Flow Cytometry for Immunophenotyping of Lung Inflammation in a Pre-Clinical Murine Model of Asthma

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Background

- Asthma is the third most common chronic disease in Canada, affecting 12% of Canadian children.
- For pre-clinical research, animal models reflecting key clinical features are needed for new therapeutic development.
- We proposed to optimize a protocol for efficient immunophenotype analysis using flow cytometry to assay immune cells in bronchoalveolar lavage fluid (BALF) and lung tissue using a murine model of allergic asthma.

Methods

- We used house dust mite (HDM)-challenged BALB/c mice (female, 8-10 weeks), randomized into groups (N=6): 2-weeks (5 days/week) challenge with HDM (25 ug, intranasal instillation) or saline (control).
- Individual BALF and lung samples were collected 48 hrs after final challenge and assessed by 5-colour immunophenotyping with an Attune-NxT flow cytometer
- We used two antibody panels for cell specific markers: ***Granulocytes*** (eosinophils, neutrophils, alveolar and interstitial macrophages); or ***Lymphocytes*** (CD3+, CD4+, CD8+, and B cells).
- The % frequency of live cells for each cell sub-type was determined using Flow-Jo. Data, and analyzed by two-tailed t-test.

Figure 1: Attune NxT flow cytometer used to perform immunophenotype analysis

Gating Sequences for Granulocyte and Lymphocyte Sub-populations

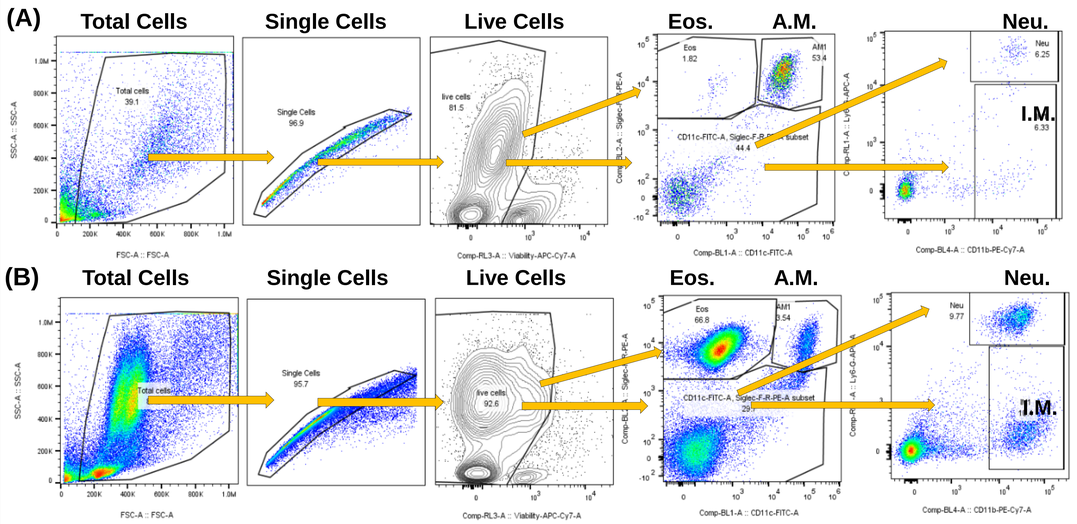

Figure 2: Gating sequence for the Granulocyte panel (eosinophils (Eos.), neutrophils (Neu.), alveolar macrophages (A.M.) and interstitial macrophages (I.M.)) shown for **A.** naïve mouse and **B**. HDM challenge mouse

