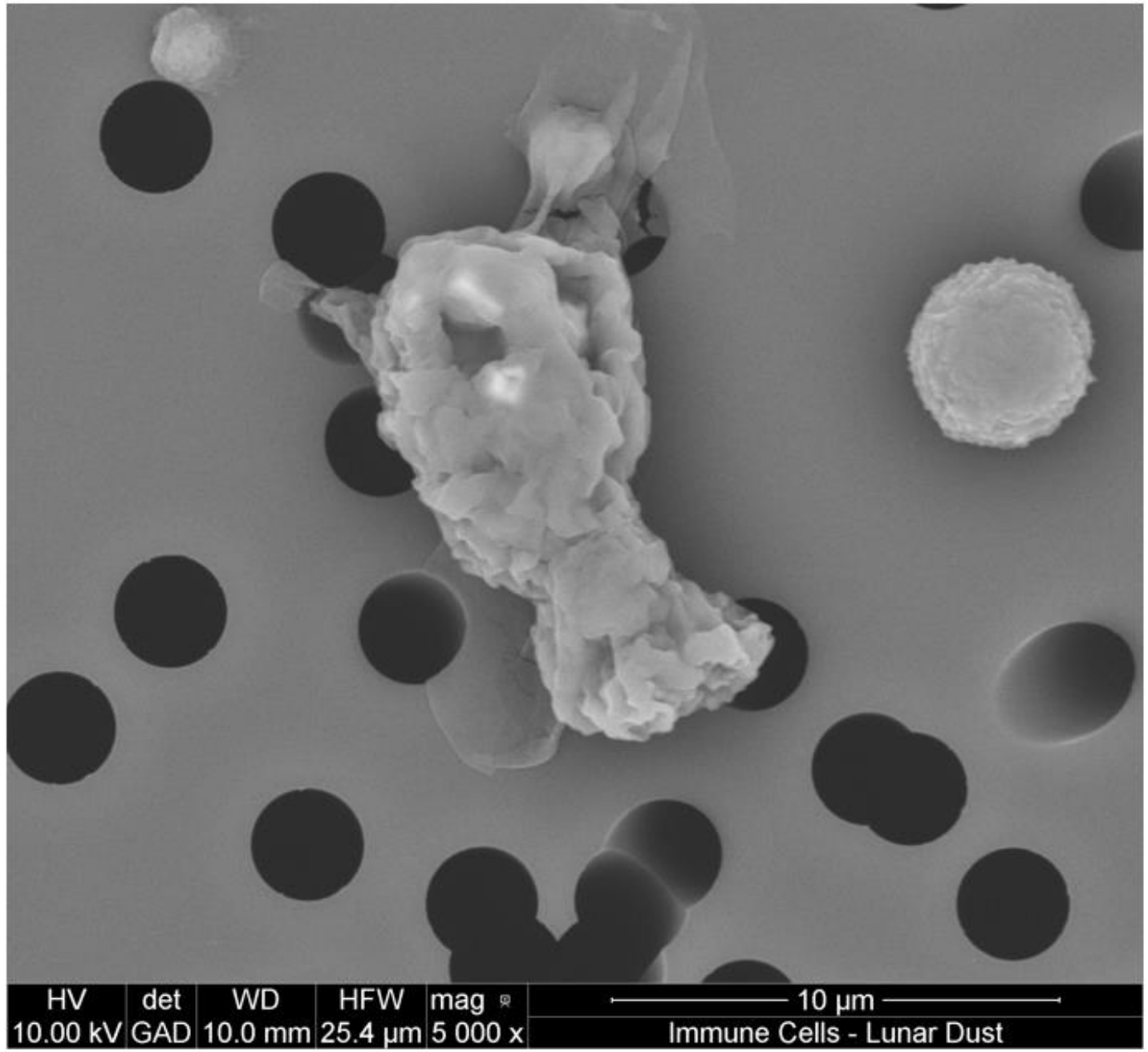
Particle Id For Size Distribution



Size distribution of lunar dust particle that was in immune cell co-culture of confirmed cellular uptake. The large particle size had a surface area of 5760 µm^2 with a width of 75.8 µm and height of 111 µm. Particles of smaller size distributions also present with the smallest particle size having a surface area of 0.472 µm^2 with a width and length of 0.709 µm. This depicts the large variability in the size distribution of the Lunar dust samples used for this study.

Section	Area (µm^2)	Width (µm)	Height (µm)
1	1.83E+02	2.06E+01	1.68E+01
2	9.93E+00	4.52E+00	3.46E+00
3	1.07E+02	3.64E+01	9.22E+00
4	5.76E+03	7.58E+01	1.11E+02
5	1.56E+00	1.42E+00	1.42E+00
6	4.72E-01	7.09E-01	7.09E-01
7	3.76E+00	1.86E+00	2.48E+00
8	9.27E-01	1.15E+00	1.06E+00

Lunar Dust PBMC Ingested Particle



Conclusions

- Lunar dust does not significantly impact activation of eosinophils, basophils, T cells, B cells, or monocytes
- Lunar did not increase lymphocyte proliferation (data not shown)
- Silica and lunar dust increased activation of eosinophils cell line
- Lunar dust increases leukotrienes and histamine in both cell lines
- The lack of response seen in primary cell cultures indicates a low risk for allergenicity of lunar dust

Acknowledgements

- **PI:**
  - Brian Crucian, *JSC NASA*
- **Co-Investigators:**
  - Cody Gutierrez, *JEStech, JSC Immunology Laboratory*
  - Mayra Nelman-Gonzalez, *KBR, JSC Immunology Laboratory*
  - Gailen Marshall, *The University of Mississippi Medical Center*
  - J. Torin Mccoy, *JSC NASA*