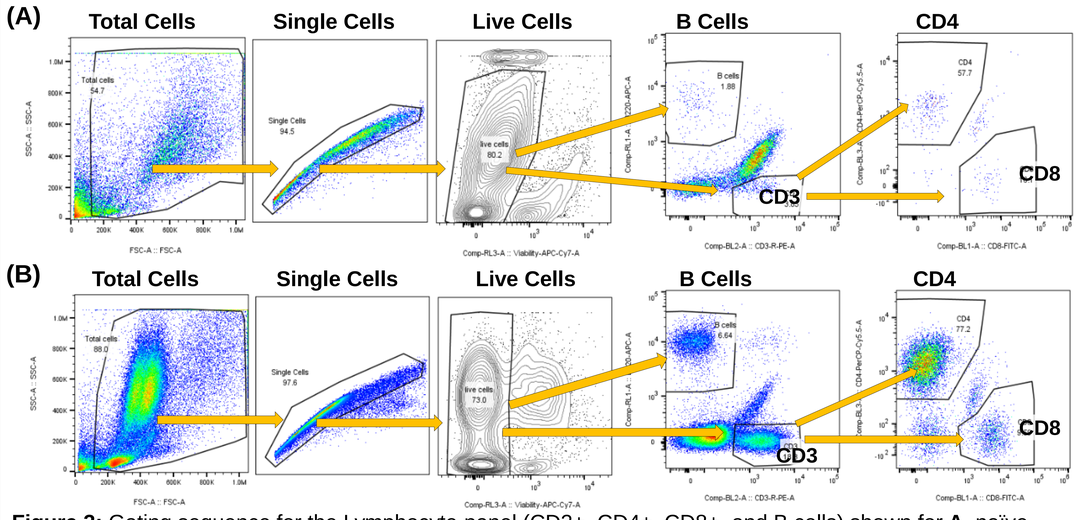

Figure 3: Gating sequence for the Lymphocyte panel (CD3+, CD4+, CD8+, and B cells) shown for **A.** naïve mouse and **B**. HDM challenge mouse

Acknowledgements

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Results

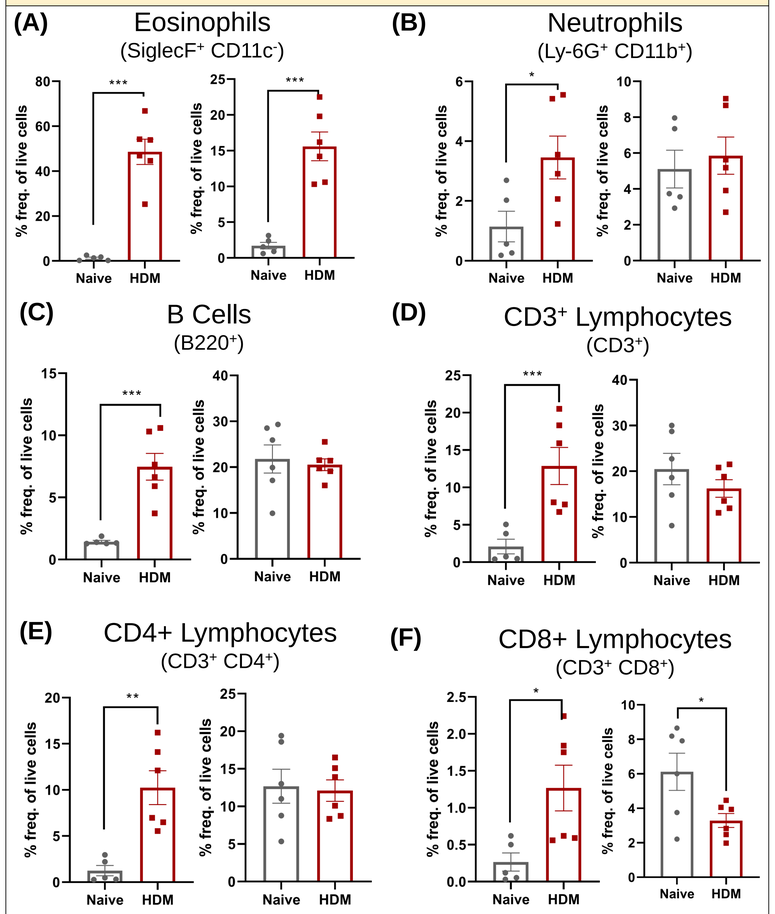

Figure 4: Comparison of the % frequency of live cells for the Granulocyte and Lymphocyte sub-types determined using FlowJo. **A.** eosinophils in BALF (left) and lung tissue samples (right), **B.** neutrophils in BALF (left) and lung tissue samples (right), **C.** B cells in BALF (left) and lung tissue samples (right), **D**. CD3 cells in BALF (left) and lung tissue samples (right), **E.** CD4 cells in BALF (left) and lung tissue samples (right), and **F.** CD8 cells in BALF (left) and lung tissue samples (right).

Conclusions

Immunophenotyping by flow cytometry is an effective means of broadly and rapidly assessing immune cells in the mouse lung. Moreover, it discriminates the immune response to allergen challenge in lung tissue and the airway compartment, and offers excellent potential to discriminate cellular mechanisms for asthma pathobiology.